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Genome Editing in rice : Current Scenario in India

Satya Sandeep Tata*[1], Sudhakar Godi[2]

*[1]Department of Biotechnology, Andhra University, Visakhapatnam.

[2]Department of Genetics, Andhra University, Visakhapatnam.

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INTRODUCTION:

Unlike the conventional breeding approaches and genome editing approaches using SSNs such as ZFNs and TALENs, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology has drawn the attention of the scientific community as it seems to be more efficient, less time-consuming, and robust as well. The profound application of this widespread technology in the field of agricultural research has changed the scenario. Rice (*Oryza sativa*. L) is a major food source for billions of people all over the world. It is very difficult to meet the food demand by following traditional agricultural methods. Hence there is a need of editing the genome to increase crop productivity. CRISPR technology is a Cas (CRISPR associated) mediated genome editing tool through which double-stranded breaks can be induced in the genome and incorporation of desired sequences for the development of agronomic traits, using short guider RNAs. This system has enabled the enhancement of the desired traits and elimination of unwanted traits in the rice plants. Rice is a flexible model system for the study of functional genomics. It is known that CRISPR/Cas approach has been applied in rice to develop the traits like nutritional quality improvement, biotic and abiotic stress tolerance, grain yield and quality improvement, stomatal density, etc. Recently it is being employed to increase the size of root hair cells, through which the phosphate intake efficiency is expected to be enhanced. CRISPR/Cas has the potential of enhancing food security, sustainable agriculture if it is successfully employed in the lab as well as in the farmers' field.

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MECHANISM:

CRISPR/Cas (CRISPR-associated protein) system is a bacterial adaptive defence system that has the potential of cleaving the genetic material. Unlike ZFNs (Zinc Finger Nucleases) and TALENs (Transcription Activator Like Effector Nucleases), it is an RNA-guided DNA cleavage mechanism. Along with the clustered short palindromic repeats, it is evident that it includes several other components such as a gRNA, crRNA, tracrRNA, and PAM motifs. Cas9 is a class 2- type II effector nuclease, that cleaves the target DNA precisely at a position where the short single guide RNA was bound. Cas9 contains several domains namely NUC, REC1, RuvC, PI facilitate the cleavage of DNA at the target site. Complementary and non-complementary strands of sgRNA were cleaved by HNH and RuvC like domains respectively.Double-stranded breaks generated as a result of the activity of Cas9 can be repaired by two mechanisms namely the Non-homologous end joining (NHEJ) pathway and HomologyDirectedRepair (HDR) pathway.

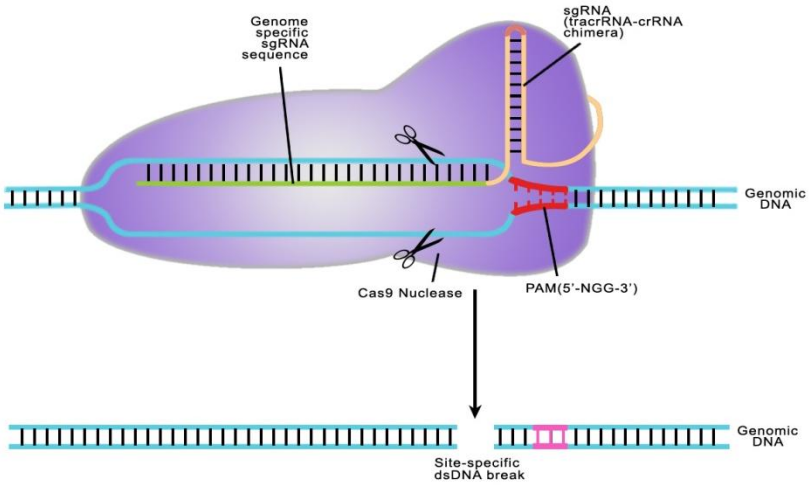

Fig 1: CRISPR/Cas9 System

CRISPR/Cas System for Crop Improvement :

CRISPR/Cas9 system was extensively used for the crop improvement of rice cultivars because of its accuracy, precision, and cost-effectiveness. After enormous efforts, researchers were able to get highly satisfying results. For instance, in order to enhance the rice yield, *DEP1* (Dense and Erect Panicle 1 gene), *Gn1a* (grain number gene), *GS3* (gene for grain size), and *IPA1* (plant architecture gene) genes were edited and obtained the desired yield

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enhancement. Similarly, a multiplex genome editing system that was employed to edit three yield-related QTL genes viz., *OsGS3, OsGW2,* and *OsGn1a* resulted in a 30-68% of yield increase per panicle. Along with the yield, quality, nutritional potential, tolerance to biotic and abiotic stresses are the major traits to be focused on. To ensure that, the *Badh2* gene was edited to develop fragrant rice in the Zhonghua 11, which is an Indica rice variety. The *OsOr* gene for increased β-carotene accumulation, *SBEIIb* gene for a considerable increase in starch and amylose contents, *OsNramp5* gene for low-cadmium rice, *OsRR22* gene for improved salt tolerance, *OsSWEET14* for increased BLB resistance and *OsERF922* gene for rice blast disease resistance were edited.

Current status of Genome Edited (GEd) crops in India:

Controversy and dilemma on approvalof Genome edited plants in India, is an issueof debate for many years. Though the Department of Biotechnology, Govt of India istaking many initiatives such as i) Indo-USGenome Engineering / Editing Technologies Initiative (GETin) Program, ii) Individual-centricand multi-institutional R&D projects on Genome Editing Technologies andtheir Applications etc., to promote genome editing research in India, thedeveloped varieties / GEd lines were not being released to the farmingcommunity. The government has framed certain regulations and risk assessmentguidelines on genome editing technologies and GEd organisms, under “rules 1989” of EP Act 1986. Based on the complexity of the functional / structuralmodification compared with the known natural organism, a Risk Evaluation Matrixwas developed and the GEd lines were categorized into three groups viz.,Group-I, II, &III also named as SDN (Site-Directed Nucleases)-1,2&3 Groups.Organisms falling under Groups-I & II do not contain any foreign geneinserted but possess minor modificationsat the target base level. Scientists argue that the first 2 groups can be considered asnon-GMO GEd lines, as they are transgene-free in nature. The 3rd groupcould be considered GMO, as it contains transgenes inserted. All the approvals, assessments andgrants are monitored by hierarchical statutory committees such as InstitutionalBio-Safety Committee (IBSC), Review Committee on Genetic Manipulation (RCGM)& Genetic Engineering Appraisal Committee (GEAC) etc.

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Conclusion:

"End hunger, achieve food security and improve nutrition, and promote sustainable agriculture" is the second listed (prioritized immediately after "zero poverty"), among the sustainable development goals by UNO. The estimated rice production in India for the Kharif season of the crop year 2020-21 was 102.36 million. In order to meet the food demands, GEd lines with high productivity should be cultivated and could be included under the National Food Security Mission (NFSM-Rice). Epigenetic modifications using CRISPR/Cas 9 systems were not reported so far in rice. Hence the development of these systems would report the trait enhancement without the integration of transgenes. This approach could abide by the biosafety norms and ensures sustainable agriculture.