Research Article
THE INTERNATIONAL JOURNAL OF BIOLOGICAL RESEARCH (TIJOBR), ISSN Online: 2618-1444
Vol. 2(2) June-Aug, 295-300; 2019
http://www.rndjournals.com

Genome Editing and Speed Breeding; Game Changers to Boost the Crop Production

Muhammad Haroon[1]✉ Rabail Afzal[2], Fahad Idrees[1], Ahmar Sunny[1], Abdul Saboor Khan[1,]
[1]National Key Lab of Crop Genetics Improvement, Huazhong Agricultural University China.
[2]Department of Plant Breeding and Genetics, University of Agriculture Faisalabad Pakistan.
*Corresponding Author: muhammadharoon974@gmail.com

ABSTRACT:
Selection of the desired plants was started as the human civilization started. In result of human civilization, population was also increased, and it will be doubled up to 2050. Increased population will need more food. To overcome the future food challenges, new high yielded and disease resistant crop varieties are being developed. For the development of new lines, conventional breeding methods are applied. These conventional methods cannot meet the food demand in a very short time as it takes 6 to 7 years for the development of a new variety. To address this problem, different gene editing techniques including ZFN, TALEN and CRISPR/Cas9 were also employed. In comparison to zinc-finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN), CRISPR technique showed more efficiency. CRISPR/Cas9 is used to edit the plant's genome efficiently and precisely. With the use of CRISPR technique, Speed breeding procedure is also adapted to decrease the crop's life cycle. By decreasing the crop cycle, a variety can be developed in a very short time. Comparison to conventional breeding methods, both new techniques can increase the yield, adaptability, biotic and abiotic resistances in a very short period.

Keywords: Speed breeding, CRISPR, Conventional breeding, Genome editing

---

INTRODUCTION:

10,000 years ago, plant breeding started when human selected more productive plants for their food. The longest plant breeding art started with the human civilization. Around the world, more attention is given to electronic technologies, instead of this inequality, if same working potential is given to agriculture, adequate food can be produced to overcome the hunger problems. Only 2 % of the American population is directly involved in the agriculture sector (Hallauer, 2011).

To overcome the food scarcity, molecular researchers tried their best and developed different suitable methods and technologies for the increment of crop productions. From plant selection to CRISPR's (Clustered Regularly Interspaced Short Palindromic Repeats) discovery, all the procedures and techniques were engaged for the pursuit of more crop production. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a kind of defensive immune system of many bacterial and archaeal species. Upon the attack of viruses, these tiny creatures recognize the foreign inserted genome in the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) cassette and target that specific genome for degradation (Makarova & Koonin, 2015).

Plant breeding is an outdated art that constitutes less increment for the crop production. With the ever-increasing number of populations, the dire need was to replace the old techniques with the newer one. That's why, in the late 1990s and early 2000s, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) drew the attention of many molecular researchers around the globe. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a defensive mechanism of tiny creatures; bacteria and archaea. These palindromic sequences serve as a safeguard against the insertion of the exogenous genome.

Due to the protection strategy of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), molecular researchers utilized this system for the betterment of mankind services. This system works based on the recognition and defensive strategy. Upon recognition of foreign bacteriophage's genome, the prokaryotic CRISPR system

recognizes the inserted genome and add them in the CRISPR cassette. Finally, by employing the guiding RNA (gRNA), it cuts the already recognized genome and proves its defensive power to demolish it (White et al., 2017).
By bringing in the use of CRISPR mechanism, the double-stranded break is done on the specified genetic part, and modification is done in the form of insertion, deletion, and knockout. Now, these genetic modifications are being controlled for improving the plant's genome Moreover, it is being utilized to enhance the production and disease resistance (Mao et al., 2013).
Why CRISPR is an interesting technique in the present scientific arena? Because, CRISPR can transform theoretical ideas into practice. CRISPR focuses to edit the targeted genome accurately. Except, CRISPR use, different kinds of techniques are used for the genetic engineering of plants, including zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN). Instead of other two nucleases systems, RNA guided nucleases (RGENs) is being adopted by more and more molecular researchers. Because it involves guided RNA (gRNA) to edit the genome precisely. Therefore, it is the foremost priority of many molecular researchers around the world (Woo et al., 2015).
The story of science never ends at one point, as it is believed that the things invented today would be outdated in the next seconds. Additionally, NASA (The National Aeronautics and Space Administration) had performed some of the controlled experiments on the growth of wheat by providing artificial growing conditions.
It gave a new hope to the scientists of the Australian Queensland University to utilize the technique to shorten the development of generation time of cereal crops. In comparison to both new techniques; CRISPR and speed breeding, simple breeding is also a useful procedure up to a limited gain of crop production but it's a slow breeding procedure. On another side, NASA (The National Aeronautics and Space Administration) had performed some of the experiments on the growth of wheat by employing artificial growth treatments. Further, Scientists from the Australian Queensland University focused their attention toward NASA's experiment and designed the research on the same idea and named it as "Speed Breeding (Shivakumar et al., 2018).
Around the globe, many molecular and conventional breeders are working leaps and bounds to feed the world by making huge increment in crop production. Though conventional breeding is making progress in the race of crop production, but it's going slow. To address the time shortage problem, CRISPR and speed breeding techniques are being practiced for the development of new crop varieties. Both are easy and efficient genome editing technique that were brought in use for the genetic engineering of plants, for making them more productive and resistant to different kinds of diseases (Wolter et al., 2019).
Day by day population is increasing. To face the food challenges, old new breeding methods are being developed. Despite of newly developed breeding methods, these cannot meet the demand of food in less time. So, in this review, we have discussed about the Collaborative approach of CRISPR and speed breeding, both can increase the production of crops up-to many times in a very narrow time.
Further, both techniques can play its versatile role to increase the efficiency of crop's adaptability, disease resistance, and high yield. Generally, this review will describe about the importance, efficiency, and reliability of CRISPR and speed breeding.

DISCOVERY OF CRISPR SEQUENCES:

In 1987, some tandem repeats were identified in *E. coli genome*, and later these were named as CRISPR (Driehuis & Clevers, 2017). During one another ongoing experiment of gene sequencing of a gene that was coding for phosphate isoenzyme in *E. coli,* discovered some repeated sequences that were exactly like the CRISPR/Cas system of *E. coli*. These related sequences were also identified in other different species of bacteria, including *Salmonella enterica, Mycobacterium tuberculosis,* and *Shigella dysenteriae*. In 1993, Archaeal species also showed the same repeated sequences (Lander, 2016).
Mojica joined the University of Alicante and was performing research on the growth of *Haloferax mediterranei* by applying saline treatments that were collected from the near marshes. Upon the application of high doses of saline treatment, it showed significant effects in the archaeal genome and identified 30 repeated sequences with some pacers. Same repeated sequences were also identified in closely related species *H. volcanii*. Moreover, 40 % bacteria and 90 % archaeal species have CRISPR sequences (Lander, 2016; Chilcoat et al., 2017; Han & She, 2017).
In the earlier period, different scientists coined different names, including LCTR (Large Cluster of Tandem Repeats), DRs (Direct repeats), SRSR (Short regularly Spaced Repeats) etc. Finally, CRISPR name was coined by Jansen and his colleagues in 2002. These discoveries motivated to Mojica to find the reason behind these repeated sequences. Moreover, during his research, he found the same sequences in other 20 different microbial species (Lander, 2016; Chilcoat et al., 2017).

CRISPR DISCOVERY AS A DEFENSIVE MECHANISM:

On this planet, there is a food war between the prey and predator. Predator influence on the prey by imposing its defensive strategies. In the same context, bacteria and archaea were considered as prey while virus as a predator. Virus attacks on these tiny creatures and destruct them 4 to 50%. Due to continuous genome variability, phage took benefit and let the archaea and bacteria to hamper the recognition. The continuous evolution and attacks urged to develop different resistant layers in bacteria and archaea. Thus, archaea and bacteria recognize the foreign genetic element and degrade it. In the past, molecular scientists studied diversely on the CRISPR system; how it works and disrupt the foreign genetic element? Several studies cleared the objectives of CRISPR. It's a defensive mechanism in the genome machinery of the archaea and bacteria. CRISPR system store the foreign sequences and incorporate into a small RNA based reporting that acts as a guiding RNA (gRNA) (Karginov & Hannon, 2010).

The CRISPR system works based on the recognition and memorization of the foreign DNA. Upon the again attack of the same organism, bacteria and archaea recognize the already memorized genome and by cleaving makes it functionless (Zhang et al., 2016). The base of this system was dependent on the repeated sequences with the spacers. This system was also studied in the dairy industry. One of these industries revealed its function by studying the influence of bacteriophage on their dairy bacteria (Driehuis & Clevers, 2017).

Mechanism of CRISPR/Cas in plant's genome:

For the sustainable food production, it's quite necessary to increase the output of plants of (Nekrasov et al., 2013). Plant growth is severely affected by the attack of different viruses (Pyott et al., 2016).

In this dire need, Molecular researchers brought the use of CRISPR technology and published its successful scientific results. (Jiang et al., 2013). CRISPR/Cas9 acts to break the double-stranded DNA in two following components; Cas9 and single guiding RNA (sgRNA) (Liu et al., 2017). Further, single guiding RNA (sgRNA) is the only RNA to lead the CRISPR/Cas system because of its efficient guiding performance on the specific part of genome. Multiple bioinformatics tools are used to design these desired sgRNAs (Cui et al., 2018). With the association of Cas9 proteins, sgRNA gets the conformational information for the performance of cleavage at the specific genome sites and cut the double-stranded DNA (Jinek et al., 2014).

By employing the CRISPR/Cas technique, we can edit the genome at precise locations. Multiple guide RNAs (gRNAs) are expressed with the use of nucleases enzyme (cas9), and mutation is created in plants (Peterson et al., 2016). SgRNA is designed synthetically, it has a length of about 100nt. Its 5′ and 3′ ends functionally identify the target and anchor these sequences respectively. 3′ end has some loop sequences which act as the anchoring. With the association of cas9, a complex is formed which cuts the double-stranded DNA and results in a double-stranded break. After cleavage of the double-stranded DNA, repairing of non-homologous end joining works. This process causes gene insertions, deletions, and knockout (Liu et al., 2017). The graphical explanation is given in the image below (Fig. 1)

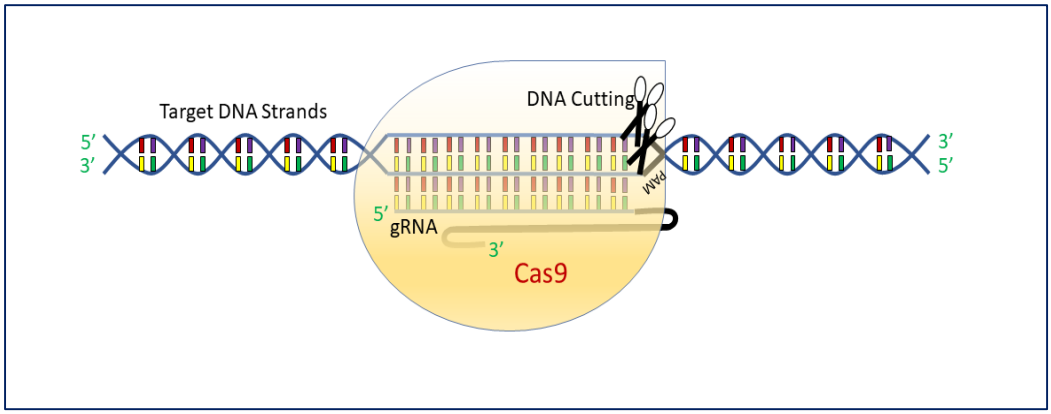

Figure 1: Mechanism of CRISPR

CRISPR/Cas 9; an efficient technique for improving the plants:

CRISPR/Cas9 system is being used for targeting the desired part of the plant genome and creates mutations accordingly (Yin et al., 2017). In the past, two of the strategies were being used for genome engineering including, zinc finger nucleases (ZFN) and transcription activator-like nucleases (TALEN). Both strategies were dependent upon DNA-protein interaction.

While CRISPR/Cas9 is the only technique that utilizes the RNA-guided endonucleases for editing the genome. The efficiency of the technique depends upon less off-target results and percentage of the desired mutations (Noman et al., 2016). Each technique has some pros and cons aspects (Brooks et al., 2014). Use of CRISPR/Cas9 is preferred over both techniques; ZFN and TALENS. In comparison to CRISPR/Cas9, ZFN and TALENS are much expensive, less efficient, and have numerous off targeted results. Therefore, based on positive characteristics, CRISPR/Cas9 is used worldwide to improve the genome. Hence, desired mutations can accelerate the productivity of plants speedily and efficiently than the other conventional breeding methods (Mushtaq et al., 2018).

With the arousal of excitement among different agricultural scientists, now the CRISPR/Cas9 is being employed for improving the plant genome and developing ideotype plant lines (Noman et al., 2016). CRISPR/Cas 9 developed the trust in the eyes of scientists due to its efficient doings than ZFN and TALEN (Wendt et al., 2013; Ma et al., 2014). Uniqueness of CRISPR/Cas9 system is Cas9; single effector nuclease that cuts the genome at a precise location, that's why it's called effective genome-editing technique. According to the different performed researches, plants showed 70% targeting efficiency (Hsu et al., 2013; Fu et al., 2014

SPEED BREEDING'S REVOLUTION:

No doubt, several techniques, and approaches have been developed to increase the crop productions. Despite the updated and effective molecular technologies, we need many new ways to increase the food production to meet the current and future challenges. Therefore, it was needed to introduce such type of technique or approach that can bring revolution in the agriculture sector.

Scientists of Australian Queensland University introduced a specific method to decrease the breeding time, and it was named as "speed breeding". Speed breeding is a specific kind of updated breeding procedure that allows to shorten the development of plant generations. According to a conducted controlled experiment, wheat can develop 6 generations in a year. In comparison to the old breeding methods, CRISPR and speed breeding saves our time. Speed breeding experiments are performed in a fully controlled environment by providing artificial conditions. By using this approach, we can develop crop varieties in a very narrow time and can also study different mutant plants (Watson et al., 2018).

Selection breeding was first introduced in durum wheat. By using the novel multi-trait phenotyping method for the F2 and F3 generations selection become easy. The scientist of Syria performed this method under speed breeding where each generation completed its cycle from sowing to harvesting within 77 days. This method under speed breeding ensure selection of those inbred lines that were enriched in desirable traits and the duration to combine these traits was reduced (Alahmad et al., 2018). Selection was done in these generations for different traits by adapting clear pot method. This method is suitable for large scale screening and show high heritability and genetic correlation among the experiment (Richard et al., 2015).

Speed breeding and genomic selection can be combined by different methods like single seed descent or by applying genomic selection to segregating populations in glass house. After this the selected lines can go for field trial and this reduces time as compared to field-based breeding and improve the desirable traits more quickly. 260 genotypes of wheat were grown for yield trials for 3 years and 8000 DArT (Diversity arrays technology) polymorphic markers were used.

The results showed that genomic selection using speed breeding have more genetic gain as compared to genomic selection done in simple breeding (Watson, 2018).

Speed breeding is successful for different crops like chickpea, barley, brassica, pea, canola, wheat, oat etc. These crops can be grown and crossed under speed breeding in single-seed descent method. Speed breeding can be used for cultivar development which requires high density planting and used to accelerate gene transformation. For cereal crops speed breeding is ideal with single-seed descent (Ghosh et al., 2018). In Barley different lines were used to develop disease resistant lines under modified backcross method using multi-trait phenotyping and speed breeding. This experiment was performed in Australia and the lines having resistant to diseases were developed within 2 years.

The scientists doing this experiment concluded that this can be used to accelerate gene pyramiding and is useful to transfer desirable genes with modified backcross (Hickey et al., 2017).

By accelerated breeding programs, a new variety of wheat named as 'DS Faraday' has been introduced in Australia that has more protein, it is highly tolerant to pre-harvest sprouting. A breakthrough in grain dormancy has been achieved. This was the major problem of wheat and the breeders of Australia were trying to solve it for 40 years (Kapiel, 2018).

CONCLUSION:

As the statistics gave the idea; how the population is increasing day be the day. Now, it's very hard and difficult for the agriculturists to meet the food requirements. To feed the world population which will be 9-10 billion in 2050 a strong breeding strategy like Speed breeding which saves the time was demanded (Hickey et al., 2017). Hence, in this hour of need, we say thanks to molecular researchers and plant breeders who developed the techniques of CRISPR and Speed breeding, respectively. By employing both the techniques, enough food can be produced to meet the food requirements of the population.

REFERENCES:

1. Alahmad, S., E. Dinglasan, K.M. Leung, A. Riaz, N. Derbal, K.P. Voss-Fels, J.A. Able, F.M. Bassi, J. Christopher and L.T. Hickey, 2018. Speed breeding for multiple quantitative traits in durum wheat. Plant methods., 14: 36.
2. Brooks, C., V. Nekrasov, Z.B. Lippman and E.J. Van, 2014. Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. J Plant Physiol., 166: 1292-1297.
3. Chilcoat, D., Z.B. Liu and J. Sander, 2017. Use of CRISPR/Cas9 for crop improvement in maize and soybean. Prog Mol Biol Transl Sci., 149: 27-46
4. Cui, Y., J. Xu, M. Cheng, X. Liao and S. Peng, 2018. Review of CRISPR/Cas9 sgRNA design tools. Interdiscip Sci., 10: 455-465.
5. Driehuis, E. and H. Clevers, 2017. CRISPR/Cas 9 genome editing and its applications in organoids. Am J Physiol Gastrointest Liver Physiol., 312: G257-G265.
6. Fu, Y., J.D. Sander, D. Reyon, V.M. Cascio and J.K. Joung, 2014. Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. Nat Biotechnol., 32: 279.
7. Ghosh, S., A. Watson, O.E. Gonzalez-Navarro, R.H. Ramirez-Gonzalez, L. Yanes, M. Mendoza-Suárez, J. Simmonds, R. Wells, T. Rayner, P. Green and A. Hafeez, 2018. Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. Nat Protoc., 13: 2944-2963.
8. Han, W. and Q. She, 2017. CRISPR history: discovery, characterization, and prosperity. Prog Mol Biol Transl Sci., 152: 1-21
9. Hallauer, A.R., 2011. Evolution of plant breeding. Crop Breed. Appl. Biotechnol., 11: 197-206.
10. Hickey, L.T., S.E. Germán, S.A. Pereyra, J.E. Diaz, L.A. Ziems, R.A. Fowler, G.J. Platz, J.D. Franckowiak and M.J. Dieters, 2017. Speed breeding for multiple disease resistance in barley. Euphytica, 213(3): 64.
11. Hsu, P.D., D.A. Scott, J.A. Weinstein, F.A. Ran, S. Konermann, V. Agarwala, Y. Li, E.J. Fine, X. Wu, O. Shalem and T.J. Cradick, 2013. DNA targeting specificity of RNA-guided Cas9 nucleases. Nat Biotechnol., 31: 827.
12. Jiang, W., H. Zhou, H. Bi, M. Fromm, B. Yang and D.P. Weeks, 2013. Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice. Nucleic Acids Res., 41: 188.
13. Jinek, M., F. Jiang, D.W. Taylor, S.H. Sternberg, E. Kaya, E. Ma, C. Anders, M. Hauer, K. Zhou, S. Lin and M. Kaplan, 2014. Structures of Cas9 endonucleases reveal RNA-mediated conformational activation. Science., 343.
14. Karginov, F.V. and G.J. Hannon, 2010. The CRISPR system: small RNA-guided defense in bacteria and archaea. Mol. Cell., 37: 7-19.
15. Kapiel, T.Y.S. Speed Breeding: A Powerful Innovative Tool in Agriculture Awareness of GM Food Proliferation in Saudi Arabia: A Case Study of Al Baha Province View Project. https://www.researchgate.net/publication/322644357.
16. Lander, E.S., 2016. The heroes of CRISPR. Cell., 164: 18-28.
17. Liu, X., S.Wu, J. Xu, C. Sui and J. Wei, 2017. Application of CRISPR/Cas9 in plant biology. Acta Pharm Sin B., 7: 292-302.
18. Ma, Y., L. Zhang and X. Huang, 2014. Genome modification by CRISPR/Cas9. FEBS J., 281: 5186-5193.

19. Makarova, K.S. and E.V. Koonin, 2015. Annotation and classification of CRISPR-Cas systems. Methods Mol Biol., 1311: 47–75.
20. Mao, Y., H. Zhang, N. Xu, B. Zhang, F. Gou and J.K. Zhu, 2013. Application of the CRISPR–Cas system for efficient genome engineering in plants. Mol plants., 6: 2008-2011.
21. Mushtaq, M., J.A. Bhat, Z.A. Mir, A. Sakina, S. Ali, A.K. Singh, A. Tyagi, R.K. Salgotra, A.A. Dar and R. Bhat, 2018. CRISPR/Cas approach: A new way of looking at plant-abiotic interactions. Plant Pathol J., 224: 156-162.
22. Nekrasov, V., B. Staskawicz, D. Weigel, J.D. Jones and S. Kamoun, 2013. Targeted mutagenesis in the model plant Nicotiana benthamiana using Cas9 RNA-guided endonuclease. Nat. Biotechnol., 31: 691.
23. Noman, A., M. Aqeel and S. He, 2016. CRISPR-Cas9: tool for qualitative and quantitative plant genome editing. Front. Plant Sci., 7: 1740.
24. Peterson, B.A., D.C. Haak, M.T. Nishimura, P.J. Teixeira, S.R. James, J.L. Dangl and Z.L. Nimchuk, 2016. Genome-wide assessment of efficiency and specificity in CRISPR/Cas9 mediated multiple site targeting in Arabidopsis. PloS ONE., 11(9).
25. Pyott, D.E., E. Sheehan and A. Molnar, 2016. Engineering of CRISPR/Cas9-mediated potyvirus resistance in transgene-free Arabidopsis plants. Mol Plant Pathol., 17: 1276-88.
26. Richard, C.A., L.T. Hickey, S. Fletcher, R. Jennings, K. Chenu and J.T. Christopher, 2015. High-throughput phenotyping of seminal root traits in wheat. Plant Methods., 11: 13.
27. Shivakumar, M., V. Nataraj, G. Kumawat, V. Rajesh, S. Chandra, S. Gupta and V.S. Bhatia, 2018. Speed breeding for Indian Agriculture: a rapid method for development of new crop varieties. Curr Sci., 115: 1241.
28. Watson, A., S. Ghosh, M.J. Williams, W.S. Cuddy, J. Simmonds, M.D. Rey, M.A.M Hatta, A. Hinchliffe, A. Steed, D. Reynolds and N.M. Adamski, 2018. Speed breeding is a powerful tool to accelerate crop research and breeding. Nat Plants., 4: 23.
29. Watson, A., 2018. Speed breeding with genomic selection to accelerate genetic gain for yield in spring wheat (Triticum aestivum). [online] GlobalRust.org. Availableat https://www.globalrust.org/content/speed-breeding-genomic-selection-accelerate-genetic-gain-yield-spring-wheat-triticum.
30. Wendt, T., P.B. Holm, C.G. Starker, M. Christian, D.F. Voytas, H. Brinch-Pedersen and I.B. Holme, 2013. TAL effector nucleases induce mutations at a pre-selected location in the genome of primary barley transformants. Plant Mol Biol., 83: 279-285.
31. White, M.K., R. Kaminski, W.B. Young, P.C. Roehm and K. Khalili, 2017. CRISPR editing technology in
32. biological and biomedical investigation. J Cell Biochem., 118: 3586-3594
33. Wolter, F., P. Schindele and H. Puchta, 2019. Plant breeding at the speed of light: the power of CRISPR/Cas to generate directed genetic diversity at multiple sites. BMC Plant Biol., 19: 176.
34. Woo, J.W., J. Kim, S.I. Kwon, C. Corvalán, S.W. Cho, H. Kim, S.G. Kim, S.T. Kim, S. Choe and J.S. Kim, 2015. DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. Nat Biotechnol, 33: 1162.
35. Yin, X., A.K. Biswal, J. Dionora, K.M. Perdigon, C.P. Balahadia, S. Mazumdar, C. Chater, H.C. Lin, R.A Coe, T. Kretzschmar and J.E. Gray, 2017. CRISPR-Cas9 and CRISPR-Cpf1 mediated targeting of a stomatal developmental gene EPFL9 in rice. Plant Cell Rep., 36: 745-757.
36. Zhang, Y., Z. Liang, Y. Zong, Y. Wang, J. Liu, K. Chen, J.L. Qiu and C. Gao, 2016. Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. Nat. Commun., 7: 12