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PERFORMANCE OF MENTIK SUSU RICE MUTANT WITH GENOME EDITING USING CRISPR/CAS9 SWEET11 CONSTRUCT T1 GENERATION

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SUMMARY

Rice (*Oryza sativa* L.) with increased resistance to bacterial leaf blight can play a vital role in maintaining stable rice production with reduced economic losses and improved food security. This study aimed to evaluate the Mentik Susu rice mutant T1 generation resulting from gene editing, focusing on molecular and phenotypic characteristics. The selected T0 mutant seeds entailed planting and observation for molecular and phenotypic analyses. Molecular analysis through PCR helped detect the hygromycin phosphotransferase (*hptII)* and *Cas9* genes and further sequenced target genes to identify the mutation. The phenotypic analysis comprised observations on plant height, panicle length, total tiller number, and flowering age. In the T1 generation, no detection of *hptII* and *Cas9* genes occurred, indicating that rice strains had undergone mutation without carrying the transgene. Sequencing analysis identified mutations in several mutant rice strains in the OsSWEET11 promoter. Nine mutant rice strains experienced mutations, eight strains mutated by substitution, and one mutant strain mutated by substitution and insertion. On phenotypes, the T1 mutant rice strains showed more tillers and longer panicle length than the wild-type rice strains. In continuing the said research, further analysis based on phenotypic variations, mutation stability, and tests on bacterial leaf blight resistance is essential.

Keywords: Rice (*O. sativa* L.), genome editing, mutation, SWEET11, molecular and phenotypic characteristics, bacterial leaf blight resistance, morphological and yield traits

Key findings: Using the CRISPR/Cas9 method in rice (*O. sativa* L.), mutant rice strains appeared in the SWEET11 promoter region, undergoing studies for molecular and phenotypic characteristics.

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INTRODUCTION

Rice (*Oryza sativa* L.) plays an important role as the main food source for more than half of the population worldwide, especially in Asia. In the context of Indonesian agriculture, Mentik Susu rice is one of the local cultivars known for its high quality and flavor. However, challenges faced by rice production due to various factors, including pest and disease attacks and climate change, eventually reduced crop yields (Rachmawati *et al*., 2023). Therefore, the development of rice cultivars resistant to environmental stress factors and diseases is crucial to ensure food security (Yuliantika *et al*., 2023).

Genome editing technology, particularly the CRISPR/Cas9 technique, has become a promising tool in improving plant morphological and yield-related traits. Using CRISPR/Cas9 can modify the effector-binding element (EBE) in the promoter region of the *OsSWEET11* gene to prevent recognition and activation by TAL effectors. Although this modification does not alter the protein-coding sequence, changes in the promoter region may influence gene expression. However, the precise editing design of the EBE will specifically disrupt pathogen-induced activation while preserving the gene's normal physiological expression and function in plant growth and development. The *SWEET11* gene editing is an advanced approach that plays a vital role in the sugar transport mechanism, which ultimately affects plant growth and resistance to various biotic and abiotic stress factors (Purwanto and Hutomo, 2023).

The CRISPR-Cas9 system represents an advancement in genetic engineering, offering customizable and efficient genome editing capabilities. Its primary function utilizes guide RNAs (gRNA) that direct Cas9 nucleases to specific genomic locations, enabling targeted cutting and repair, which can benefit various applications, including disease resistance in plants (Knott and Doudna, 2018). In the context of rice cultivation, the SWEET transporter family, specifically the *SWEET11, SWEET13,* and *SWEET14* genes, has reached key player identities in rice susceptibility to bacterial leaf blight, which is a major threat to agriculture (Eom *et al*., 2019).

This study aimed to evaluate the morphological and yield-related traits of the Mentik Susu rice T1 generation that has undergone the gene-editing process using the CRISPR/Cas9 construct against the *SWEET11* gene. By using the mutation and gene editing technology, one can expect to obtain superior Mentik Susu rice strains, which will both enhance productivity and strengthen resistance to adverse environmental factors (Joshi *et al*., 2018; Chandra *et al*., 2024). In this study, a deep dive into the agronomic characters of gene-edited Mentik Susu rice will contribute to the development of a more sustainable and productive plant breeding strategy, which will generally support the national food security program in Indonesia.

MATERIALS AND METHODS

Field research and procedure

The relevant research on rice (*O. sativa* L.) commenced in the greenhouse of the Sebelas Maret University, Surakarta, Central Java, Indonesia. It occurred under controlled environmental conditions with an average temperature of 29 °C–32 °C and a relative humidity of 70%–80%. This research ran from 2023 to 2024. The genetic materials used in this study were the seeds of five Mentik Susu rice strains with DNA editing results (T0), lithosol soil, organic and inorganic fertilizers, and sand. The tools used in the research included seedling tubs, buckets, hoses, sprayers, and picks. For observations and data collection, the tools utilized were a meter, a camera, and a pair of scissors.

Media preparation and planting

For the nursery, the planting media used was the mixture of sand and Rojokoyo organic fertilizer in a 1:1 ratio. The sand sustained sun-drying and incubation for seven days before being mixed with the organic fertilizer. After the nursery, the planting medium used

was a mixture of lithosol soil and organic fertilizer in a ratio of 1:10, put into a 17-cm-high bucket and flooded with water. The seeds of the selected rice strains entailed germination on a petri dish for two days, then transfer to seedbeds containing sand media. Each seedbed sown with 30 plants underwent maintenance for 21 days. At 21 days of age, the seedlings proceeded to their transfer to planting buckets filled with the planting medium. Each bucket received one plant that had been selected using direct PCR. The plants' maintenance included manual weeding, further fertilization with KCl and urea at 10, 31, and 59 days after planting, and the control of plant pests according to conditions. Watering continued to keep the planting media waterlogged.

The observations ensued on the growth and yield traits of rice, carried out at the time of harvesting. The harvesting took place when the rice reached full physiological maturity, the 90%–95% panicles became yellow, the flag leaf was dry, the grains were full and pithy, and the petiole and panicle began bowing.

Laboratory research

The research proceeded at the Laboratory of Plant Physiology and Agricultural Biotechnology, Faculty of Agriculture, Sebelas Maret University, Indonesia. Tools used in this study included micropipettes, microtips, Eppendorf and PCR tubes, electrophoresis, and PCR machines. Materials included rice leaf samples, a DNA isolation kit, Kappa 2G primers, nuclease-free water, and hptII, flanking, and Cas primers. Rice DNA isolation succeeded in using the Genomic DNA Mini Kit (Plant) Geneaid DNA isolation kit.

Molecular analysis of Mentik Susu rice T1 mutants

The PCR to detect the *hptII* gene used 5 µl Kappa 2G, 0.3 µl forward hptII primer (5 µM), 0.3 µl reverse hptII primer (5 µM), 1 µl template (isolated DNA of T1 milk paddy), and 3.4 µl NFW. The PCR program used for the *hptII* gene detection with 32 cycles comprised pre-denaturation at 95 °C for 4 min, denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, elongation at 72 °C for 45 s, and final elongation at 72 °C for 5 min. PCR results received electrophoresis before visualization with Geldoc. PCR for *Cas9* gene detection used 5 µl Kappa 2G, 0.3 µl Cas9 forward primer (5 µM), 0.3 µl Cas9 reverse primer (5 µM), 1 µl template, and 3.4 µl NFW. The PCR program used for *Cas9* gene detection with 25 cycles included pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, elongation at 72 °C for 30 s, and final elongation at 72 °C for 5 min. PCR results entailed electrophoresis and then visualization with Geldoc. The PCR kit consisted of 5 µl Kappa 2G, 0.3 µl forward primer apit (5 µM), 0.3 µl reverse primer apit (5 µM), 1 µl template, and 3.4 µl NFW, with a total reaction of 10 µl. The PCR program for the *SWEET11* gene was initial denaturation at 92 °C for 4 min, denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, elongation at 72 °C for 45 s, and the final elongation at 72 °C for 5 min with 35 cycles. PCR results achieved verification by sequencing to ensure the results were in accordance with the expected target. Genetic mutation analysis by comparing the DNA sequences of the samples to the wild-type sequence used bioinformatics software. This technique involves multiple sequence alignment (MSA) using the Clustal Omega application to detect base substitutions, insertions, and deletions, as well as BLAST for rapid homology searches to assess the functional impact of mutations at the structural level. The list of five Mentik Susu rice strains used for testing appears in Table 1.

RESULTS AND DISCUSSION

***HPTII* gene detection in Mentik Susu rice T1 mutant**

Mentik Susu rice (*O. sativa* L.) mutants were successful in their growing, with the young leaf samples collected for DNA isolation, which can serve as a template in the PCR analysis. The molecular analysis proceeded to identify the presence of transgenes (*hptII* and *Cas9* genes)

Table 1. Mutant Mentik Susu strains used for testing.

No.	Batch No.	Target gene	Mutation type	Number of seeds
1	MS-SW-C 3.1	OsSWEET11	Sub 1 base (promoter)	30
2	MS-SW-A 4.1	OsSWEET11	Sub 1 base (promoter)	30
3	MS-SW-A 21.2	OsSWEET11	Sub 1 base (promoter)	30
4	MS-SW-C 48.1	OsSWEET11	Sub 1 base (promoter)	30
5	MS-SW-C-137.1	OsSWEET11	Sub 1 base (promoter)	30

Description: Sub = substitution

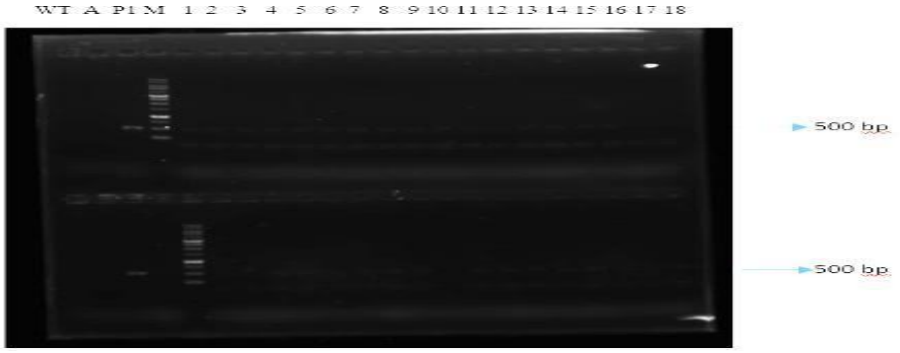

Figure 1. DNA amplification results of Mentik Susu mutant targeting the *SWEET11* gene with the *hptII* gene primer pair. The *hptII* gene amplicons are shown with a size of 500 bp. Notes: P1 = vector pDIRECT_25H, M = 1kb marker, and 1-36 = Mentik Susu mutant rice sample number.

using specific primers, with an amplicon target size of 500 bp. Molecular analysis was crucial to select the T1 generation rice mutant strains, whether to carry transgenes or no longer contain transgenes. The expected results were for mutant rice strains to no longer carry the transgenes (*hptII* and *Cas9* genes).

The CRISPR/Cas9 technology application in rice, especially those that rely on hygromycin phosphotransferase (HPTII) selection markers, has attracted significant attention due to its ease of supporting genome editing. The *HPTII* gene serves to select transgenic plants that successfully integrate the CRISPR/Cas9 construct during the Agrobacterium-mediated transformation process. Wu *et al*.'s (2019) findings showed the *HPTII* gene could be applicable effectively to select plants with edited genomes and free of CRISPR/Cas9 transgenes, using a hydrogen peroxide-based method. The technology could play a vital role in ensuring the elimination of transgenes from the next generation, thereby facilitating the regulatory process and market acceptance of genetically modified crops.

Additionally, the results showed chromatin accessibility plays an important role in the efficiency of CRISPR/Cas9 editing in rice. Liu *et al*. (2019) reported the chromatin structure with variations can improve Cas9 editing efficiency and highlights the importance of designing sgRNA that target promoter and exon regions to achieve optimal editing results. Findings suggested, in addition to the molecular tools used in genome editing, the epigenetic context of the target gene also needs deliberation to maximize the effectiveness of editing.

The PCR analysis using the hptII gene primer pair showed all the studied samples have not produced amplicons with a size of 500 bp (Figure 1). Mentik Susu mutant T1 generation, which had previously gone through mutation in generation T0, did not produce amplicons with a size of 500 bp. The analysis revealed the mutant rice no longer carries the *hptII* gene. This may occur because the mutant T1 generation rice has experienced genetic segregation. Genetic segregation has a close association with the distribution of alleles into

gametes during meiosis, which can affect the inheritance of traits between the generations. In crop plants, the genetic segregation can appear as a deviation from the expected Mendelian ratio, which often leads to consideration of a new inheritance pattern due to the influence of various genetic and environmental factors (Coulton *et al*., 2020). Deviations from Mendel's expected ratio can be because of sampling errors, interactions between genotypes, and environmental influences impacting the differential expression of traits. The presence of null alleles and errors in the interpretation of genetic markers also complicate the analysis of genetic segregation patterns in crop plants (Danner *et al*., 2013). For confirmation that the T1 generation mutant rice strains no longer carry the *hptII* gene and still have mutation in the gene target area, it is necessary to verify the same through sequencing analysis.

Cas9 gene detection in Mentik Susu rice T1 mutants

The CRISPR/Cas9 system is a highly beneficial tool for genome editing in various plant species, and the rice crop is no exception. Among the important genes targeted by rice genome editing, the *SWEET11* gene has considerably played a vital role in sugar transportation and can impact grain yield and stress resistance. The exploration of gene modification receives great aid from advances in CRISPR/Cas9 technology, which allows for considerable specificity and efficiency in genetic modification compared with previous methods.

The *Cas9* gene's presence in mutant rice T1 generation plants sustained analysis using PCR with four different primer pairs on each gene target. Primer pairs Cas9-SWEET-F and Cas9-SWEET-R for detection of the Cas9 in the promoter part of the *OsSWEET* gene had designs to produce amplicons with a size of 500 bp. For the *Cas9* gene presence in the T1 generation of Mentik Susu mutant rice plants, the results enunciated all rice plants no longer carry the *Cas9* gene, as confirmed by the absence of amplicons with the targeted size (Figure 2).

Sequencing analysis in Mentik Susu rice T1 mutants

The CRISPR-Cas9 system represents a significant advancement in genetic engineering, offering customizable and efficient genome editing capabilities. Its primary function utilizes the guide RNAs (gRNA) that direct Cas9 nucleases to specific genomic locations, enabling targeted cutting and repair, which can be beneficial for various applications, including disease resistance in crop plants (Knott and Doudna, 2018).

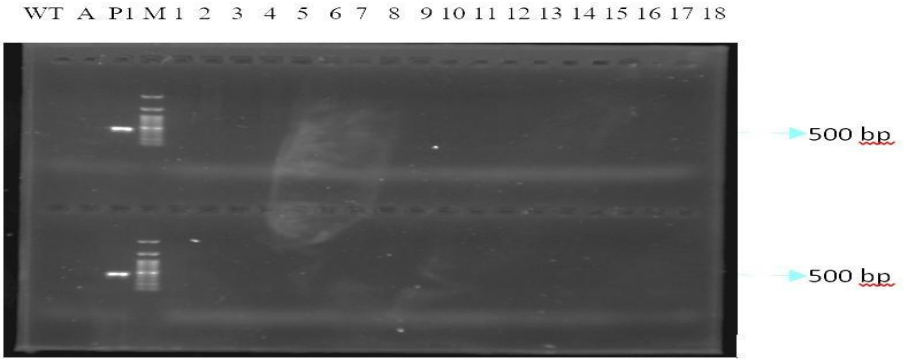

Figure 2. DNA amplification results of Mentik Susu plants with *Cas9* gene primer pairs to detect plasmid pDIRECT25H. Plants that positively contain the *Cas9* gene are indicated by amplicons of 500 bp. Notes: A = Air, WT = Wild type, P1 = vector pDIRECT25H, M = Marker 1 kb, and 1-18 = Sample number of Mentik Susu rice mutant.

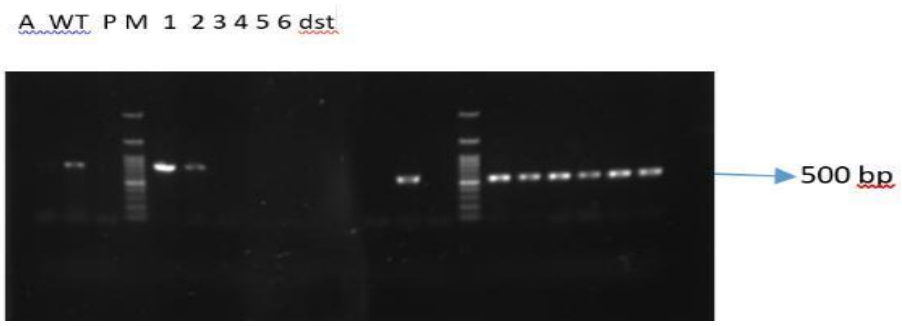

Figure 3. DNA amplification results of Mentik Susu rice T1 generation mutant at the target gene region. Notes: A = Air, WT = Wild type, P = vector plasmid, M = Marker 100 kb, and 1-18 = Sample number of Mentik Susu rice mutant.

Amplification of the gRNA flanking region of the *SWEET11* gene continued for sequencing analysis. The sequencing analysis determines the occurrence of mutation in the target gene region. The rice DNA amplification using primers flanking the *SWEET11 g*ene gRNA each produces the PCR products with a target size of 500 bp (Figure 3). Numerous studies have successfully used CRISPR-Cas9 technology to knock out these *SWEET* genes, resulting in increased resistance to pathogens, such as *Xanthomonas oryzae* (Xoo), which secrete effectors, i.e., transcriptional activators that bind to SWEET gene promoters, thereby increasing susceptibility (Joshi *et al*., 2018; Eom *et al*., 2019). Previous studies further exhibited that knockout of OsSWEET13 has shown an association with improved plant growth and resistance to bacterial leaf blight in rice (Chandra *et al*., 2024).

Mutation occurred in nine rice plants derived from five mutant Mentik Susu-SWEET11 rice strains. The types of mutations that occurred were the substitutions and insertions (Tables 2, 3, and 4). The SWEET11 is a member of the *SWEET* gene family that acts as a sugar transporter, which contributes to plant metabolism as used by *Xoo* to obtain nutritional resources from its host (Oliva *et al*., 2019; Balone *et al*., 2024). *Xoo* relies on the transcription activator-like effector (TALE) effector proteins that can bind to the SWEET11 promoter, activating its expression and increasing susceptibility to bacterial infection (Carpenter *et al*., 2020).

The occurring mutation in the *SWEET11* gene can result in variants that alter how the protein interacts with *Xoo* effector proteins. For example, the mutation that causes variations to binding sequences in the SWEET11 promoter can inhibit TALE binding, thus reducing SWEET11 expression. This can also reduce sugar availability to *Xoo,* which, in turn, can inhibit bacterial leaf blight infection and disease development (Oliva *et al*., 2019; Carpenter *et al*., 2020).

Enhancement of the CRISPR/Cas9 system through modifications, such as the inclusion of ubiquitin-associated domains (UBAs), has been evident to enhance the stability of the Cas9 protein. Zheng *et al*.'s (2020) findings showed this UBA fusion did not affect the cutting mechanism of Cas9; however, it considerably improved the editing efficiency in various rice target genes. These results suggested similar adaptation could be beneficial for targeting the *SWEET11* gene in future studies. These improvements were crucial as they can enhance the success rate of editing and produce more precise variations without causing undesirable side effects on the rice genome.

Furthermore, Banakar *et al*. (2020) highlighted that the delivery of CRISPR components as ribonucleoprotein (RNP) complexes enhances the transient expression of the desired editing results while avoiding the integration of unwanted elements into the plant genome. This method has particularly appeared useful for selective gene editing,

Table 2. SWEET11 promoter sequence in Mentik Susu rice plants that show the presence of mutations.

Strain	Nucleotide sequence of gRNA flanking the promoter of *OsSWEET11* gene	Mutation type
WT_MS_SW	CAGAGTGAAAAAGAAATATCAAGCACAAGAAAAAAAAGCAAAGGTTAAATATGCATCT	*Wild type* sequence
MS_SW_F8	CAGAGTGAAAAAGAAATATCAAGCACAAGAAAAAAAAGCAAAGGTTAGATATGCATCT	1-base substitution
MS_SW_G29	CAGAGTGAAAAAGAAATATCAAGCACAAGAAAAAAAAGCAAAGGTTAGATATGCATCT	1-base substitution
MS_SW_F4	CAGAGTGAAAAAGAAATATCAAGCACAAGAAAAAAAAGCAAAGGTTAGATATGCATCT	1-base substitution
MS_SW_G15	CAGAGTGAAAAAGAAATATCAAGCACAAGAAAAAAAAGCAAAGGTTAGATATGCATCT	1-base substitution
MS_SW_F5	CAGAGTGAAAAAGAAATATCAAGCACAAGAAAAAAAAGCAAAGGTTAGATATGCATCT	1-base substitution

Table 3. Sequence of the SWEET11 promoter in Mentik Susu rice plants that show the presence of mutations.

Strain	Sequence of gRNA-flanking nucleotide of *OsSWEET11* gene promoter	Mutation type
WT_MS_SW	CCAAGGCCAAACCACACATGCAGTTGTAGTAGCACTTAAGCCTTCCTCTCTAGCTAGCAT	*Wild type* sequence
MS_SW_G20	CCAAGGCCAAACCACACTTGCAGTTGTAGTAGCACTTAAGCCTTCCTCTCTAGCTAGCAT	1 base substitution
MS_SW_F8	CCAAGGCCAAACCACACATGCAGTTGTAGTAGCACTTAAGCCTTCCTCTCTACCTAGCAT	1 base substitution
MS_SW_H20	CCAAGGCCAAACCACACATGCAGTTGTAGTAGCACTTAAGCCTTCCTCTCTAGCTACCAT	1 base substitution
MS_SW_H12	CCAAGGCCAAACCACACATGCAGTTGTAGTAGCATTTAAGCCTTCCTCTCTAGCTACCAT	2-base substitution

Table 4. Sequence of the SWEET11 promoter in Mentik Susu rice plants that show mutations.

Strain	Sequence of gRNA-flanking nucleotide of *OsSWEET11* gene promoter	Mutation type
WT_MS_SW	CTCTTGTGTCAGGAAGTTGGAAGGGATTTCTGGCTAGTTTCTA-GCTGGTGTCTCCTCTC	*Wild type* sequence
MS_SW_G20	CTCTTGTGTCAGGAAGTTGGAAGGGATTTCTGGCTAGTCTCTAAACTGGGGTCTCCTCTC	3-base substitution, 1 base insertion
MS_SW_H20	CTCTTGTGTCAGGAAGTTGGAAGGGATTTCTGGCTATTTTCTA-GCTGGTGTCTCCTCCC	2-base substitution
MS_SW_E29	CTCTTGTGTCAGGAAGTTGGAAGGGATTTCTGGCTAGTTTCTA-GCTGGGGTCTCCTCTC	1 base substitution
MS_SW_H12	CTCTTGTGTCAGGAAGTTGGAAGGGATTTCTGGCTATTTTCTA-GCTGGGGTCTCCTCTC	2-base substitution

such as SWEET11, as it can minimize the risk of off-target mutation, which is a common concern in stable transformation methods. These methods help facilitate cleaner genetic modifications, allowing researchers to focus on the functional implications of their edits.

In addition to technical improvement, understanding the biological context of the *SWEET11* gene is crucial. Its remarkable role in sugar transport and regulation suggests that its editing could directly affect rice productivity and plants' response to stress conditions. He *et al*. (2020) noted that combining gene editing with visual markers for transgene detection, such as those related to anthocyanin biosynthesis, can facilitate the identification of edited plants. This will further boost the process of isolating plants successfully edited for genes, such as *SWEET11,* as well as accelerate the gene function and its impact on plant physiology under various environmental conditions.

SWEET11 is a sugar-transport-regulator gene that considerably alters rice grain yield and response to environmental stress conditions. Mutation in the promoter of this gene can regulate *SWEET11* gene expression, which has direct implications on rice agronomic performance. In improving the efficiency of the CRISPR/Cas9 system, proper promoter selection is essential. Hu *et al*. (2018) reported that by replacing the commonly used 2×35S promoter with endogenous promoters, such as UBIQUITIN1 (UBI1) or ACTIN1 (ACT1), the efficiency of genetic editing could reach significant improvement. In the context of SWEET11 promoter mutation, the use of endogenous promoters, such as UBI1, can provide more appropriate regulation in rice cells, increasing the likelihood of successful mutation.

Figure 4. Comparison of CRISPR/Cas9 SWEET mutant strains of Mentik Susu and wild-type rice on the characters of plant height and panicle length.

For gRNA and Cas9 expression, the promoter selection plays an influential role in regulating the frequency of the resulting mutations (Mikami *et al.,* 2015). Their results further revealed that in rice cell culture, mutation efficiency can be higher by using promoters that have better expression in rice cells, emphasizing the importance of proper promoter selection in rice gene editing projects. Thus, designing a system that targets SWEET11 using an endogenous promoter can enhance the possibility of obtaining mutants with desirable traits.

Phenotypic analysis of the Mentik Susu rice T1 mutants

The results showed CRISPR/Cas9 SWEET Mentik Susu mutant rice strains and the wild type have significant differences for phenotypic traits, and the rice mutants have more tillers than the wild-type rice. In rice mutants, the panicle length was longer than the wild type (Figure 4). The *SWEET11* gene in rice has received extensive studies for its role in sucrose transport and its implications in various developmental processes and disease resistance. SWEET11 mutants were evident to exhibit phenotypic traits, such as an increased tiller number and panicle length, as compared with wild-type rice plants. This improvement in rice mutant strains could refer to the increased sucrose transport rate facilitated by SWEET11, which is critical for phloem unloading processes, eventually influencing plant growth and development (Gao *et al*., 2018). Therefore, one can conclude that functional enhancement of SWEET11 can lead to increased tiller number and panicle length in rice mutants compared with wild-type rice.

Moreover, in rice the physiological role of SWEET11 has support from the regulation of its expression in response to various biotic stress factors, which correlates with structural development and sugar accumulation in crop plants (Huang *et al*., 2016; Oliva *et al*., 2019). Regulatory network involving SWEET11 displays responses to pathogens, such as *Xanthomonas oryzae*, emphasizing trade-offs where attributes that promote disease susceptibility can also enhance growth performance under certain conditions (Carpenter *et al*., 2020). In Table 5, one can see that the fastest flowering in the Mentik Susu rice mutant T1 generation was with the code G15, where this mutant strain has a mutation in the *OsSWEET11* gene promoter. In general, the mutant rice strains were notable for their increased number of panicles and filled grains per panicle compared with wild-type rice.

Mutations in the *SWEET* gene promoter led to variations in key agronomic traits, including panicle number, filled grains, and total grain weight across almost all mutant rice strains. Depending on the type and location of the mutation, panicle number could either increase or decrease, reflecting alterations in carbon metabolism that influence panicle formation. Since the SWEET promoter

Table 5. Agronomic characteristics of mutation in the *SWEET* gene in Mentik Susu rice.

Strains	Number ofPanicle panicles	length (cm)	Number of filled grains	100-grain weight (g)	Total weight	Flowering time (Days)	Height (cm)
WT	18	23.91	1927	3.34	43.24	78	92
F4	16	24.89	2703	1.71	38.69	80	99
F5	24	21.98	2413	1.65	38.22	85	88
F8	29	23.61	2127	1.81	36.94	80	104
G15	25	22.28	3466	1.82	55.99	65	95
G20	23	19.95	2458	1.89	41.17	72	110
G 29	33	23.36	4207	2.18	70.3	74	77
H12	51	17.88	1830	1.66	23.027	80	100
H20	31	20.50	2900	1.82	47.73	80	102

regulates sugar transport, these mutations also affect energy distribution to developing grains, thereby impacting grain-filling efficiency. Consequently, total plant weight and grain yield incur direct influences from the promoter's role in carbon resource allocation throughout plant growth and development.

Certain genes, such as the *SWEET* gene, regulate panicle formation and development in rice and can increase or decrease the panicle number. The number of filled grains gained changes from energy availability during the reproductive stage, which has a direct link to SWEET's function in seed filling. If mutation in the SWEET promoter enhances the sugar transport to the grains, the number of filled grains will increase, as more resources were available for seed filling. However, if sugar transport is defective, many grains will be empty because of not enough energy for the grain filling. Promoter mutation can alter the allocation of sugar among the plant organs, such as panicles, grains, and stems. Errors in determining location can cause the number of filled grains to decrease, although panicles may remain numerous.

CONCLUSIONS

This study demonstrated that CRISPR/Cas9 technology successfully edited the genome of Mentik Susu rice (*O. sativa* L.) in the T1 generation, with the *HPTII* gene undetectable, indicating the elimination of the transgene. Detection of the *Cas9* gene also did not produce amplicons, indicating the absence of this gene in the T1 mutant rice strains. Mutations in the *SWEET11* gene have a potential association with resistance mechanisms in rice against bacterial leaf blight by altering its interaction with TALE proteins. The phenotype of the T1 generation mutant rice showed increased tiller number, panicle length, and filled grains, indicating increased grain-filling efficiency. Overall, CRISPR/Cas9 technology has great potential in efforts to improve rice resistance to bacterial leaf blight, which paves the way for superior rice varieties.

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