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OPINION PAPER

Genome editing for banded leaf and sheath blight resistance in Indian maize genotypes

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Abstract: Maize is the third most significant grain crop in India and is used as food, feed, and industrial raw material. Banded Leaf and Sheath Blight (BLSB) disease of maize caused by the necrotrophic fungal pathogen *Rhizoctonia solani* causes significant yield losses frequently between 10-40 per cent. To date, a few maize genotypes having mild/partial resistance to this destructive fungus have been reported. Additionally, a small number of quantitative trait loci (QTLs) for resistance to BLSB have been identified globally; yet, none of these QTLs have been found to confer strong resistance to BLSB. Therefore nonavailability of completely field-resistant or immune maize genotypes is of great concern and poses a major challenge for plant breeders. To manage this disease, a variety of agrochemicals, agronomic practices, and integrated pest management strategies are being used; nonetheless, sufficient and sustainable control could not be achieved. Further, there are negative ecological effects from the widespread usage of fungicides. Moreover, the management of BLSB is highly ineffective because of the broad host range of *R. solani*, the great genetic heterogeneity of the pathogen, the long survival ability in the form of sclerotia, and the lack of stable resistant maize cultivars against this disease. This implies that novel strategies should be used to develop BLSB-resistant Indian maize genotypes. In view of the above facts, developing a transgene-free genome-edited Indian maize genotype resistant to BLSB via the CRISPR/Cas- mediated targeted mutagenesis of major regulator gene i.e. the F-box protein-encoding gene, *ZmFBL41* would be a viable approach. It is anticipated that precise editing of this key gene would potentially address a major factor causing yield penalty in Indian maize i.e. BLSB disease.

Keywords: BLSB · Tropical maize · CRISPR-Cas9 · F-box protein ZmFBL41

Introduction

Banded Leaf and Sheath Blight (BLSB) is one of the most devastating diseases in maize caused by the most widespread, destructive and versatile necrotrophic fungal pathogen *Rhizoctonia solani* f. sp sasakii which claims heavy yield loss (generally 10-40% which may go up to 100% in severe cases) (Mehra *et al.*, 2012; Hooda *et al.*, 2015; Kaur *et al.,* 2020). This fungus is widely distributed and also responsible for causing sheath blight in rice. The disease cannot be adequately and sustainably controlled by traditional management techniques/measures involving cultural practices and the use of fungicides (Hooda *et al.*, 2015). To date, a natural and stable source of host resistance is not available in the Indian maize germplasm (Kaur *et al.,* 2020); hence resistance breeding has not been very effective in developing BLSB-tolerant maize cultivars. This suggests the need to devise novel strategies for engineering BLSB resistance in Indian maize. Although maize varieties exhibiting mild/moderate resistance to *R. solani* have been reported in the temperate and sub-tropical germplasm, however, complete field-resistant or immune maize genotypes have not been found. In this context, genetic engineering-based strategies such as genome editing can prove to be an effective approach to managing this menacing disease in maize.

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So far, several resistant quantitative trait loci (QTLs) and defense-related genes conferring partial resistance to this fungus, have been identified and functionally characterized mostly from rice and a few in maize. However, to date, none of the BLSB-resistant QTLs are being utilized effectively in practical maize breeding programs. Recently, a breakthrough discovery has been made which has led to the identification of a major negative regulator of BLSB resistance i.e. *ZmFBL41* encoding an F-box protein and functions via modulating the proteasomal degradation of cinnamyl-alcohol-dehydrogenase, ZmCAD, resulting in decreased lignin synthesis rendering the plant susceptible to *R. solani* (Li *et al.*, 2019). Further, two amino acid substitutions in *ZmFBL41* restrict lesion expansion (fungus infection) by preventing its interaction with ZmCAD while overexpression of this key gene in rice elevated susceptibility to the fungus (Li *et al.*, 2019). These findings confirm that *zmfbl41* is a recessive gene responsible for BLSB resistance. Hence, we hypothesize that precise editing to create either a loss-of-function mutant or generate a weak allele through amino acid substitution of the dominant allele *ZmFBL41* may lead to the development of the BLSB-resistant maize genotype. Considering, the significant yield penalty caused by the BLSB disease and the non-availability of natural and stable resistance sources in Indian maize germplasm, precise editing of *ZmFBL41* would be a novel and effective approach to tackle this disease.

Promising QTLs and genes identified for BLSB tolerance

Maize (*Zea mays* L.) is an important crop for billions of people as food, feed, and industrial raw material. In the recent past, it has gained much importance for ethanol production as well (Shah *et al.,* 2016). It's known as the "queen of cereals" because of its exceptionally high yield potential. One of the main deterrents to high grain yield in maize is its vulnerability to several diseases. BLSB is one of the most devastating diseases in maize which can cause wipeout of the entire crop (nearly 100% grain loss) in the ear rot phase of the disease thus resulting in significantly high economic losses in maize-growing areas of the world, particularly in China, South Asia, and Southeast Asian countries (Huang *et al.,* 2007; Yang *et al.,* 2017; Sagar and Bhusal, 2019). It has also been reported that heavily infected maize cobs did not produce grains (Sagar and Bhusal, 2019). Therefore, this disease is a major constraint to maize production and productivity. Besides maize, the same fungus attacks several other crops, rice is one of them where it causes sheath blight disease (Zheng *et al.,* 2013), thus disease intensity aggravates in rice maize cropping pattern. In recent years, BLSB outbreaks have occurred in many countries assuming epidemic dimensions. In India, this disease is prevalent in many states such as Himachal Pradesh, Uttarakhand, Uttar Pradesh, Haryana, Punjab, Madhya Pradesh, Rajasthan, Jharkhand, West Bengal, Meghalaya, Assam, and Orissa (Kaur *et al.*, 2020). This disease is spreading to new areas under intensive farming practices and the yield losses becoming greater year by year.

To date, few maize cultivars having quantitative/partial resistance to this destructive fungus have been reported globally (Chen *et al.,* 2013). Further, a small number of studies have been reported on the identification of quantitative trait loci (QTLs) for BLSB resistance. For instance, Yang *et al.* (2005) and Zhao *et al.* (2006) identified three and six QTLs, respectively, conferring relative resistance to BLSB in maize. These QTLs are shown to be distributed on six different chromosomes, and none of these QTLs were found to impart high or complete resistance against BLSB. Since sources of stable field resistance are not reported in the Indian tropical maize, there is no study on the identification of QTLs/genes associated with BLSB resistance from India so far. Therefore non-availability of completely field-resistant or immune maize cultivars is of great concern and poses a major challenge for plant breeders.

As we know that the same fungus causes sheath blight (ShB) in rice, an array of studies has been carried out in rice related to the identification of QTLs, genes, and functional characterization of some of these genes. So far, more than 40 QTLs for ShB resistance in rice distributed on all 12 chromosomes have been identified (Taguchi-Shiobara *et al.*, 2013). Several pathogenesis-related (PR) and defense-related genes have been utilized in rice for overexpression studies to develop resistant cultivars (Li *et al.,* 2009; Helliwell *et al.,* 2013; Mao *et al.,* 2014; Pan *et al.,* 2014; Wang *et al.*, 2015). In India, the natural sources having partial/quantitative resistance to this fungus have been reported in some rice germplasm (Kumar *et al.*, 2003) and many QTLs have been identified to confer partial resistance in different Indian rice cultivars (Channamallikarjuna *et al.*, 2010; Yadav *et al.*, 2015b). Further, attempts have been made to identify and

characterize a few antifungal genes in rice by transgenic approach (Richa *et al.,* 2016). However, to the best of our knowledge, so far, no ShB-resistant genetically engineered rice is available for commercial cultivation or near commercialization at national and international levels.

In 2019, a major candidate gene, ZmFBL41 encoding F-box protein, which negatively regulates BLSB resistance i.e. responsible for maize susceptibility to *R. solani* was discovered through a genomewide association (GWAS) approach (Li *et al.*, 2019). In this breakthrough study, the higher expression of *ZmFBL41* has been shown to result in susceptibility to fungus, while transposon-insertion in the 52 UTR of *ZmFBL41* (novel allele, *zmfbl41*) leading to reduced expression (up to 28%) confer BLSB resistance significantly in the W22 temperate line, strongly suggesting its negative regulatory role in BLSB resistance. The transposon-insertion line exhibited weaker disease symptoms compared to W22 after *R. solani* infection, and the disease index was reduced by approximately 29 per cent than W22. Further, its overexpression in rice has shown enhanced susceptibility to this fungus and confirmed its negative regulatory role. Authors have demonstrated that FBL41 interacts with S-phase kinase-associated protein 1 (SKP1) through its F-box domain to form the SKP1–cullin–F-box (SCF E3 ubiquitin ligase) complex, and recruits cinnamyl-alcohol dehydrogenase (CAD) for 26S proteasome-mediated degradation. ZmCAD encodes the final enzyme in the monolignol biosynthetic pathway, and its degradation results in reduced lignin synthesis and consequently increased susceptibility to *R. solani* (Li *et al.*, 2019). The same has been confirmed in rice by targeted knocking out the *ZmCAD*-homologous gene, *OsCAD8B,* that showed elevated susceptibility to *R. solani.* Furthermore, it has been demonstrated that the mutated *ZmFBL41* allele (having two amino acid substitutions i.e. E214G and S217R) in Chang 7-2 Chinese

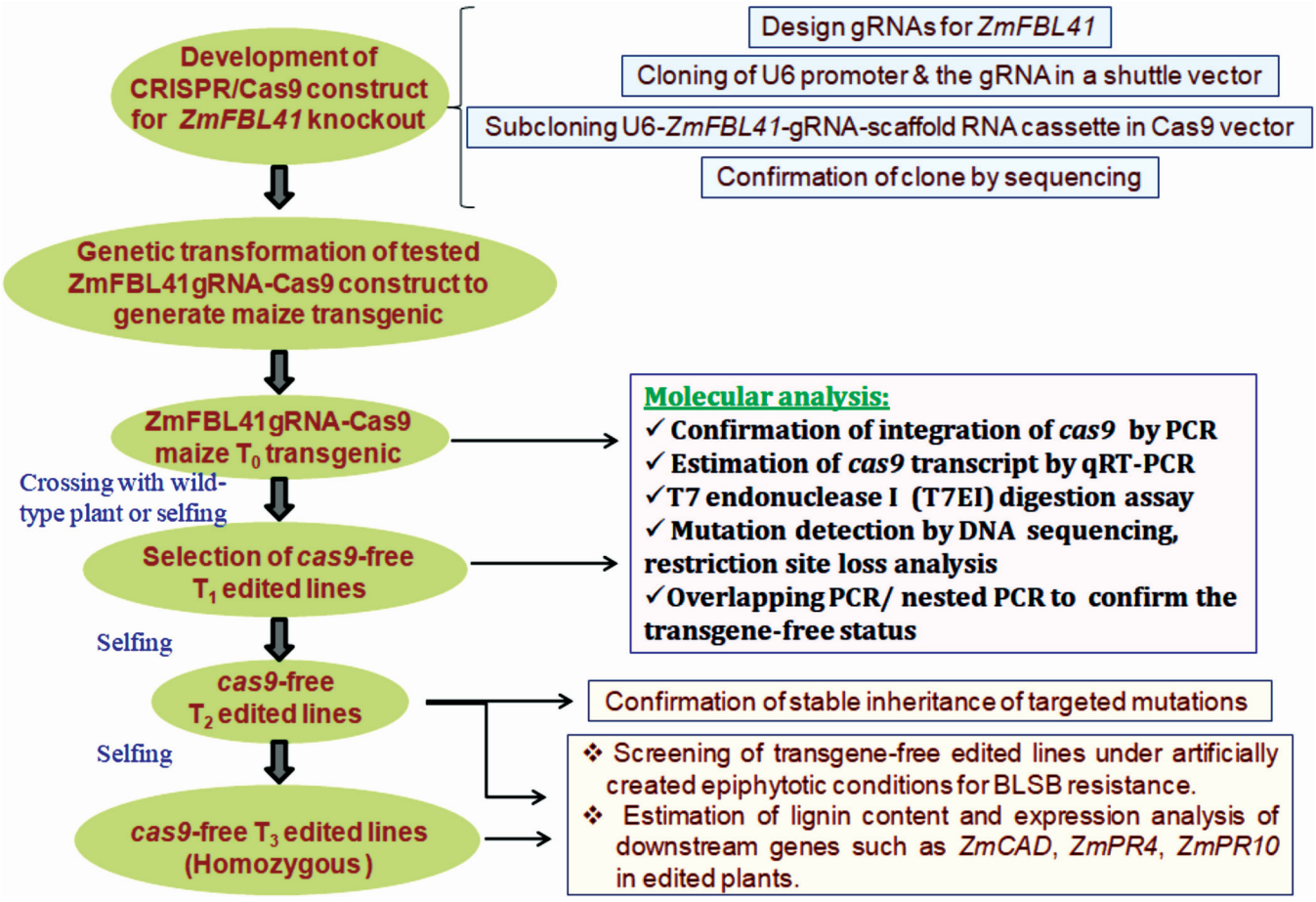

Figure 1. Flow diagram depicting the methodology to be followed for generating transgene-free knockout alleles of *ZmFBL41* gene using the CRISPR/Cas9-mediated targeted mutagenesis. For generating weak alleles through amino acid substitution suitable constructs for base editing (Cytosine base editors and Adenine base editors i.e. CRISPR/Cas9 nickases having cytidine deaminase and deoxy-adenosine deaminase fusion, respectively) or prime editing to be used instead of knockout construct.

maize line cannot interact with ZmCAD, which in turn, resulted in the inhibition of ZmCAD degradation and, consequently, the accumulation of lignin and restriction of lesion expansion (fungus infection) (Li *et al.*, 2019). The expression level of downstream pathogenesis-related genes has been increased in *zmfbl41* lines. Thus, this study also revealed the molecular mechanism by which the ZmFBL41–ZmCAD interaction regulates BLSB resistance in maize.

Developing BLSB-resistant Indian maize genotypes

Considering the non-availability of stable sources of high BLSB resistance in Indian maize genotypes to date and the recent breakthrough discovery of the regulatory role of the *ZmFBL41* gene in imparting BLSB resistance, we hypothesize that the BLSB-resistant Indian maize genotype could be developed via either CRISPR/Cas9-based knockout of this key gene or by generating a weak allele through amino acid substitution by employing base editors/ prime editors (Figure 1). The developed genome-edited lines could be subjected to crossing with wild-type plants in the T_0 generation to increase the frequency of getting *cas9*-free genome-edited plants in the T_1 generation which further will be selfed to increase the homozygosity in T_2 and T_3 generations. Simultaneously, the genome-edited lines should be functionally validated for resistance against *R. solani* under artificially created epiphytotic conditions using various physiological, biochemical, and molecular analyses. Later, genome-edited transgene-free BLSB-resistant maize could be utilized, in the National maize germplasm improvement program for resistance breeding, as a donor for introgression of the BLSB-resistant trait in various agronomically important tropical maize cultivars.

Conclusion

Targeted knocking out and base substitution of the *ZmFBL41* gene via CRISPR/Cas9 and base editing/prime editing, respectively, may impart BLSB resistance traits to maize. So this would be a novel and effective approach to tackle this disease via precise genome editing tools (SDN-1). To date, there is no report about developing gene editing-based mutants of this gene globally. Doing such interventions would also advance the knowledge base in BLSB pathogenesis and resistance. Taken together, the development of such genotypes is expected to address a major factor causing yield penalty in Indian maize i.e. BLSB disease.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author contribution statement

KK and BK conceptualized the idea. KK wrote the manuscript. AKJ, PP and Neha contributed to the preparation schematic diagram. KK and BK contributed to critically revising the draft. All authors read and approved the manuscript.

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