

Estimation Model of *OSMYB2-19* Gene Expression Regulation with Correlation of Rice Growth Under Drought Conditions in Sandy Soil



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ABSTRACT

Drought is one of the major abiotic factors limiting crop productivity, particularly in rice. Sandy soils have low water-holding capacity, making them vulnerable to drought stress, which negatively affects plant growth and yield. One of the plant adaptation strategies to drought involves genetic regulation, including genes from the MYB family. This study aimed to analyze the expression regulation of the *OsMYB2-19* gene and its correlation with rice plant growth under drought stress conditions in sandy soil. Sandy soil's limited water retention impacts plant development, including that of rice. The *OsMYB2-19* gene, part of the MYB family, is known to play a role in drought tolerance; however, its expression regulation mechanisms remain largely unexplored. The experiment was conducted in a greenhouse using two rice varieties: IR64 (drought-sensitive) and Hawara Bunar (drought-tolerant), under both normal and drought conditions for two weeks. Plant growth was assessed through morphological parameters (plant height, tiller number, leaf number, leaf area), and gene expression was measured using RT-qPCR. The analysis showed that *OsMYB2-19* expression significantly influenced plant height ($R^2 = 0.251$) and leaf area ($R^2 = 0.190$), but not leaf number or tiller number. Positive correlations were found between gene expression and plant height ($r = 0.501$) as well as leaf area ($r = 0.436$). K-means clustering analysis revealed that plants with higher gene expression exhibited better morphological traits. These findings suggest that *OsMYB2-19* may serve as a key candidate gene for improving rice drought tolerance by enhancing critical morphological characteristics.

ABSTRAK

Kekeringan merupakan salah satu faktor abiotik utama yang membatasi produktivitas tanaman, terutama padi. Tanah berpasir memiliki kapasitas menahan air yang rendah, sehingga rentan terhadap stres kekeringan, yang berdampak negatif pada pertumbuhan dan hasil tanaman. Salah satu strategi adaptasi tanaman terhadap kekeringan melibatkan regulasi genetik, termasuk gen dari famili MYB. Penelitian ini bertujuan untuk menganalisis regulasi ekspresi gen *OsMYB2-19* dan korelasinya dengan pertumbuhan tanaman padi dalam kondisi stres kekeringan di tanah berpasir. Retensi air yang terbatas pada tanah berpasir berdampak pada perkembangan tanaman, termasuk padi. Gen *OsMYB2-19*, bagian dari famili MYB, diketahui berperan dalam toleransi kekeringan; namun, mekanisme regulasi ekspresinya sebagian besar masih belum dieksplorasi. Percobaan dilakukan di rumah kaca menggunakan dua varietas padi: IR64 (sensitif kekeringan) dan Hawara Bunar (toleran kekeringan), dalam kondisi normal dan kekeringan selama dua minggu. Pertumbuhan tanaman dinilai melalui parameter morfologi (tinggi tanaman, jumlah anakan, jumlah daun, luas daun), dan ekspresi gen diukur menggunakan RT-qPCR. Analisis menunjukkan bahwa ekspresi *OsMYB2-19* secara signifikan memengaruhi tinggi tanaman ($R^2 = 0,251$) dan luas daun ($R^2 = 0,190$), tetapi tidak memengaruhi jumlah daun maupun jumlah anakan. Korelasi positif ditemukan antara ekspresi gen dan tinggi tanaman ($r = 0,501$) serta luas daun ($r = 0,436$). Analisis pengelompokan K-means menunjukkan bahwa tanaman dengan ekspresi gen yang lebih tinggi menunjukkan sifat morfologi yang lebih baik. Temuan ini menunjukkan bahwa *OsMYB2-19* dapat menjadi kandidat gen kunci untuk meningkatkan toleransi kekeringan tanaman padi dengan meningkatkan karakteristik morfologi kritis.

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KEYWORDS

Drought, Growth, Rice, OsMYB2-19

INTRODUCTION

Sandy drylands are dry lands with a higher sand content than clay. These drylands are often found in dry fields. Based on the physical properties of the soil, sandy drylands have the potential to reduce the growth and development of cultivated plants if soil improvement efforts are not implemented. Not all plants can be cultivated on sandy soils due to the numerous constraints encountered in sandy drylands as cultivation areas. In this type of soil, the permanent wilting point is often reached even though the soil water content is still close to or slightly below field capacity, usually at around 10-12% water content depending on the texture. This causes plants to quickly experience water stress, even after rainfall or light irrigation.

Rice, a major food crop in many tropical and subtropical countries, is highly sensitive to water shortages. Water shortages trigger biological stress that can disrupt physiological processes and functional activities in the organism. The rice plant's first response to water shortage is to close its stomata. Reduced leaf bulk flow and increased free abscisic acid also cause stomatal constriction. Stomata closure and narrowing can inhibit photosynthesis, disrupting the flow of carbon dioxide to the leaves, which in turn disrupts starch mobilization (Mudor *et al.*, 2022).

Drought stress is an environmental factor that significantly affects the growth and development of rice plants. The plant's morphological response to this condition can be observed through several parameters, one of which is stomatal density. A high stomatal density indicates that the plant is still in normal or unstressed condition, while a low stomatal density indicates that the plant is experiencing significant drought stress. This reduction in stomata number is a form of plant adaptation to reduce water loss through evaporation. Furthermore, another morphological symptom that appears due to drought is leaf rolling, which aims to reduce the surface area exposed to sunlight, thereby reducing the transpiration rate (Mudor *et al.*, 2025). Drought itself is defined as a condition of prolonged water shortage caused by persistently low rainfall or its absence for a long period (Rammadhan, 2021). In the long term, this condition can result in reduced plant height, reduced tiller number, and reduced grain yield (Oktaviani *et al.*, 2021). To survive, plants also exhibit physiological responses such as the accumulation of osmoprotectant compounds, including total sugars and proline. These compounds function to maintain cell turgor pressure, maintain enzyme stability, and help cells adjust osmotic pressure to maintain optimal function even in water-limited conditions (Naazirah in Mudhor *et al.*, 2025).

Plant tolerance to drought stress is influenced by genetic factors, one of which is through the expression of genes from the MYB family, particularly the R2R3-MYB subfamily. These genes function as transcription factors that regulate the activation of various target genes involved in stress responses, including drought stress. One example that has been studied is the *BpMYB123* gene from *Betula platyphylla*. Overexpression of this gene has been shown to increase the activity of antioxidant enzymes such as peroxidase (POD) and superoxide dismutase (SOD), and reduce levels of free radicals such as H_2O_2 and O_2^- . Furthermore, this gene also suppresses cell damage by reducing malondialdehyde (MDA) levels and electrolyte leakage (EL) levels. *BpMYB123* works by binding to the MYB1AT element in the *BpLEA14* gene promoter, thereby inducing the expression of the LEA (Late Embryogenesis Abundant) gene, which encodes a protein that protects cells from drought stress damage (Liu *et al.*, 2021). Furthermore, MYB also regulates hormonal signaling pathways such as abscisic acid (ABA), which is crucial in drought adaptation mechanisms, as well as the biosynthesis of secondary metabolites such as epicuticular wax and proline, which play a role in maintaining osmotic balance and reducing oxidative stress. Overall, MYB is a key regulator linking drought signals with plant physiological and molecular responses, thereby enhancing plant adaptation and survival in dry environmental conditions (Yan *et al.*, 2025).

One gene involved in drought tolerance is *OsMYB2-19*. This gene belongs to the MYB-related transcription factor family, known to play a role in regulating the expression of genes related to responses to abiotic stresses such as drought, salinity, and extreme temperatures. According to information from the RAP-DB rice genome database, *OsMYB2-19* is located on chromosome 1 on the positive strand. This gene shows similarity to the MCB2 protein and is classified as MYBR17 by RAP-DB. Although it has been structurally annotated and is known to belong to the transcriptional regulator group, the exact regulation pattern of *OsMYB2-19* gene expression remains unknown, specifically whether it is upregulated or downregulated under drought stress. This makes *OsMYB2-19* an interesting candidate for further study in gene expression studies specific to drought stress. The aim of this study was to analyze the estimated regulation of *OsMYB2-19* gene expression and its correlation with rice growth under drought conditions.

METHODS

The experiment used a completely randomized design. The experiment was conducted in a greenhouse, with a two-week drought treatment (unwatered) and a watered control. The experiment used Hawara Bunar rice plants, known to be drought-tolerant, and IR64, a sensitive variety. Twenty experimental units were conducted, with five replications for

each treatment-variety combination. Plants were grown to uniform height after two weeks. One plant was then selected and grown optimally for four weeks. Afterward, the drought treatment was administered for two weeks.

Plant growth under drought stress was observed based on morphology, including plant height, number of tillers, number of leaves, and leaf area during the vegetative phase. Gene expression was assessed through RNA isolation, cDNA synthesis, and RT-qPCR analysis. Prior to the procedure, primer design for the OsMYB2-19 gene was performed using sequences from RAP-DB (<https://rapdb.dna.affrc.go.jp>). RNA isolation procedures were carried out following the TRIzol method. Then, cDNA synthesis followed the RevertAid First Strand cDNA Synthesis Kit procedure. The cDNA synthesis results were measured with a nanodrop spectrophotometer to see its purity and concentration at wavelengths of 260 and 280 nm as a template for RT-qPCR analysis. After the cDNA was obtained, RT-PCR analysis was run with QuantStudio 5 and the SYBR Lo-Rox Kit using cDNA as the template. Gene expression was carried out with three biological replicates and two technical replicates. Growth data were collected every five days per observation unit during the 2-week drought treatment and Ct data were obtained from RT-qPCR. Ct data were calculated using the $2^{-\Delta\Delta C_t}$ method to determine the level of gene expression. Growth data were analyzed statistically using two-way ANOVA to see the interaction between treatment and cultivar, DMRT to see growth under drought, regression to obtain an estimation model from the obtained equation, Pearson correlation to see the relationship between gene expression and growth under drought stress, and k-mean clustering to see the role or contribution of genes to growth under drought stress.

RESULTS AND DISCUSSIONS

Based on the results of the two-way ANOVA analysis, it was found that the plant height (PH) variable showed a significant effect of variety ($p = 0.000$), while treatment ($p = 0.059$) and the interaction between the two ($p = 0.282$) were not significant. For the number of tillers (JA), the variety factor had a significant effect ($p = 0.031$), but neither treatment ($p = 0.119$) nor the interaction ($p = 0.119$) were significant. For the number of leaves (LN), neither the variety, treatment, nor the interaction factor showed a significant effect ($p > 0.05$). Meanwhile, for leaf area (LA), the variety factor had a significant effect ($p = 0.009$), while treatment ($p = 0.825$) and the interaction ($p = 0.410$) were not significant (Table 1). In general, these results indicate that rice variety has a greater effect on plant morphological responses than drought treatment, at least under these experimental conditions. The absence of a significant interaction also indicates that the effects of variety and treatment occur independently of the observed morphological characters.

Table 1. ANOVA Two Way of variety, treatment, and variety x treatment to variables of morphology

Variables of Morphology	Variety		Treatment		Variety x Treatment	
	F	p-value	F	p-value	F	p-value
Plant Height	17.3	0	3.71	0.059	1.18	0.282
Tiller Number	4.9	0.031	2.5	0.119	2.5	0.119
Leaf Number	0.85	0.36	0.85	0.36	1.14	0.291
Leaf Area	7.4	0.009	0.05	0.825	0.69	0.41

Rice growth in sandy soil under drought stress conditions resulted in a decrease in yield in sensitive rice varieties compared to tolerant varieties. Drought-tolerant varieties showed adaptive abilities in morphological characteristics that were almost the same as the control treatment, while drought-sensitive varieties struggled to grow due to disturbances in their growth.

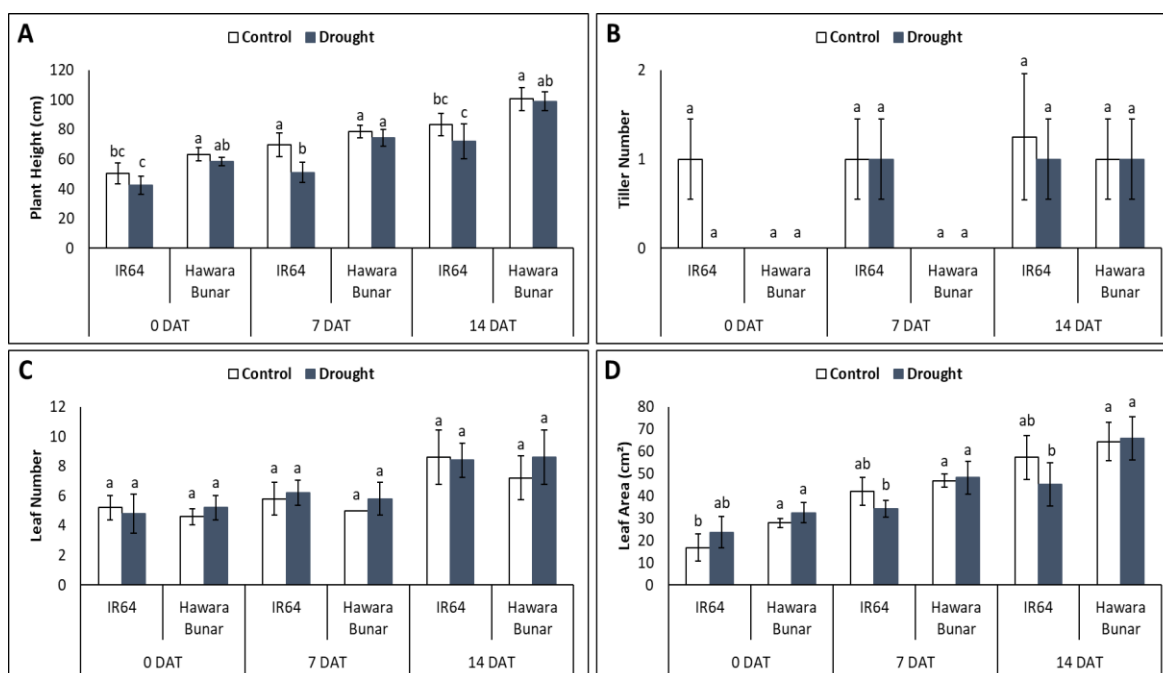


Figure 1. Rice morphological traits under drought stress

Plant height growth during drought stress conditions showed a significant decrease in IR64 compared to Hawara Bunar. The height growth of Hawara Bunar under drought stress was almost the same as the control. Drought conditions on plant height growth showed only IR64 had a significant effect, while Hawara Bunar did not (Figure 1a). The number of tillers showed no significant effect during drought in both varieties. Similarly, the number of leaves did not have a significant effect. The number of tillers in IR64 rice under drought stress showed lower growth compared to Hawara Bunar (Figure 1b). The number of leaves showed that Hawara Bunar under drought stress was still able to grow leaves than IR64 (1c). This is suspected because Hawara Bunar conserves photosynthates for leaf growth and the role of the *OsMYB2-19* gene which may contribute to leaf growth because the correlation is 0.59 (Figure 5). Leaf area showed a decrease in growth under drought stress conditions for IR64 compared to Hawara Bunar (Figure 1d). It is possible that leaf area growth is involved with the *OsMYB2-19* gene, as indicated by a correlation of 0.49 (Figure 5).

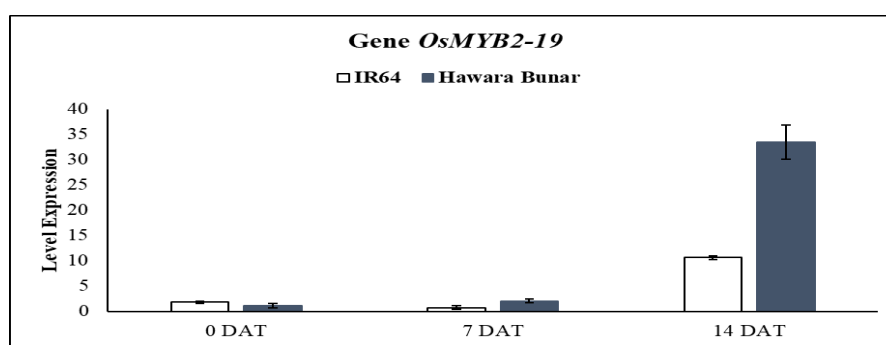


Figure 2. Level expression *OsMYB2-19* gene under drought stress

Gene expression levels were higher in Hawara Bunar than IR64 (Figure 2). At 0 DAT, the initial drought stress condition showed higher gene expression in IR64 compared to Hawara Bunar. After a week of drought stress, gene expression levels began to increase, indicating that genes began to respond to the drought environment. Gene expression levels spiked after two weeks under drought stress. This very high level of gene expression has the potential for further study, such as its role in physiology, biochemistry, metabolites, and others, so that plant traits and trait ontology can be identified and then used in further research.

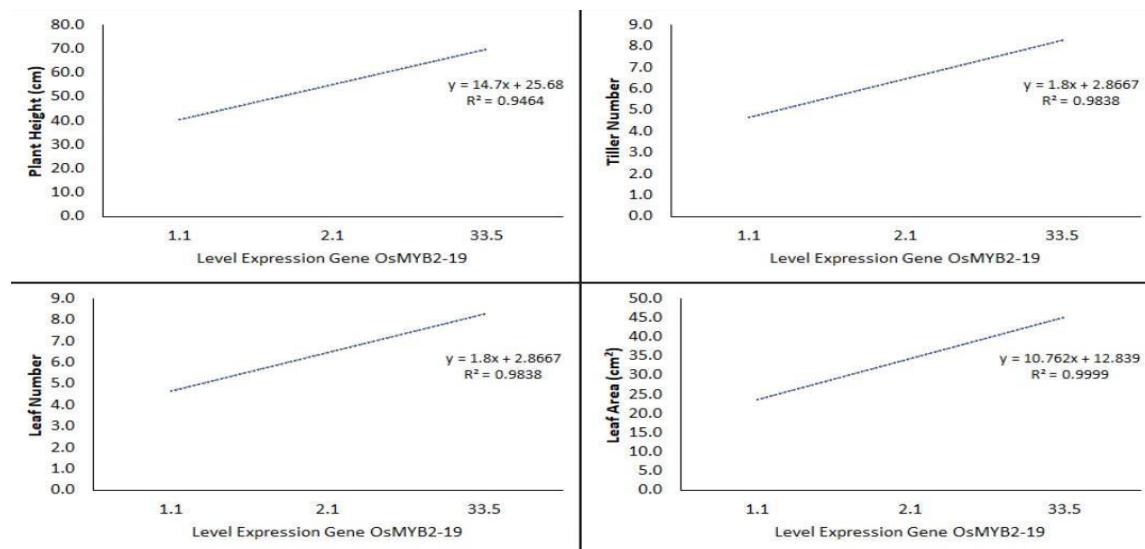


Figure 3. Regression of *OsMYB2-19* gene expression on IR64 rice growth parameters

Based on the results of linear regression analysis on IR64 rice growth, it is known that exogenous variables have different effects on each observed plant growth parameter (Figure 3). For the plant height variable (PH), the analysis results show that exogenous variables have a significant effect, with an R^2 value of 0.9464. This indicates that approximately 94.6% of the variation in plant height can be explained by exogenous variables. The resulting regression model is: $PH = 14.7x + 25.68$. This model shows that each increase in exogenous units will be followed by an increase in plant height of 14.7 units. Conversely, in the number of tillers (TN) variable, a significant effect of exogenous variables was found, with an R^2 value of 0.9838. The regression equation obtained is $TN = 1.8x + 2.8667$. This indicates that the increase in *OsMYB2-19* gene expression is closely related to the increase in the number of tillers. Similarly, the number of leaves (LN) also did not show a significant influence from exogenous, with a regression model of $LN = 1.8x + 2.8667$. The R^2 value was 0.9838. This indicates that approximately 98% of the variation in the number of leaves can be explained by the increase in the expression of the *OsMYB2-19* gene, so this gene plays a major role in driving the development of the number of leaves in rice plants. Different from the two previous parameters, in the leaf area variable (LA), exogenous has a significant influence, with an R^2 value of 0.9999. The regression model obtained was $LA = 10.762x + 12.839$. This model shows that the increase in exogenous is very strongly related to the increase in leaf area. In general, these results indicate that exogenous *OsMYB2-19* plays an important role in increasing plant morphological growth, especially height, number of tillers, number of leaves, and leaf area.

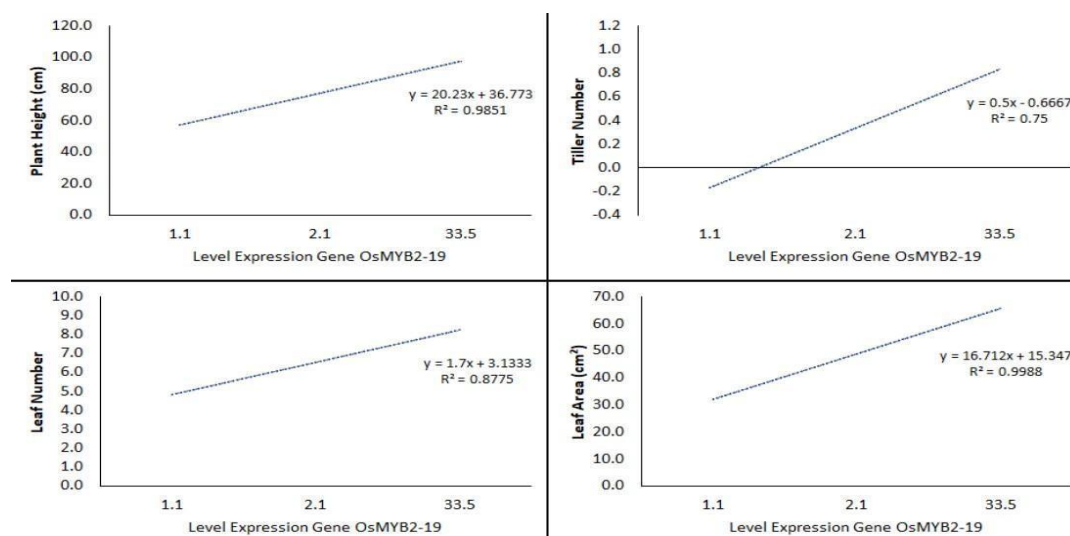


Figure 4. Regression of *OsMYB2-19* gene expression on Hawara Bunar rice growth parameters

Based on the results of the linear regression analysis on the growth of Hawara Bunar rice, it is known that exogenous variables have different effects on each plant growth parameter (Figure 4). For the plant height (PH) variable, the analysis results show that exogenous variables have a significant effect, with an R^2 value of 0.9851. This indicates that approximately 98.5% of the variation in plant height can be explained by exogenous variables. The resulting regression model is $PH = 20.23x + 36.773$, which shows that each increase in exogenous units will be followed by an increase in plant height of 20.23 units. For the number of tillers (TN) variable, the regression equation is $TN = 0.5x - 0.6667$ with $R^2 = 0.75$. This value is lower than other parameters, which indicates that exogenous variables are only able to explain approximately 75% of the variation in the number of tillers. This indicates that other factors outside of exogenous variables also play a role in influencing tiller formation. For the number of leaves (LN), the equation obtained is $LN = 1.7x + 3.1333$ with $R^2 = 0.8775$. These results indicate that increasing exogenous *OsMYB2-19* expression is quite closely related to increasing the number of leaves, although the effect is not as strong as the effect on plant height or leaf area. Meanwhile, on leaf area (LA), exogenous has the strongest effect with an R^2 value of 0.9988. The regression model obtained is $LA = 16.712x + 15.347$ which shows a nearly perfect relationship between exogenous increase and increase in leaf area. In general, these results indicate that exogenous *OsMYB2-19* plays an important role in increasing the morphological growth of rice plants, especially on plant height and leaf area, while its effect on the number of tillers and the number of leaves is relatively smaller.

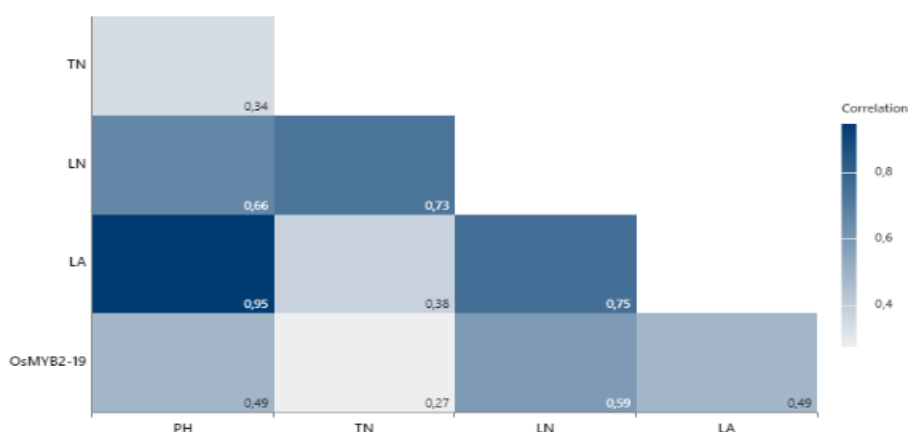


Figure 5. Correlation of *OsMYB2-19* gene expression with rice morphological growth

Based on Pearson correlation analysis, it was found that *OsMYB2-19* gene expression was significantly positively related to plant height ($r = 0.501$; $p < 0.01$) and leaf area ($r = 0.436$; $p < 0.01$). This indicates that increased *OsMYB2-19* gene expression correlates with increased plant growth in terms of height and leaf area. However, no significant relationship was found between gene expression and the number of leaves and tillers ($p > 0.05$) (Figure 5).

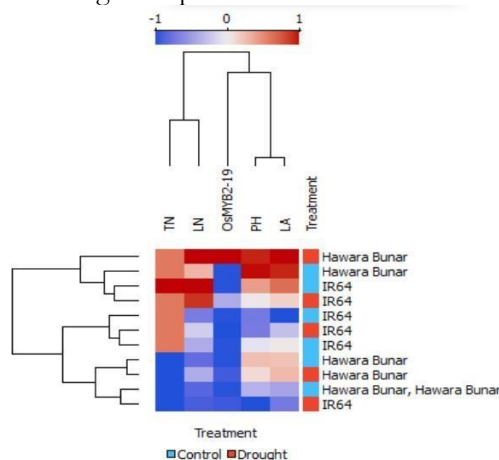


Figure 6. K-means clustering on *OsMYB2-19* gene expression with rice morphological growth

Based on k-means clustering, it is known that exogenous factors have a strong relationship with plant height and leaf area (Figure 6). Plants in Cluster 1 (with higher exogenous factors) tend to have greater height and leaf area. Conversely, the number of tillers and the number of leaves are not the main differentiators between clusters, as their values are relatively uniform. In general, the formation of two clusters in this study illustrates a clear separation between the groups of plants with and without exogenous treatment, particularly in terms of key morphological growth performance.

CONCLUSION

The *OsMYB2-19* gene has strong potential as a molecular marker for selecting drought-tolerant rice cultivars. Cultivars with higher gene expression tend to maintain better growth under drought conditions, and molecular approaches can support future rice breeding programs in the face of climate change. Conversely, cultivars with lower gene expression tend to show reduced morphology, making them more susceptible to drought. This suggests that *OsMYB2-19* gene expression can be used as an important indicator in the selection of drought-tolerant rice varieties and provide a basis for the development of biotechnology-based breeding strategies to improve plant resilience to environmental stresses.

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