AGRI MAGAZINE
(International E-Magazine for Agricultural Articles)
Volume: 03, Issue: 07 (July, 2026)
Available online at http://www.agrimagazine.in
©Agri Magazine, ISSN: 3048-8656

Site Directed Mutagenesis and It's Applications in Crop Improvement

*Bhavesh D. Bhatiya[1], Shivrajsinh V. Chauhan[1], Naresh R. Makwana[2] and K. V. Patel[3]
[1]Ph. D. Research Scholar, Department of Genetics and Plant Breeding, Anand Agricultural University, Anand (Gujarat), India-388110
[2]Agriculture Officer, Main Rice Research Station, Anand Agricultural University, Nawagam (Gujarat), India-387540
[3]Associate Research Scientist and Head, Medicinal and Aromatic Plants Research Station, Anand Agricultural University, Anand (Gujarat), India-388110
*Corresponding Author's email: bhaveshbhatiya11@gmail.com

Site-directed mutagenesis (SDM) is a precise genetic engineering technique that enables targeted modification of specific DNA sequences to alter gene function and expression. With the advent of advanced genome-editing tools such as CRISPR/Cas systems, TALENs, and Zinc Finger Nucleases (ZFNs), SDM has become an important strategy for crop improvement. It facilitates the development of crop varieties with enhanced yield, improved nutritional quality, resistance to diseases and pests, and tolerance to abiotic stresses such as drought, salinity and heat. Unlike conventional breeding, SDM allows rapid and accurate genetic modifications without introducing undesirable traits. Additionally, it serves as a valuable tool for functional genomics and precision breeding. The integration of SDM with emerging genome-editing technologies offers significant potential for developing climate-resilient and high-performing crops, contributing to sustainable agriculture and global food security.

Key words: Site-directed mutagenesis, Genome editing, CRISPR/Cas9, Crop improvement, Targeted mutagenesis, Functional genomics, Climate-resilient crops

Introduction

To enhance the speed of crop improvement programs there is ever-greater need to develop new technologies for multifaceted and integrated strategies of crop improvement. Mutation breeding can play a significant role in creating noble genetic variation which can be utilized in crop-plant improvement. But the major drawback of mutation breeding is that mutations are random in nature and very little frequency of desirable mutations observed. Site-directed mutagenesis is among the recent technologies from the molecular crop improvement methods. Site-directed mutagenesis is aimed at precise change of any coding sequence of DNA which can result in desirable phenotypic changes. Agronomic traits such as yield, quality and stress tolerance have been improved using site-directed mutagenesis. In addition to this selectable marker elimination was also reported from transgenic crops by targeted mutation. Most of the reports on site-directed mutagenesis is focusing on cereal crops (58.33%) followed by horticultural crops (22.92%) (Bezie *et al*., 2021).

Basic Concepts

Genome-editing tools such as Meganucleases, Zinc Finger Nucleases (ZFNs), TALENs and CRISPR/Cas9 act as molecular scissors that recognize specific DNA sequences and generate a double-strand break (DSB) at a targeted genomic location. The progression from meganucleases to CRISPR/Cas9 highlights the increasing simplicity, efficiency and precision

of genome-editing technologies. Once the DNA is cut, the cell activates one of two natural repair pathways. The first pathway, **Homology-Directed Repair (HDR)** functions like a "copy and paste" system. Using a homologous DNA template, HDR enables precise insertion, replacement, or correction of genetic sequences, making it ideal for targeted gene modification and trait improvement (Iqbal *et al*., 2020)

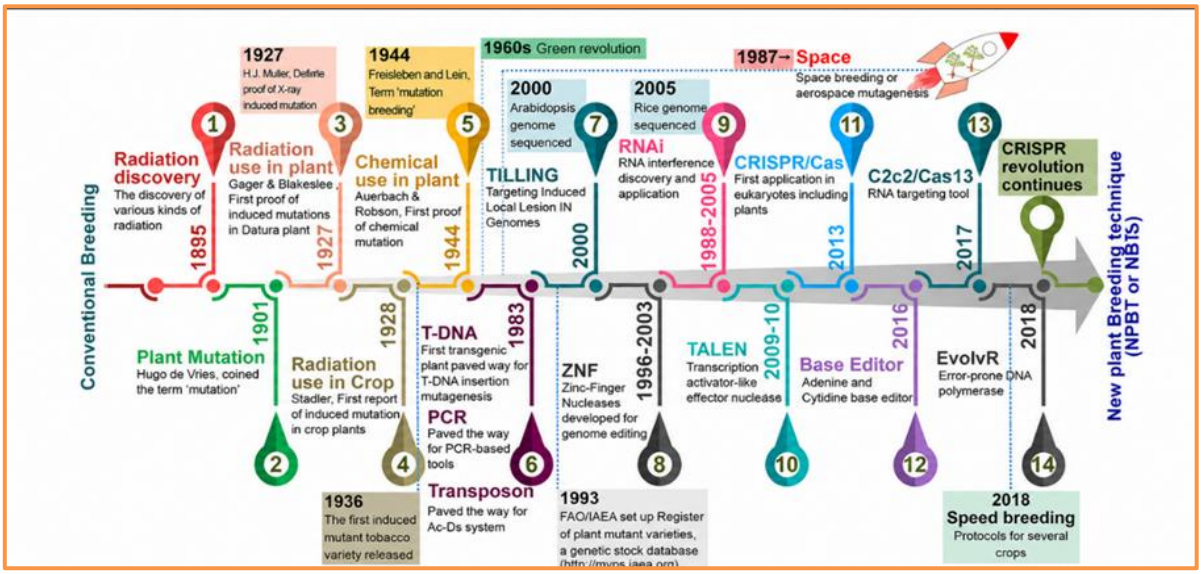

Fig 1: History of induced mutagenesis development in plants (Shelake *et al.*, 2019)

The second pathway, **Non-Homologous End Joining (NHEJ)**, acts as a rapid "cut and join" mechanism. Because it repairs DNA without a template, it often introduces small insertions or deletions (indels) at the break site. These mutations can disrupt gene function, effectively creating gene knockouts for functional studies or crop trait enhancement.

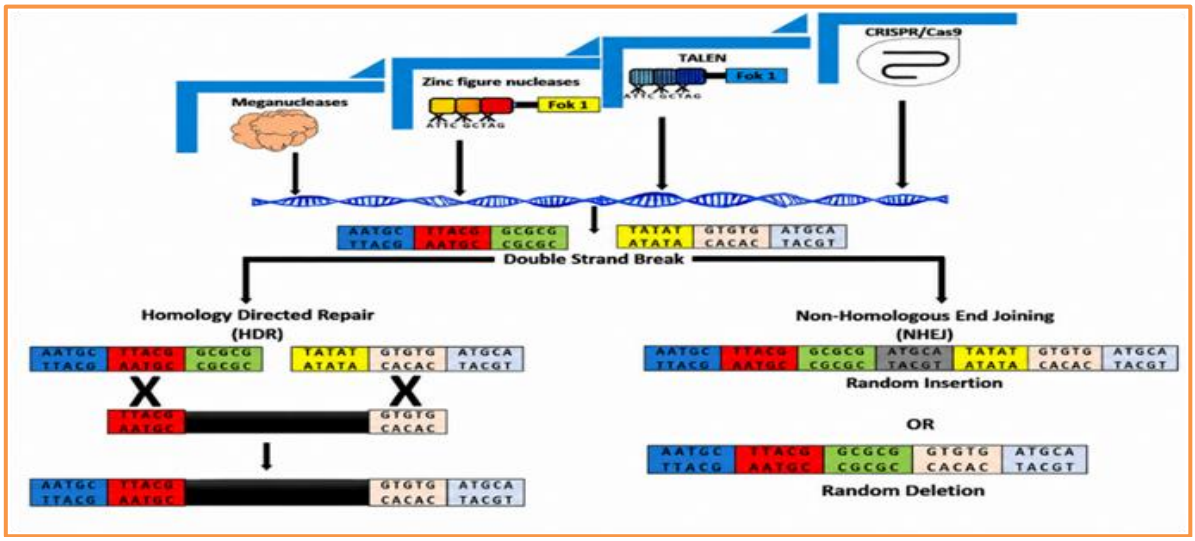

Fig 2: Engineered nucleases induce double stranded break's (DSB) in DNA at particular locus.

Types of Nuclease Used

Basically there are the following four types of SDNs:

1. Zinc Finger Nucleases (ZFNs)
2. Meganucleases (Homing endonucleases)
3. Transcription Activator Like Effector Nucleases (TALENs)
4. Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)-Cas9

Among the four mutagenic tools that have been reported, the CRISPR/Cas-9 technology was found to be most frequently used (66.67%) followed by TALENs. CRISPR/Cas-9 is potential as it is efficient in inducing targeted mutagenesis and less likely off-target effects, so it is

frequently utilized in various research works. TALENs were used to knock-out the genes with undesirable traits.

Criteria for an engineered DNA endonuclease: It must recognize a long DNA sequence with high specificity in order to avoid cytotoxic off-target DNA cleavage; It must be readily designed to recognize and cleave a defined sequence in the genome.

Zinc Finger Nuclease (ZFN)

ZFNs are the chimeric restriction enzymes. The chimeric structure of ZFNs consists of an N-terminal DNA-binding domain derived from Zinc Finger Proteins. These are then connected *via* a peptide linker to a C-terminal Fok1 nuclease domain. Each ZF domain recognizes and binds to 3 tandem nucleotides. The dimerization of the restriction enzyme Fok1 is pivotal for the enzymatic activity of ZFN.

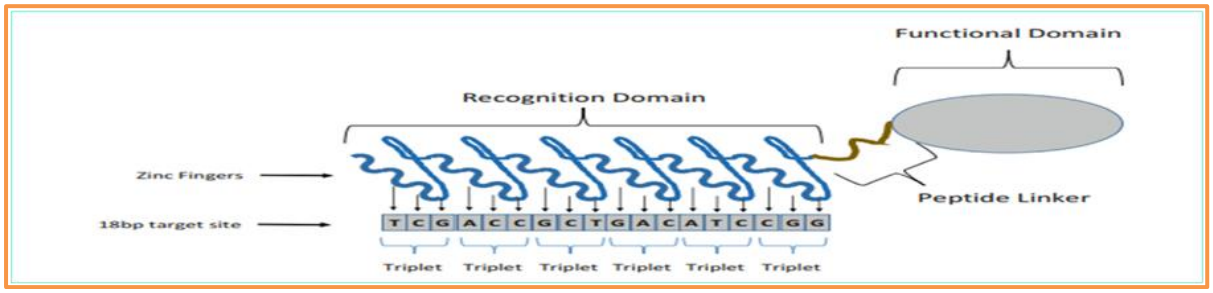

Fig3: Chimeric structure of a ZFP

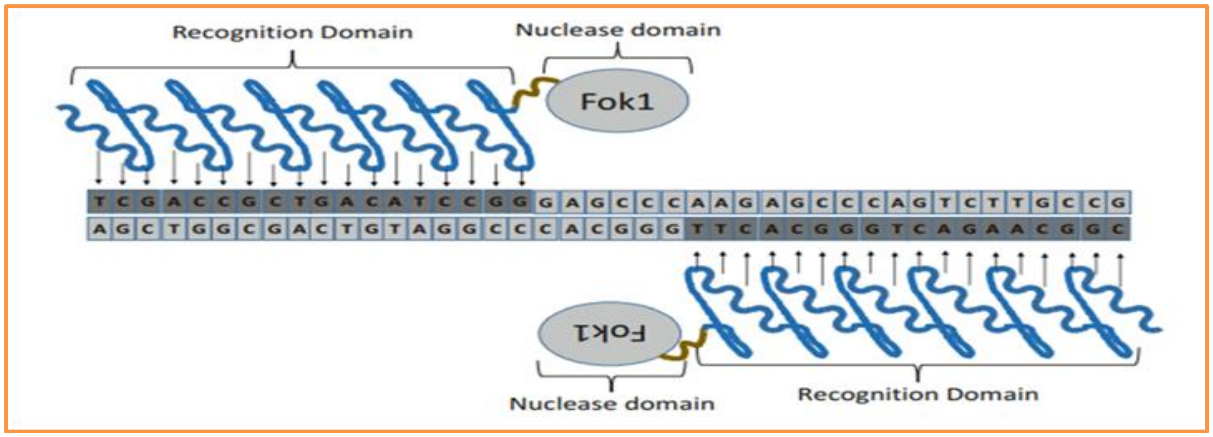

Fig 4: Mechanism Zinc finger nuclease action.

ZFN are effectively deployed when two different ZFNs are oriented in an inverted orientation which allows for each arm of the ZFN to make contact with a different strand of the DNA. Each ZFN specifically recognizes and binds to a segment of DNA (dark gray). The overlapping Fok1 nuclease domains nonspecifically cleave the DNA in the space between the two different zinc finger binding regions.

Meganucleases (Homing endonucleases)

Meganucleases or homing endonucleases were the first sequence specific nucleases deployed for and are found in prokaryotes, archaea and unicellular eukaryotes. It have specific recognition sites of around 14-40 bp and are considered as most specific naturally occurring restriction enzymes. They are comprised of DNA binding and DNA cleavage domains. The formation of DSB at the recognition site of meganucleases, repairs the lesion by HR. The large recognition sites make meganucleases perfect tools for genome editing, in addition to this they have benefit of being less toxic in cells as compared to other methods still Meganucleases have not been widely adopted as genome

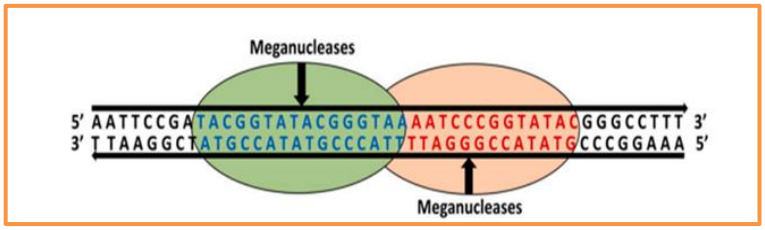

Fig 5: Schematic representation of plant genome editing with meganucleases

editing tools because, the number of naturally occurring meganucleases is limited and not sufficient to cover all potentially interesting loci and high sequence specificity is hard to engineer. A meganuclease identifies a DNA sequence to generate sticky end DSBs by cutting both the DNA strands at specific loci.

Transcription Activator Like Effector Nucleases (TALENs)

TALE are virulence factors derived from *Xanthomonas* plant pathogenic bacteria. Similar to ZFNs, TALENS are chimeric nucleases formed by coupling of 13-28 transcriptional activator-like effector (TALE) repeats (comprises the DNA binding domain) with FokI endonuclease (comprises the cleavage domain). Unlike ZFNs, each TALE repeat targets only one nucleotide which allows flexible target design. This increases the number of potential target sites in comparison to ZFNs.

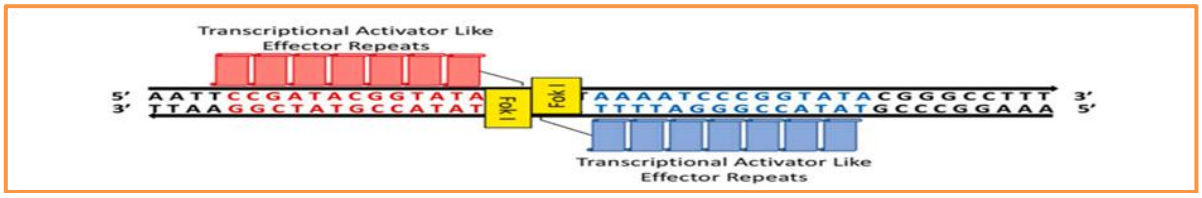

Fig 6: Schematic representation of plant genome editing with TALENs.

The DNA binding domain of TALENs consists of 13-28 TALE Repeats. TALENs generates sticky DSBs.

Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated proteins (CRISPR-Cas)

It has been developed in 2013. Initially, CRISPR/Cas9 editing system was mainly classified into three categories (type I, II and III) on the basis of proteins and accessory RNAs. Among three classes most widely employed simple, efficient and versatile system is the type II clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 (CRISPR-associated) system from *Streptococcus pyogenes*.

Components of CRISPR-Cas nuclease:

1. Protospacer adjacent motif (PAM)
2. CRISPR-RNA (crRNA)
3. trans-activating crRNA (tracrRNA) (guide RNA).
4. Cas proteins

Mechanism of CRISPR-Cas nuclease

- Integration of short fragments of foreign DNA (called spacer) between two adjoining repeats of the CRISPR locus.
- These CRISPR arrays along with the spacers are transcribed upon combating foreign DNA and are processed to form 40 nucleotides long small interfering CRISPR RNAs (crRNAs). They then associate with transactivating CRISPR RNA (tracrRNA) to stimulate and guide the Cas9 nuclease (Abdallah *et al*., 2015).

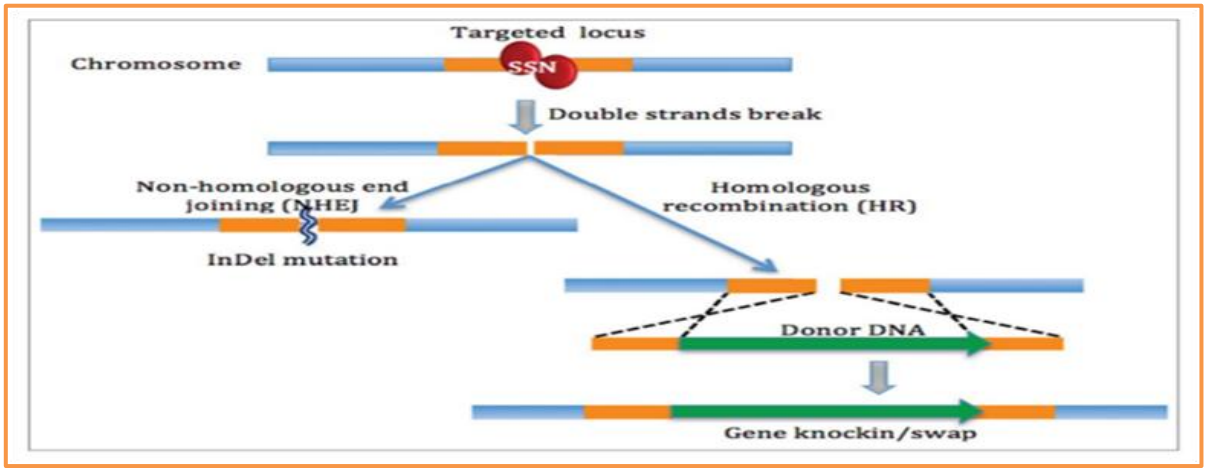

Fig 7: Mechanism of Double Strand Break (DSB) repair

- The homologous double-stranded DNA (protospacers) is cleaved. The condition necessary for this cleavage is the presence of a conserved protospacer-adjacent motif (PAM). PAM is usually located downstream of the target DNA. PAM generally possess the sequence 5′ -NGG-3′ and less commonly NAG.

Table 1: Applications of site-directed mutagenesis in crop improvement

Crops	Improved Trait	Techniques used	Detail	References
Rice	Disease resistance (bacterial blight)	CRISPR/Cas9	Knockout of OsSWEET13 and OsSWEET14 genes	Zhou *et al.*, 2015
	Increase fragrance	TALENs	High fragrant rice by knocking of osBADH2 gene	Ashokkumar *et al.*, 2020
	Improved grain weight	CRISPR/Cas9	Gene knockout	Li *et al.*, 2020
	Yield/biomass	CRISPR/Cas9	Mutations in Gn1a, DEP1, GS3, IPA1 genes	Li *et al.*, 2020
Wheat	Disease resistance (powdery mildew)	TALENs	Editing of MLO genes	Wang *et al.*, 2014
	Increased grain number spikelet	CRISPR/Cas9	mutation on either B or D genome	Wang *et al.*, 2019
Maize	Sterile male maize developed	CRISPR/Cas9	On zmtms5 gene (chr 2 with exon number 5)	Shi *et al.*, 2017
	Drought tolerance	CRISPR/Cas9	Changing the promoter of the ARGOS8 gene	Shi *et al.*, 2017
	Heritable genome modification	TALENs	Evaluation of mutation efficiency maize glossy2 (gl2) locus	Char *et al.*, 2015
Barley	Generating homozygosity in transgenic barley	CRISPR/Cas9	producing homozygous mutants, with the Nud gene knockout resulting in naked grains	Gasparis *et al.*, 2018
	Improve malting quality	CRISPR/Cas9	Mutated the D hordein gene in the cultivar, ‘Golden Promise’.	Li *et al.*, 2020

Challenges and future outlook

Although site-directed mutagenesis (SDM) offers precise genome modification for crop improvement, its application is limited by challenges such as off-target mutations, variable editing efficiency, difficulties in delivering editing components and regulatory constraints. However, advancements in high-fidelity CRISPR systems, base editing, prime editing and transgene-free genome editing are enhancing its precision and efficiency. With these developments, SDM is expected to play a vital role in developing climate-resilient, high-yielding and disease-resistant crops, contributing to sustainable agriculture and global food security.

Conclusion

Site-directed mutagenesis has emerged as a transformative technology in modern crop improvement, enabling precise and targeted genetic modifications that were previously difficult to achieve through conventional breeding. Its ability to accelerate the development of improved crop varieties with desirable agronomic traits makes it a valuable tool for sustainable agriculture. As genome-editing technologies continue to advance, site-directed mutagenesis is expected to become an integral component of precision breeding, supporting future food security and agricultural resilience in the face of global challenges.

References

1. Abdallah, N.A., Prakash, C.S. and McHughen, A.G. (2015). Genome editing for crop improvement: Challenges and opportunities. *GM Crops Food*, **6**(4):183-205.
2. Ashokkumar, S., Jaganathan, D., Ramanathan, V., Rahman, H., Palaniswamy, R., Kambale, R. and Muthurajan, R. (2020). Creation of novel alleles of fragrance gene OsBADH2 in rice through CRISPR/Cas9 mediated gene editing. *PloS one*, **15**(8): e0237018.
3. Bezie, Y., Tilahun, T., Atnaf, M. and Taye, M. (2021). The potential applications of site-directed mutagenesis for crop improvement: A review. *Journal of Crop Science and Biotechnology*, **24**(3): 229-244.
4. Char, S.N., Unger-Wallace, E., Frame, B., Briggs, S.A., Main, M., Spalding, M.H. and Yang, B. (2015). Heritable site-specific mutagenesis using TALEN s in maize. *Plant biotechnology journal*, **13**(7): 1002-1010.
5. Gasparis, S., Kała, M., Przyborowski, M., Łyżnik, L.A., Orczyk, W. and Nadolska-Orczyk, A. (2018). A simple and efficient CRISPR/Cas9 platform for induction of single and multiple, heritable mutations in barley (*Hordeum vulgare* L.). *Plant methods*, **14**(1): 111.
6. Iqbal, Z., Iqbal, M.S., Ahmad, A., Memon, A.G. and Ansari, M.I. (2020). New prospects on the horizon: genome editing to engineer plants for desirable traits. *Current Plant Biology*, **24**: 100171.
7. Li, Y., Liu, D., Zong, Y., Jiang, L., Xi, X., Cao, D. and Liu, B. (2020). New D hordein alleles were created in barley using CRISPR/Cas9 genome editing. *Cereal Research Communications*, **48**(2): 131-138.
8. Shelake, R.M., Pramanik, D. and Kim, J.Y. (2019). Evolution of plant mutagenesis tools: a shifting paradigm from random to targeted genome editing. *Plant Biotechnol Rep*,**13**: 423-445.
9. Shi, J., Gao, H., Wang, H., Lafitte, H.R., Archibald, R.L., Yang, M. and Habben, J.E. (2017). ARGOS 8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant biotechnology journal*, **15**(2): 207-216.
10. Wang, W., Pan, Q., Tian, B., He, F., Chen, Y., Bai, G. and Akhunov, E. (2019). Gene editing of the wheat homologs of TONNEAU 1-recruiting motif encoding gene affects grain shape and weight in wheat. *The Plant Journal*, **100**(2): 251-264.
11. Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C. and Qiu, J. L. (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature biotechnology*, **32**(9): 947-951.
12. Zhou, J., Peng, Z., Long, J., Sosso, D., Liu, B.O., Eom, J.S. and Yang, B. (2015). Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. *The Plant Journal*, **82**(4): 632-643.