

Molecular Markers and its Applications in Plant Breeding

Bedadyuti Mohanty*

Department of Biotechnology, SRM Institute of Science & Technology, Chennai, India

Editorial

Various biotic and abiotic factors cause different plant diseases and number of stresses leading to low crop productivity and huge yield losses thereby causing global food scarcity. Hence, sustainable crop development and improvement requires breeders to produce crops continuously with disease and pest resistance varieties and with other potentials such as stress tolerance, high nutritional value crop and better yield, etc. Though there have been many conventional strategies to improve breeding in crops (conventional breeding, gene bank, etc.), molecular marker technology have surpassed all these methods in terms of efficiency.

Molecular biomarkers

Molecular biomarkers are short sequence of DNA linked with a specific region of the genome. Marker molecules can be of both the types; shorter DNA sequence, like sequence surrounding a single nucleotide polymorphism, where a single base-pair change occurs and longer DNA sequences, like the microsatellites (10 to 60 base pairs long).

Molecular biomarkers are designed for various biophysical properties that allow them to be measured in different biological samples. They have wide range of applications as nucleic acid-based biomarkers in gene mutations or polymorphisms, lipid metabolites, proteins, peptides and other small molecules gene expression analysis. Recently, researchers have discovered a wide range of applications of molecular markers in the field of molecular and genetic breeding including gene selection and editing [1,2].

CRISPR/Cas9

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9) has been used extensively in technologies such as genome editing technology for its versatility, efficiency and simplicity. CRISPR-Cas9 system possesses most reliable target specificity function which is based on Watson and Crick model where the off-target sites are recognized through sequence analysis. Cas9 and single guide RNA are the two component of CRISPR/Cas9 which cleaves the foreign DNA, where Cas9 is basically a DNA endonuclease which is extracted from bacteria like the *Streptococcus pyogenes*, *Brevibacillus laterosporus*, *Streptococcus thermophilus*, *Staphylococcus aureus*. The most commonly used bacteria is *Streptococcus pyogenes* for Cas9 isolation [3-6].

CRISPR/Cas9 is the latest introduced technology in genome editing and is being extensively appreciated worldwide for its contribution in science and now the applications have been extended to crop plant improvement technology. As mentioned earlier, the world needs more production of crops and also with some genetic modification to counter different biotic and abiotic stresses. This technology has enabled the researchers throughout the world to perform DNA free insertions, gene knock-ins or insertions, and gene knock-out-outs.

CRISPR/Cas9 generally known as CRISPR is a specific designed sequence of DNA which is associated with an enzyme called Cas9 which is

basically working as molecular scissors for cleaving the target DNA. CRISPR was first identified in bacteria and archaea, where their main function was to defend the cell from viral DNA and RNA or to perform the role of acquired immunity mechanism. There are uncountable applications of this technology and in terms of plant breeding, this technology has boosted the plant genetic engineering field by introducing novel plant therapeutic and crop improvement applications.

CRISPR/Cas9 mechanism

The mechanism initiates with the development of an editing complex followed by pairing of Cas9 (molecular scissor enzyme) with the guide RNA. The guide RNA or gRNA possess the complementary sequences and helps in the delivery of Cas9 to the genome. Further this Cas9, guide RNA and complementary sequence (complex) pairs with the targeted gene in the genome. Then, this target gene (on the genome) is then cleaved by the Cas9 enzyme. Though numbers of attempts are made by the cell in order to repair the DNA, the attempts fail and thus, it creates a mutation causing its function to get disabled permanently. In the next step the original gene gets finally replaced by inserting the desired gene with target specific function for filling the gap. Finally, it leads to the production of desired specific protein.

Recent application of CRISPR in plant genetic engineering includes disease resistant wheat, healthier soy crop with higher soya oil production and long term storage potatoes. Genetic engineering is the most suitable method among all other breeding techniques as in gene editing a single desired character can be inserted without hampering the background. Similar kind of alteration can be introduced in different varieties or cultivars produced in different environment without being resulted into monoculture [7-10]. The applications of genomic editing that take in consideration the use of homology directed repair (HDR) mechanism of CRISPR/Cas are also of magnificent interest in the plant research industry. The HDR tool functions to insert or knock-in the DNA fragment, like tags or new domains, as well as allele replacements and recoded genes. Despite of novel desirable molecular breeding techniques for DNA free editing, CRISPR/Cas9 being a revolutionary editing system promises for better complex genomic relocation.

Further improvement CRISPR/Cas9 technology and the formation of an evaluation system can enable more researchers to introduce an optimistic behavior toward production of CRISPR-edited crops.

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*Address for Correspondence: Mohanty B, Department of Biotechnology, SRM Institute of Science & Technology, Chennai, India, E-mail: bedadyutimohanty7@gmail.com

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