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Opinion

Recent Milestones Achieved in Rice Genomes: Hurdles and Future Strategies

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Abstract

Rice is an important crop among the other cereals and considered as the model crop for function genomic studies. The rice genome size is very small 389m. The simplicity protocol of genetic transformation, physical and molecular map also developed. The recent advancement in genome sequencing and genome editing technologies has enabled us to demonstrate the potential and function of various genes for rice improvement. This spotlight presents the comprehensive overview the modern tools and resources for advance in rice genome to develop elite rice genotype which have potential tolerance against multi stresses. However, we argue the next step of rice functional genome improvement, draft genome refinement and resequencing of rice broad diversity panel genome with highly efficient technology and multidisciplinary integrated approaches to inferring gene function and future rice improvement program.

Keywords: CRISPR/Cas9; Rice; Genome; TALENs; CRISPR/Cpf1; Targeted mutation

Introduction

Rice (*Oryza sativa* L) is staple food after wheat and maize. It is the major source of the dietary energy supply of the globe. It is estimated that three billion people utilize rice grain daily and almost 20% of the globe energy source [1]. The rice crop expanded worldwide due to diverse resistance and wider potential to survive at different ecological conditions. Food and agriculture organization considered it the world strategic crop in terms of food security due to wider potential of adaptability [2]. The rice consumption enhanced from 450 million tons (2011) to 490 million tons (2020) and will exceed to 650 million tons in 2050 [3]. It is estimated that population will demand approximately 40% more to fulfill their needs in 2050 [4]. The combined conventional and molecular approaches have made toward rice productivity in last few years. In the recent decades, the rice production declined gradually. There are other many challenges such as rapid population growth, emergence of pathogens, globe climate change and other environmental factors which are the main cause of reduction in rice yield. In the current scenario, there is urgent need of modern technologies which enable the breeders to develop verities with higher potential of yield and excellent tolerance against biotic and abiotic stress.

Modern era in Rice Genome

In the modern era of crop improvement, the hurdle of conventional breeding curtailed with emergence and advancement in modern genome editing technology. Genome editing technology enable us to modify gene at specific location in the genome with the usage of SSNs (engineered site of specific nucleases) [4-8]. The applications of genome editing tools have extended rice research to develop new varieties which have better yield and quality. In current review, we emphasis on genome editing approaches, their applications, major challenges and future prospects of genome editing in rice crop. We also emphasized the emergence of CRISPR/Cpf1 and CRISPR/Cas9 system for rice crop development [8].

The limitation of traditional breeding method has replaced by recent genome editing technologies that leads to new era of crop enhancement. The site-specific nucleases (SSNs) like zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) break the targeted DNA and repaired by cells of homologous recombination (HR) or non-homologous end joining (NHEJ) through natural repair mechanism [9]. The NHEJ repair is the error prone pathway while HR pathway is much more precise in the exchange of homologous sequence leading to gene knock in or gene replacement [10,11].

Researcher have published many articles from last 5 year to indicate effective targeted mutagenic in variety of crops by just using CRISPR/Cpf1, CRISPR/Cas9, and TALENs systems [12-16].

First time targeted mutagenic reported in rice crop in early 2012 when gene of bacterial blight susceptible Os11N3 (also famous as OsSWEET14) was usually targeted for TALEN-based distraction for making disease resistant plant lines [17]. Then further studies done by using TALENs for targeting multiple susceptible genes against blight disease in rice crop [18-20]. TALEN technology was not only used for targeting disease but also used for enhancement of fragrance by interrupting Oryza sativa betaine aldehyde dehydrogenase 2 (OsBADH2) gene in rice [21]. In a study, it was confirmed that Lig4 plays significant role in the cNHEJ pathway in rice and lack of DNA lig4 knockout rice lines can improve the frequency of TALEN-mediated targeted mutagenesis [22].

During 2013, there were published almost five articles related to effective type of targeted mutagenic by using CRISPR/Cas9 system in rice [13,21,23,24]. A single modified sgRNA was used to induce target mutations in three gene of rice, OsMPK2, Os02g23823, and OsBADH2 resulted in high frequency of mutation in CRISPR/Cas9 construct designed for OsSWEET14 and OsSWEET11, which caused deletion of nine and seven nucleotides from the promoter of the OsSWEET14 and OsSWEET11 genes [24]. In the similar year, a CRISPR/Cas9 concept was used for immediate targeting of three rice genes, young seedling albino (YSA), stromal processing peptidase (SPP) and rice outmost cell-specific gene 5 (ROC5) resultant in homozygous or bi-allelic mutants with the high mutation frequency up to 84 percent in the T0 and T1 rice lines [23]. Furthermore, four sugar efflux transporter genes also targeted by CRISPR/Cas9 named (OsSWEET11, OsSWEET12, OsSWEET13, and OsSWEET14) resultant in large chromosomal deletions between two nuclease-targeted loci [8]. As such, the concepts were suggested for knockout screening of whole rice genome with sgRNA libraries for mutant rice populations with greater heritable variability and precision. These studies evidently showed that CRISPR/Cas9 system can be used as real tool for chromosomal engineering, production of insertion, deletion, substitution, and translocation lines which showing greater efficiency for the advancement of new cultivars with better novel traits. In rice targeted mutation was successfully used to knockout the multi paralogous gene with the help of CRISPER/Cas9 system [15].

Targeted mutation of three rice genes, namely, OsMPK2, Os02g23823 and phytoene desaturase (OsPDS) exposed a high co-mutation rate with the range of mutation frequency between 66.4 and 81 percent. In second study, a high-efficiency multiplex genome editing was tried in rice by producing multiple sgRNA cassettes [25]. In rice genome up to 46 target sites were edited with an average 85.4% mutation frequency. As many as 46 target sites were edited in the rice genome with an average mutation frequency of 85.4%. The study also confirmed immediate editing of three sites within the gene OsWaxy, which caused of amylose content reduction (up to 14 percent). Multiplex genome editing was also testified with the help of endogenous tRNA processing system in rice, wherever each sgRNA was flanked by tRNA and processed into single sgRNAs which caused of large deletions in genomic sequences of T0 generation [26]. Likewise, it was reported a new strategy in rice for CRISPR/Cas9-sgRNA multiplex editing system where 21 sgRNAs were designed and the equivalent Cas9/sgRNAs expression vectors were created [27]. The successfully edition of transformed rice plant were significantly edited and up to 82 percent of the desired target sites represented deletion, insertion, substitution, and inversion, thus exhibiting high editing efficiency. All these reports clearly show that the CRISPR/Cas9 system is highly effective to create multiple gene mutations by using conventional strategy that could be subsequently used for the rice breeding in near future.

Challenges and Ways Forward

In addition, several species of rice have been sequenced which was cultivated in old world (Africa, Asia and Europe). These rice species contain disease resistance qualities such as fungal, viral diseases and also it is drought resistant [28]. These sequences are of such types which can recognize the genes handling the biotic and abiotic factors in which genome editing platforms can be applied to on the cultivated rice such as clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9. Now it is possible to modify the rice crop as “marker free” and “transgenic free” editing [29]. However, there are some barriers such monitoring affairs and obstinate nature of rice on the basis of which it is impossible for the breeder, scientists and biotechnologists are limited to adopt these technologies. The other alternative way is to use the marker assisted ‘speed breeding”. Specifically, there is need to improve the traits by an alternative way such as yield, quality and adaptation of the other practices such as biotic and abiotic factor stresses on the basis of it is possible to bring some changes. Although there has some innovation been developed in the genetic world which enables us to make some formal changes in the genes of crop to modify it against different stresses, while resolving the green super rice is still not solved. There is a basic need to bring more accuracy in the genome assembling and long-read sequencing technologies and designed to self-learn and resolve biotic and abiotic stress tolerance issue is a way forward to solve this problem.

The mutlidisplinary approaches are required to harnessing the complete potential of rice functional genome and assimilated the knowledge of biological and other molecular process in response to gene exploitation. One approach unable to inferring the gene function and mutlidisplinary approaches are required to mitigate this problem. The different regulatory pathway of underlying different traits will be explored and critically investigated through the additional information which is obtained from proteomics, genomics, transcriptomics, and epigenomics studies. The high potential software and bioinformatics tools underlying good resolving power need to be developed for the improvement and categorically explore the rice functional genome. The refinement of huge date comes from different high through put techniques should be stored and interconnected with fundamental updated database. The linked data with updated data bases easily available for comparison that will enhance the understanding and open the gate for future advancement in rice genome.

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Conclusion

It is concluded that the genome editing technology developed the similar modified plant to conventional breeding. CRISPR/Cas9 and other tools improve the rice genome through revolutionary change which meet the curial requirement and demand of rice for future generations. The further research is requiring to optimized refine the CRISPR/Cas9 protocol in rice and need more effort for making freely accessible and user friendly in the practical application. The efficient transformation technology would facilitate the development biotic and abiotic stress crop. However, the multidisciplinary and integrated approaches allow the complete characterization of rice function genome, inferring the single gene function and talking full benefits. The advance in rice genome will integrated with developing high throughput technology, information of proteomics, transcriptomics, bioinformatics, epigenomics and genomics in the future breeding program.

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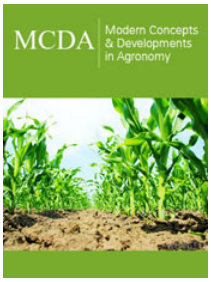

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