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Evaluation of Agronomic Performance of Mutant Rice Lines of Mentik Wangi Variety (Oryza sativa L.) Resulting from OSSWEET11 Gene Editing

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ABSTRACT

Bacterial leaf blight, caused by *Xanthomonas oryzae* pv. oryzae (Xoo), is a major disease that significantly reduces rice yields. A local variety of Central Java, Mentik Wangi, has several unique characteristics that are favored by consumers, such as a soft texture and a distinctive fragrant aroma. Despite its advantages, Mentik Wangi is susceptible to bacterial leaf blight because Xoo targets the susceptibility gene *OsSWEET11*. CRISPR/*Cas9* technology can induce resistance by mutating the gene's promoter, preventing its recognition by Xoo. This study aimed to observe the phenotypic traits of T1 mutant lines of Mentik Wangi rice edited using CRISPR/*Cas9*. T1 seeds derived from T0 parents were analyzed for agronomic traits, including plant height, tiller number, flowering time, and grain weight. Dunnett's test showed no significant differences between mutant lines and their wild-type parents, suggesting no pleiotropic effects from the *OsSWEET11* mutation. These results indicate that mutating susceptibility genes can be a viable approach for developing bacterial leaf blight-tolerant rice without compromising agronomic performance. However, molecular analyses are needed to confirm the inheritance of mutations and correlate them with agronomic traits in the T1 generation. This study demonstrates the potential of CRISPR/*Cas9* technology in breeding disease-resistant rice varieties.

1. INTRODUCTION

There are various rice varieties, including Mentik Wangi, an elite variety from Central Java. This type of rice has a unique aroma and produces soft-textured rice [1]. However, Mentik Wangi is a local variety that is vulnerable to bacterial leaf blight. Bacterial leaf blight initially appears as spots on the leaves, which later expand, causing the leaves to turn pale yellow [2, 3]. Bacterial leaf blight is a plant disease that causes significant losses. It affects rice plants and is caused by the gram-negative bacterium *Xanthomonas oryzae* pv. *oryzae* (Xoo), which typically infects through wounds and/or hydathodes, moves and colonizes the xylem vessels of the leaves and creates grey to white lesions along the leaf vessels [4]. In Indonesia, yield losses due to bacterial leaf blight are also influenced by weather conditions, with losses reaching 21-36% during the rainy season and ranging between 18% and 28% during the dry season [5]. Globally, yield losses from bacterial leaf blight can reach up to 50% [6]. This indicates that bacterial leaf blight not only affects specific countries but is also a global concern.

The bacterium *Xanthomonas oryzae* pv. *oryzae* (Xoo) secretes Transcription Activator-Like (TAL) effectors, which specifically target the Effector Binding Element (EBE) in the promoter region of the dominant *Xa13* gene (also known as *Os8N3* or *OsSWEET11*), leading to the coding of the *Xa13* protein. This protein facilitates sugar efflux (transporting sugars from inside the cell to the apoplast), which Xoo utilizes as a food source. Additionally, the *Xa13* protein interacts with the COPT1 and COPT5 proteins, which are involved in redistributing the Cu mineral from xylem tissues. Copper is crucial for plant resistance to Xoo, and this redistribution allows the pathogen to freely colonize the xylem tissues of rice plants and cause disease [7].

Mutations in the recessive *OsSWEET11* and *OsSWEET14* genes, particularly in the EBE region of their promoters, prevent the TAL effectors from hijacking the expression of these genes. As a result, the sugar transporter proteins are not produced, preventing sugar efflux and depriving the Xoo pathogen of the nutrients it needs from its host plant. The recessive *OsSWEET11* gene (*Xa13*) also prevents copper redistribution and maintains copper levels in the xylem vessels, inhibiting Xoo growth and providing resistance to *Xanthomonas oryzae* pv. *oryzae* (Xoo) [7-9].

To obtain *OsSWEET11* and *OsSWEET14* genes with mutations in their promoter EBEs, Xu et al. [10] used CRISPR/*Cas9* technology on the Kitaake rice variety to induce mutations in the PthXo1-EBE region of the *OsSWEET11* promoter and the PthXo3-EBE region of the *OsSWEET14* promoter. Based on sequencing data, they successfully

obtained four T2 homozygous lines, one of which, MS14K, showed a five-nucleotide homozygous mutation in both the PthXo1-EBE and PthXo3-EBE regions.

The use of CRISPR/*Cas9* to induce disease resistance has also been widely explored in previous studies. For example, CRISPR/*Cas9* was utilized to edit three susceptibility genes, *SWEET11*, *SWEET13*, and *SWEET14*, in two major rice varieties, Ciherang and IR64, resulting in nine Xoo-resistant lines [11]. Another study by Huang et al. [12] induced mutations in rice susceptibility genes against the nematode *Meloidogyne graminicola*, successfully obtaining two transgene-free mutant lines and five mutant lines containing transgenic elements, all of which exhibited higher resistance to *M. graminicola* compared to their wild-type parent. Additionally, Yang et al. [13] targeted two susceptibility genes, *Pi21* and *OsSULTR3;6*. A single *Pi21* mutant showed increased susceptibility to blast disease during both the tillering and reproductive phases. Furthermore, a single homozygous *OsSULTR3;6* mutant line, when inoculated with *Xanthomonas oryzae* pv. *oryzicola* (Xoc), exhibited a disease lesion length 75% shorter than the wild type. These various studies demonstrate that CRISPR/*Cas9* is a reliable method for inducing mutations in susceptibility genes, thereby conferring passive resistance to pathogens.

Class III SWEET protein family members are also involved in various biological processes, such as pollen and seed development [14]. Inactivating these genes has been shown to cause detrimental pleiotropic effects, such as reduced endosperm development and seed filling in Kitaake *OsSWEET11* and *OsSWEET11- OsSWEET15* mutants [15]. Moreover, RNA-mediated gene silencing of *OsSWEET14* or *OsSWEET11* in Kitaake bacterial leaf blight-resistant lines negatively impacted seed [16, 17]. However, reported reductions in yield and quality are not universal occurrences. Duy et al. [18] found no significant differences in agronomic performance among three CRISPR/*Cas9*-induced *OsSWEET14* homozygous mutant lines compared to their wild-type parents, indicating that limited promoter region modifications did not affect the normal expression of the *OsSWEET14* gene.

Given these findings, evaluating the agronomic performance, particularly yield-related components, of SWEET mutant lines in subsequent generations is crucial. Several studies have previously conducted agronomic performance evaluations on individuals within mutant lines. For instance, Duy et al. [18] evaluated the agronomic performance of the mutant line *OsSWEET14* to determine whether mutations in the *OsSWEET14* promoter affect the agronomic traits of rice. In this study, Duy et al. [18] edited the *OsSWEET14* gene in the Vietnamese rice variety *TRB225* and evaluated its agronomic performance under greenhouse conditions. Similarly, Achary and Reddy [19] conducted an agronomic performance evaluation of mutant wheat *GW2-KO* under field conditions, comparing various vegetative variables and yield components between the mutant wheat and its wild type.

Rifhani et al. [20] have constructed a CRISPR/*Cas9*-gRNA construct targeting the *OsSWEET11* promoters in Mentik Wangi rice and successfully transformed the rice with this construct. This research yielded 129 putative T0 mutant Mentik Wangi plants. Rifhani et al. [20] also conducted molecular analysis on T0 generation plants and revealed that there were four lines confirmed to have mutations in the *OsSWEET11* gene. To test the effect of promoter mutations in *OsSWEET11* in the T1 generation on agronomic performance, all T0 transgenic mutants were self-pollinated to produce T1 seeds. This study aims to evaluate the impact of editing the *OSSWEET11* gene through CRISPR/*Cas9* technology on the agronomic traits of the T1 generation of the Mentik Wangi variety, and explore whether the mutation will cause negative phenotypic changes. It is hypothesized that the mutation will have no significant effect on agronomic performance, providing a promising outlook for further development.

2. MATERIAL AND METHOD

2.1 Study location and variables observed

This study was conducted in the greenhouse of the Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, at an altitude of 96 meters above sea level (masl) with an astronomical location of 7° 33' 39.5" S and 110° 51' 31.4" E. The research took place from February to August 2024. The seeds used in this study were T1 generation rice seeds, resulting from a study by Rifhani et al. [20]. These seeds were obtained through genome editing using the CRISPR/*Cas9* method on the Mentik Wangi rice variety. The mutant lines obtained were selected based on the presence of mutations in the *OsSWEET11* gene in the T0 generation. Four mutant lines were successfully selected: line 85, line 88, line 113, and line 121. This study was designed as a simple experiment to compare dependent variables obtained from the mutant lines with their wild-type parent. Data were analyzed using ANOVA, followed by Dunnett's test to compare the wild-type parent with each mutant line. The observed variables included plant height, number of tillers, flowering time, total number of grains per clump, total number of filled grains per clump, grain weight per clump, and percentage of unfilled grains per clump. The selection of vegetative variables, such as plant height, in this study is based on the research by Liu et al. [21], which revealed that the expression of sugar transporter genes also affects plant phenotypes, such as height and flowering time. Similarly, Zhang et al. [22] demonstrated that the expression of a sugar transporter protein-coding gene influences the tillering response in rice. Therefore, in this study, where we observe the impact of mutations in *OsSWEET11*, a sugar transporter protein-coding gene, on the agronomic performance of plants, it is necessary to evaluate the aforementioned variables, which are important agronomic traits for rice growth and are closely related to breeding goals.

2.2 Research implementation and plant management

The rice seeds were soaked for 24 hours, drained, wrapped in tissue, and incubated until the seeds developed radicles. The seeds with radicles were then planted in seedling trays. The seedlings were sown in trays containing soil, with four mutant Mentik Wangi lines to be sown. Each line was sown in a separate group in the same tray, resulting in 4 seedling trays for the four mutant lines. After 18 days, the seedlings were ready to be transplanted into research buckets. The rice plants were planted in buckets filled with soil that had been chemically sterilized. Each bucket contained one rice seedling. For each line, 25 plants were grown, so for the four lines, a total of 100 individual plants were grown, all of which served as samples for this study. The rice plants were watered periodically, about every 2-3 days, and their growth stages

were monitored to collect data based on predetermined variables. Fertilization was carried out according to the recommended dosage from Kurniadie's [23] study, with an NPK dosage of 300 kg/hectare and a ZA fertilizer dosage of 333 kg/hectare, which was then converted to calculate the fertilizer dose for one bucket with 6 kg of soil per bucket. The NPK fertilizer was applied twice: half at planting and the other half when the plants were 3 weeks old. Meanwhile, the ZA fertilizer was applied all at once during planting [23].

2.3 PCR analysis of transgenic element in T1 generation

The *Cas9* gene detection used a total reaction mixture of 10 µl, consisting of 5 µl Kappa 2G Polymerase, 0.3 µl forward *Cas9* primer (5 µM), 0.3 µl reverse *Cas9* primer (5 µM), 1 µl template (isolated DNA from Mentik Wangi T1 rice), and 3.4 µl NFW. The PCR program used for detecting the *Cas9* gene consists of 35 cycles, as follows: pre-denaturation at 95°C for 10 minutes, denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, elongation at 72°C for 45 seconds, and final elongation at 72°C for 5 minutes. The PCR products are subjected to electrophoresis and then visualized using a gel documentation system. Samples positive for the *Cas9* gene are identified by the presence of an amplicon with a size of 444 bp.

3. RESULT

3.1 Height

The ANOVA results of all variables showed that there were significant differences between the tested strains as shown in Table 1, we then conducted a further Dunnett test to confirm the accuracy of the ANOVA test results. The CRISPR/*Cas9*-based mutations produced rice plants with an average medium height ranging from 116 to 124 cm. Figure 1 shows that MW 113 mutant line population have an average height of 116.32 cm, while the MW 121 mutant line population have an average height of 124.58 cm. Figure 1 also indicates that the wild-type plants had an average height of 118.06 cm. The shortest individual plant was found in the MW 113 line with a height of 98 cm, while the tallest individual was in the MW 121 line with a height of 133 cm (Table A1). Statistical analysis using Dunnett's test showed that the plant height data of the mutant lines, when compared to the wild-type plants as a control, did not differ significantly, with a significance value of >0.05.

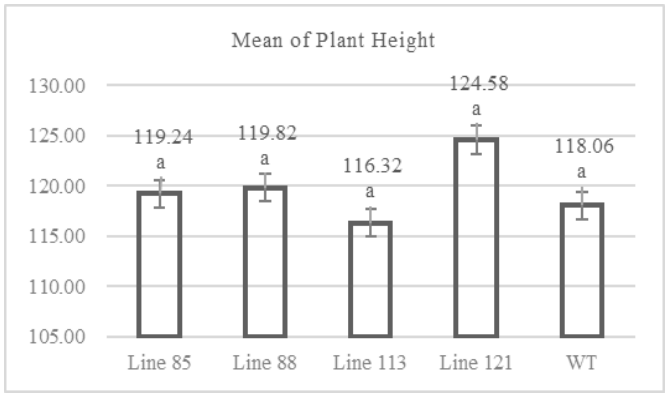

Figure 1. Graph of plant height
Numbers followed by the same letter indicate results that are not significantly different according to the Dunnet test at p> 0.05.

Table 1. ANOVA test of quantitative variables

Variable	df1	df2	F Value	α =5%	
				F Table	Significance
PH	4	103	4.631	2.46	*
NT	4	103	9.624	2.46	*
FT	4	103	4.211	2.46	*
NG	4	104	11.537	2.46	*
NFG	4	104	15.046	2.46	*
WG	4	103	18.227	2.46	*
PUG	4	104	11.968	2.46	*

Note: PH: Plant Height, NT: Number of Tillers, FT: Flowering time, NG: Number of Grains per clump, NFG: Number of Filled Grains per clump, WG: Weight of Grains per clump, PUG: Percentage of Unfilled Grains per clump.

3.2 Number of tillers

The observation of the number of tillers showed that the mutant rice lines produced an average range of 8.8 to 12.16 tillers. Figure 2 indicates that MW 113 mutant line population have an average of 8.8 tillers. On the other hand, the highest number of tillers was observed in the MW 85 mutant line population, with an average of 12.16 tillers. Figure 2 also shows that wild-type plants had an average of 9.75 tillers. The individual plant with the fewest tillers was found in the MW 113 line with 5 tillers, while the individual with the most tillers was in the MW 85 line with 18 tillers (Table A1). Data analysis using Dunnett's test method revealed that the average number of tillers in the mutant lines did not differ significantly from the control population (wild-type), with a significance value of >0.05.

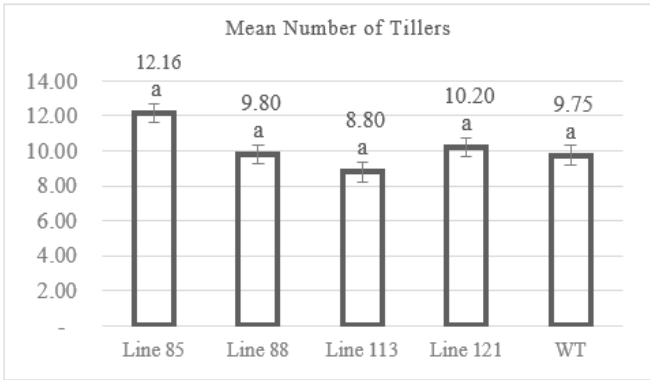

Figure 2. Graph of number of tillers
Numbers followed by the same letter indicate results that are not significantly different according to the Dunnet test at p> 0.05.

3.3 Flowering time

The observation of flowering time in the Mentik Wangi mutant rice lines showed that the flowering time across the four mutant line populations ranged between 68.12 to 71.04 days after planting (DAP) in average. The shortest flowering time was found in two lines, MW 85 and MW 113, both with the same average of 68.12 DAP (Figure 3). However, the individual plant with the shortest flowering time among these two lines was in the MW 85 line (Table A1), with a flowering time of 67 DAP. The line with the longest flowering time was MW 88, with an average of 71.04 DAP (Figure 3), and the individual with the longest flowering time took 85 DAP (Table A1). Figure 3 shows that the wild-type population had an average flowering time of 64.75 DAP, ranging between 63 and

65 DAP. The Dunnett post-hoc test comparing the average flowering time of the mutant lines to the control population (wild-type) showed significance values >0.05 for all comparisons, indicating no significant differences in the average flowering time between the mutant lines and their wild-type parent.

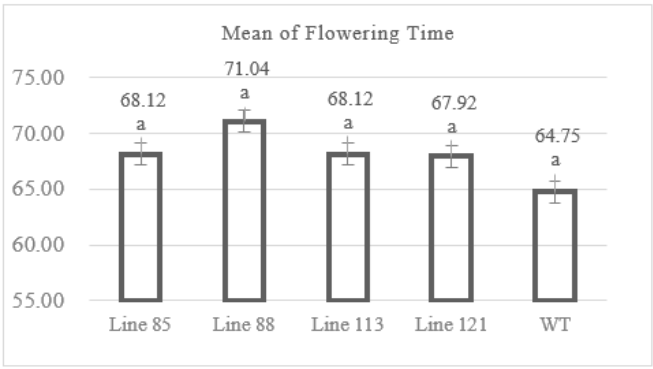

Figure 3. Graph of flowering time
Numbers followed by the same letter indicate results that are not significantly different according to the Dunnet test at p> 0.05.

3.4 Total grains number per clump

Observations of the total grain number per clump revealed that the mutant rice lines produced a total grain count ranging from 993 to 1,289.96 grains per clump in average. Figure 4 indicates that the lowest total grain number was recorded in the mutant line MW 113, with an average of 993 grains per clump. In contrast, the highest total grain number was observed in the mutant line MW 85, with an average of 1,289.96 grains per clump. Figure 4 also shows that the wild-type plants had an average total grain number per clump of 996.56 grains. The individual plant with the lowest total grain number per clump was found in line MW 121, with 448 grains, while the individual with the highest total grain number per clump was in line MW 85, with 1,631 grains (Table A1). Data analysis using the Dunnett test showed that the average total grain number per clump among the mutant lines did not differ significantly from the control population (wild type), with significance values >0.05.

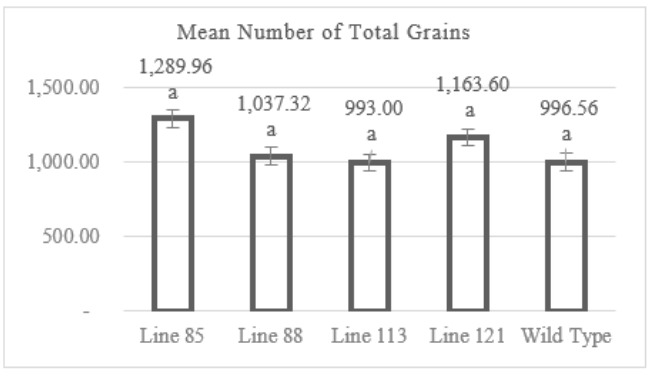

Figure 4. Graph of number of total grains
Numbers followed by the same letter indicate results that are not significantly different according to the Dunnet test at p> 0.05.

3.5 Filled grain number per clump

Observations of filled grain number per clump showed that the mutant rice lines produced filled grain count ranging from 673.84 to 938.24 grains per clump in average. Figure 5 indicates that the lowest filled grain number was recorded in the mutant line MW 113, with an average of 673.84 grains per clump. In contrast, the highest filled grain number was observed in the mutant line MW 85, with an average of 938.24 grains per clump. Figure 5 also shows that the wild-type plants had an average filled grain number per clump of 687.44 grains.

The individual plant with the lowest filled grain number per clump was found in line MW 121, with 292 grains, while the individual with the highest filled grain number per clump was in line MW 85, with 1,172 grains (Table A1). Data analysis using the Dunnett test showed that the average filled grain number per clump among the mutant lines did not differ significantly from the control population (wild type), with significance values >0.05.

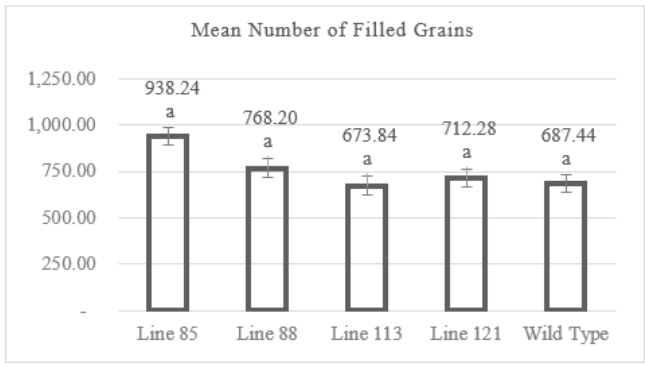

Figure 5. Graph of number of filled grains
Numbers followed by the same letter indicate results that are not significantly different according to the Dunnet test at p> 0.05.

3.6 Weight of grains per clump

Observations of grain weight per clump revealed that the mutant rice lines produced average grain weights ranging from 15.68 to 24.05 grams per clump. Figure 6 indicates that the lowest grain weight per clump was recorded in the mutant line MW 113, with an average of 15.68 grams per clump. In contrast, the highest grain weight per clump was observed in the mutant line MW 85, with an average of 24.05 grams per clump. Figure 6 also shows that the wild-type plants had an average grain weight per clump of 18.07 grams.

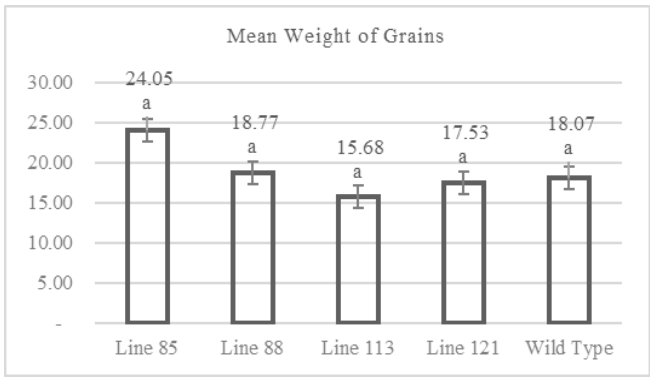

Figure 6. Graph of weight of grains
Numbers followed by the same letter indicate results that are not significantly different according to the Dunnet test at p> 0.05.

The individual plant with the smallest grain weight per clump was found in line MW 121, with a weight of 6.84 grams, while the individual with the highest grain weight per clump

was in line MW 85, with a weight of 31.14 grams (Table A1). Data analysis using the Dunnett test showed that the average grain weight per clump among the mutant lines did not differ significantly from the control population (wild type), with significance values >0.05.

3.7 Percentage of unfilled grains per clump

Observation of the percentage of empty grains per clump showed that the mutant rice lines produced an average percentage of empty grains of 25.93% to 38.62% per clump. Figure 7 shows that the lowest percentage of empty grains was recorded in the mutant line MW 88, with an average percentage of 25.93%. In contrast, the highest percentage of empty grains was observed in the mutant line MW 121, with an average of 38.62%. Figure 7 also shows that wild-type plants had an average percentage of empty grains per clump of 31.12%. The individual plant with the smallest percentage of empty grains per clump was found in line MW 88, with a percentage of 9.75%, while the individual with the highest percentage of empty grains per clump was in line MW 113, with a percentage of 53.08% (Table A1). Data analysis using the Dunnett test showed that the average percentage of empty grains per clump among the mutant lines did not differ significantly from the control population (wild type), with significance values > 0.05.

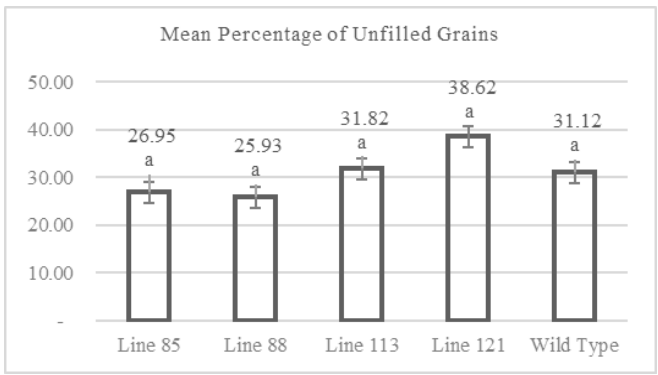

Figure 7. Graph of percentage of unfilled grains
Numbers followed by the same letter indicate results that are not significantly different according to the Dunnet test at p> 0.05.

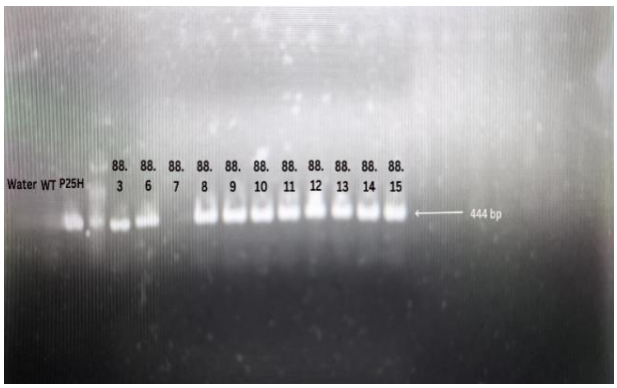

Figure 8. Visualization results of *Cas9* gene PCR
Water: Negative control. WT (Wildtype): Wild-type sample (non-transgenic control). P25H: Plasmid as a positive control.

3.8 Detection of the *Cas9* gene

We also analyzed the presence of the *Cas9* gene in all tested individuals. The visualization in Figure 8 showed that nearly all individuals tested in this study still contained the *Cas9* gene, except for one individual from line 88, number 7 (designated as individual 88.7). This indicates that the majority of the plants tested have not yet been freed from transgenic elements. Furthermore, it can be assumed that most of the tested individuals inherited the CRISPR/*Cas9* construct from their parent and likely also inherited mutations from the parent.

4. DISCUSSION

The analysis of plant height and the number of tillers in four CRISPR/*Cas9*-gRNAOsSWEET mutant lines revealed no significant differences compared to their parental lines. This aligns with research by Duy et al. [18], which involved editing the *OsSWEET14* gene's promoter and found no differences in height or tiller number between mutant lines and their wild-type parents. Similarly, Oliva et al. [11] Found no differences in plant height between mutant plants and their parental lines. According to Sulicantini et al. [24], rice plants can be categorized into three groups based on their height: short (<110 cm), medium (110-130 cm), and tall (>130 cm). The data analysis showed that the average height of the mutant lines ranged between 116-124 cm, indicating that these mutant lines fall into the medium height group (110-130 cm). Additionally, Peng et al. [25] stated that the ideal rice plant height should be between 90-100 cm. Based on this standard, the four mutant lines do not qualify as ideal-height rice plants.

The number of tillers is an important agronomic trait influencing rice [26]. Tiller characteristics can be observed from early growth until the maximum tillering phase, which is achieved during the grain-filling stage. Primary tillers emerge from the main stem, secondary tillers arise from primary tillers, and tertiary tillers emerge from secondary tillers, and so on [27]. Therefore, an increase in tiller number positively correlates with crop yield. Data analysis revealed no significant differences in the number of tillers between mutant lines and control plants. This indicates that the editing limited to the promoter region did not affect the normal expression of SWEET genes [18], thus not disrupting the growth or tillering of mutant lines. Additionally, other genes like OsGA3ox2, OsGA20ox2, and OsMADS57 play vital roles in regulating plant height [28]. Therefore, these genes should be the focus of research to regulate plant height.

Flowering time is when a plant begins to flower [29]. According to Ismachin and Sobrizal [30], earlier flowering results in earlier harvest time. Flowering time is positively correlated with harvest time [31]. Mutations in the *OsSWEET11* gene do not significantly affect flowering time, as evidenced by the lack of significant differences in flowering time between rice plants with and without mutations in this gene. This may be due to other vital factors influencing flowering time, such as photoperiod sensitivity [32], temperature [33], genetic factors like Hd1, Hd3a, RFT1, and Ghd7 [34], phytohormones [35], water and nutrient availability [36, 37], and stress factors [38].

Variables such as total grain number and filled grain number are significantly and positively associated with yield [39, 40]. Additionally, grain weight per clump also positively correlates with yield per unit area [41]. Ikhwani et al. [42] stated a positive relationship between grain weight and filled grain number per clump, making these three variables useful indicators of genotypic yield. This study aligns with findings from Zeng et al. [43], who also observed no difference in yield between *OsSWEET14* mutant rice lines and the Zhonghua 11

wild type. The absence of distinct phenotypic changes may result from compensation by other genes involved in grain filling. As stated by Fei et al. [44], the minimal phenotypic changes in *OsSWEET14* mutants compared to wild types suggest that other members of the clade III SWEET gene family, such as *OsSWEET15*, might compensate for the loss of *OsSWEET14* function. Similarly, Fei et al. [44] also analyzed the tissue-specific expression of *OsSWEET14* and *OsSWEET15*, revealing that both genes are expressed in developing rice caryopses, further supporting the hypothesis of functional compensation for the *OsSWEET11* gene by these two genes. Genetic redundancy may underlie this compensatory phenomenon.

The percentage of unfilled grains variable reflects the proportion of unfilled or underdeveloped grains. This measurement is crucial for understanding and improving crop yields due to its direct correlation with overall productivity. According to Majidimehr [45], the empty grain number is negatively correlated with yield, indicating that higher empty grain percentages result in lower yield per clump. The empty grain number can be converted into a percentage by dividing the number of empty grains by the total grains per clump. Breeders prefer lines with a lower empty grain percentage, making this variable essential for line selection. In this study, no significant differences were observed in the empty grain percentage, likely for similar reasons as other variables: functional compensation by other genes involved in grain filling. As reported by Scofield et al. [46], knocked out the *OsSUT1* gene resulted in mutant lines with significantly reduced grain filling. Hu et al. [47] highlighted that *OsSUT1* is vital for rice plant development during reproductive stages and is strongly expressed in rice stems and developing caryopses, indicating its role in grain filling. This gene is specifically expressed massively in the aleurone layer of rice caryopsis or grain [48, 49].

In this study, we also examined the presence of transgenic elements, specifically the *Cas9* gene, in the genome of T1 generation rice plants that had been cultivated. Detecting the *Cas9* gene in progeny generations is crucial, as *Cas9* expression in progeny can result in unwanted new mutations, which differ from those obtained in the T0 generation [50]. Furthermore, Ishizaki [50] emphasized that eliminating the *Cas9* gene is essential to ensure the stability of mutation inheritance and to prevent chimerism in later generations.

Based on PCR analysis of the *Cas9* gene, it was found that all individuals still carried the *Cas9* gene except for one individual from line 88 number 7 (namely individual 88.7), in which the *Cas9* gene was no longer detected in its genome. During reproduction, if plants or animals carrying CRISPR/*Cas9*-induced mutations reproduce, there is a possibility that the offspring inherit a version of the genome without the *Cas9* transgene. This aligns with the findings of Yang et al. [51], who reported that 11.3% of CRISPR/*Cas9* mutant *Brassica napus* T1 generation individuals did not show a *Cas9* band.

A possible explanation for this phenomenon is that the CRISPR/*Cas9* cassette is lost during cell division, resulting in the absence of detectable *Cas9* in the T1 generation. The relatively small sample size in this study might have contributed to the limited number of individuals confirmed to be transgene-free, leaving other unplanted seeds potentially free of the *Cas9* transgene unidentified.

Moving forward, individuals detected to carry the *Cas9* gene as well as those without it need to undergo further testing through flanking PCR and sequencing to confirm the presence of mutations induced in the target gene *OsSWEET11*.

Overall, it can be concluded that mutations in the *OsSWEET11* promoter region did not significantly affect phenotypic traits in the genetic background of the Mentik Wangi variety. Possible reasons for this include:

1. Mutations limited to the promoter region did not significantly impact SWEET gene expression [18], allowing SWEET genes to still be transcribed and translated into proteins.
2. Genetic redundancy compensated for the loss of *OsSWEET11* function during grain filling. References indicate that other genes, such as *OsSWEET14*, *OsSWEET15* [44], and *OsSUT1* [47], can also facilitate grain filling.

Future studies should include molecular analysis of the mutant lines especially DNA sequencing of the target gene to confirm whether the mutation occurs in the *OsSWEET11* target gene and examine the mutation form. Additionally, gene expression analysis using qRT-PCR on specific tissues is necessary to determine the impact of mutations on *OsSWEET11* transcription in the Mentik Wangi genetic background.

5. CONCLUSION

Agronomic variables in the mutant lines did not differ significantly compared to their wild-type parents. This could be due to several possible factors, such as:

1. CRISPR/*Cas9*-induced mutations in the T0 generation were not inherited by the T1 generation.
2. Editing limited to the promoter region of the *OsSWEET11* gene did not significantly influence the mutant phenotype.
3. Genetic redundancy actively compensated for the loss of *OsSWEET11* function due to mutations in the grain-filling process.

Furthermore, if the mutation is indeed inherited from the T0 generation, this demonstrates that editing the *OsSWEET11* gene does not negatively affect the agronomic performance of the mutant plants. Thus, the gene-editing method to engineer bacterial leaf blight resistance in the Mentik Wangi rice variety is a viable approach without causing significant yield penalties.

Because this study has not yet conducted a disease resistance test on individuals in the mutant line, it is not yet known how mutations in the gene promoter impact the resistance of the mutant line to bacterial leaf blight. Molecular analysis in the form of DNA sequencing is still needed to confirm the presence of mutations in the mutant plant genome. Additionally, disease resistance analysis should be performed by inoculating the confirmed mutant plants with the bacterial pathogen causing bacterial leaf blight.

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APPENDIX

Table A1. Supplementary table

Individual Number	PH (cm)	NT	FT (days)	WG (g)	NG	NFG	PUG (%)
MW 85.1	114	12	67	17.59	1178	699	40.66
MW 85.2	124	16	67	23.58	1305	886	32.11
MW 85.3	126	18	67	23.91	1631	984	39.67
MW 85.4	111	12	67	21.13	1267	979	22.73
MW 85.5	123.5	10	67	23.83	1203	925	23.11
MW 85.6	124.5	11	67	18.95	1269	768	39.48
MW 85.8	124	13	67	24.89	1118	940	15.92
MW 85.9	110	12	67	17.47	1179	779	33.93
MW 85.10	116	12	67	24.09	1153	972	15.7
MW 85.11	112	10	67	17.85	1202	779	35.19
MW 85.12	120	11	67	24.91	1179	957	18.83
MW 85.13	114	12	67	26.8	1326	1052	20.66
MW 85.14	121	11	67	21.15	1347	864	35.86
MW 85.15	126	12	67	26.27	1547	978	36.78
MW 85.16	117	13	67	31.14	1576	1172	25.63
MW 85.17	128	10	67	23.78	1367	901	34.09
MW 85.18	127.5	12	67	25.89	1363	997	26.85
MW 85.19	110	15	69	26.43	1286	1000	22.24
MW 85.20	120	13	67	28.76	1361	1083	20.43
MW 85.21	123.5	12	67	27.16	1207	932	22.78
MW 85.23	122	7	85	21.13	1099	807	26.57
MW 85.24	124.5	11	69	25.63	1322	1028	22.24
MW 85.25	107	14	68	28.67	1401	1053	24.84
MW 85.26	120.5	15	67	26.61	1244	1016	18.33
MW 85.27	115	10	72	23.56	1119	905	19.12
MW 88.1	127.5	10	67	19.78	1155	796	31.08
MW 88.2	128.5	12	67	18.69	1212	787	35.07
MW 88.3	118	10	67	17.62	1024	695	32.13
MW 88.4	131	9	67	23.2	1341	932	30.5
MW 88.5	128.5	12	67	16.03	1068	688	35.58
MW 88.6	124	10	67	11.46	758	456	39.84
MW 88.7	124	10	67	20.15	1005	822	18.21
MW 88.8	122.5	10	67	17.55	1079	730	32.34
MW 88.9	124	9	67	18.97	1156	794	31.31
MW 88.10	121	12	67	23.36	1178	929	21.14
MW 88.11	126	6	67	13.97	844	611	27.61
MW 88.12	121	8	67	20.33	1097	848	22.7
MW 88.13	120	14	67	24.02	1167	987	15.42
MW 88.14	122	10	67	19.54	994	795	20.02
MW 88.15	119.5	12	67	20.96	1165	811	30.39
MW 88.16	111	8	85	16.78	995	703	29.35
MW 88.17	110	8	85	15.95	834	688	17.51
MW 88.18	108	7	74	24.9	1274	1032	19
MW 88.21	120	8	70	11.06	569	433	23.9
MW 88.22	112.5	9	74	16.1	888	664	25.23
MW 88.23	113	6	85	15.6	892	673	24.55
MW 88.24	114.5	10	74	17.66	959	709	26.07
MW 88.25	121	13	67	24.33	1046	944	9.75
MW 88.27	120	11	72	22.61	1220	902	26.07
MW 88.28	108	11	85	18.64	1013	776	23.4
MW 113.1	124	9	67	11.9	1070	502	53.08
MW 113.2	127	10	67	16.12	1081	647	40.15
MW 113.3	119	11	67	20.39	1239	830	33.01
MW 113.4	124	11	67	16.64	965	628	34.92
MW 113.5	121	11	67	18.02	1142	783	31.44
MW 113.6	109	9	67	14.54	1020	673	34.02
MW 113.7	122	8	67	12.88	795	585	26.42
MW 113.8	126	8	67	15.52	981	671	31.6

MW 113.9	121	12	67	17.87	1077	756	29.81
MW 113.10	124	10	67	19.38	1069	831	22.26
MW 113.11	128	7	69	13	959	581	39.42
MW 113.12	121	8	69	17.14	1008	719	28.67
MW 113.13	127	8	69	19.58	1107	792	28.46
MW 113.14	113	8	68	13.42	921	625	32.14
MW 113.15	109	9	70	14.65	953	659	30.85
MW 113.16	131	7	69	14.12	852	609	28.52
MW 113.18	107	6	68	19	1081	763	29.42
MW 113.19	124	11	67	19.22	1076	808	24.91
MW 113.21	121	11	70	13	940	645	31.38
MW 113.22	100	8	69	14.16	932	612	34.33
MW 113.24	106	8	68	15.18	1147	721	37.14
MW 113.25	101	7	74	10.75	646	490	24.15
MW 113.27	105	9	68	18.91	1091	792	27.41
MW 113.28	98	5	68	14.33	724	595	17.82
MW 113.29	100	9	67	12.31	949	529	44.26
MW 121.1	122	9	69	14.64	859	570	33.64
MW 121.2	122	11	67	20.75	1105	805	27.15
MW 121.3	123	9	67	17.36	1135	691	39.12
MW 121.4	127	12	67	19.62	1236	766	38.03
MW 121.5	128	10	67	19.99	1120	807	27.95
MW 121.6	125	11	68	22.2	1334	874	34.48
MW 121.7	126	13	67	13.22	888	520	41.44
MW 121.8	124	11	67	17.07	1491	714	52.11
MW 121.9	120	12	67	21.98	1377	879	36.17
MW 121.10	129	11	67	15.13	982	624	36.46
MW 121.11	132	8	67	14.79	1144	628	45.1
MW 121.12	117	8	67	16.92	1278	692	45.85
MW 121.13	116	6	85	12.09	843	508	39.74
MW 121.14	123	13	67	19.65	1465	854	41.71
MW 121.15	120.5	8	67	15.95	1019	638	37.39
MW 121.16	123	12	67	25.94	1463	1036	29.19
MW 121.17	133	9	68	22.33	1388	914	34.15
MW 121.18	128	9	67	17.31	1474	724	50.88
MW 121.19	128	12	67	21.22	1268	852	32.81
MW 121.20	128	9	67	21.43	1271	863	32.1
MW 121.21	128	10	67	21.74	1304	893	31.52
MW 121.22	123	13	67	11.79	869	460	47.07
MW 121.23	122	10	67	14.53	1175	615	47.66
MW 121.24	120	10	67	6.84	448	292	34.82
MW 121.25	127	9	68	13.78	1154	588	49.05
WT.1	122	10	65	19.94	1076	743	30.95
WT.2	121	9	65	15.29	1035	755	27.05
WT.3	125	9	63	24.2	783	564	27.97
WT.4	121.5	11	65	17.13	1261	880	30.21
WT.5	110	11	65	19.6	878	637	27.45
WT.6	117	10	65	19.14	1099	733	33.3
WT.7	116	8	65	12.01	985	733	25.58
WT.8	112	10	65	17.27	848	475	43.99
WT.9	114	12	67	17.59	1004	667	33.57

Note: PH: Plant Height, NT: Number of Tillers, FT: Flowering time, NG: Number of Grains per clump, NFG: Number of Filled Grains per clump, WG: Weight of Grains per clump, PUG: Percentage of Unfilled Grains per clump.