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RESEARCH ARTICLE

Screening for submergence tolerant varieties via association of *Sub1* gene related markers in rice (*Oryza sativa* L.)

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Abstract

Complete submergence from flooding and stagnant water seriously injures the plant's growth and survival. The aim of the study was to identify local rice varieties that can be used as breeding materials for submergence tolerant varieties development using SSR and CAPS markers. Phenotypic screening revealed a significant difference whereby the mean elongation was higher for susceptible lines compared to tolerant lines. A strong negative correlation at -0.933 existed between survival percentage and elongation height in the submerged group. The elongation for the control group was also higher at 24.541 ± 5.119 cm than the submerged group at 14.806 ± 8.196 cm. Genotypic screening using the GNS2 CAPS marker has revealed the presence of the Sub1 gene and the gene polymorphism in the identified tolerant varieties with SSR markers, RM8303 that showed the highest PIC value (0.5174) and gene diversity (0.5816). It is expected that the results obtained may significantly help the development of better varieties in the upcoming rice breeding programmes.

Keywords: Genotypic screening, rice, submergence, *Sub1* gene, SSR

Introduction

Rice (*Oryza sativa* L.) is classified as a vital carbohydrate source providing sustenance to the 3 billion world population. The demand for rice is expected to rise by 1.7% annually, setting its requirement to add 13 mt from 1990 to 2025 (Aryalakshmi et al. 2021). The challenges rice production faces globally are the increase in demand for rice and the decrease in supply as the population grows (Shi et al. 2023). Thus, the supply of rice needs to be sufficient by increasing local rice production or via imported rice as biotic and abiotic stresses also affect rice cultivation (Nair 2019).

Abiotic stresses such as drought stress, salinity stress, submergence stress, and temperature are often beyond the control of human intervention and are caused by climate change (Aryalekshmi et al. 2020). Flash flooding is recognized as one of the three most important abiotic limitations on rice yields by 51% of respondents in a survey of rice breeders' concerns in south and southeast Asia (Panda et al. 2021). Annual flooding and inundation challenge rice cultivation in the rainfed lowland rice ecosystem as the floodwater remains for an estimated range of two weeks. Malaysia, especially during the monsoon season, is subjected to considerable rainfall. Both heavy redundant rainfalls and flash floods damage the rice field and lead to economic loss (Afrin et al. 2018). Although rice is capable of adapting to lowland or submerged conditions, the fact remains that it will die if submerged for more than three days due to oxygen shortage (Aryalekshmi et al. 2020). Substantial rice yield reduction in these submergence-prone areas drives the need to identify rice genotypes containing submergence-tolerant genes in different rice varieties (Singh et al. 2017).

Early evidence suggested that submergence tolerance is governed by a small number of partial or full dominant genes

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(Toojinda et al. 2003) based on the segregation analysis of offspring from crossings between tolerant and intolerant types. A single, dominant locus made it easy to incorporate the tolerance trait into an agronomically beneficial cultivar by traditional plant breeding. A QTL mapping study (Xu and Mackill 1996) identified a region on chromosome 9 (named Sub1) that accounted for 69% of the variation in tolerance and later verified rice's tolerance to submergence. The presence of the Submergence 1 (Sub1) gene near the centromeric region of chromosome number 9 has already been identified to be linked with the submergence tolerance trait in several rice varieties (Neeraja et al. 2007). *Sub1* is one of the major quantitative trait loci (QTL) for submergence tolerance in rice (Basu et al. 2024; Bailey-Seires et al. 2010). This gene has been proven to possess the ability to increase plant survival rate and better yield under submergence stress for a period of 14 days (Panda et al. 2021).

It is also crucial to identify DNA diagnostic markers that are tightly related to the traits of interest, which will result from a fine mapping of the identified QTLs, to construct a more successful marker-assisted selection (MAS) breeding system (Fukao et al. 2009). Variations in restriction fragment lengths brought on by Single Nucleotide Polymorphisms (SNPs) or Insertion-Deletions (INDELs) that add or remove restriction endonuclease recognition sites in PCR amplification generated by locus-specific oligonucleotide primers are known as Cleaved Amplified Polymorphic Sequences (CAPS) polymorphisms (Amiteye 2021). CAPS markers are co-dominant, locus-specific, flexible in being scored and interpreted, easier in data sharing between laboratories, and do not require the use of radioactive isotopes in assays (Wang et al. 2014). Meanwhile, in plant genome assessment, these CAPS markers assist in preparing genetic maps, genetic linkage, and fine mapping of studied plant genes (Sandhu et al. 2019). It is very important to identify submergence tolerance germplasm at the seedling stage of different selected rice cultivars and to find out the molecular markers to be used in submergence tolerance breeding (Amiteye 2021). The present study was, therefore, conducted to identify the submergence tolerance at the seedling stage of different selected rice cultivars using Simple Sequence Repeats (SSR)/microsatellites and CAPS markers, which could be used further in phenotype screening and submergence tolerance breeding of superior rice genotypes.

Materials and methods

Evaluation of submergence tolerance

Fourteen rice varieties obtained from MARDI's Rice GenBank, namely, Abong, Bunga Tebu, ChatekKuning, Cheloring, Katimun, Kepak, Tingkang, Dular, Mekbujangkelsom, Guanera15, Pokkali, Chempaka173, IR 64 and Swarma *Sub1* were used in the study. The seeds from different rice varieties were soaked in Clorox solution for 20 minutes for sterilization. The seeds were transferred into a 20 x 10 tray with soil filled ¾ quarter in each column. The experiments conducted followed as split plot-complete randomized design (CRD) with two treatments (control and submerge condition). The seeds germinated for 14 days under light exposure in the glasshouse at the surrounding temperature. The seedlings were submerged for another 14 days before de-submergence. On the 15th day, the plants were de-submerged and allowed for recovery for 7 days. Measurement of the shoot lengths from the base to the tip of the shoots at the end of the 14-day germination and 14-day stress period was taken. Meanwhile, the control sets were cultivated under a normal irrigation system. Evaluation of the shoot elongation pre- and post-submergence and the post-submerged survival rate (%) of each seed was also carried out according to Sukiran et al. (2022). Biometric observations for seedling vigor, such as leaf count and length of the shoot, were recorded. The performed submergence treatment and phenotypic screening was performed following the standard protocol of the International Rice Research Institute (IRRI 2006).

DNA isolation and PCR amplification

DNA buffer preparation DNA extractions were carried out by preparing an extraction buffer mixture using 2 g polyvinylpolypyrrolidone (PVP), 1 g sodium sarkosyl, 0.9 g diethyldithiocarbamiacid (DIECA), 0.088 g ascorbic acid, 28.0 mL 5M NaCl, 10.0 mL 1M tris hydrochloric acid (HCl) (pH 8.0) and 4.0 mL 0.5M ethylene diamine tetraacetic acid (EDTA) for the composition of 1 plate. Double-distilled water (ddH_2O) was added to produce a total volume of 200 mL of extraction buffer. This method was modified from the proposed procedure by Harisha (2007). PCR amplification was done using DNA marker particular primers for both SSR and CAPS (GNS2) markers, as stated in Table 1. This procedure was adapted and modified by Oladosu et al. 2020. The PCR-amplified products were then allowed to undergo capillary analysis in an ABI3730xl DNA analyzer. For multiplexing of GNS2, 0.2 µL of GNS2 and 0.2 µL of matK 18 were added for a total volume of 0.4 µL dye. The PCR was run to obtain PCR products amplified through multiplexing. For restriction enzyme treatment, 5 µL of the GNS2 multiplexed by matK PCR amplicons was added to 5 µL of the treatment mix containing 3.85 µL ddH2O, 1.0 µL buffer, and 0.15 µL Alu1. The DNA band output was resolved on a 10%w/v agarose gel for both pre-treated and post-treated amplicons amplified by GNS2 and matK primers.

DNA scoring and marker analysis

GeneMapper Version 4.0 was used to score peaks produced from electropherogram output to determine the allele size range for the SSR markers. Meanwhile, for the GNS2 marker, the DNA band output was used to detect the presence of

Table 1. The DNA marker particular primers used for submergence analysis

Primer ID	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	Temperature (˚C)
RM8300	GCTAGTGCAGGG TTGACACA	CTCTGGCCGTTT CATGGTAT	58.3
RM8303	AGGGGAGAGGA CACACACAC	GGATCCTCCTGC AAAATCAA	64.4
RM23805	GAGACAGATGTG TACGGTTTGGTG	TTGACAAGGAAG GAGAAG	51.0
RM23917	CTCAGCTGTCTG TTCAGCTCTCAC	CTTTGGTGCTGA GGTAGGTATTGG	41.6
RM23922	TGGAGGGAGTAT CATTATTAGCCG	CTTGGATAGATT TGGTGGGATGAC	55.0
GNS2	CTTCTTGCTCAA CGACAACG	TCGATGGGGTCT TGATCTCT	63.0

a band for each variety, whereby data was scored from gel electrophoresis. PowerMarker Version 3.25 software was used to compute major allele frequency, number of alleles per locus, genetic diversity, and Polymorphism Information Content (PIC) values. Pairwise genetic distance and relatedness of the varieties, phylogenetic reconstruction, and a dendrogram via an unweighted pair group method with UPGMA were also built from PowerMarker. This procedure was adapted and modified from Mojulat et al. (2017).

Statistical analysis

The ANOVA was used to analyze mean, standard deviation, standard error, and correlation using statistical analysis software for pre/post-submergence plant height of both control and submerged groups and post-submergence survival rate. SPSS was used to compute ANOVA analysis on the plant height for control and submerged groups. Pearson correlation was also computed for the plant height and survival percentage with a *p-value* of 0.01. R studio was utilized to code for the violin plot output between the two groups, using Fisher-LSD. The data was then analyzed and evaluated based on Masuduzzaman et al. (2016)**.**

Results and discussion

Phenotypic screening of rice varieties

Data was collected for the 14 varieties on the biometric observations, such as shoot height, and survival score before pre-submergence for both treatments, under control and submerged conditions, which showed major differences for both treated and control groups. Pearson correlation between shoot elongation and survival percentage calculated for the submerged group showed a strong correlation. Statistical analyses showed significant differences between the two conditions for the elongation height.

Shoot elongation and survival percentage of rice varieties

The results were presented as the mean elongation over ten growths of seedlings for each variety under both the treatments, control, and submerged conditions (Fig. 1). The results showed that the mean height for shoot elongation was significantly higher for control than for submerged varieties. Figure 1 shows the difference in shoot elongation between the control group and the submerged group. The average shoot elongation rate was observed to be lower for the submerged group compared to the control group. This is in accordance with the findings by Santos et al. (2017). In this study, the positive tolerant check and negative susceptible check among the varieties are Swarna-Sub1 and IR64, respectively. The genotypes that presented the highest shoot elongation post-submergence were Abong, Bunga Tebu, Chatek Kuning, Cheloring, Katimun, Kepak, Tingkang and IR64, ranging from 17.033 ± 1.134 to 26.660 ± 1.304 cm. This shows these varieties underwent rapid elongation to escape the induced flood conditions. Their upward growth is enhanced as part of their "escape strategy," thus allowing rapid decrease and utilization of carbohydrate and energy reserves. A study by Aryalekshmi et al. (2021) confirmed that a rapid increase in shoot elongation indicates that these rice genotypes are among the susceptible lines. Another study (Yusoff 2018) reported that tolerant varieties preserve carbohydrates as an energy source, contributing to their suppressed elongation during submergence. The presence of the ethylene-response factor (ERF) gene is seen in these tolerant varieties with limited shoot elongation after submergence. The tolerant varieties, namely, Dular, Mekbujangkelsom, Guangera15, Pokkali, Chemapaka173, and Swarna-Sub1 showed a mean shoot elongation ranging from 10.840 ± 1.708 to 1.025 ± 0.126 cm showing a lower elongation compared to the varieties Abong, Bunga Tebu, Chatek Kuning, Cheloring, Katimun, Kepak, Tingkang and

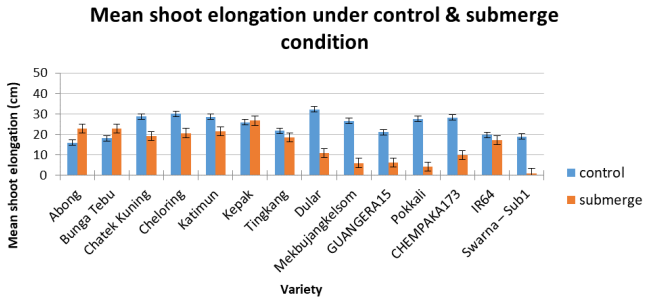


Fig. 1. Shoot height before and after submergence and survival percentage of 14 rice varieties under submerged conditions

IR64 (Fig. 1). The low elongation length indicates that these varieties possess the ethylene-response factor gene that incurs tolerance to submergence. Thus, they preserve carbohydrates for the regrowth and development of the plant after the flood recedes. No other varieties have the lowest elongation length than Swarna-Sub1, which served as the tolerant check variety with a value of 1.025 ± 0.126 cm.

The post-submerged survival percentage is low for varieties Abong, Bunga Tebu, Chatek Kuning, Cheloring, Katimun, Kepak, Tingkang and IR64, showing that these varieties are unlikely to recover after 14 days of submergence due to complete utilization and exhaustion of carbohydrate and other nutrient reserves leading to death Varieties Abong, Bunga Tebu, Chatek Kuning, Cheloring, Katimun, Kepak and IR64 did not survive the 14-day submergence except Tingkang, which showed only 10% survival (Fig. 2). There is a strong negative correlation between shoot elongation and survival percentage in these varieties, whereby Pearson correlation showed the relationship between these variables (Table 2). Stem elongation during flash flooding is not desirable, and it has been discovered that cultivars' propensity to withstand flash flooding is correlated with their level of stem elongation growth. Most species that elongate more quickly underwater use entrapped ethylene as their signal (Sukiran et al. 2022). Commonly noted is a strong negative link between elongation growth and survivability. Accordingly, elongation or growth in plant height implies acceleration in the elongation of the sensitive line under stress, making a phenotypic assessment of plant height essential (Aryalekshmi et al. 2020).

It can be observed that intolerant varieties, i.e., Abong, Bunga Tebu, Chatek Kuning, Cheloring, Katimun, Kepak and IR64, showed 0–10% of survivability with higher shoot elongation (Fig. 2). Tolerant varieties such as Dular, Mekbujangkelsom, Guanera15, Pokkali, Chempaka173, IR 64 and Swarma Sub1, showed more than 50% survival except for variety Chempaka173. This explained the existence of different levels of tolerance in rice varieties ranging from completely resistant to medium tolerant. Pokkali and Swarna-Sub1 have shown the highest survival percentage upon submergence, 90 and 75%, respectively. Hence, it can be deduced that Pokkali has complete tolerance to submergence, followed by Swarna-Sub1. According to a previous study (Santos et al. 2017), recovery is more prominent upon submergence for tolerant genotypes containing Sub1 gene, whereby morphological damage induced by free radicals is lower in these genotypes.

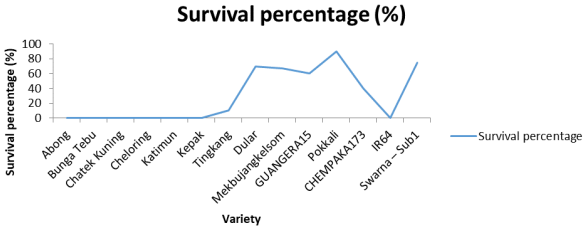


Fig. 2. Survival percentage of 14 rice varieties under submerged condition

Overall, the control group exhibited a higher mean in shoot elongation of 24.541 ± 5.119a cm. as compared to the submerged group with a mean elongation of 14.806 ± 8.197b cm. The significant difference is denoted with different letters (i.e. a, b etc.) according to LSD. These results could infer that the physiological injury brought upon by submerged conditions, slow rates of gaseous exchange, severe turbidity contributing low rate of photosynthesis, and low concentration of dissolved oxygen, carbon dioxide and ethylene gas possibly affect the low shoot elongation in the submerged group (Hong et al. 2020).

Analysis of genetic distance

The genetic distance between the 14 rice varieties was assessed from six DNA markers (Table 2). The genetic distances among all pairwise comparisons showed that the varieties Abong, Bunga Tebu, Chatek Kuning, Cheloring, Katimun, Kepak and Tingkang have a lower pairwise value for genetic distance compared with IR64. Based on the six DNA marker data, these varieties are closely related to the susceptible check, IR64. The varieties Dular, Mekbujangkelsom, Guanera15, Pokkali, and Chempaka173, showed a lower pairwise value for genetic distance when compared with Swarna-Sub1. The relative distance between these varieties towards Swarna-Sub1 indicates they are closely related. A high genetic distance shows a high degree of dissimilarities between these varieties. The genetic dissimilarity between varieties Dular, Mekbujangkelsom, Guanera15, Pokkali, and Chempaka173 and varieties Abong, Bunga Tebu, Chatek Kuning, Cheloring, Katimun, Kepak and Tingkang is high showing that their source of origin varies from each cluster. The genetic dissimilarity range is narrower between the tolerant lines compared to susceptible lines showing that they had recently diverged. This can also be due to the use of local varieties from the same environment, presenting a lack of genetic diversity (Serif et al. 2019). Values presenting ≥0.500 show a distant relation among varieties. Based on the computed pairwise distances, the lowest pairwise value of '0' was found between varieties Dular, Mekbujangkelsom, Guanera15, Pokkali, and Chempaka173 and Swarna-Sub1, indicating that these varieties are the closest to Swarna- Sub1, and display similar genetic characteristics as the tolerant check. Meanwhile, for the susceptible line, varieties Abong, Bunga Tebu, Chatek Kuning show the lowest pairwise value of 0.1667 with IR64, showing a close relationship with the susceptible check and indicating the absence of *Sub1* gene

Phylogenetic analysis of rice varieties

The genetic relationship is shown for the 14 varieties through UPGMA cluster analysis based on six markers data (Fig. 3).

Table 2. Summary of genetic distances across 14 rice varieties showing genetic similarity among different varieties

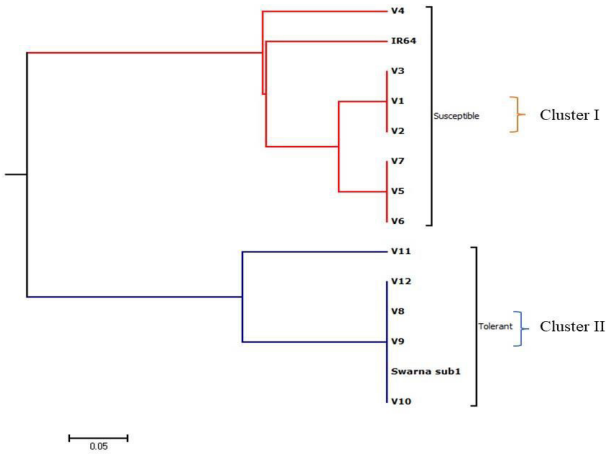
OTU	IR64	SS1	V1	V10	V11	V12	V2	V3	V4	V5	V6	V7	V8	V9
IR64	0.0000	0.7500	0.1667	0.7500	0.7500	0.7500	0.1667	0.1667	0.2500	0.2500	0.2500	0.2500	0.7500	0.7500
SS1	0.7500	0.0000	0.5833	0.0000	0.2500	0.0000	0.5833	0.5833	0.7500	0.5000	0.5000	0.5000	0.0000	0.0000
V1	0.1667	0.5833	0.0000	0.5833	0.7500	0.5833	0.0000	0.0000	0.1667	0.0833	0.0833	0.0833	0.5833	0.5833
V10	0.7500	0.0000	0.5833	0.0000	0.2500	0.0000	0.5833	0.5833	0.7500	0.5000	0.5000	0.5000	0.0000	0.0000
V11	0.7500	0.2500	0.7500	0.2500	0.0000	0.2500	0.7500	0.7500	0.8333	0.7500	0.7500	0.7500	0.2500	0.2500
V12	0.7500	0.0000	0.5833	0.0000	0.2500	0.0000	0.5833	0.5833	0.7500	0.5000	0.5000	0.5000	0.0000	0.0000
V2	0.1667	0.5833	0.0000	0.5833	0.7500	0.5833	0.0000	0.0000	0.1667	0.0833	0.0833	0.0833	0.5833	0.5833
V3	0.1667	0.5833	0.0000	0.5833	0.7500	0.5833	0.0000	0.0000	0.1667	0.0833	0.0833	0.0833	0.5833	0.5833
V4	0.2500	0.7500	0.1667	0.7500	0.8333	0.7500	0.1667	0.1667	0.0000	0.2500	0.2500	0.2500	0.7500	0.7500
V5	0.2500	0.5000	0.0833	0.5000	0.7500	0.5000	0.0833	0.0833	0.2500	0.0000	0.0000	0.0000	0.5000	0.5000
V6	0.2500	0.5000	0.0833	0.5000	0.7500	0.5000	0.0833	0.0833	0.2500	0.0000	0.0000	0.0000	0.5000	0.5000
V7	0.2500	0.5000	0.0833	0.5000	0.7500	0.5000	0.0833	0.0833	0.2500	0.0000	0.0000	0.0000	0.5000	0.5000
V8	0.7500	0.0000	0.5833	0.0000	0.2500	0.0000	0.5833	0.5833	0.7500	0.5000	0.5000	0.5000	0.0000	0.0000
V9	0.7500	0.0000	0.5833	0.0000	0.2500	0.0000	0.5833	0.5833	0.7500	0.5000	0.5000	0.5000	0.0000	0.0000

Two clusters are presented in the dendrogram: Cluster I and Cluster II. Observations are made based on genetic distance and similarity indices computed previously, showing that varieties Katimun, Kepak, Tingkang have the highest similarity with varieties Abong, Bunga Tebu and Chatek Kuning. Meanwhile, compared with the susceptible check, IR64, a high similarity was found between IR64 and varieties Abong, Bunga Tebu and Chatek Kuning. The lowest similarity was set between Cheloring and IR64, confirming the distant relation towards IR64 and other varieties Abong, Bunga Tebu, Chatek Kuning, Katimun, Kepak and Tingkang. Overall, these varieties are categorized under cluster I, which carries the susceptible line.

For the tolerant line, under cluster II, the highest genetic similarity was found between Swarna-sub 1 and varieties Dular, Mekbujangkelsom, Guangera15 and Chempaka173, indicating a close relation between varieties. Variety 11 confers the lowest genetic similarity among the other tolerant varieties. This observation shows that a number of *O. sativa* varieties, such as these varieties Dular, Mekbujangkelsom, Guangera15 and Chempaka173, indeed possess *Sub1A* on their polygenic locus since it has been previously known that only a restricted number of *O. sativa* possesses the gene (Santos et al. 2017). A previous study (Jambhulkar et al. 2018) also deduced that the percentage of tolerant varieties, including complete and medium tolerant, is higher and often clustered together as one in a phylogenetic analysis, confirming their close evolutionary relatedness.

Overall polymorphism of DNA markers

In this study, the marker polymorphism for SSR markers (RM8300, RM8303, RM23805, RM23917 and RM23922) and CAPS marker (GNS2) is assessed based on major allele frequency, genetic diversity, allele number and polymorphism information content (PIC). The PIC value corresponds negatively to the major allele frequency, indicating that a higher PIC value gives a lower major allele frequency. A marker that shows high polymorphism should possess a higher PIC value with a lower major allele frequency value. Based on the DNA marker summary (Table 3), RM8300, RM8303, and GNS2 markers show the lowest allele frequency. However, only RM8303 exhibits the highest PIC value among the other markers. According to a study by Nair and Shylaraj (2021), RM8303 was used as one of the



Cluster I consists of Cheloring (V4), IR64, Chatek Kuning (V3), Abong (V1), Bunga Tebu (V2), Tingkang (V7), Katimun (V5) and Kepuk (V6). Cluster II consists of Pokkali (V11), Chempaka173 (V12), Dular (V8), Mekbujangkelsom (V9), Guangera15 (V10) and Swarna-Sub1.

Fig. 3. The UPGMA cluster dendrogram displaying genetic relationship across 14 rice varieties

Table 3. Summary of 6 DNA markers used across 14 rice varieties

Marker	MAF	Chr. No.	Allele No.	Gene Diversity	PIC	Type of marker
RM8300	0.5714	9	2.0000	0.4898	0.3698	polymorphic
RM8303	0.5714	9	3.0000	0.5816	0.5174	polymorphic
RM23805	0.8571	9	4.0000	0.2577	0.2464	polymorphic
RM23917	1.0000	9	1.0000	0.0000	0.0000	monomorphic
RM23922	0.7143	9	3.0000	0.4388	0.3862	polymorphic
GNS2	0.5714	9	2.0000	0.4898	0.3698	polymorphic
Mean	0.7143	NA	2.5000	0.3763	0.3150	NA

Chr.No. = Chromosome number, PIC = Polymorphism Information content, MAF = Major Allele Frequency, NA = Not Applicable

Table 4. Summary of SSR and CAPS markers that amplified alleles in 14 rice varieties

Variety	RM8300	RM8303	RM2380	RM2391	RM23922	Allele size (bp)	GNS2* Sub1**	Reaction
Abong	215/215	147/147	281/281	172/172	242/242	100	0	Susceptible
Bunga Tebu	215/215	147/147	281/281	172/172	242/242	100	0	Susceptible
Chatek Kuning	207/207	139/139	281/281	172/172	242/242	100	0	Susceptible
Cheloring	215/215	136/147	283/283	172/172	0/0	100	0	Susceptible
Katimun	215/215	139/145	281/281	172/172	242/242	100	0	Susceptible
Kepak	222/222	147/147	281/281	172/172	242/242	100	0	Susceptible
Tinkang	207/207	147/147	281/281	172/172	242/242	100	0	Susceptible
Dular	215/215	147/147	281/281	172/172	242/242	111	1	Tolerant
Mekbujang Kelsom	207/207	139/145	281/281	172/172	242/242	111	1	Tolerant
Guangera 15	207/207	147/147	281/281	172/172	242/242	111	1	Tolerant
Pokkali	215/215	136/136	281/289	172/172	235/235	111	1	Tolerant
Chempaka 173	207/207	139/139	281/281	172/172	242/242	111	1	Tolerant
IR64	207/207	147/147	279/281	172/172	242/242	100	0	Susceptible
Swarna Sub1	215/215	139/145	281/281	172/172	242/242	111	1	Tolerant

The presence and absence of band for GNS2* are denoted as 111, 100, respectively. Presence and absence of **sub 1 gene denoted as: 1, 0, respectively.

recombinant markers for the selection and introgression of the *Sub1* gene, as it showed polymorphism between parents during a genotypic screening of every plant generation.

The lowest PIC value is presented by the marker RM23917, with a value of 0.000, which shows that only RM23917 is monomorphic. This can be further validated through the allele size computed for RM23917, whereby RM23917 only presented an allele size of 172/172 bp in all 14 varieties, showing it is less valuable in discriminating individuals on homozygotic and heterozygotic information. The genetic diversity has a mean of 0.3763 and ranges from zero to 0.5816 for RM23917 and RM8303 (Table 4). The highest diversity is set for RM8303 and the lowest for RM23917. The higher genetic diversity of RM8303 indicates that the genetic variation is high, giving valuable information on the polymorphism of the marker.

Using a CAPS marker is another way to validate and confirm the presence of the *Sub1* gene, as it is used as a functional marker. This marker is best used with flanking markers such as SSR markers to accurately distinguish tolerant and susceptible varieties (Neeraja et al., 2007). The presence of the *Sub1* gene is indicated by the DNA band in agarose gel amplified by the CAPS marker, GNS2. The presence of the band was detected in varieties Dular, Mekbujangkelsom, Guanera15, Pokkali, Chempaka173 and Swarna-Sub1, which indicates the tolerance trait to submergence. Overall, varieties Abong, Bunga Tebu, Chatek Kuning, Katimun, Kepak and Tingkang show an

absence of the *Sub1* QTL, which was observed in accelerated shoot height and through the detection of DNA markers, respective to the susceptible check, IR64. On the other hand, varieties Dular, Mekbujangkelsom, Guanera15, Pokkali, and Chempaka173 show the presence of the *Sub1* QTL through minimum shoot elongation respective to the tolerant check, Swarna-Sub1 (Table 4).

Authors' contribution

Conceptualization of research (PK, NAB, MSFAR, NAS); Designing of the experiments (NAB, MSFAR); Contribution of experimental materials (MSFAR, AMAM); Execution of field/lab experiments and data collection (PK, AMAM, MZS); Analysis of data and interpretation (MSFAR, NAB, PK); Preparation of the manuscript (PK, NAB, SYS, NAS).

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