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Current Status of Targeting Induced Local Lesions IN Genomes (TILLING) and its Relevance to Plant Functional Genomics, Polymorphism Assessment and Plant Breeding with Relevant Case Studies on Different Crops

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Abstract

One of the most important breakthroughs in the history of genetics was the discovery that mutations can be induced. TILLING (Targeting induced local lesions in genomes) a newly developed general reverse genetic strategy helps to locate an allelic series of induced point mutations in genes of interest. It was first explored and introduced 17 years ago in the early 2000's by the efforts of Claire McCallum and her collaborators. TILLING allows the rapid and inexpensive detection of induced point mutations in populations of physically/chemically mutagenized individuals. EcoTILLING is an extension of TILLING, which uncover natural alleles at a locus contrary to induced mutations. In This review the over view of history, techniques, of TILLING and EcoTILLING and their application in functional genomics, DNA polymorphism assessment and crop breeding with relevant case study in different crops illustrated. Furthermore, this review describes the potentials, challenges and future prospects of TILLING.

Keywords: EcoTILLING; TILLING; Reverse genetics

Introduction

One of the most important breakthroughs in the history of genetics was the discovery that mutations can be induced. The high frequency with which ionizing radiation and certain chemicals can cause genes to mutate made it possible to perform genetic studies that were not feasible when only spontaneous mutations were available. Crop improvement has a long history as the key agronomic traits have been selected over hundreds of years during the domestication of crops [1].

Novel DNA sequence information allows the development of additional molecular markers for breeding of important traits and provides targets for transgenic alteration of gene expression. While very powerful, transgenic approaches have been met with a high level of public disapproval and the use of the methods for food production is currently banned in many countries [2]. And the forward genetics can hardly meet the demand of a high-throughput and large-scale survey of gene functions as most of the phenotypes are obscure. This calls for an alternative, non-transgenic, targeted approach for crop improvement in order to meet the increasing demand in food production. The sequence information available in public database has highlighted the need to develop genome scale reverse genetic strategies for functional analysis [3].

TILLING (Targeting induced local lesions in genomes) a newly developed general reverse genetic strategy helps to locate an allelic series of induced point mutations in genes of interest. It allows the rapid and inexpensive detection of induced point mutations in populations of physically/chemically mutagenized individuals [4]. In addition to allowing efficient detection of mutations by TILLING approach, EcoTILLING technology is also ideal for examining natural variation. It can be performed more inexpensive than full sequencing the methods currently used for the most single nucleotide polymorphism (SNP) discovery. SNP variation can provide clues to the adaptive strategies and population history that undoubtedly played roles in specie's evolution. It is also used for screening and detection of plants with desired traits by knockdown and knockout mutations in specific genes. This makes TILLING and EcoTILLING an attractive strategy for a wide range of applications from the basic functional genomics study to practical crop breeding [5].

An extension of TILLING is EcoTILLING, which uncover natural alleles at a locus contrary to induced mutations. In this strategy, each ecotype is pooled in 1:1 ratio with the standard. Moreover, in open pollinated crops, EcoTILLING can be used to find the heterozygosity level within a gene fragment. As the CEL I can digest small proportion of the hetroduplexes, it can be used to find the multiple polymorphisms in a single gene of interest. Furthermore, phylogenetic diversity estimates, selection and linkage disequilibrium can be estimated. It can be used to detect the DNA polymorphism in satellite repeat numbers. It can also be effectively used as an efficient, rapid technique to identify DNA polymorphisms in populations with high genetic similarity and to mine for SNPs in collections of plant germplasm.

Sequence information alone may be sufficient to consider a gene of interest, because sequence comparison tools that detect protein sequence similarity to previously studied genes often allow a related function to be inferred. Hypotheses concerning gene function that are generated in this way must be confirmed empirically. Experimental determination of gene function is desirable in other situations as well, for example, when a genetic interval has been associated with a phenotype of interest. In such cases, the functions of genes in an interval can be inferred by using reverse genetic methods. This review

illustrate the over view of history, techniques, of TILLING and EcoTILLING and their application in functional genomics, DNA polymorphism assessment and crop breeding with relevant case study in different crops. Furthermore, this review discusses the potentials and challenges of TILLING in Ethiopia.

Current Status of TILLING and EcoTILLING

Since the year of its development both TILLING and EcoTILLING have been used for different plants. TILLING was developed in 2000 while the EcoTILLING method was developed by Comai et al. [6] for detecting multiple types of polymorphisms in natural populations. EcoTILLING technique is particularly useful for plants requiring a long time for reproduction and for crops propagated vegetatively where creation of mutagenized populations is very difficult [7].

TILLING and EcoTILLING technique have been adapted in diverse species including rice, maize, Lotus, poplar, Arabidopsis, wheat, barley, potato, tomato, sunflower, common bean, Field Mustard, clover, melon, pea, peanut, sorghum, rapeseed, soybean, melon, poplar, sugarcane, brassica and other for the purpose of gene detection, functional genomics, polymorphism assessment, plant breeding as described in case study part. The protocol of EcoTILLING is slightly different from TILLING as it uses a mixture of the genomic DNA from a queried individual and a reference individual. SNPs discovery for indexing variation is essentially the same as that used in TILLING.

Potentials and Challenges of TILLING in Ethiopia

TILLING new tool for creating genetic variability is Tilling. The method combines a standard and efficient technique of mutagenesis using a chemical mutagen such as Ethyl methanesulfonate (EMS) with a sensitive DNA screening-technique that identifies single base mutations in a target gene. According to Ethiopian Institute Agricultural Research National Crop Biotechnology Research Strategic Plan (2017-2030) the Potentials of TILLING only limited to research effort on tef with the collaboration of foreign institution.

Challenges Encounters in Application of TILLING

According to different scholars, there are some scientific challenges encountered in TILLING experiments; TILLING uses the different concentration of the chemical mutagen to assess lethality to find an optimal concentration for conducting the experiments in the first step of generating a population [8]. The lethality of physical/chemical mutagens is different from species and varieties and required two to three years to develop high-quality mutant population [7,9,10]. More than 50% survival of the mutant population is the prerequisite for an ideal population but generating a mutant population in vegetatively propagated plants are very slow [7,11]. In addition, the production and maintenance of clones of vegetatively propagated plants for future analysis are somewhat problematic.

Mutation detection for species that are highly heterozygous is confusing for researchers due to natural polymorphisms in the genome, which may frighten in the finding of rare induced mutations [12]. After the creation of mutant population, it is essential that all DNA extracts be equivalent in concentration so that they are all correspondingly characterized in the pools being investigated. Otherwise, unique induced mutations may not be recognized as the amount of mutant DNA decreases in contrast to others in a pool of DNA.

The selection of target genes that sometimes exist as a single copy throughout the genome is an assignment particularly in plants as experimenting on polyploid plants that have complex genomes such as wheat or peanut leads to a problem. To overcome this challenge primer need to be designed that is precise to a single gene of interest, which may entail some extra effort to sequence the multiple alignments of the homologous target genes to find restriction site differences between the target genes [13]. The DNA can be digested, which may leave the irritating target leaving the desired gene unbroken for analysis in TILLING [14].

The identification, scoring, and tracking of cleaved fragments become more challenging as TILLING may increase the number of SNPs per fragment. Since large numbers of SNPs exist in a gene portion care should be taken during scoring fragments [6,14,15]. In Eco-TILLING or TILLING experiment, the selection of the nuclease is very important to digest the mismatches in the heteroduplexes. CEL I identify and cleave mismatched fragments in a heteroduplex and have 5' to 3' exonucleolytic activity that can digest the full-length PCR product starting with the 5' fluorescent label [8,16].

The last challenge is allocating a particular phenotype to a genotype and supposing the putative function of a gene. Chemical mutagenesis sometimes creates background mutations, which can make phenotype analysis more difficult [17]. This may take several generations of outcrossing or backcrossing [8,18]. Obviously, to assign a function to a gene will be more challenging if there is any epistasis or pleiotropic effects created from the background mutations [19].

Future Perspectives of TILLING

Mutagenesis is one of the few biotechnologies currently that is used much more in developing countries than elsewhere. Both radiation and chemical mutagenesis have been used for crop improvement since the 1930s. The TILLING method has continued to gain in popularity since its first description in 2000. There are many active TILLING projects; some are at the level of fully operational TILLING services, while others are just at the beginning of platform development for a new species. TILLING has been adapted to over 20 species, and many groups host websites describing projects and progress.

TILLING projects can be grouped into two broad categories: internally focused projects aimed at addressing specific biological problems, and service-based projects aimed at providing screening services to one or more research community. The first publicly available TILLING service was the Arabidopsis TILLING Project run by the Seattle TILLING Project (http://Tilling.fhcrc.org/).

In addition to public screening services, large-scale projects devoted to specific biological questions have been developed. For example, the Centre for Novel Agricultural Products (CNAP) at the University of York has recently initiated a project to obtain high yielding cultivars of Artemisia annua, the source of the anti-malarial component artemisinin, using the TILLING technique. Because of its high demand, the drug is becoming more expensive and is not affordable in poorer countries where the epidemics of malaria are high. Thus TILLING could have a major impact in the production of medicines, exemplifying the utility of TILLING for crop improvement for non-food production. TILLING can also be envisioned as a tool for the production of superior crops for other non-food uses such as biofuel production [20].

The Joint FAO/IAEA Programme has therefore initiated a TILLING project in cassava as a means for directly querying the putative mutants for lesions in target genes before further field trialing. The mutants developed jointly with International Centre for Tropical Agriculture (CIAT) are an important resource for developing the cassava TILLING and EcoTILLING platforms. The cassava genome is currently being sequenced and the imminent increase in sequence information will lead to a routine integration of TILLING in cassava induced mutagenesis as a crop improvement strategy. Conversely, induced mutagenesis and efficient reverse genetics strategies such as TILLING will contribute significantly to a rapid use of the bourgeoning sequence information in functional genomics studies in cassava. Parallel to setting up the cassava TILLING platform, efforts are also being directed at mitigating the effects of chimerism in cassava mutants through the development of validated protocols for the integration of somatic embryogenesis in cassava mutagenesis.

Tef (*Eragrostis tef* (Zucc.) Trotter) is grown annually on over 2.5 million hectares of land mainly in Ethiopia. The plant adapts to diverse climatic and soil conditions and grows better than other cereals both under drought and water-logged conditions. Unlike other cereals, the seeds of tef can be stored easily without losing viability under local storage conditions, since it is not attacked by storage pests. Tef is free of gluten hence safe for people with severe allergies to wheat gluten. Compared to other cereals, however, the average seed yield from tef is one of the lowest.

The tef TILLING project based at the University of Bern in Switzerland is recently initiated with financial support from Syngenta Foundation for Sustainable Agriculture and University of Bern, and scientific collaboration with University of Georgia, FAO/IAEA Programme, and Ethiopian Institute of Agricultural Research. The main goal of the project is to obtain semi-dwarf tetraploid tef lines which are resistant to crop lodging. Since tef has a tall and tender stem, it is susceptible to damage by wind and rain. In addition, when the optimum amount of fertilizer is applied to increase the yield, a high incidence of lodging occurs. As a consequence, the yield from the crop is severely reduced in terms of total grain yield and quality. In general, the yield loss due to lodging is estimated to about 30% for tef. So far, the project has generated over 4,000 M2 mutagenized lines to be utilized in TILLING [20].

The genes to be investigated are selected based on the information from other cereals. The dwarf plants of wheat that tremendously increased the yield during the Green Revolution in 1960s and 1970s contain the mutated reduced height-1 (Rht-B1 and Rht-D1) gene. The commercially popular rice cultivar known as semi-dwarf (sd-1) is also defective in a gene involved in gibberellins biosynthesis. In addition, the maize brachytic2 (br2) mutants and its ortholog in sorghum dwarf3 (dw3) are also characterized by compact lower stalk internodes. The height reduction in these plants results from the loss of a P-glycoprotein (PGP) that modulates polar auxin transport in maize stalk. The Tef TILLING Project will identify from tef several of the orthologous genes indicated above and use the sequence information to screen the mutagenized population [20].

Conclusion

TILLING (Targeting induced local lesions in genomes) a newly developed general reverse genetic strategy helps to locate an allelic series of induced point mutations in genes of interest. It allows the rapid and inexpensive detection of induced point mutations in populations of physically/chemically mutagenized individuals. EcoTILLING, which is the extension of TILLING, is used to mine natural variation in a population. It is used for the first time in 2000 by McCallum and her co-worker. Technically TILLING involves mutagenesis of M0 with traditional mutagen and production of M2 for DNA isolation pooling of DNA from 2, 4, 6 and 8 and finally mutation recovery.

It is recommended as non-GMO technology, so when using TILLING, GMO procedures and controversies are avoided. Moreover, TILLING is not technically demanding and can be performed at a relatively low cost. TILLING technology involves simple procedure, high sensitive and efficient techniques. It is widely applied in functional genomics, gene discovery DNA polymorphism assessment and crop breeding. Till recent time TILLING plat form have been developed for almost 20 species of plant and ongoing project for some agronomically important crop like tef and cassava.

Cultivated crop, subjected to intensive genetic and genomic studies. Although classical mutagenesis has successfully been applied to Helianthus genus in the past, Sabetta et al. [21] have developed the first sunflower TILLING resource.

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