Article ID 4240214

Base Editing: The Next Frontier in Genome Editing

Somsole Bharath[1], SuprithaRaj DS[2] and Shridhar Ragi[3]

[1,3]Ph.D. Research Scholar, Division of Genetics, ICAR - Indian Agricultural Research Institute, New Delhi-110012.

[2] Ph.D. Research Scholar, Department of Genetics and Plant Breeding, UAS, Dharwad-580005.

*Corresponding Author: bharathsomsole@gmail.com

Elite alleles, found in local cultivars, wild relatives, or other plant species, carry specific genetic differences that enhance important agricultural traits. Most of these differences in the alleles are because of one or more single nucleotide polymorphisms (SNP) or insertions and deletions (InDel) in the coding region of the genome. However, introgressing these alleles to commercial crops without unwanted linkage drag is difficult and time-consuming. Traditional methods take a long time to introduce just one elite allele, and it's hard to get rid of unwanted traits. Precision genome editing offers a promising solution to swiftly introduce elite alleles, thereby expediting plant research and crop breeding. "**Base editing**" has emerged as a novel approach which enables precise nucleotide substitutions in a programmable manner, without disruption of a gene or requiring a donor template.

Generations of Base Editors

Base editing is one of the amazing approaches among the several genome editing methods which can introduce direct and irreversible single base changes at the DNA level without causing deleterious double strand breaks. Four generations of base editors (BE) were reported by Komor and co-workers in their ground breaking research paper published in Nature in 2016 (Komor *et al*., 2016). The first-generation BE was engineered by fusing a rat cytidine deaminase rAPOBEC1(apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) to the N-terminus of a dead Cas9 (dCas9) to generate rAPOBEC1-dCas9 and designated as CBE1(Cytosine Base Editors 1). The substitution of Cytosine (C) to Thymine (T) in DNA is created by deaminating the cytosine (C) into uracil (U) in the exposed non-target DNA strand, and the subsequent DNA repair and replication results in C to T base conversion. The cellular base excision repair (BER) mechanism enables C:G to T:A transition in vivo, while recognizes any G:U base pair as a mismatch. The BER activity eliminates the uracil with the help of uracil N-glycosylase (UNG), resulting in a low efficiency of the CBE1 system.

To improve base editing efficiency, the second-generation BE, CBE2 (rAPOBEC1-dCas9-UGI), was constructed by binding a uracil DNA glycosylase inhibitor (UGI) to the C-terminal of CBE1 to prevent the activity of UNG. CBE2 improves editing efficiency by three times and creates few unexpected indels (<0.1%). To further improve the efficiency, third generation base editors (CBE3) were developed, which constituted the nickase cas9 instead of dead cas9. Nickase cas9 nicks guide RNA complementary strand. CBE3 creates a U/G mismatch with an adjacent nick in the complementary strand, serving as a preferred substrate for cellular mismatch repair. The remaining U-containing non complementary strand as a template to establish a U/A pair, eventually converting to a T/A pair post DNA replication or repair. By capitalizing on the endogenous MMR system, CBE3 facilitates higher frequencies of C-to-T base substitution compared to CBE2.

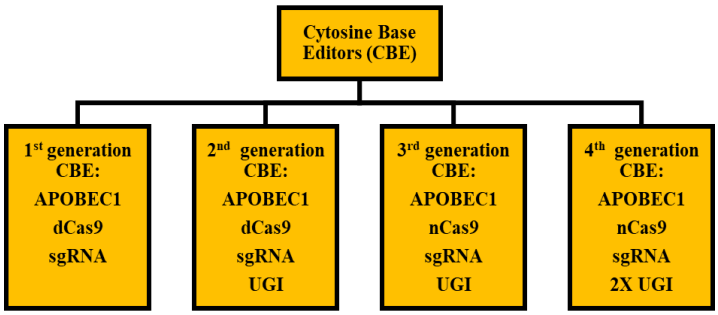

Fig. 1: Flow chart representing different generations of cytosine base editors and components in each generation of base editors. APOBEC1 (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like); dCas9 (dead cas9); nCas9 (nickase cas9); UGI (Uracil Glycosylase Inhibitors), sg RNA (Single guide RNA).

Furthermore, in order to improve the deamination activity, a fourth-generation CBE4, was developed by fusing two UGI molecules to the C-

terminal of Cas9 nickase on the basis of CBE3 to enhance the inhibition of UNG. Compared with CBE3, CBE4 not only improves the base editing efficiency but also reduces the frequency of C to A or G transversions by 2.3 times. Additional improvements were made over these existing base editors regarding narrowing down the editing window, PAM variants, and higher product purity. Swiftly adopted by plant researchers, CBEs have been incorporated into a wide array of plant species which include *Arabidopsis*, rice, wheat, maize, tomato, potato, watermelon, cotton, soybean, apple, pears, strawberry, moss, poplar, and rapeseed.

Adenine Base Editors

Theoretically, inspired by CBEs, the amalgamation of adenine deaminase and nCas9 might yield Adenine Base Editors (ABE), aiming to transform an A-T base pair into a G-C base pair. However, none of the documented naturally existing adenine deaminases exhibit activity on DNA. The advent of ABEs represents a major advancement in genome-editing technologies. Comprising an evolved TadA (tRNA adenine deaminase), Cas9 nickase, and sgRNA. ABEs capitalize on TadA's capability to convert A to inosine (I) in the anticodon loop of tRNAArg, mimicking G in cellular processes. Through genetic engineering in E. coli, Gaudelli *et al*. (2017) created TadA mutants enabling A to I conversion on DNA. ABE construction involves linking mutated TadA with a modified Cas9. The Cas9 nickase induces a single-strand DNA break precisely opposite the A to I conversion, triggering the cell to insert the correct base pair and completing the transition from A-T to G-C. Importantly, this Cas9 variant exclusively nicks DNA, avoiding the typical double-strand break associated with the native enzyme in CRISPR/Cas9 gene editing. To facilitate deamination, TadA operates as a dimer. Scientists devised a heterodimeric protein, by pairing a wild-type TadA non-catalytic monomer with an engineered catalytic monomer (TadA*). ABEs, efficiently converting A to G in mammalian cells with high purity, were generated by combining this heterodimer with nCas9. Unlike uracil excision repair, cellular inosine excision repair displayed weak activity and did not hinder the A-T to G-C conversion. So, developing ABEs didn't require an additional glycosylase inhibitor protein. Similar to CBEs, ABEs found rapid application and validation in diverse plant species, such as rice, wheat, *Arabidopsis, Brassica napus, Nicotiana benthamiana*, poplar, and moss.

Applications in Crop Improvement

Cytidine base editors have proven successful in editing a variety of plants, including rice, maize, tomato, wheat, cotton, and watermelon. Rice plants acquired resistance to multiple herbicides through multiplex base editing, as demonstrated by Shimatani *et al*. (2017). Using the Target-Activation-Induced Cytidine Deaminase (Target-AID) system, researchers fused either dCas9 or nCas9 with Petromyzon marinus cytidine deaminase (PmCDA1) and sgRNAs to target the Acetolactate synthase (ALS) gene. This gene, when mutated, confers herbicide resistance, as observed in the C287T mutation resulting in an A96V amino acid substitution and resistance to imazamox (IMZ) in rice. Employing Target-AID-based base editing, researchers introduced a similar point mutation in the ALS gene. Spontaneous resistance mutations occurred at 1.56%, while nCas9OsPmCDA1At transformants exhibited 3.41% IMZ tolerance. Seven out of 14 edited lines displayed the ALS-A96V mutation, with no detected off-target effects.

ABEs, akin to CBEs, have demonstrated successful applications in various crops for precise base editing. Adapted from their effective use in mammalian cells, ABEs have been optimized to establish an adenine base-editing system in plants, creating targeted point mutations. ABE7-10, known for highly efficient A-T to G-C conversions in mammalian cells, served as a basis for ABE-P1, a modified version utilized for precise editing in rice plants. Evaluating the editing efficiency in rice, ABE-P1 targeted IPA1 (OsSPL14), a key gene influencing plant architecture and grain yield. A designed sgRNA successfully induced T.C substitutions at the OsmiR156 binding site in OsSPL14, with an editing efficiency of 26% observed in 6 out of 23 transgenic lines. Importantly, predicted off-target sites did not exhibit any base-editing events. ABE-P1 demonstrated a broader base-editing window (4-7) in rice compared

to ABE7-10 in mammalian cells, emphasizing the specificity and efficiency of ABEs in rice.

Limitations and Methos to Overcome it

There are certain limitations in using base editors. One of the limitation is bystander edits. In case of bystander edits when multiple Cs or As are present within or near the target region all the Cs or As will be converted to Ts or Gs respectively which is undesirable. To mitigate the frequency of bystander mutations, one effective approach is to narrow the editing window. The width of this window is dictated by the DNA base editor deaminase. Introducing specific mutations in the deaminase can effectively reduce the size of the editing window without significantly affecting deaminase activity. Successful base editing requires the presence of a specific PAM sequence (NGG PAM for SpCas9) and the target base must be within a narrow base-editing window. This specific PAM requirement is a severe limitation which lowers the editing efficiency in plants. To broaden the PAM compatibility and expand the scope of base editing, several research groups have developed novel ABE and CBE base editors using Cas9 variants which recognize PAMs other than the NGG motif. Unpredictable genome wide off target effects were also reported in various studies. This can be minimized by using optimized Cas9 domains.

Conclusion

Base editing, revolutionizing agricultural studies, allows for precise manipulation of genomes in plants. Widely utilized, this innovation enhances the agricultural significance of various crops. Despite notable advancements, there are still gaps in effectiveness that require additional improvements to achieve comprehensive genome manipulation in plants. However, as crucial agronomic traits are often controlled by multiple genetic loci, targeting individual genes may not suffice to bring about the desired phenotypic alterations. Therefore, it is imperative to develop more efficient base editing techniques capable of combining mutated alleles. Further enhancements are advised to optimize the breadth and effectiveness of base editing tools.

References

Gaudelli, N. M., Komor, A. C., Rees, H. A., Packer, M. S., Badran, A. H., Bryson, D. I., & Liu, D. R. (2017). Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature*, *551*(7681), 464–471.

Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A., & Liu, D. R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*, *533*(7603), 420–424.

Lu, Y., & Zhu, J. K. (2017). Precise Editing of a Target Base in the Rice Genome Using a Modified CRISPR/Cas9 System. *Molecular plant*, *10*(3), 523–525. https://doi.org/10.1016/j.molp.2016.11.013

Mishra, R., Joshi, R. K., & Zhao, K. (2020). Base editing in crops: current advances, limitations and future implications. *Plant biotechnology journal*, *18*(1), 20–31.

Shimatani, Z., Kashojiya, S., Takayama, M., Terada, R., Arazoe, T., Ishii, H., Teramura, H., Yamamoto, T., Komatsu, H., Miura, K., Ezura, H., Nishida, K., Ariizumi, T., & Kondo, A. (2017). Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. *Nature biotechnology*, *35*(5), 441–443.

* * * * * * * *