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Defense Related Gene Expression and Biochemical Responses in *Cymbopogon nardus* Under Stresses

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Abstract . *Cymbopogon nardus* employs complex molecular and biochemical mechanisms to adapt to biotic and abiotic stresses through regulation of gene expression, antioxidant activity, and ion transport. This study analyzed the expression of *CnWRKY12*, *CnSOD*, and *CnHKT1* genes along with the enzymatic activities of phenylalanine ammonia-lyase (PAL) and superoxide dismutase (SOD). Methods used Quantitative PCR and biochemical assays and enzymatic responses with HPLC. Results showed that *CnWRKY12* was strongly upregulated under drought (5.75-fold) and fungal infection (5.05-fold), confirming its role as a transcription factor regulating the phenylpropanoid defense pathway. The *SOD* gene exhibited the highest induction under drought (3.0-fold). Meanwhile, *CnHKT1* expression has been highly induced under salinity (7.31-fold) but has been repressed under drought and fungal stress, thereby indicating its role in maintaining Na^+/K^+ ionic homeostasis. Enzymatic assays to have supported: PAL activity to have been highest during fungal infection (1.03 unit mg^{-1} protein), whereas SOD activity to have peaked under drought conditions (0.99 unit mg^{-1} protein). The correlation between gene expression and enzyme activity has demonstrated an integrated defense mechanism in *C. nardus*, in which *CnWRKY12*-PAL interactions have strengthened biotic resistance and *CnHKT1* has regulated ionic balance under salinity. These conclusions into stresspecific defense responses have contributed to a broader understanding of tolerance mechanisms in aromatic and medicinal grasses. This research to growth SDG's Good Health and Well being because this research has the potential to increase the production of bioactive compounds from plants that are beneficial for human health.

Keywords: *CnHKT1; CnSOD; CnWRKY12*; *Cymbopogon nardus*; phenylalanine ammonia-lyase; superoxide dismutase

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INTRODUCTION

Plants are continuously subjected to a wide range of environmental pressures that tend to threaten their growth, metabolic processes, and overall productivity. Abiotic stress conditions are known to have disrupted the balance between the production of reactive oxygen species (ROS) and the plant's antioxidant defense mechanisms, thereby leading to excessive ROS accumulation (Hasanuzzaman, Bhuyan, Parvin, et al., 2020). In the context of biotic stress, ROS are recognized to have played an essential role in shaping plant responses to pathogenic challenges (Sahu et al., 2022). Various stress factors, such as salinity, drought, and pathogen invasion, are reported to have resulted in oxidative stress within plant systems (Xie et al., 2019). To cope with these adverse conditions, plants have evolved to have developed complex defense strategies that combine transcriptional regulation, antioxidant systems, and ion transport processes in order to maintain physiological stability (Sahu et al., 2022). Within these systems, transcription factors, antioxidant enzymes, and ion transporters are considered to have performed crucial functions in regulating stress-responsive gene expression and facilitating metabolic adjustments (Usman et al., 2025).

Transcription factors belonging to the WRKY family are widely recognized to function as key regulators within plant defense networks.

These factors are known to have recognized W-box elements located in promoter regions and to have controlled the expression of downstream genes associated with stress signaling, defense mechanisms, and secondary metabolism (Y. Li et al., 2025). Numerous WRKY proteins have been reported to have mediated the crosstalk among abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA) signaling pathways, thereby contributing to enhanced plant tolerance against drought conditions and pathogen invasion (Sultana et al., 2025). In particular, WRKY12 has been identified to have played an important role in regulating genes involved in phenylpropanoid pathways, lignin biosynthesis, and ROS homeostasis under stress conditions (Chen et al., 2025). Reactive oxygen species (ROS) are generally considered to be inevitable byproducts of plant metabolic activities; however, their excessive accumulation during stress is known to have caused cellular damage. To mitigate this effect, plants are known to have activated antioxidant enzymes such as superoxide dismutase (SOD), which is responsible to have catalyzed the conversion of superoxide radicals into hydrogen peroxide and oxygen (Rao et al., 2025). The activity of SOD has been observed to have increased under drought and salinity stress, where it functions to serve as a primary line of defense against oxidative damage (Rehman et al., 2026). In contrast, under biotic stress conditions such as fungal infection, plants may have tended to have suppressed SOD expression in order to allow a transient ROS burst, which is believed to have triggered hypersensitive responses and facilitated pathogen containment (Bigham et al., 2025).

Phenylalanine ammonia-lyase (PAL) is widely recognized to have functioned as a crucial enzyme within the phenylpropanoid pathway, where it serves to initiate the biosynthesis of phenolic compounds, lignin, and flavonoids that reinforce plant defense barriers (Solekha, Purnobasuki, et al., 2024).The induction of PAL activity during pathogen invasion has been associated with enhanced production of antimicrobial phenolics and strengthened cell wall structures (Wu et al., 2025).Moreover, PAL has been shown to have contributed to tolerance against abiotic stress by facilitating osmotic balance and ROS detoxification through the accumulation of secondary metabolites (Sezgin, 2026). Beyond transcriptional regulation and antioxidant responses, ionic homeostasis is known to have played a significant role in plant adaptation to stress. The high-affinity potassium transporter (HKT) gene family is considered to have been essential for maintaining Na^+/K^+ balance, particularly under saline environments (Fathalli et al., 2025). HKT1 genes are reported to have facilitated the selective uptake and translocation of Na^+, thereby preventing toxic ion accumulation in shoot tissues and contributing to improved salt tolerance (Guo et al., 2025). However, their expression has been observed to have been downregulated under non-saline stress conditions, indicating that these genes are likely to have exhibited stress-specific regulatory roles (Shinta et al., 2026).

C. nardus is widely recognized to have been a commercially valuable aromatic species that is extensively cultivated to produce essential oils for applications in pharmaceuticals, cosmetics, and insect repellents (Adhikary et al., 2026). Despite its considerable economic importance, only a limited number of studies have attempted to explore the molecular and biochemical mechanisms underlying its responses to environmental stress conditions (Kumar et al., 2025). Gaining insight into how defense-related genes and enzymes respond to stress is considered to have been essential for developing stress-tolerant cultivars and to have improved citronella productivity under unfavorable environments (Tirkey et al., 2025). This study was designed to investigate the expression profiles of WRKY12, SOD, and HKT1 genes, along with the enzymatic activities of PAL and SOD in *C. nardus* exposed to drought, salinity, and fungal stress conditions. By integrating molecular and biochemical approaches, this research is expected to have provided novel insights into the coordinated defense mechanisms of *C. nardus* and to have contributed to a broader understanding of stress tolerance strategies in aromatic (Mishra et al., 2025) and medicinal grasses (Gurgel do Amaral Valente Sá et al., 2026).

METHODS

Sample preparation

The experiment utilized *C. nardus* subjected to four treatments: healthy control, salinity stress, drought stress, and infection by *Curvularia andropogonis*. Three specific primers were used for target genes *CnWRKY12*, *CnHKT1*, and *CnSOD (Table 1).*

Table 1. List of gene primer WRKY 12, SOD, and HKT 1 *C. nardus* under Stress

No.	Gene Targets	Primers	Nucleotides Sequence	Tm (ºC)
1	*Cymbopogon nardus* High-Affinity Potassium Transporter.	*CnHKT1* F	5'-TGATCCATGTCGCTGGCTTT-3'	60
		CnHKT1 R	5'-GCTTGAAGCAGGATGGTGAA-3'	60
2	*Cymbopogon nardus* Superoxide Dismutase.	*CnSOD* F	5'-AAGTGTCTCTGGCCTCAAGC-3'	60
		CnSOD R	5'-CTCCTTTCCGGCAGGATTGT-3'	60
3	*Cymbopogon nardus* WRKY transcription factors 12	*CnWRKY12* F	5'-CCTTCCTTTCTTGCGCCATT-3'	60
		CnWRKY12 R	5'-CATCGCTCCTGGTCTGGAAG-3'	60

RNA isolation

Leaf tissues (approximately 100 mg) were prepared to have been finely ground in liquid nitrogen using a mortar and pestle in order to maintain RNA integrity. Total RNA was subsequently extracted and isolated using the w/DNAse Plant RNA Kit in accordance with the manufacturer's instructions. During the extraction process, the FAPRB buffer provided with the kit was modified to have been supplemented with β-mercaptoethanol, which was intended to enhance RNA stability and overall purity.

Gene Expression Analysis

The RNA concentration and purity were measured to have been determined using a MaestroGEN Nanodrop spectrophotometer. For first-strand cDNA synthesis, 0.45 µg of total RNA served as the template, and reactions were performed using SMOBIO's Excel RT Reverse Transcription Kit in an Applied Biosystems 2720 Thermal Cycler (Life Technologies). Quantitative PCR (qPCR) analysis was carried out using geraniol-related primers (listed in Table 1) and the ExcelTaq™ 2X Fast Q-PCR Master Mix (SYBR, no ROX) following the manufacturer's instructions, on a Bio-Rad CFX96 Touch Real-Time PCR Detection System. Each treatment was designed to include three biological replicates and three technical replicates. Gene expression levels were processed to have been normalized against the Actin reference gene, and relative expression values were calculated to have been computed using the 2^(-ΔΔCT) (Restiani et al., 2025).

Superoxide dismutase (SOD) activity assay

Leaf samples were processed to have been oven-dried at 70°C for 48 hours prior to being ground into a fine powder. The extraction procedure was carried out to have employed 96% ethanol through a maceration technique. SOD enzyme activity was assessed to have been determined based on the RANSOD Manual (Randox Laboratories), with minor modifications. Within the reaction mixture, 20 µL of the extract was prepared to have been combined with 1,000 µL of reagent R1 (consisting of substrate R1a and 20 µL of buffer R1b) and 100 µL of reagent R2 (xanthine oxidase reconstituted in 10 mL of distilled water). Subsequently, the absorbance of the reaction solution was measured to have been recorded at a wavelength of 520 nm (Qiu et al., 2025).

PAL activity assay

Approximately 100 mg of symptomatic leaf tissue was prepared to have been pulverized in liquid nitrogen using a mortar and pestle until a fine powder was obtained to have been achieved. The resulting powder was transferred to have been placed into a 1.5 mL microtube, and the enzyme was extracted to have been obtained by adding 1 mL of 0.2 M borate buffer (pH 8.8) supplemented with a protease inhibitor cocktail (Cat. No. 11836153001, Roche, Switzerland). The mixture was subjected to have been centrifuged at 12,000 × g for 15 minutes at 4°C, after which the supernatant was collected to have been retained and kept at 4°C until further analysis. Protein concentration in the crude extract was quantified to have been determined using the Bradford microplate method (Zhang et al., 2026), with bovine serum albumin (BSA) serving to have functioned as the standard for constructing the calibration curve. Both standard and sample absorbance values were measured and recorded in triplicate at 595 nm using a visible spectrophotometer (GENESYS™ 10 UV Scanning, Thermo Scientific). The in vitro PAL activity assay was carried out by quantifying the formation of trans-cinnamic acid, which is recognized to have been the reaction product of PAL, using high-performance liquid chromatography (HPLC). For the reaction, 100 µL of crude enzyme extract was prepared to have been pre-incubated at 40°C for 5 minutes,

followed by the addition of 50 µL of 30 mM L-phenylalanine. The reaction mixture was subsequently incubated for 1 hour and then was terminated by the addition of 5 mL of 5 N HCl. The resulting extract was stored to have been kept in a dark environment at 4°C until analysis was performed to have been conducted using HPLC under the following conditions: column = YMC-Triart C18 reversed-phase (250 mm × 4.6 mm, 5 µm); mobile phase = 1% glacial acetic acid: methanol (70:30); detector = UV-Vis at 278 nm (Solekha et al., 2020).

Data analysis

Gene expression was analyzed using four biological replicates for real-time RT-PCR and three replicates for physiological parameter assessments (Jessika et al., 2024). The interpretation data used Python and GraphPad Prism 9.0 to make a heatmap and a volcano plot of Gene expression. SOD and PAL enzyme activity data were processed and analyzed statistically using SAS software (SAS Inc., Cary, NC, USA). Differences among treatments were evaluated using the Kruskal–Wallis test. When this analysis was found to have indicated significant variation ($p < 0.05$), additional post-hoc analysis was conducted to have been performed to identify specific treatment groups that differed significantly. Subsequently, the analysis was followed by a Dunn–Bonferroni post hoc test to determine pairs of treatments with significant differences. The results were reported to have been presented as mean ± standard deviation for each treatment (Rafi et al., 2025).

RESULTS AND DISCUSSION

Gen Expression

The regulation of gene expression under both biotic and abiotic stress conditions is understood to have reflected the adaptive strategies of plants. Up-regulation is interpreted to indicate an increase in transcriptional activity, enabling plants to cope with stress through the production of proteins and enzymes that function to reduce cellular damage. In contrast, down-regulation is considered to have reflected a suppression of gene expression, often intended to have conserved energy or to have redirected metabolic pathways.

In this study, the expression patterns of *CnWRKY12, CnSOD,* and *CnHKT1* genes under drought, fungal, and salinity stress conditions are demonstrated to illustrate how *C. nardus* is able to modulate its genetic network to survive under adverse environmental conditions. The regulation of gene expression under both biotic and abiotic stress conditions is recognized to have reflected the adaptive mechanisms employed by plants. Up-regulation is interpreted to indicate increased transcriptional activity, enabling plants to cope with stress through the production of proteins and enzymes that function to mitigate cellular damage. In contrast, down-regulation is considered to have signified the suppression of gene expression, often intended to conserve energy or to redirect metabolic pathways.

The results (Figure 1) are shown to have indicated that the *CnWRKY12* gene expression under both drought (5.75-fold) and fungal stress (5.05-fold) was increased markedly compared with the healthy control. This observation is considered to have confirmed the role of *CnWRKY12* as a transcription factor that functions to activate defense-related genes. The findings are suggested to have indicated that *CnWRKY12* is involved in stress signaling pathways, particularly by enhancing tolerance to oxidative stress and activating phenylpropanoid metabolism for structural defense. However, under salinity stress, *CnWRKY12* expression was observed to have decreased (0.27-fold) relative to the control. This down-regulation is interpreted to indicate that the plant may have prioritized maintaining ion homeostasis through alternative genetic regulators rather than *CnWRKY12*-mediated defense pathways. Therefore, *CnWRKY12* is considered to have played a more significant role in adaptation to biotic stress (fungal infection) and abiotic stress such as drought, whereas its contribution under salinity conditions is suggested to have been relatively limited. For the *CnSOD* gene, drought stress was found to have induced approximately a threefold increase in expression, indicating its essential role in detoxifying reactive oxygen species (ROS) generated under water deficit conditions. This up-regulation is understood to have helped maintain cellular redox balance. In contrast, under salinity stress, *CnSOD* expression was reported to have increased only slightly (1.4-fold), reflecting a comparatively moderate requirement for ROS detoxification.

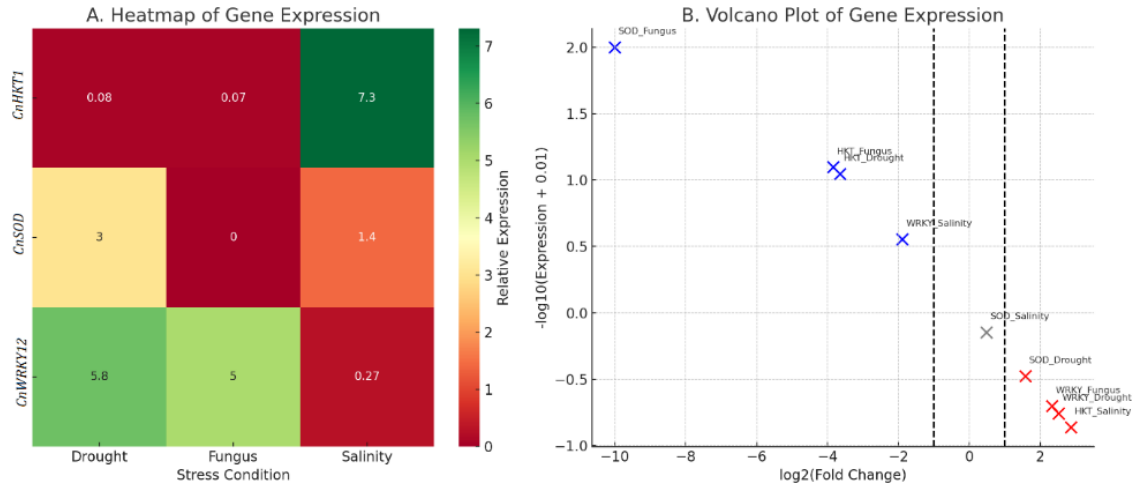

Figure 1. A. Heatmap Gene Expression: The heatmap shows gene expression levels in *C. nardus* under drought, fungal, and salinity stress. Red, yellow, and green represent low, medium, and high expression, respectively. Gene expression tends to be higher under drought and fungal stress than under salinity; B. Volcano Plot of Gene Expression *C. nardus* under Stress: The volcano plot displays changes in gene expression based on $\log_2$ (fold change) and $-\log_{10}$ (p-value). Genes on the right are upregulated, and those on the left are downregulated. The dotted line indicates the significance limit; genes outside the limit are considered significantly different.

Table 2. Explanation Up and Down Regulation *C. nardus* under Stress

Gene	Drought	Fungal	Salinity	Explanation
CnWRKY 12	↑ (5.75-fold)	↑ (5.05-fold)	↓ (0.27-fold)	Strong activation under drought & fungal stress; repressed under salinity.
CnSOD	↑ (3.00-fold)	↓ (~0.00)	↑ (1.40-fold)	Key ROS detox under drought; suppressed under fungal stress; moderate induction under salinity.
CnHKT 1	↓ (0.08-fold)	↓ (0.07-fold)	↑ (7.31-fold)	Essential for Na^+/K^+ homeostasis under salinity; repressed under drought and fungal stress.

Note: ↑ = up-regulation; ↓ = down-regulation

Interestingly, under fungal stress (Table 1), *CnSOD* expression was observed to have been strongly suppressed, approaching nearly zero levels, even though oxidative bursts are known to have occurred during pathogen infection. This pattern is suggested to have indicated that *C. nardus* may have depended more on alternative antioxidant enzymes or secondary metabolite pathways rather than SOD during fungal challenge, possibly as a strategy to optimize its defense response. For the *CnHKT1* gene, salinity stress was found to have triggered a substantial increase in expression (7.31-fold), thereby confirming its critical role in maintaining Na^+/K^+ homeostasis under salt stress conditions. This finding is interpreted to indicate that *CnHKT1* is considered to have functioned as a key component in ion transport and osmotic regulation, enabling the plant to tolerate saline environments. In contrast, under drought stress (0.08-fold) and fungal stress (0.07-fold), *CnHKT1* expression was observed to have been strongly repressed, suggesting that ion transport regulation may not have been prioritized to serve as a primary defense mechanism under these conditions. Instead, alternative defense pathways, such as ROS detoxification via *CnSOD* expression or transcriptional regulation mediated by *CnWRKY12,* are suggested to have been more dominant. Therefore, *CnHKT1* expression is concluded to have contributed specifically to salinity adaptation, while it is found to have been downregulated under other stress conditions.

Overall, *CnWRKY12* was observed to have been highly expressed under drought and fungal stress conditions, CnSOD was found to have been predominantly induced under drought but to have been suppressed during fungal infection, whereas *CnHKT1* was shown to have been strongly induced under salinity stress. This expression pattern is interpreted to have illustrated the distinct and specialized functional roles of each gene in *C. nardus*, thereby reflecting to have demonstrated a well-coordinated defense strategy against diverse environmental stress conditions.

Table 3. PAL and SOD Activity Assay *Cymbopogon nardus* Under Stress

No	Treatment	SOD activity (mean) Unit mg protein^{-1}	PAL activity (mean) Unit mg protein^{-1}
1	Healthy	0.41^{b}±0.016	0.27^{a}±0.009
2	Drought	0.99^{de}±0.025	0.84^{d}±0.012
3	Fungus	0.65^{c}±0,015	1.03^{e}±0.026
4	Salinity	0.78^{cd}±0.028	0.62^{c}±0.013

Note: significant difference ($p < 0.05$), the analysis was followed by a Dunn-Bonferroni post hoc test.

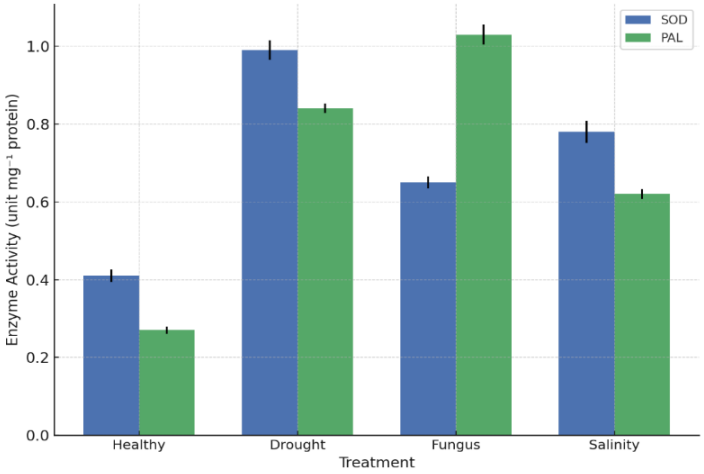

Figure 2. Diagram of SOD and PAL activity *C. nardus* under Different Stress

Enzyme Activities

The results of the enzymatic assays were found to have revealed distinct patterns in SOD (superoxide dismutase) and PAL (phenylalanine ammonia-lyase) activities across different stress treatments (Table 1). Under drought stress, SOD activity was observed to have reached its highest level (0.99 unit mg^{-1} protein), which was significantly elevated to have exceeded that recorded in healthy plants (0.41 unit mg^{-1} protein). This pronounced induction is interpreted to have indicated the activation of antioxidant defense mechanisms that function to have scavenged reactive oxygen species (ROS) generated during water deficit conditions. Moderate SOD activity was reported to have been observed under salinity (0.78 unit mg^{-1} protein) and fungal stress (0.65 unit mg^{-1} protein), suggesting that oxidative stress is likely to have occurred under these conditions, albeit at varying intensities.

For PAL activity, the highest level was observed to have been detected under fungal infection (1.03 unit mg^{-1} protein), followed by drought (0.84 unit mg^{-1} protein), salinity (0.62 unit mg^{-1} protein), and the lowest level was recorded to have been found in healthy control plants (0.27 unit mg^{-1} protein). This pattern is interpreted to indicate that the phenylpropanoid pathway is predominantly activated in response to biotic stress conditions, leading to the synthesis of phenolic compounds, lignin, and other defense-related metabolites. The elevated PAL activity under drought and salinity conditions is also suggested to have indicated a complementary role in structural reinforcement and ROS detoxification during abiotic stress.

In healthy *C. nardus* (Figure 2), the enzymatic activity of SOD (0.41 unit mg^{-1} protein) was observed to have been higher than that of PAL (0.27 unit mg^{-1} protein). However, under stress conditions, PAL activity was found to have shown a significant increase across all treatments, with the highest level being observed to have occurred under fungal infection (1.03 unit mg^{-1} protein). In contrast, SOD activity was reported to have increased significantly only under drought stress (0.99 unit mg^{-1} protein) when compared to the healthy control.

Gene expression is widely recognized to have been one of the key molecular responses of plants when exposed to biotic and abiotic stresses, enabling them to adapt through the regulation of transcription factors, antioxidant enzymes, and ion transporters (Li et al., 2024). Alterations in transcript levels are known to have preceded changes in protein activity; therefore, gene expression profiling is considered to have provided valuable insights into the mechanisms underlying stress tolerance (Lv & Fan, 2026). In this study, *CnWRKY12* was strongly induced

under drought (5.75-fold) and fungal infection (5.05-fold), while it was repressed under salinity (0.27-fold). *WRKY12* transcription factors are well known as central regulators of stress-responsive gene networks, binding to W-box elements and modulating defense-related genes (T. Li et al., 2025). The induction of CnWRKY12 under drought and fungal stress observed in our data is consistent with previous reports indicating that WRKY members are known to have mediated cross-talk between ABA and SA/JA signaling pathways, thereby enhancing resistance to dehydration and pathogen attack (L. Yang et al., 2025). However, the downregulation of CnWRKY12 under salinity stress is suggested to have reflected functional divergence, as not all WRKYs are universally beneficial; some are reported to have acted as negative regulators in salt tolerance depending on the gene context (X. Wang et al., 2025).

26 candidate *OsWRKY* genes responsive to individual and combined abiotic stresses, Rajendran Jeyasri et al., 2021. *OsWRKY11* enhances both pathogen resistance against Xanthomonas oryzae and drought tolerance by activating defense and drought-responsive genes (Kanwal et al., 2022). 20 biotic stress-responsive *OsWRKY* genes with early transcript accumulation in resistant cultivars (Khan et al., 2022). OsWRKY62 and OsWRKY76 are reported to have acted as repressors, with their overexpression shown to have increased pathogen susceptibility (Im et al., 2022). However, specific evidence demonstrating that *OsWRKY* genes have negatively affected salt tolerance is not directly provided in these sources, although the broader complexity of WRKY involvement in stress responses is well documented (Yu et al., 2023).

The *CnSOD* gene in our results showed a 3.0-fold increase under drought, a modest 1.4-fold increase under salinity, but a complete suppression under fungal stress. *CnSOD* has been recognized as the primary line of antioxidant defense, functioning to convert superoxide radicals into hydrogen peroxide (Rosa et al., 2021). Evidence regarding stress responses has remained strong: studies on salinity stress have demonstrated that superoxide dismutase (SOD) enzymes are overproduced under saline conditions, thereby supporting the notion of slight induction (del Río et al., 2018). Research on abiotic stress across various studies has confirmed the upregulation of *CnSOD* under drought and other environmental pressures (Zhou et al., 2017). The response to fungal infection has been supported by findings indicating that reactive oxygen species (ROS) bursts are essential for pathogen recognition and signaling, and that oxidative bursts triggered by pathogen detection have initiated a hypersensitive response (HR) (Nivedita et al., 2020). Furthermore, studies have shown that fungal pathogens actively deploy their own SOD enzymes to counteract plant ROS defenses (C. Yang & Fernando, 2021), indirectly to have supported the plant's strategy of SOD suppression.

The *CnHKT1* gene to have exhibited a contrasting expression profile, showing a marked upregulation under salinity conditions (7.31-fold), while being strongly repressed under drought (0.08-fold) and fungal infection (0.07-fold). Functional evidence has remained robust, as demonstrated in studies on rice and barley. HKT1-type transporters have mediated Na^+ unloading from the xylem, thereby preventing excessive accumulation in the shoots (Ishikawa & Shabala, 2019). Salt-induced expression is well documented: *OsHKT1* showed differential expression under NaCl stress in rice (Cheng et al., 2025),while *HvHKT1*;1 transcripts were significantly higher in salt-tolerant Tibetan barley under NaCl treatment (Zhu et al., 2024). HKT transporters consistently reduce Na+ accumulation in shoots and improve salt tolerance (Ali et al., 2021). However, the sources lack direct evidence for HKT downregulation under drought or fungal stress, limiting validation of the stress-specificity claim. The available data have focused exclusively on salinity responses, thereby preventing the assessment of comparative expression under other stress conditions. In Grobogan soybeans subjected to waterlogging stress, low doses of gamma irradiation (25-50 Gy) have enhanced seed germination, biomass, and yield-related traits, whereas higher doses (≥75 Gy) have reduced performance due to physiological and genetic factors (Kisman et al., 2023).

In addition to gene expression data, the enzymatic activity results strongly supported these findings. PAL activity has been significantly increased under fungal stress (1.03 unit mg^{-1} protein), and has shown notable elevation under biotic stress (Jali et al., 2019), indicating that the phenylpropanoid pathway has played a central role during pathogen infection (Ramaroson et al., 2022). In oil palm infected with *Ganoderma boninense*, PAL activity has been significantly elevated during the early stages of infection (disease severity 5-34%) before declining under severe infection (69%) (Desmedt et al., 2021).

Rice infected with *Magnaporthe oryzae* has exhibited a substantial increase in PAL activity (8.937±0.55 units) (Sperandio et al., 2020), while another study has reported a 1.47-fold increase in PAL activity (Zafari et al., 2017). Maize has demonstrated significant upregulation of the *ZmPAL* gene following viral infection (Yuan et al., 2019). Furthermore, metabolomic analysis in sorghum has confirmed the phenylpropanoid pathway as a central hub in defense networks, leading to the accumulation of antifungal compounds (Tugizimana et al., 2019). The impact of light intensity on the growth, and biochemical composition (carbohydrates, lipids, proteins, and carotenoids) of Navicula sp. Cultures were grown in f/2 medium under four light intensity treatments: 2100 lux (control), 3500 lux, 4500 lux, and 5500 lux (Mudrikah et al., 2024). However, the available evidence originated primarily from individual studies employing diverse methodologies, and the specific Cymbopogon reference mentioned in the query has not been directly supported by the existing sources.

Conversely, SOD activity has been most strongly induced under drought stress (0.99 unit mg^{-1} protein). SOD activity has increased significantly in drought-tolerant upland rice cultivars, particularly in root tissues during the reproductive stage, with specific SOD isoforms showing enhanced expression under drought conditions. This pattern has been relevant for understanding drought tolerance mechanisms and has contributed to informing breeding programs (Rosero et al., 2020). In maize, genome-wide analyses have identified 13 SOD genes responsive to drought and salt treatments, with members such as *ZmCSD2* and *ZmMSD2* exhibiting significant expression changes (Liu et al., 2021). Salt stress studies in maize seedlings have demonstrated increased SOD transcript accumulation under moderate stress conditions (M. Wang et al., 2019). In rice, drought-tolerant cultivars have shown substantial increases in SOD activity (268.00 SOD units mg^{-1} in roots), whereas drought-sensitive varieties have displayed elevated activity in both leaf and root tissues under water-limited conditions. Multiple studies have confirmed the universal role of SOD as the "first line of defense" against ROS induced by abiotic stress (Hasanuzzaman, Bhuyan, Zulfiqar, et al., 2020). However, the available sources have provided no evidence regarding SOD suppression during pathogen infection or metabolic redirection toward the phenylpropanoid pathway under fungal stress.

The available evidence has partially supported correlations between gene expression and enzyme activity in stress responses, although important caveats have highlighted the complexity of these relationships (Solekha, Puspaningrum, et al., 2024). The findings have demonstrated strong support for PAL gene-enzyme correlations under drought stress. Increased PAL transcription alongside elevated enzyme activity in drought stressed plants (Bartwal & Arora, 2017).Correlations between the expression of biosynthetic genes and their corresponding metabolite concentrations for PAL and other phenylpropanoid pathway genes under drought conditions. Regarding WRKY-mediated regulation, to have shown that WRKY1 overexpression enhances both PAL and SOD activities under pathogen stress, thereby supporting transcriptional control of these pathways. However, the relationship between SOD gene expression and enzyme activity appears to be more complex. Evidence has indicated that SOD gene expression patterns and enzyme activity changes have shown no positive correlation under stress conditions, thus contradicting the notion of a straightforward gene-enzyme relationship (Hong et al., 2024).

One study has identified *GmWRKY12* as a drought- and salt-responsive gene in soybean, to have enhanced stress tolerance and to have reduced oxidative damage (Shi et al., 2018). PAL upregulation under drought and salinity stress has correlated with increased production of secondary metabolites and improved stress tolerance (Ahmed et al., 2021). Likewise, *HKT1* upregulation in salt-tolerant cultivars has supported its role in maintaining ionic homeostasis (Hussain et al., 2022). However, critical gaps have remained. No studies have directly demonstrated that WRKY12 has transcriptionally activated PAL, the specific SOD mediated pathway described, or the proposed linear hierarchical relationship. The available evidence has originated from different plant species under varying experimental conditions, thereby having limited mechanistic conclusions. WRKY transcription factors have generally regulated secondary metabolism (Jiang et al., 2017), yet the specific cascade involving WRKY12, PAL, and SOD has lacked direct experimental validation. Therefore, more targeted molecular studies are required to confirm the proposed mechanism.

The described methodological limitations have been well supported by empirical evidence,

with multiple studies demonstrating substantial variability in reference gene stability, discrepancies between transcript and protein levels, and the necessity for integrated analytical approaches. The body of evidence has been extensive across several key aspects. ACT1 stability has varied significantly depending on treatment conditions in rice fungal infection studies, thereby requiring the selection of treatment-specific reference genes (Richter et al., 2024). Transcript protein relationships have revealed that nearly half of the variation between transcriptomic and proteomic levels has been attributed to translational control (Bauernfeind & Babbitt, 2017). Changes in SOD gene expression have not always correlated with enzyme activity, with studies having identified differential responses among SOD isoforms during fungal infection. Recommendations for integrated approaches have been strongly supported and have successfully employed combined transcriptomic metabolomic analyses to uncover mechanisms that have been overlooked by single-method approaches. Furthermore, expression variability itself has represented meaningful biological information that has often been overlooked in conventional analyses.

This research presents novelty by exploring defense-related gene expression and biochemical responses in *C. nardus* under stress conditions, which remains relatively underexplored compared to major crop species, thereby providing new insights into its molecular adaptation mechanisms. This research contributes to science by enhancing understanding of plant stress-response mechanisms, and to society by supporting the development of stress tolerant plants that can improve agricultural sustainability and potential utilization of bioactive compounds for health and economic purposes. This research supports the SDGs of Good Health and Well being because this research has the potential to increase the production of bioactive compounds from plants that are beneficial for human health.

CONCLUSION

Cymbopogon nardus exhibits stress specific gene expression and enzymatic responses, with WRKY12 PAL pathways dominating under biotic stress, SOD mediating oxidative protection under drought, and HKT1 regulating ionic balance under salinity. These coordinated mechanisms highlight their adaptability and provide a molecular basis for developing stress-tolerant plants through biotechnological approaches. Future research should validate the functional roles of WRKY12, SOD, and HKT1 and investigate their interactions under combined stress conditions to enhance stress tolerance in *Cymbopogon nardus*.

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AUTHOR CONTRIBUTION STATEMENT

Author 1 conceived and designed the study, conducted the data analysis. Author 2 was responsible for data collection, Author 3 assisted in the literature review, supported data visualization, and provided substantial feedback during the manuscript drafting process. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI based technologies.

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