IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

CRISPR FOR REVIVAL OF AROMA IN TRADITIONAL BASMATI RICE

[1]Kirti Sharma, [2]Anil Kanaujia, [3]Aparajita Das, [4]Puneet Pathak, [5]Mohan Ji Saxena, [6]Anup Kalra

[1]R&D Executive, [2]Head- R&D, [3]Principal Scientist, [4]Principal Scientist, [5]Managing Trustee, [6] Director Corporate Affairs (Ayurvet)
[1]Research & Development,
[1]Ayurvet Research Foundation, Sonipat, India

Abstract: The aroma in basmati rice grains is one of the most preferred quality characteristics (next to cooking quality, taste and elongation after cooking) for the determination of its market acceptance by the consumers which in turn determines the adoption of the variety by the farmers and marketability. Among 200 volatile compounds associated with aroma in rice grains, 2-acetyl-1-pyrroline (2-AP) is considered as the main compound responsible for the aroma. Presently, researchers are focusing to revive the basmati rice aroma using Gene editing technologies like Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) system via identified mutations in betaine aldehyde dehydrogenase (OsBADH2) gene to produce aroma in rice by increasing the level of 2-acetyl-1-pyrroline (2-AP). This technology has opened new paths for the enhancement of basmati rice grain quality through targeted mutagenesis.

Index Terms - **Rice, Aroma, CRISPR, BADH2, 2-acetyl-1-pyrroline (2-AP)**

I. INTRODUCTION

Rice is the main food for more than 50% of the world's population. Rice with aroma is attaining popularity among consumers globally. Therefore, greater attention is being given to developing high-yielding scented cultivars because native scented rice varieties have undesirable agronomic characteristics like low yield, and are susceptible to pests and diseases. The price of this rice is higher than the rice without aroma. The aroma of rice grains is due to one of the volatile compounds 2-acetyl-1-pyrroline (2-AP) which is found in all parts of the plants (except roots) of aroma-containing rice varieties. The aroma is also an important parameter as other grain quality traits such as milling, appearance, grain size, elongation after cooking, cooking quality, taste, etc. According to Agriculture Minister, Mr. Narendra Singh Tomar, a drought-resistant rice variety is developed through the application of genome-edited technology for the first time in India and is expected to be available for field evaluation by 2024 and commercial cultivation by farmers by 2026 [**1, 2, 3**].

Modern agriculture is a prime example of how technology and science can work together to increase crop productivity and quality. Although traditional breeding is now considerably faster than it was 50 years ago, it is likely unable to keep up with the rising food demand and the issues that the world's population experienced. Plant breeding remains fully dependent on finding plant populations with sufficient variation and on conventional crossing approaches to introduce traits into target crops then time and resource limitations to crop improvement will persist. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technologies could surmount these limitations and accelerate plant breeding beyond what was previously imaginable [**4**]. Muller (2021) and his team surveyed one small group of 20 farmers in Germany about their perspective on using CRISPR technology, they evaluate the benefits and perils of using CRISPR-Cas9 [**5**].

CRISPR Technology

Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR-Cas9), is a gene-editing technology that can add, remove or alter the genetic material at particular locations in the genome of an organism (**Fig. 1**). A naturally occurring genome editing system that bacteria deploy as an immunological response is the basis for CRISPR-Cas9. Bacteria that are virus-infected capture tiny fragments of the viruses' DNA and splice it into their DNA in a specific pattern to form sections known as CRISPR arrays. The bacteria can "remember" the viruses owing to the CRISPR arrays (or closely related ones). In the event of a subsequent virus attack, the bacteria produce RNA segments from CRISPR arrays that can recognize and bind to particular sections of the viral DNA. The virus is then destroyed completely by the bacteria's activation of Cas9 or a related enzyme to split the DNA.

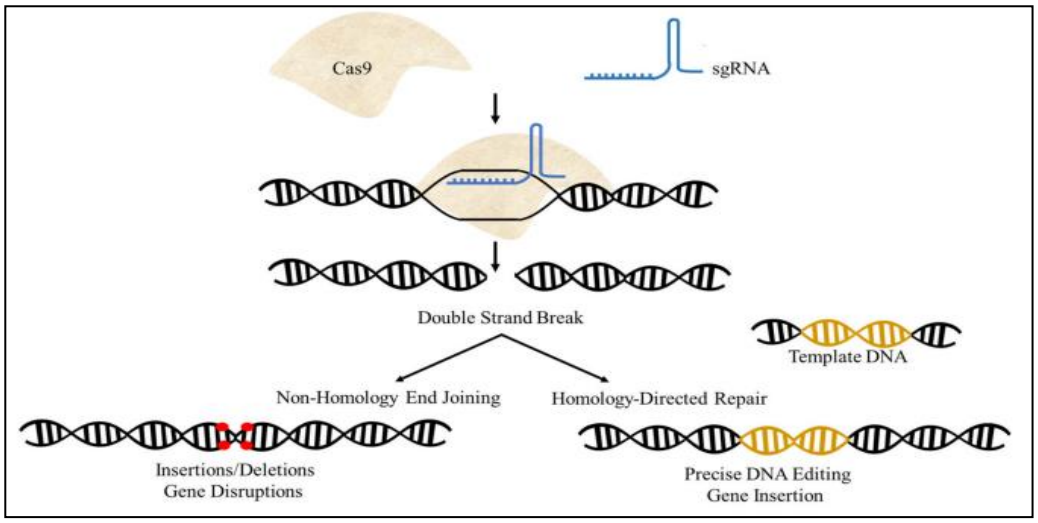

Figure 1: Mechanism of CRISPR-Cas9 [**6**]

Researchers adapted this immune defense system to edit DNA by creating small pieces of RNA with a short "guide" sequence that attaches (binds) to a specific target sequence in a cell's DNA, much like the RNA segments bacteria produce from the CRISPR array. Additionally, this guide RNA binds to the Cas9 enzyme. Similar to how the Cas9 enzyme works in bacteria when the guide RNA is delivered into cells, it detects the desired DNA sequence and allows the DNA to be cut at the desired spot. Other enzymes, such as Cpf1, can also be employed, albeit Cas9 is the most frequently used. Researchers employ the cell's DNA repair mechanism to add or remove genetic material after the DNA has been cut. They can also edit the DNA by exchanging an existing segment with a customized DNA sequence. [**7**].

Environmental problems will have an impact on farmers in the context of climate change on both an economic and agronomic level, affecting crop quality and productivity as well as negatively affecting plant tolerance to both abiotic and biotic stress. Utilizing innovative technologies based on the state-of-the-art in biotechnology, we can combat these environmental risks and increase our chances of achieving sustainable industrial manufacturing. [**8**].

Contrary to traditional breeding methods, CRISPR-Cas technology offers a quick means to produce perfect germplasm by eradicating harmful genetic components that cause undesirable phenotypes or introducing gain-of-function mutations through precise genome editing. The CRISPR-Cas genome editing technology is a flexible tool that has been utilized to enhance key crop properties, including yield, quality, disease resistance, and herbicide tolerance. Enzymatic browning in potatoes is a significant issue for producers as well as the business since it lowers the quality of both the raw and processed product. Gonzalez et al. [**9**] report on a successful application of CRISPR ribonucleoproteins to reduce enzymatic browning in potato tubers by targeting the Polyphenol Oxidase 2 gene (StPPO2). The authors achieved a dramatic reduction in tuber PPO activity (up to 69%) and enzymatic browning (73%).

CRISPR-Cas has been widely utilized to improve traits in cereals (monocot crops), such as rice and maize, in addition to dicot crops. For instance, Zafar et al. [**10**] have reported on improving Xanthomonas oryzae pv disease resistance. Oryzae (Xoo), a pathogen, causes the rice to develop bacterial blight by altering the susceptibility (S) gene's promoter. Transcription activator-like effectors (TALEs), which are secreted by Xoo, activate host S genes. The promoter of the OsSWEET14 gene in the Super Basmati elite cultivar was altered using CRISPR-Cas to introduce deletions that overlapped with effector binding elements (EBEs) recognised by AvrXa7/PthXo3 or TalF TALEs. Gao et al. reported on a method for stacking biotech characteristics within complicated trait loci in maize using CRISPR-Cas9 and recombinase (CTLs). These findings show many opportunities that the CRISPR-Cas systems hold for agriculture such as gene discovery of oil (Virdi et al.) [**11**] and disease genes (Zhang et al.), [**12**] as well as improved agricultural outcomes e.g., via decreasing potato browning (Gonzalez et al.) [**9**], improving disease resistance (Zafar et al.) [**10**] , mitigating volunteer rice (Komatsu et al.) [**13**] , and stacking biotech traits (Gao et al.) [**14**], etc. [**15**, **16**].

Usman et al. 2020 prepared rice that had been mutagenized using the OsSPL16/qGW8 gene using CRISPR/Cas9, and proteome analysis was utilized to uncover changes caused by mutations that result in larger grains of rice. The most significant characteristics of fragrant rice that set it apart from other forms of rice are its scent and cooked kernel elongation. Genetic investigations in the past have revealed a connection between the genes underlying these two features [**17**] . Faruq et al. (2010) evaluated the expression of aroma, kernel elongation, and their association in 55 fine rice genotypes, and concluded that aroma concentration was significantly different in the highest kernel elongation ratio [**18**].

Among more than 200 volatile reported compounds associated with aroma in rice grains, 2-acetyl-1-pyrroline (2-AP) is the main compound. Most of these compounds belong to classes of hydrocarbons, aldehydes, ketones, esters, alcohols, phenols, etc. contributing to rice aroma. The biosynthesis and accumulation of 2-AP are also affected by various environmental stress conditions and crop management practices like shading during the grain filling stage, irrigation regimes, application of nitrogenous fertilizers, temperature, drought, and salinity. Additionally, plant growth regulators, harvesting time, and postharvest handling is also reported to affect 2-AP content in rice plant. Storage of rice for flavor enhancement at high temperatures can degrade its quality due to increased amounts of nearly 100 volatile compounds such as aldehydes, ketones, and furans. Flavor variations in fragrant rice might result from different storage conditions. There aren't many medium/short slender grain rice variations with aroma on the market,

aside from the long slender grain Basmati genotypes. These short/medium slender fragrant genotypes have various drawbacks, including low yield. It can take many years to develop aromatic rice varieties with excellent grain qualities using traditional or molecular breeding methods, and in some situations, it can be difficult to maintain the superior grain qualities of elite genotypes. In this perspective, using CRISPR/Cas9 to introduce mutations causing the fragrance in high-yielding elite genotypes appears to be a promising approach to bypass the lengthy development time for desired genotypes. [**1, 2, 3, 17, 18, 19**].

Studies by Lorieux et al. [**20**] & Amrawathi et al. [**21**] led to the identification of a single recessive gene encoding betaine aldehyde dehydrogenase 2 (*BADH2*) on chromosome 8 to be responsible for aroma in scented rice. According to reports, BADH2 produces gamma-aminobutyric acid (GABA) from gamma-aminobutyraldehyde (GABald). GABald is transformed into the aromatic molecule 2-Acetyl 1-Pyrroline at the end when there is no functional BADH2 present, i.e., when there is a non-functional BADH2 with an 8 bp deletion in exon 7 of the BADH2 gene. Later, CRISPR/Cas9 was used to enhance OsBADH2, which resulted in the aroma in rice. Based on the aforementioned data, a study was conducted to show how CRISPR/Cas9 can speed up the formation of aromatic rice genotypes. To do this, CRISPR/Cas9 tool was used to introduce mutations into OsBADH2 of non-aromatic rice variety ASD16. Given that the rice genome contains two homologs of BADH, BADH1, and BADH2, the sgRNA targeting BADH2 was carefully constructed after examining the sequences of both homologs. They have a sequence similarity of 75.94%. Results showed that using the CRISPR/Cas9 tool has reduced the time needed to develop aromatic lines that will serve as novel genetic stocks/donors in breeding programs for developing non-basmati aromatic rice varieties. BADH1 was reported to be involved in modulating abiotic stress responses, and BADH2 was found to be involved in the production of aroma [**1**].

Through map-based cloning and complementary experiments, Chen et al. (2008) [**22**] confirmed that mutation in BADH2 gene is responsible for the fragrant phenotype. Additionally, the aroma was produced in the rice grains as a result of RNA interference (RNAi)-directed down regulation of OsBADH2 (Niu et al., 2008) [**23**] and artificial micro RNA-induced down regulation of OsBADH2 (Chen et al., 2012) [**24**]. Variations in 2-AP accumulation resulted from different kinds of BADH2 mutations. New BADH2 mutants from various genetic origins and alleles have been found and identified, and this genetic resource is crucial for future studies on rice fragrance enhancement. Hui et al. (2021) used CRISPR/Cas9 gene editing technology to introduce new BADH2 alleles into the genetic background of the japonica and indica rice varieties, which also improved the aroma of the non-fragrant japonica and indica kinds. The newly created BADH2 alleles also offered significant genetic resources for grain flavor enhancement in three-line hybrid rice. In conclusion, the results of the above studies have demonstrated that site-directed mutagenesis of BADH2 by CRISPR/Cas9 system can successfully create new alleles of BADH2 with improved aroma in the rice grain [**2**].

II. Conclusion

Aroma is considered as one of the most preferred quality parameters, which is due to the formation of 2-acetyl-1-pyrroline (2-AP) as the principal compound among several volatile compounds, whose biosynthesis and accumulation is influenced by various environmental stress conditions, crop management practices, shading during the grain filling stage, irrigation regimes, application of nitrogenous fertilizers, temperature, drought, and salinity, etc. which results in the reduction of aroma in one of the traditional heritage crops - rice. In this context, creating mutations leading to the aroma in high-yielding elite genotypes through CRISPR/Cas9 is helpful. A single recessive gene encoding betaine aldehyde dehydrogenase 2 (*BADH2*) on chromosome 8 is responsible for the aroma in scented rice. *BADH2* converts GABald to GABA then; GABald is converted into an aromatic compound 2-Acetyl 1-Pyrroline at the end. Subsequently, CRISPR/Cas9 was applied for improving *OsBADH2* leading to the aroma in rice. The discovery and identification of new BADH2 mutants from different genetic backgrounds and different alleles provide an important genetic resource for further research on fragrance improvement in rice.

III. Acknowledgment

We would like to thank Ayurvet Research Foundation - Managing Trustee Mr. Mohan Ji Saxena for his heartfelt guidance and support.

IV. References

[1] Ashokkumar, S., Jaganathan, D., Ramanathan, V., Rahman, H., Palaniswamy, R., Kambale, R., & Muthurajan, R. (2020**)**. Creation of novel alleles of fragrance gene OsBADH2 in rice through CRISPR/Cas9 mediated gene editing. *PloS one*, *15*(8), e0237018.

[2] Hui, S., Li, H., Mawia, A. M., Zhou, L., Cai, J., Ahmad, S., Lai, C., Wang, J., Jiao, G., Xie, L., Shao, G., Sheng, Z., Tang, S., Wang, J., Wei, X., Hu, S., & Hu, P. (2022). Production of aromatic three-line hybrid rice using novel alleles of BADH2. *Plant biotechnology journal*, *20*(1), 59–74.

[3]https://geneticliteracyproject.org/2022/08/10/india-likely-to-see-drought-resistant-crispr-gene-edited-rice-by-2026/#:~:text=A%20drought%2Dresistant%20rice%20variety,Narendra%20Singh%20Tomar%20has%20said.

[4] Gao, C. (2018). The future of CRISPR technologies in agriculture. *Nat Rev Mol Cell Biol.*, 19(5):275-276.

[5] Müller, R., Clare, A., Feiler, J., & Marco, N. (2021). Between a rock and a hard place: Farmers' perspectives on gene editing in livestock agriculture in Bavaria. *EMBO reports*, *22*(7), e53205.

[6] Ahmad, A., Munawar, N., Khan, Z., Qusmani, A. T., Khan, S. H., Jamil, A., Ashraf, S., Ghouri, M. Z., Aslam, S., Mubarik, M. S., Munir, A., Sultan, Q., Abd-Elsalam, K. A., & Qari, S. H. (2021). An Outlook on Global Regulatory Landscape for Genome-Edited Crops. *International journal of molecular sciences*, *22*(21), 11753.

[7] https://medlineplus.gov/genetics/understanding/genomicresearch/genomeediting/

[8] Camerlengo, F., Frittelli, A., Pagliarello, R. (2022). CRISPR towards a sustainable agriculture. *Encylopedia*, 2, 538-558.

[9] González, M. N., Massa, G. A., Andersson, M., Turesson, H., Olsson, N., Fält, A. S., Storani, L., Décima Oneto, C. A., Hofvander, P., & Feingold, S. E. (2020). Reduced Enzymatic Browning in Potato Tubers by Specific Editing of a Polyphenol Oxidase Gene *via* Ribonucleoprotein Complexes Delivery of the CRISPR/Cas9 System. *Frontiers in plant science*, *10*, 1649.

[10] Zafar, K., Khan, M. Z., Amin, I., Mukhtar, Z., Yasmin, S., Arif, M., Ejaz, K., & Mansoor, S. (2020). Precise CRISPR-Cas9 Mediated Genome Editing in Super Basmati Rice for Resistance Against Bacterial Blight by Targeting the Major Susceptibility Gene. *Frontiers in plant science*, *11*, 575.

[11] Virdi, K. S., Spencer, M., Stec, A. O., Xiong, Y., Merry, R., Muehlbauer, G. J., & Stupar, R. M. (2020). Similar Seed Composition Phenotypes Are Observed From CRISPR-Generated In-Frame and Knockout Alleles of a Soybean *KASI* Ortholog. *Frontiers in plant science*, *11*, 1005.

[12] Zhang, N., Roberts, H. M., Van Eck, J., & Martin, G. B. (2020). Generation and Molecular Characterization of CRISPR/Cas9-Induced Mutations in 63 Immunity-Associated Genes in Tomato Reveals Specificity and a Range of Gene Modifications. *Frontiers in plant science*, *11*, 10.

[13] Komatsu, A., Ohtake, M., Shimatani, Z., & Nishida, K. (2020). Production of Herbicide-Sensitive Strain to Prevent Volunteer Rice Infestation Using a CRISPR-Cas9 Cytidine Deaminase Fusion. *Frontiers in plant science*, *11*, 925.

[14] Gao, H., Mutti, J., Young, J. K., Yang, M., Schroder, M., Lenderts, B., Wang, L., Peterson, D., St Clair, G., Jones, S., Feigenbutz, L., Marsh, W., Zeng, M., Wagner, S., Farrell, J., Snopek, K., Scelonge, C., Sopko, X., Sander, J. D., Betts, S., … Chilcoat, N. D. (2020). Complex Trait Loci in Maize Enabled by CRISPR-Cas9 Mediated Gene Insertion. *Frontiers in plant science*, *11*, 535.

[15] Zhu, H., Li, c., Gao, C. (2020). Applications of CRISPR–Cas in agriculture and plant biotechnology, *Nature Reviews Molecular Cell Biology*, *21(11)*, 661-677.

[16] Kumar, S., Rymarquis, L. A., Ezura, H., & Nekrasov, V. (2021). Editorial: CRISPR-Cas in Agriculture: Opportunities and Challenges. *Frontiers in plant science*, *12*, 672329.

[17] Usman, B., Nawaz, G., Zhao, N., Liao, S., Qin, B., Liu, F., Liu, Y., & Li, R. (2020). Programmed Editing of Rice (*Oryza sativa* L.) *OsSPL16* Gene Using CRISPR/Cas9 Improves Grain Yield by Modulating the Expression of Pyruvate Enzymes and Cell Cycle Proteins. *International journal of molecular sciences*, *22*(1), 249.

[18] Golam, F., Norzulaani, K., Jennifer, A.H., Subha, B., M Z, Osman, M., Nazia, A.M., Zulqarnian, M., Mohammad, O. (2010). Evaluation of kernel elongation ratio and aroma association in global popular aromatic rice cultivars in tropical environment. *African Journal of Agricultural Research*. *Vol. 5(12)*, pp. 1515-1522.

[19] Zhao, Q., Yousaf, L., Xuea, Y., Shen, Q. (2020). Changes in flavor of fragrant rice during storage under different conditions, *J Sci Food Agric*, 100: 3435–3444.

[20] Lorieux, M., Petrov, M., Huang, N., Guiderdoni, E., & Ghesquière, A. (1996). Aroma in rice: genetic analysis of a quantitative trait. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik*, *93*(7), 1145–1151.

[21] Amaravathi, Y., Singh, R., Singh, A., Singh, V., Mohapatra, T., Sharma, T., Singh, N. (2008). Mapping of quantitative trait loci for basmati quality traits in rice (Oryza sativa L.). *Molecular Breeding*. *21*. 49-65. 10.1007/s11032-007-9108-8.

[22] Chen, S., Yang, Y., Shi, W., Ji, Q., He, F., Zhang, Z., Cheng, Z., Liu, X., & Xu, M. (2008). Badh2, encoding betaine aldehyde dehydrogenase, inhibits the biosynthesis of 2-acetyl-1-pyrroline, a major component in rice fragrance. *The Plant cell*, *20*(7), 1850–1861.

[23] Niu, X., Tang, W., Huang, W., Ren, G., Wang, Q., Luo, D., Xiao, Y., Yang, S., Wang, F., Lu, B. R., Gao, F., Lu, T., & Liu, Y. (2008). RNAi-directed downregulation of OsBADH2 results in aroma (2-acetyl-1-pyrroline) production in rice (Oryza sativa L.). *BMC plant biology*, *8*, 100.

[24] Chen, M., Wei, X., Shao, G., Tang, S., Luo, J., & Hu, P. (2012). Fragrance of the rice grain achieved via artificial microRNA-induced down-regulation of OsBADH2. *Plant breeding*, *131*(5), 584-590.