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REVIEW ARTICLE

A Review: TILLING Technique Strategy for Cereal Crop Development

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ABSTRACT

Increase in global warming, changing the rainfall pattern, spread of disease, and sedates the production of crops varieties adapted to new habitats has turned out hunger situation in certain developing countries. Gene-editing technologies such as Zinc Finger Nucleases (ZFNs), Transcriptional Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9) systems ensure the rescue. But they have limitations including cytotoxicity, undesired mutations, and chromosomal aberrations, creating problems in the procedure at lab to the ground soil. These are required for the modernization of novel approaches for crop improvement against abiotic and biotic stresses. One that non-transgenic approach, called Targeting Induced Local Lesions IN Genomes (TILLING) is used for increasing sequence data and merges it with classical and advanced methods. Moreover, TILLING produces high-rate genome-wide variations with no off-target effects, and high output. Hence, the TILLING approach is useful for scientist involved in crop improvement especially cereal crops like rice and wheat around the world.

Keywords: Gene-editing techniques, TILLING, mutation, cereal crops, CRISPR

1. INTRODUCTION

Increasing population is one of the world's biggest problems with passing year. According to the United Nations Food and Agriculture Organization, the world's population is projected to grow by 9 billion people by 2050, resulting in an increase in hunger [1]. As a result, agricultural areas must be capable of rapidly responding to changing weather patterns, natural disasters, and risks from natural disasters. The primary constraint on crop

development is the loss of genetic diversity and the erasure of geographical differences, which prevent breeders from developing new species that are either resistant to biological or abiotic stresses. Genetic deterioration is further exacerbated when crop nutritional quality and output are increased to address supply-demand imbalances [2]. To meet human food requirements for an extended period of time while minimizing environmental impact, sources of long-lived heritable varieties must be discovered and adopted immediately. Some scientists think that by using the method of mutagenesis, they may enhance the genetic variations of cereal plants. Chemical mutagenesis is the process by which beneficial genetic characteristics are created in plants via the application of DNA-modifying chemicals that they are exposed [3]. As a consequence of random point mutations, missense/nonsense mutations, and minor insertions or deletions, among other things, a succession of altered alleles is resulted [4-5]. A vast variety of cereal crop types have been developed through mutagenesis and made available to the public. However, random mutagenesis is a long and challenging method since it requires exposing a large population to mutagenesis, and undesirable variants must be eliminated while desirable background changes cannot be removed during the transition phase. Cereal crops were developed using molecular techniques for variation and mutation in desired genes. *Agrobacterium tumefaciens* is well recognized in the area for its application in targeted mutagenesis [6]. As previously stated, stable gene insertion into plants was achieved via the use of biolistic and electroporation techniques [7]. Numerous plant gene editing or processing techniques are developed like zinc finger nucleases (ZFN) [8]. While animals can be edited using CRISPR/Cas systems, excluding few species for which it is not feasible. The disadvantage of these techniques is that they rely on agrobacterium-mediated transformation methods, which prioritize regeneration of transformed cells above stable DNA transformation. These techniques are not used in the stability-oriented methods of DNA transformation [9]. In terms of cultivation prior to transgenic recovery, which is required, and current processing methods, which have a low success rate, a number of crops fail to satisfy this criterion [10]. In this instance, genetic engineering was carried out using Agrobacterium-based delivery methods, such as transient transformation and DNase and RNase targeting. Due to the direct administration of the Cas9-sgRNA combination to the target cell, transitory activity and minimal off target effects were detected [11]. Inherent features such as tissue culture and transformation make genetic engineering techniques for plant improvement difficult or impossible. Additionally, transgenics are characterized as unpredictable and unreproducible. A shift in behaviour occurs based on the number of transgenic copies present in the host DNA. Because both cytotoxins and transcriptional activator-like effector nucleases contain similar-length effector cleavage sequences. Both cytotoxins and TALENs induce undesirable mutations and chromosomal abnormalities, whereas TALENs cause fewer undesirable mutations and chromosomal aberrations [12]. CRISPR/Cas9, both separately and in combination has a

greater selectivity for targets than ZFNs and TALENs [13]. While the off-target effect has been shown, further study is necessary. Strict regulatory processes are implemented to prevent genetic engineering from altering food crops. Modern plant genetic editing can be made feasible via a process called mutagenesis. Mutagenesis has to be beneficial for producing a high rate of changes per genome, remaining stable over many generations, and making transgenic crops safe to consume. This research examined the technique for creating mutations in cultivations (TILLING), as well as a number of other critical processes, and found that the procedure might be utilized to enhance the number of cereal mutants.

2. TILLING (Targeting Induced Local Lesions IN Genomes)

A group of scientists studying ethyl methane sulfonate mutagenesis in Arabidopsis devised a method called Targeting Induced Local Lesions In Genomes (TILLING) for identifying induced point mutations in selected genes after treatment with the mutagen ethylene diamine sulfonate (figure 1) [14]. The novel method for finding single-nucleotide variation combines traditional whole-genome mutagenesis with contemporary, high-throughput gene-based search for single-nucleotide variation. There has been a quick movement from plants to animals due to the recent use of the TILLING method, which was originally intended for use in plants [15]. This new development led to it being utilized for many plant and animal species. The concept of TILLING may be defined as the use of various types of mutagenesis, plus the generation of new genetic population(s), with the use of mutants obtained via breeding to yield more mutants for ultimate release. Thus, one of the most critical phases in TILLING is the creation and maintenance of mutant populations, because if a target gene is successfully modified, the mutants from the population must be allowed to thrive. The mutagenized population may provide valuable study resources because of possible differences at a number of loci. Any animal with low genetic resources, such as reptiles, benefits the most from the TILLING technique.

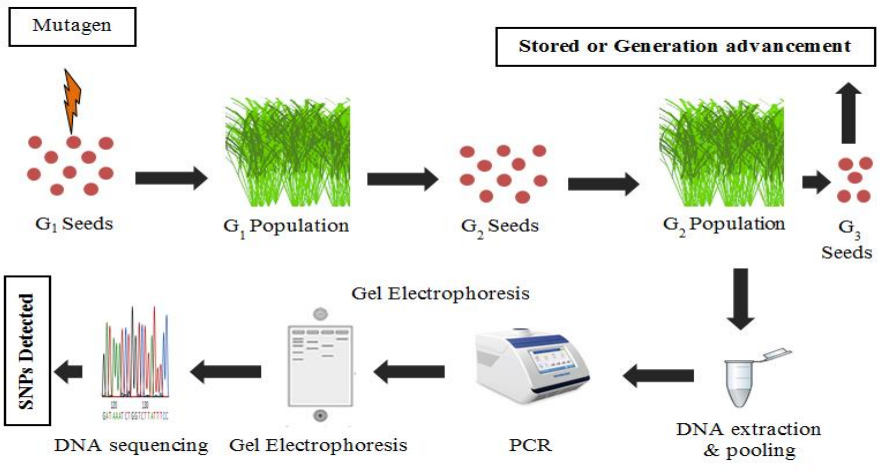

Figure 1. Schematic representation of TILLING approach

Table 1. Cereal Crops modified through TILLING (Targeting Induced Local Lesions IN Genomes) approach

Crop	Modified Phenotype	Targeted Gene	Reference
Rice (*Oryza sativa*)	Salt tolerance	*OsAKT1, OsHKT6, OsNSCC2, OsHAK11, and OsSOS1*	Hwang *et al.,* 2016 [35]
Rice (*Oryza sativa*)	Drought tolerance	*OSCP17*	Yu *et al.,* 2012 [36]
Rice (*Oryza sativa*)	Low phytic acid	*ITPK*	Kim *et al.,* 2014 [37]
Rice (*Oryza sativa*)	Arsenic tolerance	*ATT1*	Lim *et al.,* 2020 [38]
Wheat (*Triticum sp.*)	Non-waxy	*Seia*	Slade *et al.,* 2012 [39]
Wheat (*Triticum sp.*)	Vernalization	*VRN-A1*	Liang *et al.,* 2011 [40]
Wheat (*Triticum sp.*)	Karnal Hardness	*Pin a, Pin b*	Ma *et al.,* 2017 [41]
Wheat (*Triticum sp.)*	Powdery mildew disease resistance	*TaMlo*	Garcia *et al.,* 2017 [42]

3. INDUCING LESIONS

Depending on the genotype, mutagen, and dosage, the chemical mutagenesis phase of the TILLING technique may result in a high rate of genome-wide mutations (variations). Given that only a small number of mutants may be subjected to saturation mutagenesis, it makes sense to incorporate some saturation mutagenesis, even in small numbers [16]. By combining methods such as saturating mutagenesis and DNA sequencing, it may be possible to bridge the gap between genes and functional analysis [17]. The establishment of pool populations of plants is a significant advance for future TILLING genomic studies. TILLING (genetic modification of splice junctions in the transcriptional start proteins eIF4E1 and EIF4G) may be used to assist in increasing disease resistance in crops by genetically modifying splice junctions in the transcriptional start proteins eIF4E1 and EIF4G. In relation to Piron *et al.*, (2010), TILLING has also been shown to be effective in plants with numerous duplicated genes [18-19]. Polyploids (those with multiple sets of chromosomes) are more resistant to mutagenesis effects than comparable diploids in TILLING methods (with a single set of chromosomes). TILLING makes it more difficult to identify a single recessive mutation in hexaploid wheat due to the presence of wild type homologs. This method of identifying recessive variants necessitates the use of a large number of backcrosses to eliminate undesirable "off-target" mutations [20]. Even diploid populations require backcrossing, whereas polyploid mutant populations require only a few crossings.

4. DETECTING LESIONS

Detection of mutations is another critical step in the TILLING process. Adapting the high-throughput performance of the high-efficiency

liquid chromatography technique for the research of the mutant Arabidopsis population was not straightforward following it, cell's nuclear screening was completed [14, 21-22]. Direct sequencing and high-resolution melting curve analysis are effective techniques for detecting nucleic acids [23-26]. Recently, induced lesions were discovered utilizing the genome enrichment method [27]. All NGS methods need the utilization of single-stranded DNA, the detection of HETT enzymatic endonuclease, and the physical properties of single-stranded DNA. Due to their ability to isolate and extract extremely sensitive DNA, these techniques were utilized to develop improved liquid chromatography and capillary electrophoresis [28]. While utilizing non-denaturated polyacrylamide or laser scanned dermal gels, combining cell culture with agarose is simpler [29-30]. TILLING has also been proven to be effective in detecting direct DNA sequencing and the cost of analysis per base pair, the availability of specific technologies in the real world, and the sensitivity of the method employed all have an effect on whether or not a mutation detection system is viable for TILLING [31-33].

5. TILLING for cereal crops improvement

Apart from being essential for the supply of commodities for direct and indirect usage, rice (*Oryza sativa*) and wheat (*Triticum* sp.) are the two most significant staple food crops for almost half of the world's population. If demand increases, rice and wheat output must also rise. Rice and wheat must be produced to be able to tolerate stress, produce more grain, enhance grain quality, and be pest and disease resistant (table 1). This novel method may be capable of altering many genes in rice and wheat, both of which are responsible for yield and quality control. Over 2,600 genes in over 18,000 second-generation rice plants developed by combining sequencing technology and current bioinformatics techniques suggest harmful changes [34].

6. CONCLUSION

Each crop presents a unique set of challenges for breeders and molecular scientists, due to the diversity of functional genomics and chromosomal groupings. This implies that no one can successfully apply a technique to a wide variety of crops. A critical element is that the method is sufficiently adaptable to a broad range of circumstances. Physical and chemical mutagenesis has been shown to be successful in generating mutations in all cultures, and no crop has yet been shown to be immune to either form of mutagenesis. Prior to using the TILLING method for fresh crops, an early shot must be made to target induced local lesions in a gene of interest.

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8. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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