

Molecular screenings of drought tolerance QTLs and agronomic performance in selected rice (*Oryza sativa*) cultivars

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Abstract. Pohan SD, Prima HS, Jamsari, Runifah T. 2025. Molecular screenings of drought tolerance QTLs and agronomic performance in selected rice (*Oryza sativa*) cultivars. *Biodiversitas* 26: 3731-3744. This study aimed to identify Quantitative Trait Loci (QTL) associated with drought tolerance (*qDTY*) and evaluate their agronomic performance under both non-stress and stress conditions. Molecular analysis using eight SSR markers (RM236, RM276, RM511, RM520, RM28099, RM28130, RM28166, and RM1261) was conducted to detect the presence of targeted *qDTY* loci. A Completely Randomized Design (CRD) with three replications was applied to assess the effects of drought stress on rice plants. Two treatments were implemented: a non-stress control (C₁, 100% of field capacity) and a drought stress condition (C₂, 50% of field capacity). Agronomic traits such as PH, PTN, PL, NFG, NUFG, DTF, and DTM were measured to evaluate plant performance under both conditions. The SSR markers used for detecting *qDTY* loci exhibited polymorphism. The number of alleles per marker averaged 8.625, ranging from 1 (RM511) to 12 (RM236). RM236 and RM28099 showed the highest polymorphism with eight alleles each. Phenotypic evaluation found significant differences in trait performance between non-stress and drought-stress conditions. While all traits generally decreased under drought stress (C₂), the extent of reduction varied among genotypes. Most genotypes appeared to exhibit a drought escape mechanism by shortening their DTF and DTM under drought stress.

Keywords: Agronomic traits under stress, drought tolerance QTLs, molecular screening, SSR markers

INTRODUCTION

Drought is one of the most significant abiotic factors influencing the evolution and productivity of rice. It is also the main limitation of rice production in the rain-fed ecosystem (Saud et al. 2022). Drought is widely regarded as the most destructive environmental pressure, causing a greater decline in crop yield than any other ecological stressor (Ye et al. 2024). Drought has a significant impact on rice growth and development, resulting in substantial reductions in its growth and biomass accumulation. Drought in crop plants ultimately leads to a decrease in cell division and expansion, as well as leaf size, stem elongation, and root proliferation. It also alters stomatal conductance oscillations and negatively affects plant water and nutrient interactions, resulting in diminished crop yield and reduced water use efficiency (Li et al. 2017). In addition, a lack of water speeds up the production of Abscisic Acid (ABA), which in turn reduces the opening of stomata to limit the loss of water through transpiration (Meddy et al. 2023). According to Bandurska et al. (2022), under dry conditions, rice has a phenological delay in flowering, an increased level of pollen sterility, and impeded grain formation.

However, plants have a good short-term survival because they can delay or avoid tissue water shortages and endure drought by altering their growth and structural parts. These adaptations lead to three main drought response strategies: (i) absorbing and storing more water; (ii) losing less water during dry periods; and (iii) maintaining tissue

strength to prevent wilting, which can cause permanent cell damage (Haghpanah et al. 2024). Plants also adapt to drought by changing the ratio of roots to shoots and modifying traits related to root structure and anatomy (De Micco et al. 2023). Several rice varieties possess mechanisms to cope with drought stress and are called drought-tolerant. They can survive water stress with or without a decline in performance. A key biochemical process for drought tolerance is osmotic adjustment, mainly achieved by increasing the accumulation of common solutes like sugars, organic acids (for example, proline and glycine betaine), and ions, especially K⁺ (Zahra et al. 2023).

From a molecular perspective, plants adapt to drought stress through gene expression regulation, hormonal signaling, Reactive Oxygen Species (ROS) scavenging, signal transduction pathways, and epigenetic modifications (Hoang et al. 2020). Drought QTLs are gene fragments that control the coping mechanism against drought stress by regulating physiological, biochemical, and developmental processes. By identifying and mapping drought QTLs, researchers can recognize specific genome regions that are linked to drought tolerance traits. These groups of genes encode proteins that are responsible for drought tolerance in plants (Andleeb et al. 2020).

The International Rice Research Institute (IRRI) conducted thorough research and found several drought QTLs that have a significant impact on rice yield. These QTLs include *qDTY_{3.1}*, *qDTY_{2.1}*, *qDTY_{2.2}*, *qDTY_{4.1}*, *qDTY_{9.1}*, and *qDTY_{10.1}* (Dwivedi et al. 2022) and *qDTY_{12.1}* (Shaodan

et al. 2023). These *qDTYs* were incorporated and combined with mega-cultivars worldwide, and then distributed to the farmers. Shamsudin et al. (2016) reported that several QTLs such as QTLs, viz. *qDTY_{2.2}*, *qDTY_{3.1}*, and *qDTY_{12.1}*, pyramided through the marker-assisted backcrossing method, can increase the yield advantage of more than 1000 kg ha⁻¹. Furthermore, Mohd Ikmal et al. (2021) reported the effect of different combinations of the *Sub1* and *qDTY* on morpho-physiological, agronomical traits, and yield under reproductive stage drought stress (RS) and non-stress (NS) conditions. A combination of *Sub1* and *qDTY_{3.1}* increased yield up to 6954 kg Ha⁻¹.

QTLs linked to submergence tolerance and blast disease resistance had been studied before in Pulau Batu, Cantik Manis, Bungo Sungkai, Kuriak, Mundam Putih, and some local rice cultivars examined in this study (Pohan et al. 2023, 2025). However, until now, there is no information regarding QTL associated with drought tolerance in these cultivars. Therefore, this study aims to identify Quantitative Trait Loci (QTLs) associated with drought tolerance using several SSR markers in the targeted genome regions under both drought stress and non-stress conditions. Understanding these QTLs allows breeders to develop varieties that are particularly suited to certain prone regions (Ma et al. 2024). Combining multiple QTLs (QTL pyramiding) in plants can confer robust tolerance against abiotic and biotic stresses (Kumar et al. 2018; Pohan et al. 2024).

MATERIALS AND METHODS

Materials

This study utilized 8 rice (*Oryza sativa* L.) genotypes, comprising 5 traditional landraces—from West Sumatra: Pulau Batu, Cantik Manis, Bungo Sungkai, Kuriak, and Mundam Putih, and 3 modern cultivars: Inpari 48 Blas, IR64-*Sub1*, and Inpago 9. The cultivars were selected based on the farmer's reference and the authenticity of local germplasm. The study was conducted under loam soil conditions, with average temperatures of 30-34°C and low rainfall (<100 mm/h per month), indicating moderate to severe drought stress during the treatment period. Detailed characteristics and origins of the rice genotypes are summarized in Table 1.

All rice cultivars used in this study were obtained with permission from the Indonesian Ministry of Agriculture (Balitbangtan). The use of plant materials complied with national regulations on germplasm access and utilization.

Procedures

Molecular screening of drought tolerance QTLs (qDTY) in eight rice cultivars

The leaves of 14-day-old rice seedlings were collected from each genotype as samples for DNA extraction. Furthermore, DNA was extracted using the Geneaid™ Genomic DNA Mini Kit (Geneaid Biotech Ltd.) following the manufacturer's protocol. Next, we measured the DNA concentration using the BioDrop™ nanodrop spectrophotometer, standardized to 25 ng/μL, and stored it at -20°C. A total of eight Simple Sequence Repeat (SSR) markers were obtained from the previous study (Mohd Ikmal et al. 2021). These markers were used to determine the presence of *qDTY* in the tested rice genotypes (Table 2). Furthermore, PCR amplification was carried out following a protocol based on Toyobo manufacture's protocol. The mixture for the reaction included 2 μL of genomic DNA, 12.5 μL of KOD One™ PCR Master Mix-blue, 2.5 μL (10 mM) of SSR marker, and 8 μL of nuclease-free water, making a total of 25 μL. The PCR running followed the program as follows: 94°C for 5 min (initial denaturation), followed by 35 cycles at 94°C for 30 s of denaturation, annealing at 55°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 5 min. PCR products with selected markers were analyzed with a 1.5% agarose gel (stained in ethidium bromide) for electrophoresis with a voltage of 100 V for 45 minutes. SSR marker, sequences, and information were obtained from the GRAMENE database (<https://www.gramene.org/>), and relevant literature sources as detailed in Table 2. Marker IDs and primer references are provided to support reproducibility and future comparison. The migration distance of electrophoretic bands (PCR products) was compared to the DNA ladder. The DNA ladder, containing fragments of known sizes, was used to estimate the size of PCR products based on how far they travel. The estimated size of PCR products was then validated and linked to certain phenotypic traits by following the previous studies or relevant references.

Table 1. The list of rice cultivars used in the study

Genotype	Rice type	Description
Pulau Batu (PB)	Indica	Local rice, landrace, good taste, high yield
Cantik Manis (CM)	Indica	Local rice, landrace, high-density grain
Bungo Sungkai (BS)	Indica	Local rice, landrace, strong stem, many productive tillers, fast-harvested
Kuriak (K)	Indica	Local rice, landrace, good taste
Mundam Putih (MP)	Indica	Local rice, landrace, good texture and taste
Inpari 48 Blas (IB)	Indica	Modern rice, blast-resistance check, high-yield
IR64- <i>Sub1</i> (IR)	Indica	Modern rice, short-stemmed, submergence-tolerant
Inpago 9 (Ip9)	Japonica	Modern rice, highland cultivar, blast-resistant

Table 2. List of eight SSR markers used for molecular screening of *qDTY* QTLs in rice (*Oryza sativa*)

QTL	Marker	Marker type	Sequence	Expected product size (bp)	Annealing temperature (°C)	References
<i>qDTY_{2.2}</i>	RM236	SSR	F: GCGCTGGTGGAAAATGAG R: GGCATCCCTCTTTGATTCCTC	191	55	Gramene.org
<i>qDTY_{2.2,3.1}</i>	RM276	SSR	F: CTCAACGTTGACACCTCGTG R: TCCTCCATCGAGCAGTATCA	149	55	Gramene.org
<i>qDTY_{3.1}</i>	RM520	SSR	F: AGGAGCAAGAAAAGTTCCCC R: GCCAATGTGTGACGCAATAG	247	55	Venuprasad et al. (2009)
<i>qDTY_{12.1A}</i>	RM511	SSR	F: CTTCGATCCGGTGACGAC R: AACGAAAGCGAAGCTGTCTC	130	55	Dixit et al. (2012)
<i>qDTY_{12.1A}</i>	RM28099	SSR	F: TGTGCGGATGCGGGTAAAGTCC R: CCACCTGTCAACCACCGAAACC	120	55	Dixit et al. (2012)
<i>qDTY_{12.1A}</i>	RM28130	SSR	F: CAGCAGACGTTCCGGTTCTACTCG R: AGGACGGTGGTGGTATCTGG	175	55	Dixit et al. (2012)
<i>qDTY_{12.1B}</i>	RM28166	SSR	F: TGCTTGCAAACATTGCTTCTGG R: TGCTTGCAAACATTGCTTCTGG	194	55	Dixit et al. (2012)
<i>qDTY_{12.1B}</i>	RM1261	SSR	F: GTCCATGCCCAAGACACAAC R: GTTACATCATGGGTGACCCC	167	55	Dixit et al. (2012)

A Completely Randomized Design (CRD) was applied in this study, consisting of three replications. Two treatments were implemented: a non-stress control (C_1 , 100% of field capacity) and a drought stress condition (C_2 , 50% of field capacity). Agronomic traits were measured to evaluate plant performance under both conditions.

Drought tolerance phenotypic screening of eight rice cultivars

Rice plant seedlings were planted in a soil medium with a composition ratio between soil and compost fertilizer (1:1). Rice seedlings were initially germinated for 21 days before planting. The seedlings were nursery in the normal field conditions, receiving daily watering and full sunlight exposure. Furthermore, the stress treatments were administered to the plants at the age of 31 after transplanting (Mohd Ikmal et al. 2021). A field capacity measurement was carried out to determine the irrigation volume applied to the plants. Each pot was filled with 500 g of soil media, then watered until saturated and left to drain for 72 hours until no more water dripped; it was also weighed as wet weight (T_b). Furthermore, we placed the soil in an oven at 100°C for 24 hours, cooled it in a desiccator, and weighed it as dry weight (T_k). We repeated the experiment five times to obtain an accurate average result, and then calculated the field capacity of the soil using the following formula:

$$(KL) = \frac{(T_b - T_k)}{T_k} \times 100$$

Inpago 9 was used as a tolerant check in this study. Inpago 9 is a highland cultivar that is known for its tolerance to drought stress. The agronomic traits observed following treatment included the Plant Height (PH), Productive Tiller Number (PTN), Panicle Length (PL), Number of Filled Grains (NFG), Number of Unfilled Grains (NUFG), Days to Flowering (DTF), and Days to Maturing (DTM). The application of chemical fertilizers for the plant in the field follows standards by the Indonesian Ministry of Agriculture (2007): 200-275 kg/ha

urea, 75 kg/ha SP-36, 50 kg/ha KCl, and 250-350 kg/ha NPK. Meanwhile, Pest management was implemented using Regent (fipronil 80%), Plenum (pimetrozin 50%), and Filia (propiconazole 125 g/L and tricyclazole 400 g/L). Moreover, conventional hand-weeding was done to control weeds in the pots. All mentioned pesticides and fungicides were applied as specified by the Indonesian government regulations. Supplementary materials, including the SSR band scoring matrix, full agronomic trait data, and gel electrophoresis images, are provided in Table S1, S2 and Figure S1 to support data transparency and reproducibility.

Data analysis

Descriptive statistics and One-Way Analysis of Variance (ANOVA) were used to compare variation between rice genotypes with a significance level of 0.05 and 0.01. Tukey's post hoc test has been used to identify specifically which group means were significantly different from each other, after finding that there was a statistically significant difference among the group means overall. The collected data from agronomic characteristic observations were analyzed using SPSS Version 26 (IBM Corp 2019). Pearson's correlation analyses were performed to determine the correlation between traits (agronomic data of rice genotypes). R Studio software version 2022.02.1+461 was applied to generate a graphical correlation matrix, principal component, and clustering in agronomic traits of all genotypes. The built-in R Stats package was used to obtain the correlation values, while the R Corplot package was used to visualize the correlation values into a graphical correlation matrix. Meanwhile, the R ggplot package was applied to visualize the PCA biplot and results, and the R Cluster package was used for clustering analysis using the K-means method. Furthermore, the Silhouette method was used to determine the optimum number of clusters. The Euclidean method was used to compute the distances between genotypes.

RESULTS AND DISCUSSION

Genotyping screening results of several rice cultivars

This study focused on molecular screening using QTL-linked markers previously reported to be associated with drought tolerance, rather than conducting de novo QTL mapping. Therefore, the markers used are associated with putative QTL regions and serve as tools for genotyping rather than for identifying novel loci. To ensure reproducibility and minimize scoring errors in the detection of molecular markers, each PCR amplification was performed in technical duplicate. Two researchers independently evaluated the agarose gel scoring to reduce subjective bias, and any discrepancies in band interpretation were resolved through consensus.

The PCR amplification product of eight rice genotypes using SSR markers is shown in Figure 1. These markers, previously reported to be associated with drought-tolerance QTLs (*qDTYs*), were used to confirm the presence of specific QTLs in the tested genotypes. Molecular analysis revealed that the markers used for identifying *qDTYs* were

polymorphic; markers RM236 and RM276, associated with *qDTY_{2.2}*. For RM236, the targeted band size was 191 bp. Pulau Batu (PB) exhibited a closely positioned band at 194 bp, while Cantik Manis (CM), Bungo Sungkai (BS), Inpari 48 Blas (IB), and IR64-*Sub1* (IR) showed bands at 197 bp. Kuriak (K) and Inpago 9 (Ip9) exhibited bands at 200 bp, and Mundam Putiah (MP) at 184 bp. For RM276, only genotype K showed the expected 149 bp band. BS showed a band at 179 bp, IB at 183 bp, while PB, MP, and Ip9 were observed at 186 bp. CM and IR displayed higher bands at 194 bp.

Markers RM28099, RM28130, and RM511 are closely linked to *qDTY_{12.1A}* (Dixit et al. 2012; Zhou et al. 2024). Analysis using RM511 confirmed the presence of the *qDTY_{12.1A}* locus in PB and CM, which exhibited bands at 129 bp and 132 bp, respectively. Marker RM28099 revealed polymorphism among genotypes: Ip9 showed a distinct band at 112 bp, PB, CM, BS, and IR showed bands around 120 bp, while K and MP had higher bands at 124 bp and 127 bp, respectively (Figure 1).

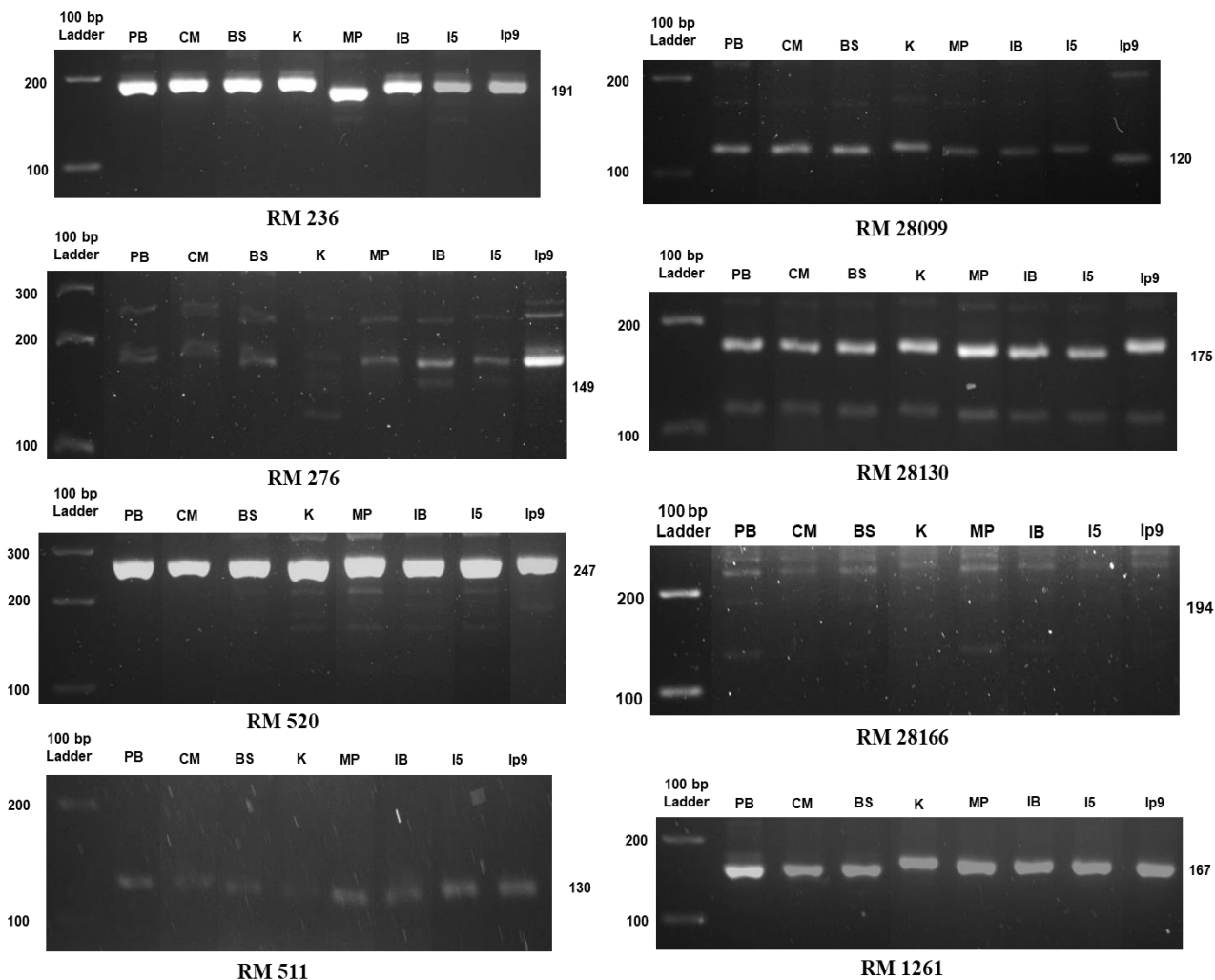


Figure 1. Visualization of PCR-amplicon of several rice genotypes amplified using eight SSR markers linked to drought tolerance

Table 3. Molecular screening result of eight rice genotypes analyzed using eight DNA markers linked to drought tolerance

QTL	Marker/Code	Expected amplicon size (bp)	Genotypes							
			PB	CM	BS	K	MP	IB	IR	Ip9
<i>qDTY_{2.2}</i>	RM236	191	+	-	-	-	-	-	-	-
<i>qDTY_{2.2,3.1}</i>	RM276	149	-	-	-	+	-	-	-	-
<i>qDTY_{3.1}</i>	RM520	247	-	-	-	-	-	-	-	-
<i>qDTY_{12.1A}</i>	RM511	130	+	+	-	-	-	-	-	-
<i>qDTY_{12.1A}</i>	RM28099	120	+	+	+	-	-	-	+	-
<i>qDTY_{12.1A}</i>	RM28130	175	-	-	-	+	-	-	-	+
<i>qDTY_{12.1B}</i>	RM28166	194	-	-	-	-	-	-	-	-
<i>qDTY_{12.1B}</i>	RM1261	167	+	+	+	-	-	-	-	+

Note: +: Targeted band exists, -: Targeted band does not exist

Table 4. Number of alleles and polymorphic bands of eight rice genotypes using eight SSR markers

Marker	Number of alleles	Number of polymorphic alleles	PIC values
RM236	12	8	0.225
RM276	9	6	0.245
RM520	11	5	0.200
RM511	1	0	0.000
RM28099	10	8	0.212
RM28130	11	0	0.000
RM28166	7	4	0.198
RM1261	8	5	0.235
Total	69	36 (52.17%)	1.315

Marker RM520, associated with *qDTY_{3.1}*, did not amplify the targeted 247 bp fragment in any of the tested genotypes, indicating the absence of this QTL (Table 3). Marker RM28166, linked to *qDTY_{12.1B}*, did not show polymorphism across the tested genotypes. None of the cultivars exhibited the targeted band at 194 bp, as previously observed in the drought-tolerant check IR81896 (Mohd Ikmal et al. 2021). PB, BS, and MP produced bands at 180 bp, and Ip9 at 184 bp. In contrast, RM1261, another marker associated with *qDTY_{12.1B}*, exhibited polymorphism among genotypes. CM and BS showed the targeted band at 167 bp, while PB and Ip9 had lower bands at 163 bp. IB and IR showed bands at 174 bp, MP at 177 bp, and K at 184 bp. These polymorphic differences suggest variability in the presence of *qDTY_{12.1B}* among the tested cultivars.

RM28166 is a flanking marker located on chromosome 12 and is closely associated with the *qDTY_{12.1B}* locus. This QTL contributes significantly to rice yield under reproductive-stage drought stress it also plays a crucial role in maintaining grain production. In addition to drought tolerance, *qDTY_{12.1B}* has also been linked to resistance against blast and bacterial blight diseases (Dixit et al. 2012). RM28166 lies near the peak of the *qDTY_{12.1}* QTL region, indicating a strong linkage to alleles conferring drought tolerance (Singh et al. 2025).

RM236 is located on chromosome 7 and is widely recognized for its association with drought tolerance. This marker has been linked to multiple QTLs, including *qDTY_{2.2}*, *qDTY_{7.1}*, and *qDTY_{7.2}*, all of which contribute to drought adaptation mechanisms in rice (Shaodan et al. 2023; Roy et al. 2025). RM276 is located on chromosome 6 and has been

used extensively in QTL mapping and marker-assisted selection for agronomic traits. It serves as a flanking marker for QTLs associated with panicle length, grain number, and yield-related traits under abiotic stress conditions, including drought (Kumar et al. 2018; Haque et al. 2021).

Pulau Batu (PB) and Cantik Manis (CM) were the most frequently occurring genotypes carrying the targeted QTLs. Pulau Batu tested positive for *qDTY_{2.2}*, *qDTY_{12.1A}*, and *qDTY_{12.1B}*, as detected by markers RM236, RM511, RM28099, and RM1261, respectively. Similarly, CM was positive for *qDTY_{12.1A}* and *qDTY_{12.1B}*, indicated by amplification with RM511, RM28099, and RM1261. Kuriak (K) exhibited the presence of *qDTY_{2.2}*, *qDTY_{3.1}*, and *qDTY_{12.1A}*, while Inpago 9 (Ip9) was positive for *qDTY_{12.1A}* and *qDTY_{12.1B}*, as confirmed by RM28130 and RM1261, the flanking markers closely linked to these QTL regions. IR64-Sub1 (IR) was confirmed to possess *qDTY_{12.1A}* based on amplification with RM28099.

As shown in Table 4, averaging 8.625 alleles, the number of alleles per marker varied from 1 (RM511) to 12 (RM236). While RM511 and RM28130 showed no polymorphism, RM236 and RM28099 had the most polymorphic alleles (8).

Of all alleles, 52.17% are polymorphic (36 out of 69). A mean Polymorphism Information Content (PIC) value, which shows the discriminatory power of each marker, ranged from 0.000 (RM511 and RM28130) to 0.245 (RM276) and was categorized as low to moderate. Markers RM276 (0.245), RM1261 (0.235), and RM236 (0.225) showed relatively higher PIC values, implying their relevance for identifying genetic variation was linked to drought tolerance.

To explore potential marker-trait associations, we compared drought stress performance of cultivars carrying specific QTL-linked markers. Cultivars positive for RM236 and RM28099 (e.g., PB, K, CM) tended to maintain higher Numbers of Filled Grain (NFG) and Productive Tiller Numbers (PTN), suggesting a possible link between marker presence and drought resilience. While not conclusive, these associations support further validation through regression or association mapping in larger populations.

The polymorphism data suggest that several SSR markers used in this study are effective in differentiating rice genotypes based on genetic variation at drought-associated loci. Among them, RM236, RM276, RM1261, and RM28099 showed the highest number of polymorphic

alleles and elevated PIC values, making them particularly valuable tools for Marker-Assisted Selection (MAS) in drought tolerance breeding programs.

Conversely, markers RM511 and RM28130 were found to be monomorphic, exhibiting no detectable polymorphism and a PIC value of 0.000, which indicates limited usefulness in assessing genetic diversity among the tested genotypes. However, when these markers are fixed within specific cultivars, they may still be useful for verifying the presence or absence of targeted alleles.

The generally low PIC values observed in this study may be due to the limited number of markers used and the small population size of rice genotypes tested, which probably restricted the observed genetic variation at the evaluated loci. Nonetheless, these markers remain useful because they are closely linked to important drought tolerance QTLs and can still be used to confirm alleles in breeding lines. However, these markers can still be valuable since they are near important drought tolerance QTLs and can be used to verify the presence or absence of a key allele. Combining low PIC values with those showing higher polymorphism could improve resolution in QTL mapping and increase the accuracy of molecular screening methods.

The dendrogram indicated that all three clusters shared a common ancestral background, with Cluster 1 and Cluster 2 diverging from the same lineage. Within Cluster 1, Ip9 further separated to form its own cluster (Cluster 2), resulting in the division of Cluster 1 into two sub-clusters: 1.A and 1.B (Figure 2).

The Jaccard Similarity Index is a measure of how similar two sample sets are based on shared traits. It provides important information on the genetic diversity of rice genotypes. Breeders can choose genetically diverse parents to promote heterosis and expand the genetic base of new cultivars by identifying pairs with low resemblance. On the other hand, maintaining desired traits within a breeding line may benefit from identifying genotypes that are genetically related. As shown in Table 5, the exact similarity value of 1.000 between genotypes MP and IB suggests that they are genetically identical or very similar.

This indicates that they share all of the molecular markers examined. The similarity between genotypes IR and Ip9 is 0.365, indicating a moderate genetic relationship. For certain breeding applications, such as the introgression of specific traits, this level of similarity may be sufficient. In contrast, the similarity value of 0.161 between PB and CM indicates a low genetic relationship. This difference demonstrates that these genotypes are genetically distinct and could contribute diverse alleles for use in breeding programs.

Phenotypic evaluation of agronomic traits under drought stress

Agronomical traits data from eight rice genotypes were collected and subjected to Analysis of Variance (ANOVA) to assess their response to drought stress. Seven agronomic characteristics Plant Height (PH), Productive Tiller Number (PTN), Panicle Length (PL), Number of Filled Grains (NFG), Number of Unfilled Grains (NUFG), Days to Flowering (DTF), and Days to Maturity (DTM) were evaluated under both non-stress (C_1) and drought stress (C_2) conditions (Table 6). The ANOVA results (Table 7) revealed significant differences ($p < 0.01$) for all traits across treatments and genotypes, except for PTN. In general, drought stress (C_2) caused a decrease in all measured traits compared to non-stress (C_1), although the magnitude of reduction varied among genotypes.

For Plant Height (PH), all genotypes exhibited significant declines. Cantik Manis (CM) showed a relatively moderate decline (from 98.87 cm to 95.43 cm), while Mundam Putiah (MP) showed the most significant reduction (from 122.20 cm to 91.37 cm). This decrease is in line with earlier research demonstrating that water stress inhibits cell division and elongation, which lowers plant stature. Most genotypes showed a sharp decline in Productive Tiller Number (PTN) with some experiencing reductions exceeding 75%. Notably, Inpago 9 (Ip9) was the only genotype that exhibited an increase in tiller number under drought (from 11.00 to 12.00) suggesting a possible drought-adaptive tillering mechanism.

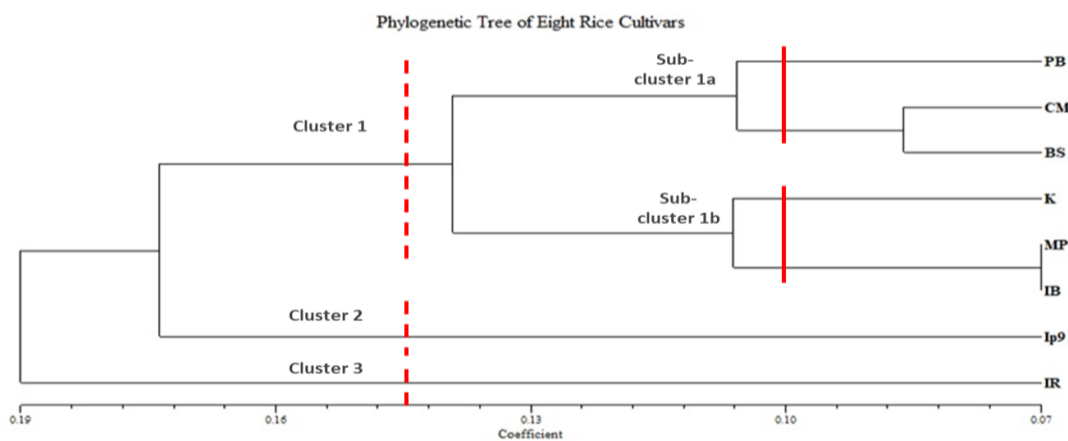


Figure 2. A dendrogram of eight rice cultivars was generated by UPGMA cluster analysis based on eight SSR markers

Table 5. Jaccard's Similarity Index between rice genotypes

Genotypes	PB	CM	BS	K	MP	IB	IR	Ip9
Pulau Batu (PB)	1.000							
Cantik Manis (CM)	0.161	1.000						
Bungo Sungkai (BS)	0.276	0.217	1.000					
Kuriak (K)	0.310	0.220	0.179	1.000				
Mundam Putih (MP)	0.204	0.175	0.196	0.132	1.000			
Inpari 48 Blas (IB)	0.204	0.175	0.196	0.132	1.000	1.000		
IR64-Sub1 (IR)	0.333	0.302	0.237	0.271	0.259	0.259	1.000	
Inpago 9 (Ip9)	0.293	0.233	0.311	0.288	0.246	0.246	0.365	1.000

In contrast, MP and IR64-Sub1 (IR) showed extreme stress sensitivity, with tiller numbers dropping to just three. Drought stress also reduced Panicle Length (PL), with variation among genotypes. CM showed a minor reduction (26.33 cm to 24.70 cm), whereas Inpari 48 Blas (IB) exhibited a more substantial drop (26.80 cm to 21.23 cm). Since panicle size influences nutrient allocation to grains, this reduction may reflect impaired reproductive development under stress. All genotypes experienced significant declines in the Number of Filled Grains (NFG), a key yield determinant. The most severe reductions were observed in MP (from 189.33 to 29.00) and IB (from 188.33 to 33.33). Conversely, Inpago 9 (Ip9) and Kuriak (K) demonstrated relatively higher NFG retention under stress (51.67 and 96.33, respectively), suggesting greater reproductive resilience.

The unfilled grains (NUFG) increased in all genotypes during drought, indicating poor grain filling and spikelet sterility. IR recorded the highest number of unfilled grains (64.00), nearly double the number observed under non-stress. This highlights the vulnerability of reproductive stages to water deficit. Most genotypes exhibited a drought escape mechanism by accelerating flowering (DTF) and maturity (DTM) under stress. For example, PB flowered 13 days earlier (106.33 to 93.33 days), and BS flowered 8 days earlier (67.33 to 59.33 days). Although early flowering can be advantageous under terminal drought, it often results in reduced biomass and yield. These results are consistent with prior studies (Radha et al. 2023; Salgotra and Chauhan 2023; Jarin et al. 2024), which reported that drought significantly reduces rice growth, development, and yield. A meta-analysis of 55 studies showed an average yield reduction of 25.4% due to drought, increasing up to 88% when drought coincides with flowering due to spikelet sterility and reduces seed set (Radha et al. 2023; Jarin et al. 2024).

The ANOVA in Table 7 shows that the interaction significantly affects most traits ($p < 0.01$) except PTN in genotype, but is significant in Intercept, DTT, and DTT Genotype. The non-significant genotype effect on PTN implies that environmental factors play a greater role in tillering than genetic differences. However, significant genotype \times drought interactions for PH, PL, NFG, DTF, and DTM highlight the differential responses of genotypes to drought stress, which is critical for selecting stress-resilient cultivars.

In this study, Pulau Batu and Kuriak exhibited stable agronomic performance under drought stress, supported by the presence of drought-related QTLs- $qDTY_{2.2}$, $qDTY_{12.1A}$,

and $qDTY_{12.1B}$. Cantik Manis showed promising performance despite the absence of detected $qDTY$ loci, highlighting the potential for phenotypic selection. K and Ip9 have a good agronomic performance and stability under stress. Ip9 exhibited an increase in tiller number and consistent maturity timing, while K retained higher NFG and exhibited only moderate reductions in other traits. These traits suggest underlying physiological mechanisms such as efficient assimilate partitioning, stress signaling pathways, or efficient water use, which warrant further investigation. In contrast, BS and IR, performed poorly under drought. While IR is known for submergence tolerance, its low adaptability under water-limited conditions highlighted the need for developing a multi-stress-tolerant rice cultivar that can withstand both flood and drought conditions.

Multivariate analysis

Correlation analysis revealed significant relationships among the seven agronomic traits under drought stress, providing insight into key factors influencing drought tolerance (Table 8 and Figure 3). Notably, plant height ($r = 0.700$), PTN ($r = 0.897$), and PL ($r = 0.728$) were strongly positive correlated with the Number of Filled Grains (NFG), a key yield component. The result suggests that genotypes capable of maintaining vegetative vigor and reproductive growth under drought stress conditions are more likely to sustain higher grain yields. On the other hand, the Number of Unfilled Grains (NUFG) exhibited strong negative correlation with several traits, particularly PL ($r = -0.829$) and NFG ($r = -0.725$) indicating that reduced panicle development and yield potential are associated with increased spikelet sterility under drought conditions. Additionally, early-flowering genotypes tended to be shorter and reached maturity more rapidly, reflecting a drought escape strategy. This trend is supported by the strong positive correlation ($r = 0.931$) between the phenological traits DTF and DTM, as well as the strong correlation ($r = 0.839$) between DTF and PH. These findings confirmed that PTN, PL, and NFG are reliable agronomic indicators of drought tolerance and are likely associated with key drought-responsive QTLs, particularly $qDTY_{2.2}$ and $qDTY_{12.1A}$. The strong inter-trait correlations further validate the selection of genotypes such as Kuriak (K) and Pulau Batu (PB), which exhibited favourable agronomic performance supported by QTL presence. These genotypes represent promising candidates for use in breeding programs aimed at improving drought resilience in rice.

Table 6. Agronomical traits data recorded in the non-stress (C₁) and drought stress (C₂) conditions of eight rice cultivars

Genotype	PH (\pm SD)		PTN (\pm SD)		PL (\pm SD)		NFG (\pm SD)		NUFG (\pm SD)		DTF (\pm SD)		DTM (\pm SD)	
	C ₁	C ₂	C ₁	C ₂	C ₁	C ₂	C ₁	C ₂	C ₁	C ₂	C ₁	C ₂	C ₁	C ₂
Pulau Batu (PB)	112.30 \pm 3.27	97.27 \pm 1.57	20.00 \pm 5.00	04.67 \pm 2.89	25.80 \pm 0.36	21.10 \pm 1.31	186.33 \pm 10.59	81.00 \pm 6.24	18.33 \pm 6.03	31.33 \pm 18.23	106.33 \pm 1.53	93.33 \pm 1.53	129.67 \pm 1.53	118.00 \pm 2.64
Cantik Manis (CM)	98.87 \pm 2.21	95.43 \pm 1.60	15.67 \pm 1.53	04.33 \pm 2.52	26.33 \pm 0.50	24.70 \pm 1.32	192.33 \pm 6.65	88.33 \pm 7.37	11.67 \pm 2.52	31.00 \pm 9.16	90.00 \pm 2.64	85.33 \pm 1.15	119.67 \pm 1.53	114.33 \pm 4.04
Bungo Sungkai (BS)	75.83 \pm 2.28	72.10 \pm 2.82	17.00 \pm 6.08	04.00 \pm 1.00	24.43 \pm 0.65	22.37 \pm 0.42	154.00 \pm 6.56	17.33 \pm 8.08	07.67 \pm 3.51	30.00 \pm 7.00	67.33 \pm 1.15	59.33 \pm 1.15	96.00 \pm 1.00	88.67 \pm 0.58
Kuriak (K)	105.23 \pm 1.83	97.80 \pm 1.13	22.33 \pm 1.53	04.67 \pm 1.15	25.97 \pm 0.41	22.00 \pm 2.55	184.00 \pm 7.00	96.33 \pm 43.1	18.33 \pm 4.93	41.67 \pm 8.38	94.33 \pm 0.58	90.33 \pm 1.53	128.00 \pm 1.73	120.33 \pm 2.08
Mundam Putih (MP)	122.20 \pm 3.99	91.37 \pm 1.82	18.00 \pm 2.00	03.33 \pm 0.58	25.93 \pm 0.35	23.00 \pm 0.44	189.33 \pm 1.00	29.00 \pm 2.52	22.67 \pm 2.52	39.00 \pm 21.70	99.00 \pm 1.00	93.00 \pm 1.17	125.33 \pm 2.31	118.67 \pm 1.15
Inpari 48 Blas (IB)	112.13 \pm 2.57	82.37 \pm 2.50	20.00 \pm 2.00	04.33 \pm 2.52	26.80 \pm 0.60	21.23 \pm 2.43	188.33 \pm 10.78	33.33 \pm 5.68	28.67 \pm 5.68	42.33 \pm 4.51	81.00 \pm 1.73	76.00 \pm 1.73	93.00 \pm 1.73	90.67 \pm 2.08
IR64-Sub1 (IR)	79.30 \pm 1.31	67.20 \pm 2.67	15.67 \pm 2.52	03.33 \pm 0.58	19.80 \pm 0.62	16.20 \pm 0.72	142.00 \pm 15.87	15.00 \pm 3.00	34.67 \pm 10.69	64.00 \pm 5.00	66.33 \pm 1.53	64.00 \pm 0.00	93.00 \pm 0.00	90.67 \pm 0.58
Inpago 9 (Ip9)	95.63 \pm 1.77	87.23 \pm 3.09	11.00 \pm 1.00	12.00 \pm 2.00	26.30 \pm 1.11	21.10 \pm 0.87	116.00 \pm 8.18	51.67 \pm 8.50	17.00 \pm 13.75	33.67 \pm 8.33	73.33 \pm 0.58	69.00 \pm 1.00	102.33 \pm 0.58	96.33 \pm 2.31

Table 7. Results of ANOVA for all traits evaluated in non-stress and drought stress treatment of eight rice cultivars

Source of variation	DF	PH	PTN	PL	NFG	NUFG	DTF	DTM
Corrected model	15	123.921 ***	21.949 ***	21.624 ***	-	5.997 ***	-	194.722 ***
Intercept	1	72628.151 ***	886.912 ***	20142.801 ***	-	433.245 ***	-	157472.212 ***
Genotype	7	177.552 ***	1.579 ns	26.531 ***	***	5.787 ***	***	394.968 ***
DTT	1	399.916 ***	267.300 ***	121.117 ***	138.101 ***	46.120 ***	***	128.847 ***
Genotype*DTT	7	30.861 ***	7.269 ***	2.504 *	***	0.474 ns	***	3.886 ***
Error	32	5.749	6.875	1.290	-	96.417	-	3.542
Total	48							
Corrected total	47							

Note: ns, *, ***: No significantly different, significantly different at $\alpha = 0.05\%$ and $\alpha = 0.01\%$, respectively

Table 8. Pearson's correlation matrix for PH, PTN, PL, NFG, NUFG, DTF, and DTM

Traits	PH	PTN	PL	NFG	NUFG	DTF	DTM
PH	1.0000000						
PTN	0.5467521	1.0000000					
PL	0.7322378	0.6146322	1.0000000				
NFG	0.6997668	0.8971732	0.7284469	1.0000000			
NUFG	-0.4387063	-0.6726296	-0.8293442	-0.7248446	1.0000000		
DTF	0.8388486	0.2912790	0.5168489	0.4966795	-0.2889239	1.0000000	
DTM	0.7277683	0.2653593	0.4842173	0.4713180	-0.3558279	0.9308166	1.0000000

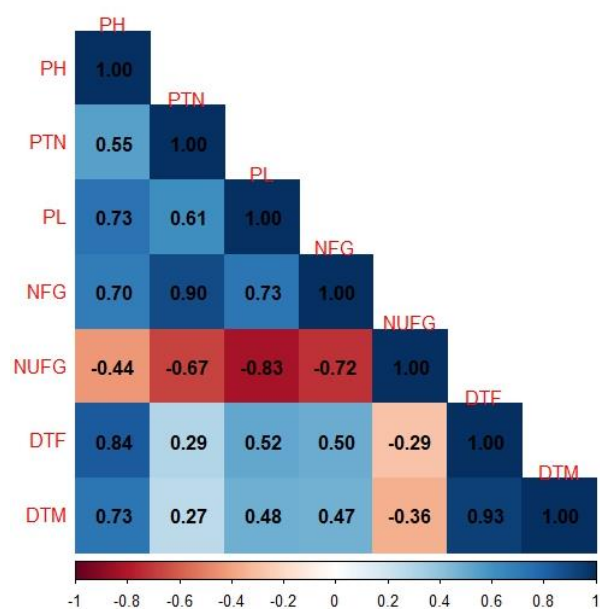


Figure 3. Correlation matrix of agronomic traits in rice cultivars under drought stress

Principal component analysis

Figure 4 presents the PCA scree plot and biplot illustrating the distribution of rice genotypes based on the seven agronomic traits evaluated. The diverse distribution of the genotypes in the PCA plot indicates substantial

phenotypic diversity, suggesting that different genotypes exhibit distinct responses to drought across the measured traits. This separation also implies the presence of significant genetic variation associated with drought-responsive characteristics. Genotypes 2 (Cantik Manis), 3 (Bungo Sungkai), 4 (Kuriak), 6 (Inpari 48 Blas), and 8 (Inpago 9) clustered closely within the same region of the biplot. This proximity suggests similar performance patterns in traits such as Productive Tiller Number (PTN), Number of Filled Grains (NFG), and Panicle Length (PL), indicating potentially similar physiological responses to drought stress.

In contrast, genotypes under drought treatment 9 (PB), 10 (CM), 12 (K), 13 (MP), 14 (IB), and 15 (IR), were closely associated with the Number of Unfilled Grains (NUFG) implying that these genotypes exhibited increased spikelet sterility and poorer grain filling under stress conditions. Meanwhile, genotypes 1 (PB) and 5 (MP) were positioned near the trait PH vector for suggesting similar expression for this characteristic under both stress and non-stress conditions.

Cluster analysis

The clustering analysis was performed using the K-means method to examine the segregation of rice genotypes based on their agronomic trait performance under drought conditions (Figures 4.A-4.C). Figure 4.A illustrates an Euclidean distance matrix which quantifies the degree of similarity or dissimilarity among genotypes. Smaller distance indicates greater similarity, whereas a larger distance reflects a greater dissimilarity.

The optimal number of clusters was determined to be four, as shown in Figure 5.B. Based on this, the K-means clustering algorithm classified the genotypes into four distinct groups (Figure 5.C). Cluster 1 included three genotypes (3-Bungo Sungkai, 6-Inpari 48 Blas, and 8-Inpago 9), Cluster 2 contained four genotypes (9-Pulau Batu, 10-Cantik Manis, 12-Kuriak, and 13-Mundam Putih), Cluster 3 comprised five genotypes, and Cluster 4 included four genotypes. This clustering reflects phenotypic similarities among the rice cultivars and provides insights into potential grouping for targeted breeding strategies.

To determine the extent of genetic control over the agronomic traits, estimates of Genotypic Coefficient of

Variation (GCV), Phenotypic Coefficient of Variation (PCV), and broad-sense heritability (H^2) were calculated. The results showed moderate to high heritability values for key traits such as grain yield, plant height, and number of tillers, indicating that genetic factors largely influenced the observed phenotypic variation. GCV and PCV values were also relatively close for most traits, suggesting minimal environmental influence. Principal Coordinate Analysis (PCoA) based on SSR data revealed clear genetic differentiation between local landraces and modern cultivars. This supports the UPGMA clustering and highlights the presence of population structure, which is important for future marker-assisted breeding.

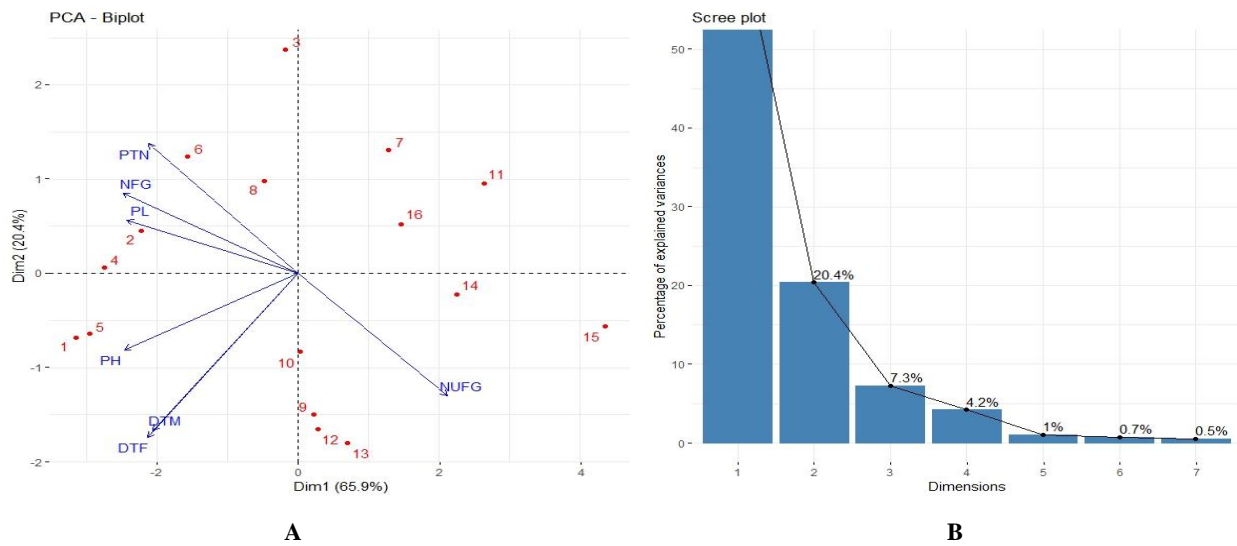


Figure 4. Principle Component Analysis (PCA) of: A. Screeplot, and B. Biplot of eight rice genotypes based on morphological performances under no stress (C_1) and drought stress (C_2): C_1 = 1-8, C_2 = 9-16. (1/9 = Pulau Batu, 2/10 = Cantik Manis, 3/11 = Bungo Sungkai, 4/12 = Kuriak, 5/13 = Mundam Putih, 6/14 = Inpari 48 Blas, 7/15 = IR-64-Sub1, and 8/16 = Inpago 9)

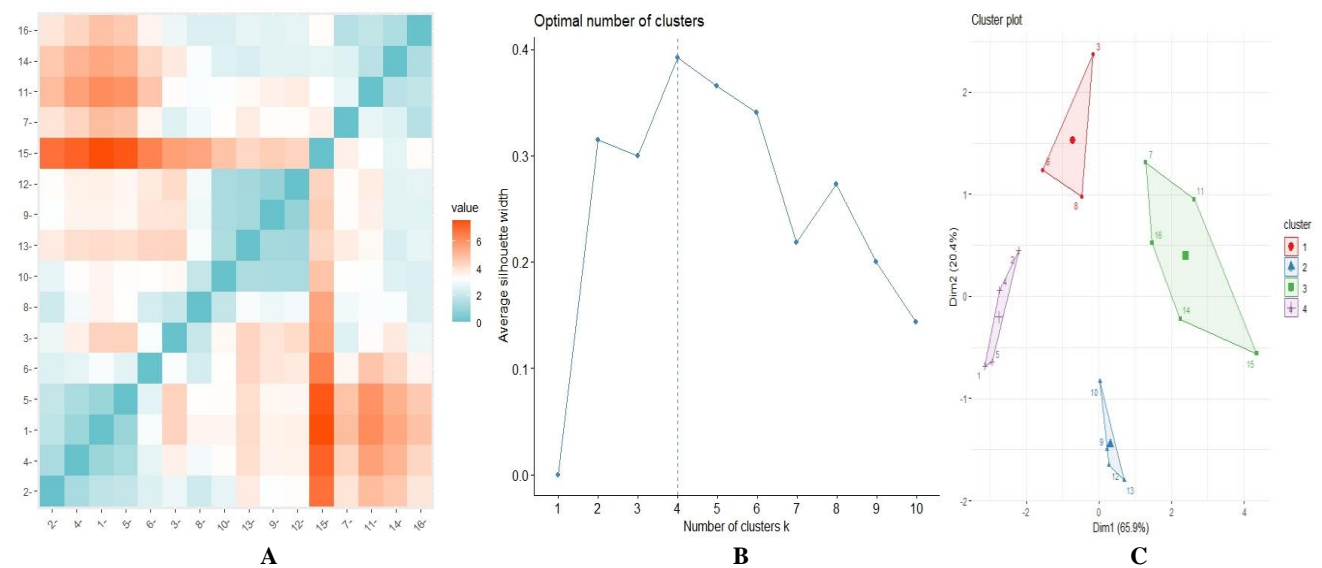


Figure 5. Clustering of 16 genotypes conditioned in non-stress (C_1) and drought stress (C_2) based on agronomical data. (C_1 = 1-8, C_2 = 9-16. (1/9 = Pulau Batu, 2/10 = Cantik Manis, 3/11 = Bungo Sungkai, 4/12 = Kuriak, 5/13 = Mundam Putih, 6/14 = Inpari 48 Blas, 7/15 = IR-64-Sub1, and 8/16 = Inpago 9)

Drought stress resulted in a general reduction across all agronomic traits, with particularly notable declines in Panicle Length (PL) and Productive Tiller Number (PTN). Most genotypes exhibited an increase Number of Unfilled Grains (NUFG) under drought, indicating impaired-grain filling and potential spikelet sterility. Among the genotypes, Kuriak demonstrated a shortened time to flowering and maturity, suggesting a drought escape mechanism and superior reproductive resilience. Pulau Batu and Kuriak also exhibited stable agronomic performance under drought stress, supported by the presence of drought-related QTLs-*qDTY_{2.2}*, *qDTY_{12.1A}*, and *qDTY_{12.1B}*. Cantik Manis showed promising performance despite the absence of detected *qDTY* loci, highlighting the potential for phenotypic selection. However, this study is limited by low marker density and the absence of physiological or gene expression data. Future research should use SNP markers, include drought-related traits like root architecture, and validate QTLs via gene expression.

The Principal Component Analysis (PCA) has unveiled a significant insight. Genotypes CM, BS, K, IB, and Ip9, with similar expression patterns for PTN, NFG, and PL traits, have clustered together. This suggests a common response to drought stress. Moreover, the significant correlations among all seven agronomic traits under drought conditions underscore the potential for future research. These findings are not just informative, but they are exciting. They pave the way for selecting promising drought-resilient genotypes and support the integration of phenotypic and molecular screening in rice breeding programs, particularly for marker-assisted selection in rice improvement. They open up new avenues for research in the field of rice genomics and breeding.

In the breeding method, researchers are advised to correlate drought resistance QTLs with the actual growth of rice plants to enhance rice yield in arid regions. Numerous genes regulate rice's drought tolerance, a feature that is highly responsive to environmental fluctuations. In this study, numerous significant-effect QTLs across various genetic backgrounds have been identified and validated, including *qDTY_{1.1}*, *qDTY_{2.2}*, *qDTY_{3.1}*, and *qDTY_{12.1}*. These QTLs were recognized for enhancing yield in plants experiencing drought stress during the reproductive phase, the critical period for grain production. These QTLs were associated with plant performance, encompassing enhanced water retention during drought, less leaf curling, deeper root systems, an elevated harvest index, and increased grain yield. For example, the incorporation of *qDTY_{3.1}* and *qDTY_{2.2}* into established rice varieties such as IR64 or Swarna has resulted in plants capable of enduring drought while still producing adequate yields. Their ecological stability renders them highly beneficial for breeding initiatives.

By employing molecular markers associated with drought tolerance QTLs, breeders can select drought-resistant rice plants at the early seedling or growth stage, rather than postponing assessments until field trials during drought conditions. This speeds up the selection process and helps identify the best candidates more quickly and accelerates the breeding process. Research has demonstrated

that combining multiple QTLs into a singular robust genetic line can enhance plants' drought tolerance. This methodology has resulted in the development of resilient cultivars capable of thriving in arid conditions. The robust framework for MAS facilitates the development of high-yielding rice varieties that are resilient to drought conditions. This expertise is essential for addressing climate change and ensuring food security in arid regions.

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Table S1. Amplicon size by genotypes using QTL specific markers for polymorphism study of several rice genotypes

QTL	Marker	Genotype	Amplicon Size (bp)	
<i>qDTY2.2</i>	RM236	PB	193.75	
		CM	197.24	
		BS	197.24	
		K	200.79	
		MP	183.64	
		IB	197.24	
		IR	197.24	
		Ip9	200.79	
		PB	186.29	
	RM276	CM	193.76	
		BS	179.10	
		K	150.03	
		MP	186.29	
		IB	182.66	
		IR	193.76	
		Ip9	186.29	
		PB	257.85	
		CM	257.85	
	<i>qDTY3.1</i>	RM520	BS	276.95
			K	267.23
			MP	281.23
IB			281.23	
IR			281.23	
Ip9			287.02	
PB			128.90	
CM			131.69	
BS			123.49	
<i>qDTY12.1A</i>	RM511	K	115.79	
		MP	113.33	
		IB	113.33	
		IR	120.86	
		Ip9	123.49	
		PB	121.81	
		CM	121.81	
		BS	119.24	
		K	124.44	
	RM28099	MP	127.12	
		IB	114.25	
		IR	119.24	
		Ip9	111.83	
		PB	171.42	
		CM	171.42	
		BS	167.79	
		K	175.11	
		MP	167.79	
	<i>qDTY12.1B</i>	RM28130	IB	157.38
			IR	157.38
			Ip9	175.11
PB			180.39	
CM			240.07	
BS			180.39	
K			257.85	
MP			180.39	
IB			257.85	
RM28166	IR	257.85		
	Ip9	183.64		
	PB	163.36		
	CM	166.70		
	BS	166.70		
	K	184.49		
	MP	177.16		
	IB	173.60		
	IR	173.60		
<i>qDTY12.1B</i>	RM1261	Ip9	163.36	

Table S2. Agronomic traits data

Code	PH	PTN	PL	NFG	NUFG	DTF	DTM
PB1	98.5	3	19.7	79	15	93	119
PB2	97.8	3	22.3	88	51	95	120
PB3	95.5	8	21.3	76	28	92	115
PB4	108.6	20	25.7	196	19	105	128
PB5	114.8	15	26.2	188	24	106	130
PB6	113.5	25	25.5	175	12	108	131
CM1	93.8	7	24.2	80	39	86	115
CM2	95.5	2	23.7	91	21	86	110
CM3	97	4	26.2	94	33	84	118
CM4	96.8	14	25.8	194	12	88	118
CM5	101.2	17	26.8	198	9	93	121
CM6	98.6	16	26.4	185	14	89	120
BS1	75.2	5	22.5	8	25	60	88
BS2	69.7	4	21.9	22	38	58	89
BS3	71.4	3	22.7	22	27	60	89
BS4	78.3	10	24.4	155	8	68	96
BS5	75.4	21	23.8	147	11	68	97
BS6	73.8	20	25.1	160	4	66	95
K1	98.5	4	19.8	74	47	92	121
K2	98.4	6	21.4	69	46	90	122
K3	96.5	4	24.8	146	32	89	118
K4	104.6	24	26.4	187	24	95	130
K5	107.3	22	25.6	176	15	94	127
K6	103.8	21	25.9	189	16	94	127
MP1	93.4	3	23.5	28	50	95	120
MP2	89.9	3	22.8	29	53	92	118
MP3	90.8	4	22.7	30	14	92	118
MP4	125.3	18	25.6	194	23	98	124
MP5	117.7	20	25.9	188	20	100	128
MP6	123.6	16	26.3	186	25	99	124
IB1	85.1	7	23.4	41	47	78	93
IB2	81.8	4	18.6	21	38	75	90
IB3	80.2	2	21.7	38	42	75	89
IB4	112.5	18	27.4	201	35	83	95
IB5	109.4	20	26.8	183	24	80	92
IB6	114.5	22	26.2	181	27	80	92
IR1	64.2	4	15.4	15	59	64	90
IR2	68.1	3	16.4	18	64	64	91
IR3	69.3	3	16.8	12	69	64	91
IR4	80.8	13	19.7	124	44	66	93
IR5	78.4	16	18.5	154	23	68	93
IR6	78.7	18	18.8	148	37	65	93
Ip91	84.5	12	22.1	58	27	70	99
Ip92	86.6	10	20.5	55	43	68	95
Ip93	90.6	14	20.7	42	31	69	95
Ip94	96.5	11	27.5	107	32	74	103
Ip95	96.8	12	25.3	118	5	73	102
Ip96	93.6	10	26.1	123	14	73	102

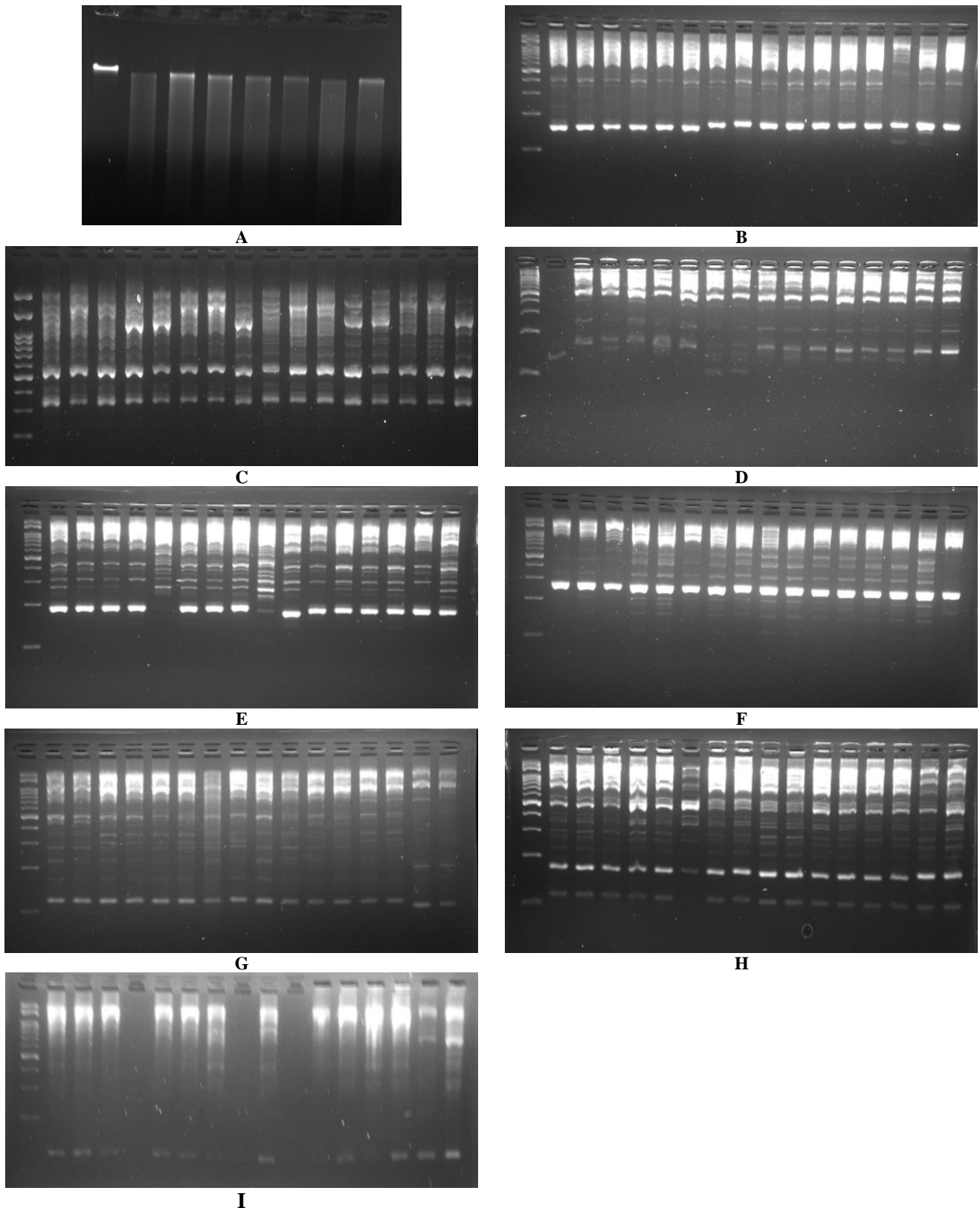


Figure S1. Gel Doc Electrophoresis Images. A. DNA Isolation (Sample order: PB, CM, BS, K, MP, IB, IR, Ip9), B. Marker RM1261 (Sample order: PB, CM, BS, K, MP, IB, IR, Ip9, two repetitions of each sample), C. Marker RM28166 (Sample order: PB, CM, BS, K, MP, IB, IR, Ip9, two repetitions of each sample), D. Marker RM276 (Sample order: PB, CM, BS, K, MP, IB, IR, Ip9, two repetitions of each sample), E. Marker RM236 (Sample order: PB, CM, BS, K, MP, IB, IR, Ip9, two repetitions of each sample), F. Marker RM520 (Sample order: PB, CM, BS, K, MP, IB, IR, Ip9, two repetitions of each sample), G. Marker RM28099 (Sample order: PB, CM, BS, K, MP, IB, IR, Ip9, two repetitions of each sample), H. Marker RM28130 (Sample order: PB, CM, BS, K, MP, IB, IR, Ip9, two repetitions of each sample), I. Marker RM511 (Sample order: PB, CM, BS, K, MP, IB, IR, Ip9, two repetitions of each sample)