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Relationship between morphological traits and expression of HKT1;5 gene in genetically modified rice genotypes under salinity stress

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Abstract

Salinity is one of the most important post-drought abiotic stresses that limits rice cultivation and productivity in all regions of the world. The level of salinity tolerance depends largely on the genetic makeup of the cultivars. This study aimed to evaluate the response of four genetically modified rice genotypes to different salinity levels by examining some morphological traits and analyzing the gene expression of the HKT1;5 gene, which is associated with salt tolerance mechanisms. The results indicated a clear variability among genotypes in their responses to salt stress. Plant height and leaf area decreased significantly at high stress (S2), with genotype V2 recording the highest plant height (97.95 cm), while cultivar V1 achieved the largest leaf area (74.84 cm^2). Different plant varieties reacted differently to salt. When salt was high, plants became shorter and had smaller leaves. Genotype V2 was the tallest (97.95 cm), and cultivar V1 had the largest leaves (74.84 cm^2). V3 recorded the highest dry weight (115.23 g). Its weight increased slightly with the addition of a small amount of salt, then decreased with increasing salt. The 1000-grain weight remained roughly constant, but the total grain and plant yield decreased with increasing salt.

The genotype V1 recorded the highest values (43.71 g/plant for grain and 136.92 g for biological yield in S1). In contrast, V4 performed poorly in all traits. Gene expression analysis revealed clear genetic variation in the induction of the HKT1;5 gene under salt stress. The expression level remained close to the control level (≈1) in S0, while it increased significantly in V1, V2, and V3, reaching (3.25-, 6.75-, and 8.16-fold, respectively) in S2. Genotype V4 also showed a limited response (1.73-fold), reflecting its poor adaptive capacity.

Therefore, genotypes V3 and V1 appear to handle salt very efficiently by controlling sodium uptake. On the other hand, the genotype V4 seems to have the most sensitive to salt. Because V3 is the strongest, it could really help with breeding rice that can stand up to salty conditions.

Keywords: HKT1;5; Rice; Genotype; Salinity Stress; Gene expression.

1. Introduction

Rice is somewhat vulnerable to salinity stress, although it is most sensitive when it is a seedling or when it is reproducing (Ali *et al*., 2013).

The productivity of rice grains is adversely impacted by soil salinity levels above a specific threshold, (3.0 dS/m), which decreases their yield along with the seed set rate, 1000 seed weight, number of effective panicles, panicle length, and grains per panicle (Hussain *et al*., 2019).

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The results obtained by Al-Ghizii *et al*. (2025) showed that both rice cultivars were negatively affected by salt stress, and treatment (S0) achieved the highest averages in plant height, leaf area, spike length, and dry weight, which were 93.72 cm, 79.00 cm, 21.40 cm, and 121.00 g plant^{-1}, respectively. Additionally, the treatment (S0) gave the maximum averages of effective tillers, grain yield, 1000-grain weight, and harvest index which were 41.00, 23.79 g, 67.04 g plant-1, and 29.15 g plant^{-1} respectively. Rice productivity is limited by salt stress, which increases the necessity of developing rice varieties that can sustain proper ion homeostasis, especially the K^+/Na^+ ratio (Kumar *et al*., 2022).

Of the many OsHKT1;5 specialized transporter genes, the OsHKT1;5 is notable for its ability to modulate Na+ flux in roots of plants, aiding in the redistribution of ions and mitigating the build-up of sodium in sensitive tissues (Ren *et al*., 2005; Kobayashi *et al*., 2017; Alnayef *et al*., 2020).

Research at the molecular level demonstrates that the specific pattern of HKT1;5 expression determines salinity tolerance because different gene sequences among cultivars lead to varying abilities to transport and redistribute sodium (Platten et al., 2006; Kobayashi et al., 2017). Genetic analysis demonstrates that single-nucleotide polymorphisms (SNPs) within the HKT1;5 gene enable specific cultivars to sustain their K+/Na+ ratios effectively which enhances their growth under salt stress conditions (Cotsaftis *et al*., 2012; Kumar *et al*., 2022).

New studies also show that HKT1;5 works with other things like OsWRKY53 and OsMKK10.2. OsWRKY53 stops genes from working, but OsMKK10.2 helps them work, so they balance each other out when there's salt around (Alnayef *et al*., 2020).

In this study, we aimed to evaluate the relationship between morphological traits, yield, and expression of HKT1;5 gene in genetically modified rice genotypes under salinity stress.

2. Materials and methods

2.1. Morphological Analysis

A field experiment was conducted on a farm in Najaf Governorate (Iraq) during the summer season of 2024. Three salinity levels were used in irrigation water (0, 7.5, and 15 dS/m), denoted by the symbol S0, S1 and S2 respectively.. The saline water treatment began irrigating the plants 45 days after germination. Seeds of the four genotypes (FR1, FR3, FR4 and FR6) denoted by the symbol V1, V2, V3 and V4 which were sown on June 15 at a rate of 116 kg/ha, according to a randomized complete block design with three replicates. The experimental unit size was 1.5 m × 1.5 m, and the distance between units was 2 m. Soil and crop maintenance, including fertilization, were carried out according to recommendations (White and Brown, 2010). At the maturity stage, the plants were harvested on December 21, 2024. Growth and yield characteristics and their components (plant height (cm), leaf area (cm^2), spike length (cm), dry weight, 1000-grain weight (g/plant), grain yield (kg/plant), and biological yield) were calculated. The data were statistically analyzed using Genstat Discovery 4, and the means were compared using the least significant difference (LSD) test with a significance level set at 5% (Alrrawi and Khalaf Allah, 1980).

2.2. Gene expression analysis

The gene expression of HKT1;5 was estimated by Real Time PCR technique in terms of the quantity and concentration of messenger RNA (mRNA), and then cDNA was synthesized by Reverse Transcription technique. The process of extracting pure DNA from the roots of rice plants of the genotypes treated with water stress was carried out according to the modified mechanical (CTAB) method (Murray and Thompson, 1980).

Using the Primer 3 Plus primer design software, the appropriate primers for the gene (HKT1;5) were designed by the BLAST database of the NCBI website https://blast.ncbi.nlm.nih.gov/Blast.cgi, which were later prepared by the South Korean company Bioneer, and the details are shown in (Table 1).

Table 1 Primer name and its nucleotide sequence

	Primer name	**Sequence (5' – 3')**	**Production size qRT–PCR (bp)**	**TM**
HKT1;5	Forward	GCCCTTGGTGCAATAGCTTTC	1639	60 °C
	Reverse	AAAATATGTCCCAGGCCAGAGTA		

2.3. qRT-PCR Technique

qRT-PCR analysis was performed according to the steps described by (Nailis *et al*., 2010). Total RNA was extracted from roots collected in the field using the Trizol kit provided by the Korean company Bioneer, according to the manufacturer's attached protocol. Complementary DNA synthesis was then performed from the extracted RNA samples to amplify the target gene using RT-PCR using the Accupower Rockscript RT Premix kit provided by the Korean company Bioneer. The kit used the Gama kit for the synthesis process.

cDNA samples were analyzed using quantitative real-time PCR (QRT-PCR) using the Accupower 2x Green Star qPCR Kit, supplied by the South Korean company Bioneer.

Table 2 The optimal thermal conditions for the qRT-PCR reaction

qRT- PCR Step	Temperature	Time	Repeat cycle
Initial Denaturation	95 °C	1 Min	1
Denaturation	95 °C	20 Sec	45
Annealing Extension	58 °C	30 Sec	
Melting curve	°C 95-60	15 Sec	1

2.4. Data Analysis

The Livak & Schmittgen (2001) method was used to analyze the data resulting from the RT-qPCR reaction to calculate the relative gene expression of the HKT1;5 gene. The mean CT was calculated (Cypress at 18°C, Cypress at 30°C, LaGrue at 18°C, and LaGrue at 30°C). Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method

with the control treatment (S0) as the baseline for each genotype. A standard curve was drawn to calculate the amount of gene expression from the cDNA synthesis product of one of the studied rice plant models.

3. Results and discussion

3.1. Effect of salt stress on vegetative growth traits

The results of the vegetative growth trait analysis (table 3) showed a different response among the four rice genotypes to salt stress. For plant height, genotype V2 outperformed the other traits, recording a plant height of 97.95 cm, while V4 recorded the lowest average of 60.64 cm. A decrease in height was observed with increasing salinity concentration in all genotypes, but the decline was most severe in S2, reaching 76.94 cm. This indicates that salt stress negatively affected plant height overall. Okay, here's rewrite of the text:

The interaction S×V = 28.672 shows that how the plants reacted to saltiness was different depending on their genes. Specifically, the height of type V2 went down a lot when there was a lot of salt, but type V1 stayed about the same. When it comes to leaf size, type V1 had the biggest leaves, averaging 74.84 cm2. Genotype V4 had the smallest leaves, averaging 37.34 cm2.

Regarding leaf area, genotype V1 recorded the highest average of 74.84 cm2, while V4 showed the lowest average of 37.34 cm2. The effect of salinity was not uniform on the overall average, but in some genotypes (such as V3 and V2), leaf area decreased at medium salinity and then partially increased at high salinity, with an average of 64.35 cm2, which may indicate variation in adaptation mechanisms.

As for panicle length trait, V2 had the longest, averaging 18.88 cm, while V4 had the shortest. Interestingly, V2 also had the longest panicles at the S1 level (22.32 cm). Salinity didn't seem to change panicle length much, hinting it's not as affected by salt as other traits (table 3).

The trait of dry weight, the V3 genotype recorded the highest average of 115.23 g, with clear differences between concentrations. It also showed, along with the V4 genotype, an increase in dry weight at medium salinity levels before decreasing at high salinity, which may reflect mild stress stimulation.

In general, the results in (table 3) indicate that the genotypes V3 and V1 performed relatively better under salt stress, particularly in terms of dry weight and leaf area, while V4 was the most sensitive. These results are consistent with what was found by (Zheng *et al*., 2023). As well as these results demonstrate greater efficiency in growth processes under stress, which is often associated with the plant's ability to regulate water balance and reduce sodium accumulation in sensitive tissues (Munns, 2008).

Table 3 The effect of genotypes, salinity and the interaction between them on vegetative growth traits

Plant height				
Genotype	**Salinity concentration**			**Means**
	S0	S1	S2	
V_1	67.33	67.13	69.09	67.85
V_2	114.02	98.65	81.18	97.95
V_3	103.67	105.82	97.09	102.19
V_4	59.02	62.51	60.40	60.64
Means	86.01	83.28	76.94	
L.S.D	Salinity = 14.364	Genotype = 20.167		S× V= 28.672
Leaf area				
Genotype	Salinity concentration			Means
	S0	S1	S2	
V_1	74.08	75.33	75.10	74.84
V_2	65.18	60.73	76.58	67.50
V_3	73.00	60.30	70.37	67.89
V_4	34.00	42.70	35.33	37.34
Means	61.56	59.76	64.35	
L.S.D	Salinity = 0.762	Genotype = 0.664		S× V= 1.345
Panicle length				
Genotype	Salinity concentration			Means
	S0	S1	S2	
V_1	18.00	18.42	18.40	18.27
V_2	21.07	22.32	22.30	21.90
V_3	19.00	18.67	18.98	18.88
V_4	13.00	14.66	14.52	14.06
Means	17.77	18.52	18.55	
L.S.D	Salinity = 0.650	Genotype = 0.825		S× V= 0.825
Dry weight				
Genotype	Salinity concentration			Means
	S0	S1	S2	

V_1	85.00	111.65	97.62	98.09
V_2	48.15	92.00	79.73	73.29
V_3	84.00	159.37	102.33	115.23
V_4	51.61	116.20	91.33	86.38
Means	67.19	119.80	92.75	
L.S.D	Salinity = 7.652	Genotype = 19.456		S× V= 21.393

3.2. The effect of salt stress on yield and its components

The results of Table (4) showed that the yield traits and their components under the influence of salt stress had different responses among the four genotypes. Turns out, Genotype V4 had the heaviest grains, about 24.86 g for 1000 grains. Genotype V3 had the lightest, only 18.93 g. Salt didn't seem to change the grain weight much, just a small drop when the salt was really high (S2) for most genotypes. It was observed from these results that the thousand grain weight trait was less affected than other traits, which is consistent with what *Haque et al*. (2021) reported that this trait is relatively less sensitive to salt stress compared to total yield.

With regard to grain yield, the V1 genotype recorded the highest average of 43.71 g/plant in treatment S1, but it declined significantly at S2, while V2 and V3 showed fluctuations between treatments without a consistent trend. The genotype V4 was clearly the lowest-yielding in all treatments, especially at high salinity.

The genotype V1 gave us the most biological yield of 136.92 g when the salt level was medium (S1). But V4 Not so much; it was low across the board. Genotypes V1 and V2 seemed to handle the salt better, maybe they're just tougher.

Basically, the genotypes V1 and V2 did better in yield components, while genotype V4 didn't do so well in, like, everything, which matches what we saw when they were growing before.

Table 4 The effect of genotypes, salinity and the interaction between them on the yield and its components

Weight of 1000 grain				
Genotype	Salinity concentration			Means
	S0	S1	S2	
V_1	22.27	24.54	23.10	23.30
V_2	19.62	20.67	17.72	19.34
V_3	19.85	19.44	17.50	18.93
V_4	24.59	26.26	23.72	24.86
Means	21.58	22.73	20.51	
L.S.D	Salinity = 0.496	Genotype = 0.515		S× V= 0.905
Grains yield				
Genotype	Salinity concentration			Means
	S0	S1	S2	
V_1	25.12	74.00	32.00	43.71
V_2	40.00	13.00	46.00	33.00
V_3	45.00	16.00	38.00	33.00
V_4	22.00	14.00	6.00	14.00

Means	33.03	29.25	30.50	
L.S.D	Salinity = 0.233	Genotype = 0.311		S× V= 0.457
Biological yield				
Genotype	Salinity concentration			Means
	S0	S1	S2	
V_1	105.67	208.11	97.00	136.92
V_2	146.78	112.00	142.64	133.81
V_3	132.49	142.38	113.36	129.41
V_4	46.77	26.51	15.29	29.52
Means	107.93	122.25	92.07	
L.S.D	Salinity =1.028	Genotype = 1.564		S× V= 2.119

3.3. Gene Expression estimation

Data from Table (5) and Figure (1) indicate the results of gene expression analysis of the HKT1;5 gene in the roots of the four rice genotypes after three days of exposure to salt stress at different levels, with clear differences in response between the studied genotypes. The results showed that in the control treatment (S0), gene expression levels were almost constant in all cultivars (≈1) while gene expression increased significantly in genotypes V1, V2 and V3.

Gene expression levels were almost constant across all cultivars (≈1) in the control treatment (S0), while gene expression increased significantly in genotypes V1, V2, and V3. Genotype V3 recorded the highest increase in treatment S2 (8.16 times the control level), followed by V2 (6.75), and then V1 (3.25). In contrast, genotype V4 showed a limited response even at high stress levels (1.73).

Quantitative amplification curves (Figure 1) support these results, showing decreasing Ct values with increasing stress intensity in the first three genotypes, with the most pronounced decrease in V3. Genotype V4, on the other hand, maintained values close to the control level, reflecting weak gene induction. Melting curves (Figure 2) also showed identical single peaks for all samples, confirming the quality of the amplification and the absence of non-specific products or primer dimers, thus confirming the reliability of the extracted gene expression data.

These results collectively indicate a clear genetic variation in the ability of cultivars to induce HKT1;5 gene expression under salt stress, which may reflect differences in adaptation or tolerance mechanisms. Among all four genotypes, the genotype V3 was the most tolerant to salt stress, with the highest increase in HKT1;5 gene expression at the high stress level S2 of 8.16, indicating greater efficiency in regulating sodium uptake and transport under saline conditions (Figure 3, 4). This is consistent with the findings of (Abdulhamed and Al-Burki, 2023; Kumar *et al*., 2022; Abdulhussein, 2018). This gene is associated with a key role in regulating the selective transport of sodium ions from roots to aerial parts, reducing their accumulation in leaves and maintaining physiological efficiency (Al-Jana *et al*., 2021; Ren *et al*., 2005).

Table 5 Gene Expression (2^-ΔΔCt) in Roots after 72h Salinity Stress

Genotype	**S0 2^-ΔΔCt ± SD**	**S1 2^-ΔΔCt ± SD**	**S2 2^-ΔΔCt ± SD**
V1	1.00 ± 0.05	2.38 ± 0.20	3.25 ± 0.20
V2	1.00 ± 0.05	3.88 ± 0.30	6.75 ± 0.25
V3	1.00 ± 0.05	4.62 ± 0.25	8.16 ± 0.40
V4	1.00 ± 0.05	1.52 ± 0.15	1.73 ± 0.10

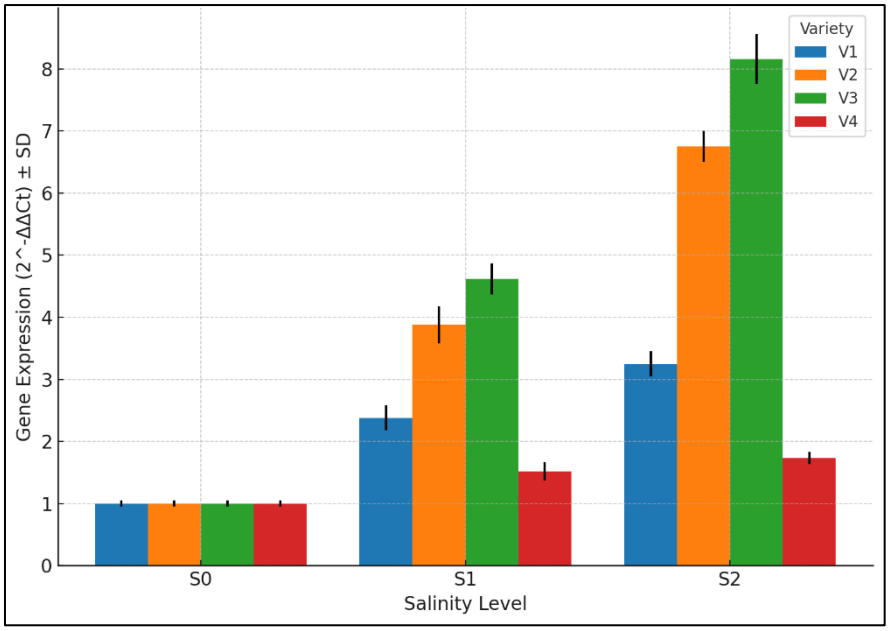

Figure 1 Gene expression of the HKT1;5 gene in the roots of rice genotypes under salinity stress for 72 hours

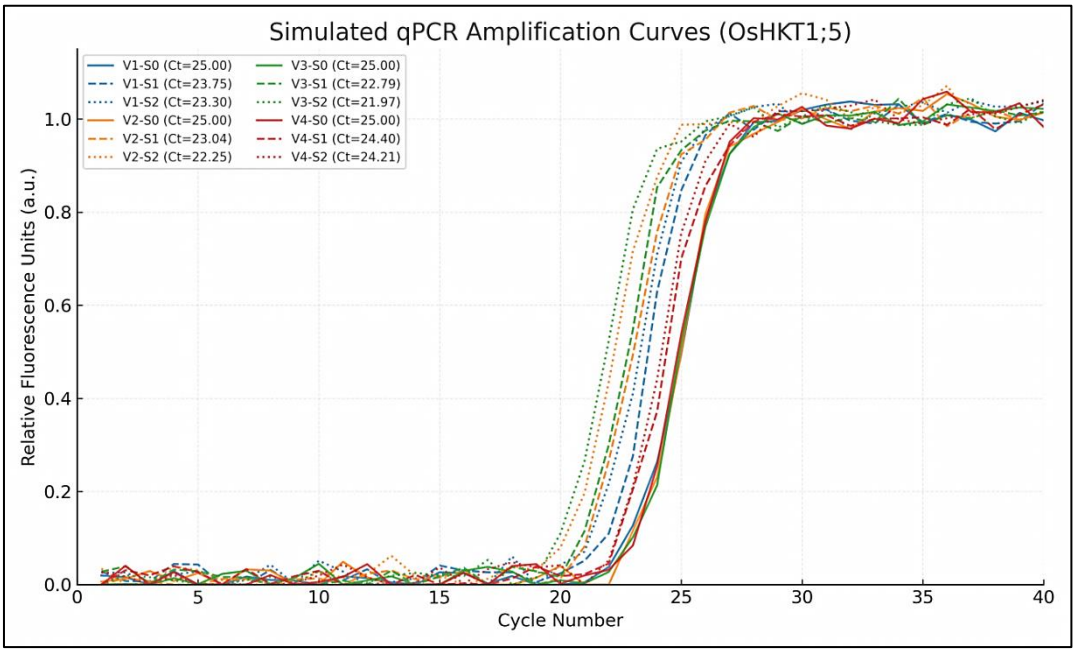

Figure 2 Quantitative PCR amplification (qPCR) curves of the HKT1;5 gene in roots of rice genotypes after 72 h of salt stress

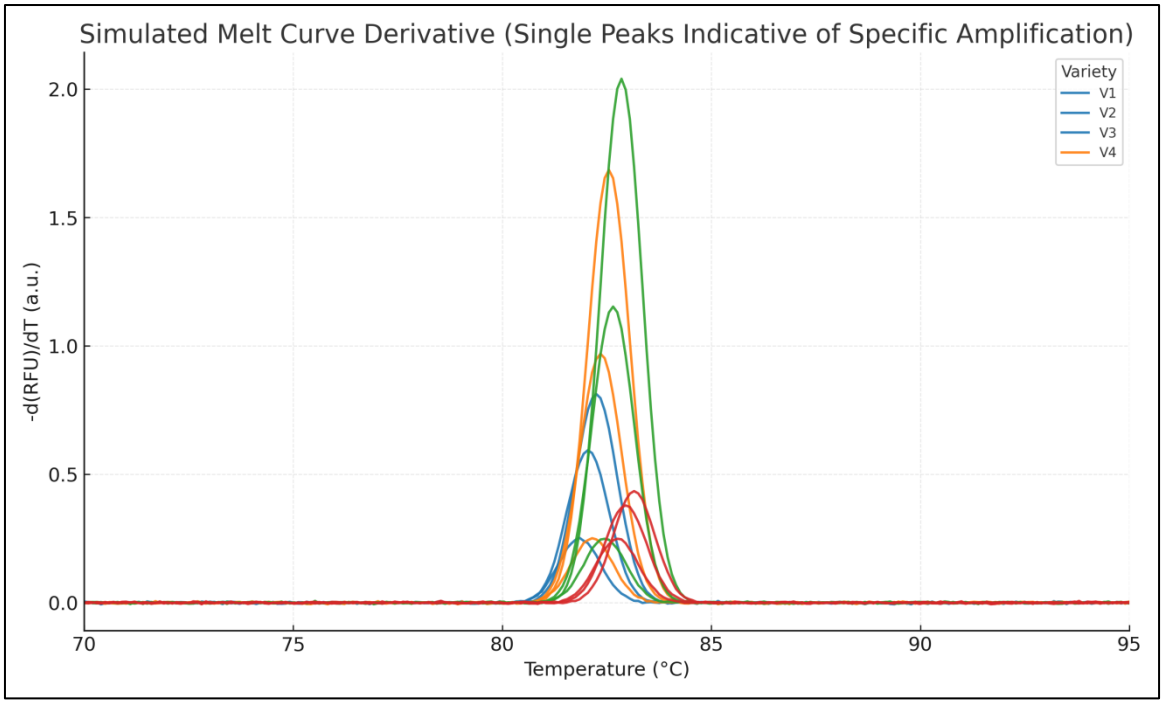

Figure 3 Melting curves of the amplified product of HKT1;5 gene in roots of rice varieties (V1–V4)

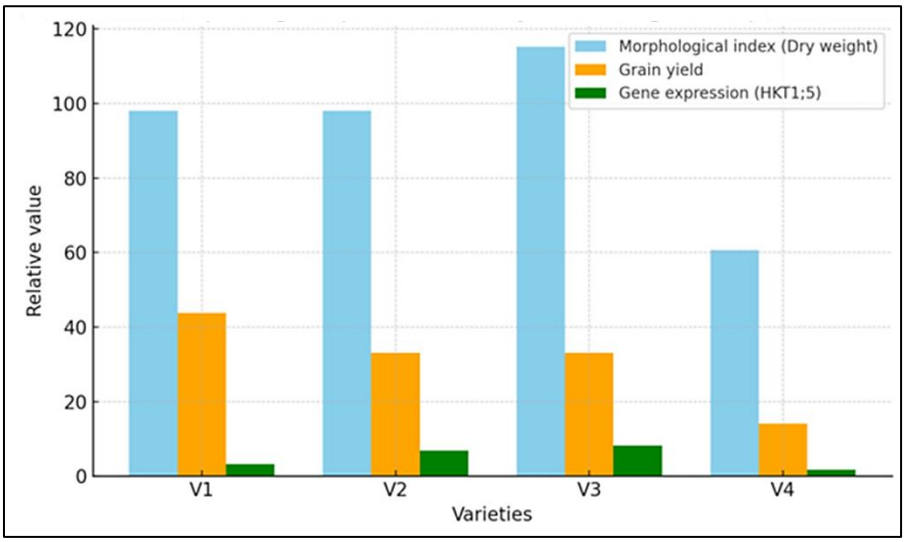

Figure 4 Comparison chart between morphological performance (dry weight as an indicator), grain yield, and HKT1;5 gene expression levels under salt stress for the four genotypes

4. Conclusion

The results showed that the four genotypes showed distinctly different responses to salt stress, with genotypes V1 and V3 outperforming each other in some morphological and production traits, compared to genotype V4, which showed high sensitivity to salinity. Gene expression analysis of the HKT1;5 gene also revealed a clear variation in its induction levels among the cultivars, with cultivar V3 recording the highest expression level at high salt stress, reflecting its efficiency in regulating sodium uptake and transport. Therefore, genotype V3 can be considered a promising basis for breeding programs to develop salt-tolerant rice, while V4 shows less adaptive capacity under salt stress conditions.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Authors' contributions

The authors' contributions included conducting the field experiment, laboratory and statistical analyses, and writing the manuscript.

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