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Role of reverse genetic approaches TILLING and EcoTILLING in crop improvement: A review

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ABSTRACT

Forward and reverse genetics are the two distinct approaches for establishing a connection between an individual's genetic makeup and their observable traits. Targeting Induced Local Lesions in Genome (TILLING) a reverse genetic method, combines chemical mutagenesis with PCR-based screening to pinpoint mutations in regions of interest (McCallum *et al.,* 2000). In TILLING, a specific endonuclease is employed to detect either artificially induced or naturally occurring DNA polymorphisms in a gene of interest. This approach involves introducing mutations into DNA, leading to changes in observable traits due to the resulting point mutations, such as insertions or deletions. The core principles of TILLING include the creation of a population of organisms mutagenized with a chemical called EMS, the use of PCR primers to target the gene of interest, and the identification of rare mutants within this population. A similar technique called EcoTILLING exists, differing only in that it seeks out natural genetic polymorphisms rather than induced ones across various germplasm (Comai *et al.,* 2004). One of the primary advantages of TILLING is its capability for genetic mapping through linkage association analysis and its ability to identify a spectrum of genetic variations, all while remaining cost-effective. ECO-TILLING serves fundamental purposes such as mapping, association analysis, mutation profiling, and the study of biodiversity. TILLING also allows for the efficient, simultaneous screening of multiple genes and enables the prediction of the expected number of genetic variations based on mutation frequency and library size. In summary, TILLING is a highly relevant technique in reverse genetics, effectively complementing the array of available methods. It serves as a valuable, non-GMO approach in molecular breeding, particularly when the desired outcome can be achieved through gene mutations without the complete knockout of the gene.

1. Introduction

The complete genome sequencing projects for *Arabidopsis thaliana* and rice (*Oryza sativa*) have ushered in a new era of genomics in plant science research. The wealth of sequence information available in public databases has underscored the necessity to develop large-scale, genome-wide reverse genetic strategies for functional analysis (Till *et al*., 2003). Given that many phenotypes remain elusive, traditional forward genetics struggles to meet the demand for high-throughput and extensive surveys of gene functions.

To bridge this gap, two distinct approaches exist for establishing a connection between an individual's genetic makeup and their observable traits: forward and reverse genetics. One such reverse genetic technique is Targeting Induced Local Lesions in Genome (TILLING), which combines chemical mutagenesis with PCR-based screening to pinpoint point mutations in specific regions of interest (McCallum *et al*., 2000). The process of discovering mutations at the DNA level and then evaluating how they alter an individual's morphology is referred to as reverse genetics (Tare *et al.*, 2023). In the TILLING approach, DNA

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undergoes mutation, resulting in changes in observable traits due to the point mutations generated by insertions or deletions.

The fundamental principles of TILLING techniques encompass the creation of a population of organisms mutagenized mostly with EMS, the precise targeting of the gene of interest using PCR primers, and the identification of rare mutants within a population. A related technique, EcoTILLING, shares similarities with TILLING but focuses on identifying natural genetic polymorphisms rather than induced ones across various germplasm (Comia *et al.*, 2004).

One of the primary advantages of TILLING lies in its ability to facilitate genetic mapping through linkage association analysis and to uncover a spectrum of genetic variations, all while remaining cost-effective. Essential applications of ECO-TILLING include genetic mapping, association analysis, mutation profiling, and the exploration of biodiversity.

2. History and Overview

The concept of TILLING originated in the late 1990s when Claire McCallum and her team embarked on their investigation of two chromo-methylase genes in *Arabidopsis thaliana* (L.) Barkley and Wang (2008). Their initial efforts involved various techniques such as T-DNA lines and antisense RNA, all of which proved unsuccessful in achieving their objectives. Ultimately, the introduction of TILLING marked a groundbreaking approach. This innovative method involves the amalgamation of densely concentrated point mutations induced through chemical mutagenesis with rapid mutation screening within pools of DNA. In their initial TILLING publication (McCallum *et al.*, 2000), they utilized Ethyl methane sulfonate (EMS) as the chemical mutagen and employed Denaturing High-Performance Liquid Chromatography (DHPLC) for detection.

There are two primary methods to link a specific allelic state within an individual's genetic makeup (such as a particular mutation) to a trait or observable characteristic: forward genetics and reverse genetics. In the context of forward genetics, the initial emphasis is placed on observed variations in traits, with the objective of identifying the underlying sequence alterations responsible for these variations. Conversely, reverse genetics commences with a modification in a DNA sequence and seeks to unveil the resulting changes in traits attributable to this sequence alteration. Historically, forward genetics has predominantly influenced the field of genetics. However, the recent rapid surge in available sequence data, doubling every nine months, has made reverse genetics more favourable for ascribing functions to genes as inferred from sequence annotations (Kahn, 2011). In comparison to reverse genetics, forward genetics faces certain limitations, including (1) the difficulty of detecting numerous mutations in standard phenotypic screenings, (2) the potential oversight of rare phenotypes or mutations contributing to a specific trait due to the large number of individuals requiring screening, and (3) the presence of many mutations that remain undetected due to factors like heterozygosity and polyploidy (Stemple, 2004).

3. Steps in the TILLING Procedure

i. Mutagenesis:

The choice of the wild type genotype is crucial and guided by two main criteria. Firstly, the chosen genotype should allow for the efficient production of many genetically identical individuals through a limited number of breeding steps. Additionally, whenever feasible, the individual with the most comprehensive sequence information should be selected. In cases starting with a homozygous genotype is not possible, adaptations to the TILLING procedure are required, as demonstrated in situations such as dioecy. The next important choice is the plant organ which should be the target of the mutation. In most cases, seeds are chosen as they are easy to handle in the mutation process, as they simply must be soaked in the mutagenizing chemical. In the original TILLING research by McCallum *et al*. (2000), ethyl methanesulfonate (EMS, $CH_3SO_3C_2H_5$) was used and remains the most employed mutagen due to its consistent mutation rate. EMS reacts with guanine in DNA by binding to the ethyl group, resulting in the formation of an abnormal base called O-6-ethylguanine. This abnormal base pairs with thymine and is later repaired to adenine.

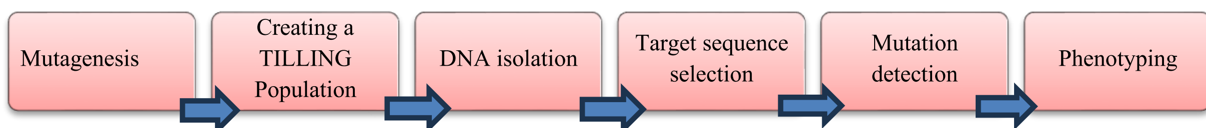

Figure 1. Different steps of TILLING Process

Consequently, about 90% of mutations induced by EMS are transitions from G/C to A/T base pairs. This specific mutation pattern is a result of the chemical properties of EMS and its interaction with DNA's guanine base.

ii. Creating a TILLING Population:

The creation of M_0 seeds can be achieved by applying the mutagen either to the seeds directly or to the pollen, followed by pollinating the female reproductive parts of a different wild-type plant. The resultant kernels are cultivated into M_1 plants. These M_1 plants are either a combination of both mutated and non-mutated cells (chimeric) or carry the mutation heterozygously, depending on whether the seed or pollen was mutagenized. In the case of pollen mutagenesis, DNA can be directly extracted from the M_1 plants for the subsequent TILLING process. Conversely, when mutagenizing the seeds, an additional self-pollination step is necessary before DNA isolation can be performed on the M_2 plants.

iii. DNA Isolation:

The primary necessity for DNA isolation in TILLING is an efficient and scalable process, as a considerable number of M_2 (M_1) individuals need to be isolated. Moreover, the DNA should exhibit adequate average size and stability under standard storage conditions. Minimizing the presence of contaminants that could interfere with subsequent reactions is also crucial. Sreelakshmi *et al*. (2010) conducted experiments to assess different methods for DNA isolation's suitability in TILLING. While the CTAB method yielded favourable outcomes, it posed challenges when applied to a 96-well format. Therefore, the quantification of DNA concentrations, either through photometric measurements or using Agarose gel analysis, becomes necessary.

iv. Target Sequence Selection:

Selecting genes with multiple copies should be avoided. In cases where this is not feasible, PCR primers must be meticulously chosen to target a distinct copy (Barkley and Wang 2008). The most valuable mutations are those that result in nonsense mutations, causing an early stop codon, or missense mutations that alter the amino acid in the protein product. These types of mutations bring about observable changes in the plant's phenotype. An ideal product length is around 800 to 1200 base pairs, depending on the mutation frequency. A greater PCR product size increases the likelihood of genetic variation, whereas detecting a single SNP in a chosen method is clearer than identifying multiple SNPs within a single fragment, as explained by Barkley and Wang in 2008.

v. Mutation Detection:

There are many different techniques used for mutation detection in TILLING (Targeting Induced Local Lesions in Genomes). The first approach used in the initial TILLING paper was Denaturing High-Performance Liquid Chromatography (DHPLC), a method based on melting temperature changes in DNA. Other methods include 30-Minor Groove Binding (MGB), Temperature Gradient Capillary Electrophoresis (TGCE), and High-Resolution Melting (HRM), all of which focus on melting temperature variations. These techniques indicate the presence of mutations but not their exact positions.

Another group of methods focuses on differences in secondary DNA structures between heteroduplexes and homoduplexes. Heteroduplex Analysis (HA) and Conformation Sensitive Capillary Electrophoresis (CSCE) rely on distinct migration patterns, while Chemical Cleavage of Mismatch DNA (CCM) utilizes differences in chemical reactivity to cleave DNA at mismatch positions.

The final set of methods involves enzymatic cleavage of mismatches. DNA N-glycolases recognize specific mismatches and create sites that can be cleaved. However, their use in TILLING is limited by specificity and the range of mismatches they can cleave.

vi. Phenotyping the Mutant(s):

After identifying a mutation, the subsequent step involves analyzing the characteristics of the genotype to understand its phenotype. Nonetheless, since most mutations exhibit recessive traits, it is generally necessary to have a genotype that is homozygous for the mutation to observe the effects. In instances of pollen-induced mutagenesis in M_1 plants, each genotype will be heterozygous. In scenarios where mutant detection occurs in M_2 plants following kernel mutagenesis, it is possible to differentiate between homozygosity and heterozygosity by conducting the mutation-detection process on individual mutant DNA instead of pooled samples. If the TILLING band appears in this context, it indicates the presence of a heterozygous genotype (Colbert *et al*., 2001). In such cases, a homozygous genotype can potentially be identified in the next generation of plants, available as seeds.

Once a change in phenotype is identified, it is essential to rule out the possibility that other background mutations might have caused the observed traits. One approach to address this concern involves crossbreeding two mutants exhibiting alterations in the same gene and observing the resulting population's segregation ratios Slade and Knauf (2005). Alternatively, one can study the segregation of the specific trait concerning the mutation within the M_2 population following pollen mutagenesis or the M_3 population following seed mutagenesis (Henikoff and Comai, 2003) to gain further clarity.

4. EcoTILLING

EcoTILLING is a molecular technique that is similar to TILLING, except that its objective is to uncover natural genetic variation as opposed to induced mutations. EcoTILLING originated in Arabidopsis research, aiming to identify genetic diversity within Arabidopsis eco-types (Comai *et al.*, 2004). Unlike TILLING, EcoTILLING does not involve cultivating mutant populations; instead, allelic variation of natural populations are studied.

In EcoTILLING, only two DNA samples are combined: one from the reference genotype and one from genotype under scrutiny. Although higher pooling depth is possible,this approach is less practical due to high anticipated diversity within natural populations utilized for EcoTILLING. Most of the modifications applied to the TILLING procedure are also relevant to EcoTILLING. However, the key limitation of EcoTILLING is that it involves a reduced pooling depth, where only one individual is characterized per reaction, and therefore reduces the technique's advantage compared to fully re-sequencing all genotypes. Further, there is a need to re-sequence the allelic variations identified through EcoTILLING to precisely pinpoint the differing DNA bases. Nevertheless, since the same coding sequence changes are likely to recur across numerous genotypes within a species, EcoTILLING still maintains a relative edge over complete re-sequencing Garvin and Gharett (2007). This advantage becomes more evident in scenarios where the genetic diversity of a particular gene within a specific population is limited.

In contrast to TILLING, EcoTILLING offers a broader array of potential applications. One of its direct applications is to evaluate genetic diversity in a plant population by scrutinizing variations in multiple genes. For example, Gilchrist *et al.* (2006) employed EcoTILLING to comprehensively document diversity levels in naturally occur-ring poplar populations across western Canada and the USA. EcoTILLING can also serve to identify Single Nucleotide Polymorphisms (SNPs) within specific genes. For instance, Hermann *et al.* (2006) employed EcoTILLING to locate SNPs in resistance gene analogs within sugarcane. By examining numerous individuals, SNPs with the highest variability or those showing diversity between specific genotype groups can be singled out. These identified SNPs can subsequently be converted into markers using simpler detection techniques. Alternatively, EcoTILLING can be leveraged to determine the alleles associated with these SNPs. This utility becomes particularly valuable when dealing with highly polymorphic sequences containing multiple polymorphic sites, as a single EcoTILLING reaction can detect numerous sequence polymorphisms simultaneously (Mejlhede *et al.*, 2006).

Table 1. Some successes studies on TILLING approaches

S. No.	Organism	Mutagen	TILLING of gene	Reference
1.	Rice (*Oryza sativa*)	EMS	*AtIPK1, AtIPK2β, AtMRP5, AtITPK1, AtITPK4, AtMIK, At5g60760* (Phytic acid metabolism)	Kim and Tai, 2014
2.	Wheat (*Triticum aestivum*)	EMS	*TaGW2* (Thousand grain weight)	Wang *et al*., 2018
3.	Maize (*Zea mays*)	EMS	*DMT102* (Chromomethylase)	Till *et al.*, 2004
4.	Barley (*Hordeum vulgare*)	EMS; Sodium azide	*GBSSI* (Granule-bound starch synthase I)	Sparla *et al., 2014*
5.	Sorghum (*Sorghum bicolor*)	EMS	*COMT* (Forage digestibility)	Xin *et al.*, 2008

Table 2. Some successes studies on EcoTILLING approaches

S. No.	Organism	Traits	Gene	Reference
1.	Rice (Oryza sativa)	Salt tolerance	OSCP17	Negrao et al., 2011
2.	Wheat (Triticum aestivum)	Kernel hardness	Pina-D1	Wang et al., 2008
3.	Brassica spp.	Erucic acid content	FAE1-A8, FAE1-C3	Wang et al., 2010
4.	Barley (Hordeum vulgare)	Chlorophyll protein	Lhcb1	Irshad et al., 2020
5.	Barley (Hordeum vulgare)	Powdery Mildew resistance genes	Mlo & Mla	Mejlhede et al., 2006

5. Applications of TILLING approach in crop improvement

TILLING can serve two main purposes: it can be employed as a reverse-genetics tool, linking known DNA sequences to observable morphological traits, or it can function as a molecular breeding tool (Slade *et al.*, 2005). An example is the Arabidopsis TILLING project (ATP), involved in bridging the gap between genetic DNA sequences and observable phenotypes in *Arabidopsis thaliana* (Greene *et al*., 2003). In *Brassica oleracea* L. (cabbage), TILLING was utilized to explore genes related to responses to abiotic stress (Himelblau *et al*., 2009). In the case of Lotus japonicus, a legume model species, TILLING results mainly revolved around genes associated with nodulation processes (Heckmann *et al.*, 2006; Horst *et al.*, 2007). Likewise, in *Medicago trunculata* (Gaertn.), another legume model species, TILLING was applied to analyze the functions of various genes (Lefebvre *et al*., 2001).

Across grass species, TILLING projects were mainly conducted for reverse-genetics perposes in species with established sequences. Notably, in rice, two separate TILLING populations were established and are currently in use (Suzuki *et al*., 2008; Till *et al*., 2007). TILLING project like the Arabidopsis TILLING initiative provides services to the community (Weil, 2009). In sorghum, TILLING yielded results pertaining to candidate genes related to lignin synthesis (Xin et al., 2008). Beyond species with complete sequences, TILLING found application in economically significant species. For example, in barley, proof-of-concept studies have been published, showcasing TILLING's role as a reverse-genetics tool (Caldwell et al., 2004 and Gottwald *et al.,* 2009). In wheat, TILLING was utilized to associate genes responsible for diverse enzymes linked to starch synthesis with mutant genotypes as a proof-of-concept (Uauy *et al*., 2009).

TILLING also functions as a molecular breeding tool, typically when required genetic variation is absent within a species' gene pool. This approach can be an alternative to using related wild species, which can be challenging in breeding. Primarily, this method involves gene knock- out, but it also has the potential to create new allelic variations for specific genes. As TILLING does not involve DNA transformation, resulting genotypes are non-genetically modified organisms (GMOs), circumventing GMO crop restrictions (Slade and Knauf, 2005). In wheat, for instance, gene knockout was used to alter starch composition by targeting granule-bound starch synthase genes (Slade *et al*., 2005). Another example involves the knockout of the *Sgp-1* gene, responsible for Starch synthase II in wheat, across all three homoeologous chromosomes (Sestili *et al*., 2010). In rapeseed (*Brassica napus*), TILLING was utilized to knock out the key gene *FAE1*, involved in erucic acid biosynthesis. Once a mutant with the anticipated phenotype is obtained through TILLING, subsequent steps involve backcrossing this mutant genotype with wild types or high-yield, high-quality varieties to eliminate background mutations (Slade and Knauf, 2005).

6. References

Barkley, N.A. and Wang M.L. 2008. Application of TILLING and EcoTILLING as reverse genetic approaches to elucidate the function of genes in plants and animals. *Curr. Genomics.,* 9:212–226.

Caldwell, D.G., McCallum, N., Shaw, P., Muehlbauer, G.J., Marshall, D.F. and Waugh., R. 2004. A structured mutant population for forward and reverse genetics in Barley (*Hordeum vulgare* L.). *Plant Journal*, 40:143–150.

Colbert, T., Till, B.J., Tompa, R., Reynolds, S., Steine, M.N., Yeung, A.T., McCallum, C.M., Comai, L. and Henikoff, S. 2001. High-throughput screening for induced point mutations. *Plant Physiol.,* 126:480–484.

Comai, L., Young, K.J., Codomo, C.A., Enns, L.C., Johnson, J.E., Burtner, C., Odden, A.R., Henikoff, S., Reynolds, S.H. and Greene, E.A. 2004. Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. *Plant J*., 37:778–786.

Garvin, M.R. and Gharett, A.J. 2007. Eco-TILLING: an inexpensive method for single nucleotide polymorphism discovery that reduces ascertainment bias. *Mol Ecol. Notes*.,7:735–746.

Gilchrist, E.J., Haughn, G.W., Ying,C.C., Otto,S.P., Zhuang, J., Cheung, D., Hamberger, B., Aboutorabi, F., Kalynyak, T., Johnson, L., Bohlmann, J., Ellis, B.E., Douglas, C.J. and Cronk, Q.C.B. 2006. Use of EcoTILLING as an efficient SNP discovery tool to survey genetic variation in wild populations of *Populus trichocarpa*. *Mol Ecol.,* 15:1367–1378.

Gottwald, S., Bauer, P., Komatsuda, T., Lundqvist, U. and Stein, N. 2009. TILLING in the two-rowed barley cultivar "Barke" reveals preferred sites of functional diversity in the gene *HvHox1*. *BMC Res Notes.,* 2(1): 1-14.

Greene, E.A., Codomo, C.A., Taylor, N.E., Henikoff, J.G., Till, B.J., Reynolds, S.H., Enns, L.C.,Burtner, C., Johnson, J.E., Odden, A.R., Comai, L. and Henikoff, S. 2003. Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in Arabidopsis. *Genet.,* 164:731–740.

Heckmann, A.B., Lombardo, F., Miwa, H., Perry, J.A., Bunnewell, S., Parniske, M., Wang, T.L. and Downie, J.A. 2006. Lotus japonicus nodulation requires two GRAS domain regulators, one of which is functionally conserved in a non-legume. *Plant Physiol.,* 142:1739–1750.

Henikoff, S. and Comai, L. 2003. Single-nucleotide mutations for plant functional genomics. *Annu Rev Plant Biol*., 54:375–401.

Hermann, S., Brumbley, S. and McIntyre, C.L. 2006. Analysing diversity in sugarcane resistance gene analogues. *Australas Plant Pathol*., 35:63-71.

Himelblau, E., Gilchrist, E.J., Buono, K., Bizzell, C., Mentzer, L., Vogelzang, R., Osborn, T., Amasino, R.M., Parkin, I.A.P. and Haughn, G.W. 2009. Forward and reverse genetics of rapid-cycling *Brassica oleracea*. *Theor Appl Genet*., 118:953–961.

Horst, I., Welham, T., Kelly, S., Kaneko, T., Sato, S., Tabata, S., Parniske, M. and Wang, T.L. 2007. TILLING mutants of *Lotus japonicus* reveal that nitrogen assimilation and fixation can occur in the absence of nodule-enhanced sucrose synthase. *Plant Physiol.,* 144:806–820.

Irshad, A., Guo, H., Zhang, S. and Liu, L. 2020. TILLING in cereal crops for allele expansion and mutation detection by using modern sequencing technologies. Agronomy., 10(3): 405.

Kahn, S.D. 2011. On the future of genomic data. Science., 331:728–729.

Kim, S.I. and Tai, T.H. 2014. Identification of novel rice low phytic acid mutations via TILLING by sequencing. Molecular breeding., 34: 1717-1729.

Lefebvre, V., Goffinet, B., Chauvet, J.C., Caromel, B., Signoret, P., Brand, R. and Palloix, A. 2001. Evaluation of genetic distances between pepper inbred lines for cultivar protection purposes: comparison of AFLP, RAPD and phenotypic data. Theor Appl Genet., 102:741–750.

McCallum, C.M., Comai, L., Greene, E.A. and Henikoff, S. 2000. Targeted screening for induced mutations. Nat Biotechnol., 18:455–457.

Mejlhede, N., Kyjovska, Z., Backes, G., Burhenne, K., Rasmussen, S.K. and Jahoor, A. 2006. EcoTILLING for the identification of allelic variation in the powdery mildew resistance genes mlo and Mla of barley. Plant Breed., 125:461–467.

Negrao, S., Almadanim, C., Pires, I., McNally, K.L. and Oliveira, M.M. 2011. Use of EcoTILLING to identify natural allelic variants of rice candidate genes involved in salinity tolerance. Plant Genetic Resources., 9(2): 300-304.

Sestili, F., Botticella, E., Bedo, Z. and Phillips, A. 2010. Production of novel allelic variation for genes involved in starch biosynthesis through mutagenesis. Mol Breed., 25:145–154.

Slade, A.J., and Knauf, V.C. 2005. TILLING moves beyond functional genomics into crop improvement. Transgenic Res., 14:109–115.

Slade, A.J., Fuerstenberg. S., Loeffler, D., Steine, M.N. and Facciotti, D. 2005. A reverse genetic, non-transgenic approach to wheat crop improvement by TILLING. Nat Biotechnol., 23:75–81.

Sparla, F., Falini, G., Botticella, E., Pirone, C., Talame, V., Bovina, R. 2014. New starch phenotypes produced by TILLING in barley. PLOS ONE., 9(10): e107779.

Sreelakshmi, Y., Gupta, S., Bodanapu, R., Chauhan, V.S., Hanjabam, M., Thomas, S., Mohan, V., Sharma, S., Srinivasan, R. and Sharma, R. 2010. NEATTILL: a simplified procedure for nucleic acid extraction from arrayed tissue for TILLING and other high-throughput reverse genetic applications. Plant Methods, 6:3-11.

Stemple, D.L. 2004. TILLING – a high-throughput harvest for functional genomics. Nat Rev Genet., 5: 145–150.

Suzuki, T., Eiguchi, M., Kumamaru, T., Satoh, H., Matsusaka, H., Moriguchi, K., Nagato, Y. and Kurata, N. 2008. MNU-induced mutant pools and high-performance TILLING enable finding of any gene mutation in rice. Mol Genet Genomics., 279:213–223.

Tare, S., Tiwari, S., Rathore, M.S., Yasin, M., Tripathi, N., Tiwari, P.N. and Tripathi, M.K. 2023. An Overview of Allele Mining for Crop Improvement: TILLING and Eco-TILLING. Plant cell biotechnology and molecular biology., 91-103.

Till, B., Cooper, J., Tai, T., Colowit, P., Greene, E., Henikoff, S. and Comai, L. 2007. Discovery of chemically induced mutations in rice by TILLING. BMC Plant Biol.,7:19.

Till, B.J., Colbert, T., Tompa, R., Enns, L.C., Codomo, C.A., Johnson, J.E., Reynolds, S.H., Henikoff, J.G., Greene, E.A., Steine, M.N., Comai, L. and Henikoff, S. 2003. High-throughput TILLING for functional genomics. In GrotewoldE (ed). Methods in Molecular Biology., 36: 205-220.

Till, B.J., Reynolds, S.H., Weil, C., Springer, N., Burtner, C., Young, K., Bowers, E., Codomo, C.A., Enns, L.C., Odden, A.R. and Greene, E.A. 2004. Discovery of induced point mutations in maize genes by TILLING. BMC plant biology., 4: 1-8.

Uauy, C., Paraiso, F., Colasuonno, P., Tran, R., Tsai, H., Berardi, S., Comai, L. and Dubcovsky, J. 2009. A modified TILLING approach to detect induced mutations in tetraploid and hexaploid wheat. BMC Plant Biol., 9:11-15.

Wang, N., Shi, L., Tian, F., Ning, H., Wu, X., Long, Y. and Meng, J. 2010. Assessment of FAE1 polymorphisms in three Brassica species using EcoTILLING and their association with differences in seed erucic acid contents. BMC plant biology., 10(1):1-11.

Wang, N., Wang, Y., Tian, F., King, G.J., Zhang, C., Long, Y., Shi, L. and Meng, J. 2008. A functional genomics resource for Brassica napus: development of an EMS mutagenized population and discovery of FAE1 point mutations by TILLING. New Phytologist., 180(4): 751-765.

Wang, W., Simmonds, J., Pan, Q., Davidson, D., He, F., Battal, A., Akhunova, A., Trick, H.N., Uauy, C. and Akhunov, E. 2018. Gene editing and mutagenesis reveal inter-cultivar differences and additivity in the contribution of TaGW2 homoeologues to grain size and weight in wheat. Theor Appl Genet., 131:2463–2475.

Weil, C.F. 2009. TILLING in grass species. Plant Physiol.,149:158–164.

Xin, Z., Li, W.M., Barkley, N., Burow, G., Franks, C., Pederson, G. and Burke, J. 2008. Applying genotyping (TILLING) and phenotyping analyses to elucidate gene function in a chemically induced sorghum mutant population. BMC Plant Biol., 8:1-14.