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Popular Article

# Zinc Finger Proteins (ZFPs): Strategy and Application in Crop Improvement

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## Introduction

Precise targeted genome editing (additions, deletions or substitutions) tools like Zinc Finger Proteins (ZFPs) in the modern era of biotechnology has broadened the chance of crop improvement in terms of resilience, adaptation, quality parameters and yield. In plant biotechnology, ZFPs have been employed as either zinc finger nucleases (ZFNs) or zinc finger protein transcription factors (ZFP-TFs). Zinc finger proteins (ZFPs) have a modular structure that provides an alluring framework for creating ZFNs. In most cases, these methods utilize site-directed nucleases (SDNs) that consist of DNA-binding domains (ZFPs) connected to a nuclease domain. A particular 3-bp DNA sequence is recognized by each designed zinc finger (ZF) with conjugated Cys2His2 motifs based on the residues in α helix. To recognize a particular DNA sequence (12–18 bp), four to six separate ZFs are often coupled together. Two ZF proteins are needed to target the appropriate DNA sequence (24–36 nucleotides), as the FokI nuclease works as a dimer to cleave double-strand DNA. The DNA-binding domain ensures specificity to particular DNA sequences, while the nuclease domain induces double-stranded DNA breaks at the intended sequence. The host repair pathways, such as nonhomologous end joining (NHEJ) and homology-directed repair (HDR), are responsible for repairing the double-stranded breaks (DSBs) caused by these reagents. ZFNs have been extensively used for genome editing of different crops in last two decades (Petolino *et al.*, 2015). In case of plants ZFN was first used in *Arabidopsis* with 0.2% mutation rate and 10% transmission rate in the next generation (Lloyd *et al*., 2005). This article describes the construct, strategies to be followed by ZFNs and its application in crop improvement.

**Construct of ZFNs**

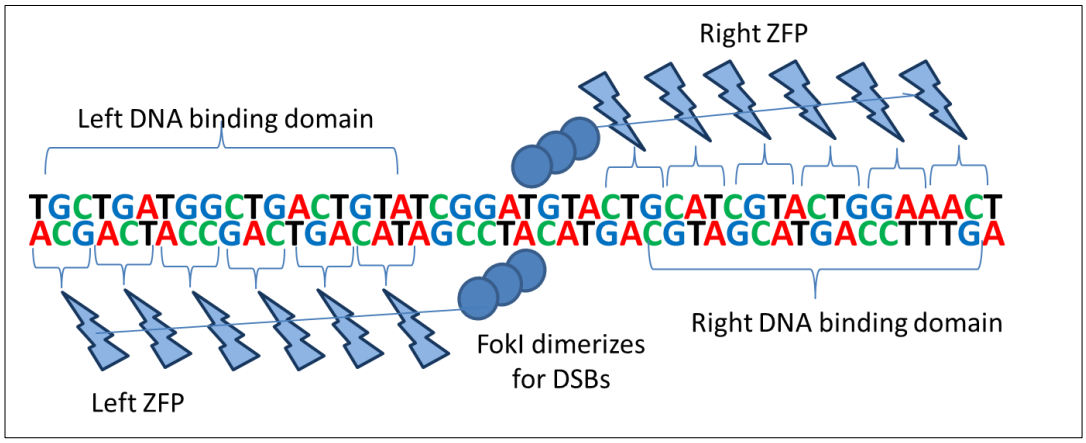


**Fig 1**: Anatomy of typical ZFP constructs

**Experimental process**

After targeting the gene to be silenced, ZFPs construct is designed followed by ZFNs constructions. In the next step, transformation is done with the help of plasmids and $T_0$ generation is cultured. The positive seedlings are then used for transgenic development and phenotypic study. ZFNGenome v2.0, ZifBASE, Zinc-Finger Database (ZiFDB) and Zinc-Finger Tool, EENdb are some popular Target genome-editing tools of ZFNs.

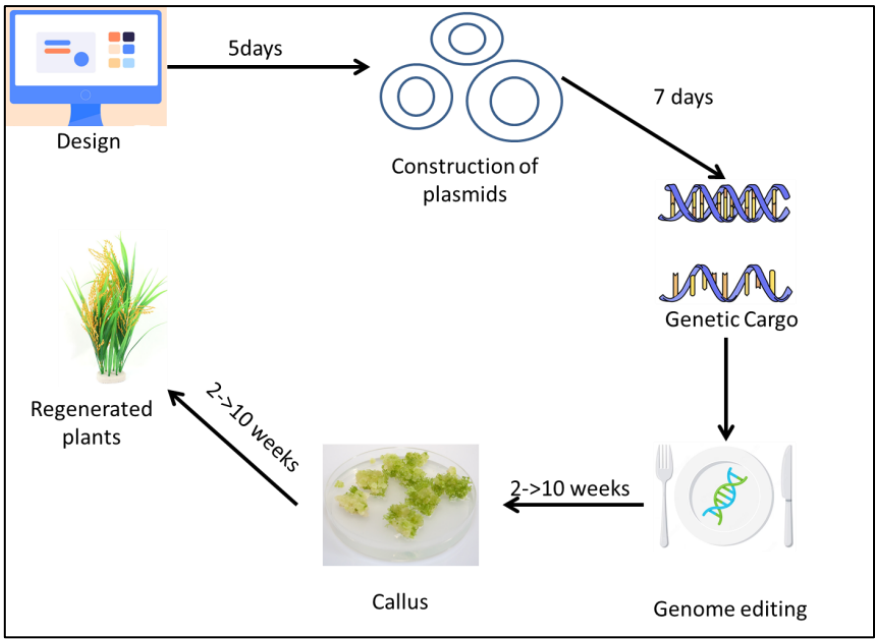


**Fig 2:** Flowchart of experimental process

**Application in plants**

ZFNs have been employed for genetic modification in various plant species, including



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*Arabidopsis*, *Nicotiana*, maize, petunia, soybean, rapeseed, rice, apple, and fig (Martínez-Fortún *et al*., 2017; Ran *et al*., 2017). Significant progress in enhancing the efficiency of transgenic trait stacking was achieved through the utilization of a system enabling selection for ZFN-induced gene targeting via nuclease-mediated cassette exchange (NMCE). The endogenous maize gene ZmIPK1 was modified through the insertion of PAT gene cassettes, leading to the development of herbicide tolerance and changes in the inositol phosphate profile during the growth of maize seeds (Shukla *et al*., 2009). In maize, ZFN-mediated targeted transgene integration has been employed for trait stacking, which involves combining multiple beneficial traits to enhance the potential for crop improvement and the endogenous targeted gene was ZmTLP (Ainley *et al*., 2013). In a subsequent study, Cantos *et al*.,2014 utilized ZFNs to identify genetically stable regions like OsQQR in rice that are suitable for safe gene integration during trait stacking. The application of ZFNs to induce deletions in tandemly arrayed genes (TAG) has been successfully demonstrated in *Arabidopsis* (Qi *et al*., 2013). Engineered zinc finger protein transcription factors (ZFP-TFs) have been utilized to enhance the expression of a native gene in canola (*Brassica napus*) (Gupta *et al*., 2014). By employing an engineered zinc finger protein transcription factor (ZFP-TF) fused with the VP16 activation domain is designed to bind to the 5'UTR of two distinct β-ketoacyl-ACP synthase II (KASII) genes and transgenic plants were generated. These plants exhibited up to a 3.5-fold increase in KASII expression. As a result, both the leaves and seeds of these transgenic plants displayed statistically significant reductions in palmitic acid content, decreased total saturated fatty acid content, and increased total C18 content. Additional modifications in gene expression and fatty acid content were achieved through both activation and repression using zinc finger protein transcription factors (ZFP-TFs) designed specifically for the fatty acid thioesterase B4 (FATB4) and fatty acid thioesterase B5 (FATB5) genes in canola (*Brassica napus*) (Johnson *et al*., 2014). In tomato mitochondrial malate dehydrogenase gene, in soybean the DCL4a and DCL4b genes, in *Arabidopsis* ABA-insensitive 4 (ABI4), alcohol dehydrogenase 1 (ADH1), and transparent testa-4 (TT4) genes have been targeted.

**Drawbacks**

However, the development of ZFNs still poses significant challenges due to its intricate, expensive and technically demanding design, often resulting in low efficacy.

**Conclusions**

Dr. Carroll was the pioneer of using ZFNs for targeted genome modifications. Based on the



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various examples provided, zinc fingers have proven to be a versatile tool in plant biotechnology, either by creating new traits through expression modulation, precision mutagenesis, gene editing, generating new alleles/germplasm through gene deletion, or expediting the deployment of transgenic trait stacks. The numerous applications demonstrate the practical utility of zinc fingers in plant biotechnology. Zinc finger nucleases (ZFNs) offer the capability to target multiple genes to the same location, reducing the number of loci involved in breeding and simplifying the development of multi-trait products. Additionally, ZFNs can be employed to introduce genes into existing commercial-grade events, enabling the creation of new gene stacks to meet the specific requirements of customers and market demands.

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