Indian Farmer
Volume 10, Issue 03, 2023, Pp. 125-129
Available online at: www.indianfarmer.net
ISSN: 2394-1227 (Online)

Original Article

OPEN ACCESS

TALENs: Strategy and application in modern agriculture

Poulomi Sen[1], Umme Salma[1], Avishek Chatterjee[2], Suprabuddha Kundu[2] and Jayita Hore[1]

[1]*Assistant Professor, School of Agricultural Sciences, Sister Nivedita University*
[2]*Assistant Professor, School of Agriculture, Swami Vivekananda University*

Corresponding email Id: *poulomi.s@snuniv.ac.in*

Received: 21 March 2023 *Published: 26 March 2023*

Abstract

Genome editing has got a key breakthrough success in the era of molecular biology. TALEN, one of the genome editing tools has played a major role in the advancements of basic biology, reverse genetics, agriculture and clinical study. The first commercially available edited crop variety was also developed through TALEN. The strategy relies on specific gene knockouts by DNA strand break by restriction enzyme followed by DNA repair system ultimately leading to predetermined site specific mutational changes. Cytoplasmic gene editing, methyl sensitivity, less off targets and not requiring a known Protospacer Adjacent Motif (PAM) has alleviated the drawbacks of another genome editing tool, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9). In different fields of agriculture especially in quality improvement of different crops TALEN technique has got huge popularity.

Keywords: TALENs, DNA, Genome, mutation, DNA repair

Introduction

TALEN (Transcription Activator-like Effector nucleases) is a potential genome editing technology that gives a diversified scope to target any DNA sequence in a wide range of organisms. TALE (Transcription Activator-like Effector) protein was reported for the first time in the plant pathogenic bacteria *Xanthomonas* spp. to alter the gene transcription of the host plant to support virulence (Boch *et al*., 2009). For inoculation of TALE, a typical type III secretion system is needed followed by colonization of bacteria in the host. TALE can bind to any DNA sequence and when combined with nuclease it can cleave double stranded DNA. This disruption is again followed by DNA repair through either non-homologous end-joining (NHEJ) or homology directed repair (HDR) leading to induced gene mutation by precisely altering the sequence. TALENs have been extensively used in modifying plant genome for any kind of improvement. In this article we have tried to briefly compile the general strategy and application of TALEN in modern agricultural field.

1. TALEN construct

The commonly used TALEN system bears two TALEN units and a typical TALEN unit comprises of DNA binding TALE repeat domains (arrays of highly conserved 30-33 amino acid repeats) with flanking N and C terminal domains and one FokI nuclease catalytic domain. Individual TALE repeat can bind to a single specific DNA sequence (A/G/T/C) but the specificity is determined by two hyper variable amino acid residues present on position 12 and 13 (Table 1; Streubel *et al*., 2012). These two variable positions are known as Repeat Variable Diresidue (RVD). The conserved 30-33 amino acid repeat

sequence is LTPDQVVAIAS**GGKQALETVQRLLPVLCQDHG where **denotes the RDV. The length of the target site corresponds to the number of repeats of an array. A thymine (T) is located at the 5' end precedes the initial base of TALE repeat.

Table 1: RDV of each TALE repeat domain with their DNA base specificity

NS	NN	NK	IG	NG	HD	NI	RDV
A/C/G/T	G/A	G	T	T	C	A	DNA base specificity

Each FokI nuclease catalytic domain attached to one TALE repeat domain finally dimerizes to form the ultimate double stranded DNA breaks (DSBs) generating domain of a final TALEN system (Fig. 1).

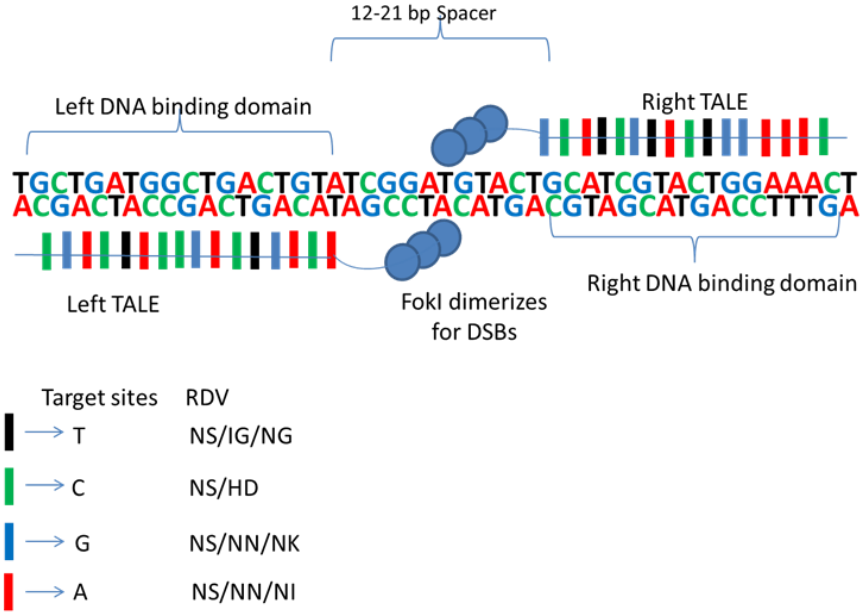

Fig 1: Schematic illustration of TALEN constructs and its function

2. Nuclease based DSBs and induced mutation

Each TALEN unit must bind specifically to the neighboring target DNA sequence with a spacer region of 12-21 bases for proper nuclease induced double stranded DNA cleavage. These DSBs are then followed by DNA repair system where DNA mutations like insertion/deletion (INDEL) can be induced. In the presence of double stranded DNA donor template, precise base substitution or insertion is commonly introduced. When two nuclease based DSBs are placed on same chromosome, it leads to INDEL and if on different chromosomes leads to chromosomal translocations conferring genome editing.

3. Benefits of TALENs

Base specificity of TALENs is highly accurate in the modern era of genome editing and this helps the biologists to reduce the off target genome alterations. When it comes to avoiding mutational changes to the genome, TALEN is considered as the most dependable among the various genome editing techniques available because of its degeneracy (certain RDVs can bind to more than one nucleotide).

TALENs give a powerful gateway towards epigenetic studies because of its methyl sensitivity (Deng *et al*., 2012). Besides genomic DNA editing, TALENs have shown perfect mitochondrial and plastid editing too in certain species (Piatek *et al*., 2018). TALENs can efficiently regulate endogenous gene expression by developing artificial transcription factors, repressors, activators. Targeted gene knock out in haploid cell by TALEN is also possible followed by regenerating homozygous lines (Gurushidze *et al*., 2014).

4. Delivery of TALENs in plant cells

Depending upon the organisms, TALENs can be delivered into living cells by several means like bacterial-based delivery (*Agrobacterium*-mediated transfer), physical methods (Microinjection, particle bombardment, electroporation), viral based delivery, chemical methods (PEG, liposomes etc.). However, for plant cell transformation in crops like Rice, wheat, *Arabidopsis*, soybean, sugarcane, barley, tomato, potato, peanut, *Brassica* sp. etc. *Agrobacterium*-mediated TALEN transfer and particle bombardment have been extensively used.

5. Applications of TALENs in agricultural crops

TALENs have been significantly used for plant breeding for improving yield, biotic and abiotic stress resistance, herbicide tolerance, nutritional quality improvement, better shelf life. Few of the achievements are furnished below.

a. Biotic and abiotic stress resistance:

The earliest TALEN edited crop was *Xanthomonas oryzae* pv. *oryzae* (causing bacterial leaf blight) resistant rice varieties. The gene OsSWEET14/Os11N3 was targeted for TALEN based disruption (Li *et al*., 2012). Development of fungal disease powdery mildew resistant barley and wheat by targeting the gene mlo1 was also possible by TALEN based editing (Wang *et al*., 2014).

b. Quality improvement:

TALEN induced mutation in the gene producing the caffeic acid O-methyltransferase (COMT) in sugarcane has improved its biofuel quality without compromising biomass and stress resistance capacity (Jung *et al*., 2016). Two fatty acid desaturase genes were mutated in soybean leading to reduced unhealthy trans-fat (linoleic acid) content and increased oleic acid content and these were the first TALEN based genome edited marketed varieties (Haun *et al*., 2014). Oleic content of peanut seeds was also improved by TALEN induced mutation in the gene fatty acid desaturase 2(FAD2) (Wen *et al*., 2018). Disruption of betaine aldehyde dehydrogenase 2 (BADH2) gene in rice through TALEN tool has improved aroma of rice though enhancing the production of the major fragrance compound 2-acetyl-1-pyrroline (Shan *et al*., 2015). Improvement of *Nicotiana benthamiana* was done by developing plants which produce quality glycoproteins devoid of plant-specific residues by knocking out two α-(1,3)-fucosyltransferase (FucT) and the two β-(1,2)-xylosyltransferase (XylT) genes (Li *et al*., 2016). ZmMRP4 (Multidrug resistance-associated protein 4), ZmIPK1A, ZmIPK, genes related to phytic acid (PA) synthesis have been mutated by TALEN for improving nutritional quality of maize seeds (Liang *et al*., 2014). Mutational change in ANT1 gene in tomato leads to a change in anthocyanin accumulation in tomato tissue (Cermak *et al*., 2015). Reduction of glycoalkaloid and cholesterol content of potato was made through TALEN induced disruption in Sterol Side Chain Reductase 2 (StSSR2) gene (Zheng *et al*., 2021).

c. Phenotypic marker:

Knock out of the glossy2 (gl2) locus of maize conferred a glossy appearance (Si *et al*., 2015). Gibberelic acid (GA) responsive tomato plants were produced by mutating the DELLA protein producing gene Procera (pro) (Lor *et al*., 2014).

d. Yield:

Genes related to yield attributing parameters of rice like Dense and erect panicle 1 (OsDEP1), Cytokinin oxidase 2 (OsCKX2) were mutated by TALENs (Shan *et al*., 2013).

e. Herbicide resistance:

TALEN induced mutation in acetolactate synthase (ALS) genes led to resistance to sulfonylurea and imidazolinone herbicide resistance in tobacco (Zhang *et al*., 2013). Glyphosate sensitivity of rice has been changed by knocking out the gene 5-Enolpyruvylshikimate-3-phosphate synthase (OsEPSPS) gene by this genome editing tool (Wang *et al*., 2015).

f. Storage tolerance:

Mutation in vacuolar invertase gene (Vlnv) in potato has improved the chips colour by reducing the acrylamide content and post-harvest shelf life (Clasen *et al*., 2015). Storage tolerance of rice seeds can be improved through mutagenesis of lipoxygenase (LOX3) gene using TALEN (Ma *et al*., 2015).

g. Mitochondrial genome editing:

Cytoplasmic male sterility (CMS) was also overcome by TALENs induce mitochondrial gene editing in rice and *Brassica* (Kazama *et al*., 2019).

h. Environment insensitivity:

Vernalization related gene FRIGIDA (FRI) in *Brassica oleracea* has been edited by TALENs (Sun *et al*., 2013).

6. Conclusions

New Breeding Techniques (NBTs) like genome editing (cluster regularly interspaced short palindromic repeats, CRISPR/Cas9, TALENs, zinc finger nucleases, ZNF), reverse breeding, cisgenesis, site directed nucleases, homing endonucleases or meganucleases etc. always offer additional benefits over the conventional procedures like selection or selection accompanied with hybridization or mutational breeding or marker assisted selection (MAS). Though CRISPR/Cas9 has tremendously helped breeders in improving crops, TALEN has its own specific potentials that make it unique. It has high specificity, low off target mutation, power to discriminate methylated and demethylated based and even it can modify cytoplasmic genes too. The key disadvantage of TALEN is its tiring construction and it can also be irrelevant if the objective can only be achieved by TALENs. Optimizing the efficient TALEN delivery into the plant cell and use of fusion proteins devoid of nuclease enzyme can be explored further.

References:

Boch, J, Scholze, H, Schornack, S, Landgraf, A, Hahn, S, Kay, S, Lahaye, T, Nickstadt, A, & Bonas, U. (2009). Breaking the code of DNA binding specificity of TAL-type III effectors. *Science,* 326, 1509–1512.

Cermak, T, Baltes, NJ, Cegan, R, Zhang, Y, & Voytas, DF. (2015). High-frequency, precise modification of the tomato genome. *Genome Biology*, 16(1), 232.

Clasen, BM, Stoddard, TJ, Luo, S, Demorest, ZL, Li, J, Cedrone, F, Tibebu, R, Davison, S, Ray EE, Daulhac A, Coffman A, Yabandith, A, Retterath, A, Haun, W, Baltes, NJ, Mathis, L Voytas, DF, & Zhang, F. (2015). Improving Cold Storage and Processing Traits in Potato through Targeted Gene Knockout. *Plant Biotechnology Journal*, 14(1), 169-176.

Deng, D, Yin, P, Yan, C, Pan, X, Gong, X, Qi, S, Xie, T, Mahfouz, M, Zhu, JK, Yan, N, & Shi, Y. (2012). Recognition of methylated DNA by TAL effectors. *Cell Research,* 22(10), 1502–1504.

Gurushidze, M, Hensel, G, Hiekel, S, Schedel, S, Valkov, V, & Kumlehn, J. (2014). True-breeding targeted gene knock-out in barley using designer TALE nuclease in haploid cells. *PLoS ONE,* 9(3), e92046.

Haun, W, Coffman, A, Clasen, BM, Demorest, ZL, Lowy, A, & Ray, E. (2014). Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. *Plant Biotechnology,* 12, 934–940.

Jung, JH, & Altpeter, F. (2016). TALEN mediated targeted mutagenesis of the caffeic acid O-methyltransferase in highly polyploid sugarcane improves cell wall composition for production of bioethanol. *Plant Molecular Biology,* 92, 131–142.

Kazama, T, Okuno, M, Watari, Y, Yanase, S, Koizuka, C, Tsuruta, Y, Sugaya, H, Toyoda, A, Itoh, T, Tsutsumi, N, Toriyama, K, Koizuka, N, & Arimura, SI. (2019). Curing cytoplasmic male sterility via TALEN-mediated mitochondrial genome editing. *Nature Plants,* 5(7), 722-730.

Li, J, Stoddard, TJ , Demorest, ZL, Lavoie, PO, Luo, S, Clasen, BM, Cedrone, F, Ray, EE, Coffman, AP, Daulhac, A, Yabandith, A, Retterath, AJ, Mathis, L, Voytas, DF, D'Aoust, MA, & Zhang F. (2016). Multiplexed, targeted gene editing in *Nicotiana benthamiana* for glyco-engineering and mono- clonal antibody production. *Plant Biotechnology Journal,* 14, 533–542

Li, T, Liu, B, Spalding, MH, Weeks, DP, & Yang, B. (2012). High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nature Biotechnology*, 30, 390–392.

Liang, Z, Zhang, K, Chen, K, Gao, C. (2014). Targeted mutagenesis in Zea mays using TALENs and the CRISPR/Cas system. *Genetics Genomics,* 41(2), 63–68.

Lor, VS, Starker, CG, Voytas, DF, Weiss, D, & Olszewski, NE. (2014). Targeted mutagenesis of the tomato PROCERA gene using transcription activator-like effector nucleases. *Plant Physiology,* 166, 1288–1291.

Ma, L, Zhu, F, Li, Z, Zhang, J, Li, X, Dong, J, & Wang, T. (2015). TALEN-based mutagenesis of lipoxygenase LOX3 enhances the storage tolerance of rice (*Oryza sativa*) seeds. *PLoS ONE,* 10(12), e0143877.

Piatek, AA, Lenaghan, SC, & Neal, SC. (2018). Advanced editing of the nuclear and plastid genomes in plants. *Plant Science,* 273, 42–49.

Shan, Q, Zhang, Y, Chen, K, Zhang, K, & Gao, C. (2015). Creation of Fragrant Rice by Targeted Knockout of the OsBADH2 Gene using TALEN Technology. *Plant Biotechnology,* 13, 791-800.

Shan, Q, Wang, Y, Chen, K, Liang, Z, Li, J, Zhang, Y, Zhang, K, Liu, J, Voytas, DF, Zheng, X, Zhag, Y, & Gao, C. (2013). Rapid and efficient gene modification in rice and Brachypodium using TALENs. *Molecular Plant,* 6(4), 1365–1368.

Sun, Z, Li, N, Huang, G, Xu, J, Pan, Y, Wang, Z, Tang, Q, Song, M, & Wang, X. (2013). Site-Specific Gene Targeting Using Transcription Activator-Like Effector (TALE)-Based Nuclease in *Brassica oleracea. Journal of Integrative Plant Biology,* 55 (11), 1092–1103.

Si, NE, Unger-Wallace, B, Frame, S, Briggs, M, Main, M, Spalding, E, Wang, VK, & Yang, B. (2015). Heritable Site-specific Mutagenesis using TALENs in Maize. *Plant Biotechnology Journal,* 13(7), 1002-1010.

Streubel, J, Blücher, C, Landgraf, A, & Boch, J. (2012). TAL effector RVD specificities and efficiencies. *Nature Biotechnology,* 30(7), 593–595.

Wang, M, Liu, Y, Zhang, C, Liu, J, Liu, X, Wang, L, Wang, W, Chen, H, Wei, C, Ye, X, Li, X, & Tu, X. (2015). Gene editing by co-transformation of TALEN and chimeric RNA/DNA oligonucleotides on the rice OsEPSPS gene and the inheritance of mutations. *PLoS ONE,* 10(4), e0122755.

Wang, Y, Cheng, X, Shan, Q, Zhang, Y, Liu, J, Gao, C, & Qui, JL. (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology,* 32, 947–951.

Wen, S, Liu, H, Li, X, Chen, X, Hong, Y, Li, H, Lu, Q, & Liang, X. (2018). TALEN-mediated targeted mutagenesis of fatty acid desaturase 2 (FAD2) in peanut (*Arachis hypogaea* L.) promotes the accumulation of oleic acid. *Plant Molecular Biology,* 97, 177–185.

Zhang, Y, Zhang, F, Li, X, Baller, JA, Qi, Y, Starker, CG, Bogdanove, AJ, & Voyras, DF. (2013). Transcription Activator-Like Effector Nucleases Enable Efficient Plant Genome Engineering. *Plant Physiology,* 161(1), 20-27.

Zheng, Z, Ye, G, Zhou, Y, Pu, X, Su, W, & Wang, J. (2021). Editing sterol side chain reductase 2 gene (StSSR2) via CRISPR/Cas9 reduces the total steroidal glycoalkaloids in potato, *All Life,* 14(1), 401-413.