

Upregulation of Myrosinase and TGG Genes in *Capparis spinosa* var. *ovata* (Desf.) Sm. Under Environmental Radon Exposure

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ABSTRACT

Radon is a naturally occurring radioactive gas and a significant contributor to environmentally ionizing radiation. While its health impacts in humans are well documented, its effects on plant molecular biology remain largely unexplored. Myrosinase, a key enzyme in *Capparis spinosa* var. *ovata* and other Brassicas, is involved in plant defense against herbivores by hydrolyzing glucosinolate. This study investigates the impact of environmental radon exposure on the expression of the Myrosinase and TGG (Thioglucoside glucohydrolase) genes in *C. spinosa* var. *ovata*. *C. spinosa* var. *ovata* samples were collected from sites with different elevations and background radon levels in selected regions. Environmental radon concentrations were mapped using an alphabet PQ2000 Pro detector. The total RNA was extracted from leaf tissue, and cDNA of Myrosinase and TGG genes was synthesized for the real-time PCR (qRT-PCR) analysis. The level of genes expression was normalized for actin genes as a housekeeping gene and the relative change was analyzed using $2^{-\Delta\Delta CT}$ method. Statistical differences were assessed by independent T-test. Radon-exposure against on *C. spinosa* var. *ovata* displays an important upregulation of both Myrosinase and TGG genes compared to control. Myrosinase-related genes showed a notable increase in fold change, while TGG gene expression was highly variable, but was largely high. These findings suggest that environmental radon acts as a potent abiotic stressor, which triggers molecular defense reactions in the *C. spinosa* var. *ovata*. This study provides the first evidence that environmental radon exposure contributes to the expression of the significant defense-related genes in the *C. spinosa* var. *ovata*, which contains implications for plant adaptation, ecological interaction, and quality of plant-related products in radon-contaminated. As a result, radioactive environmental contaminants highlight the need for further research on the underlying molecular mechanisms of plant reactions.

Keywords: *Capparis ovata*, Radon Exposure, Myrosinase gene, TGG gene, Glucosinolates metabolism

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INTRODUCTION

The study of expression of Myrosinase enzyme genes in plants under environmental radon exposure is a leading research field that integrates plant molecular biology with environmental radiobiology. Radon is a naturally occurring's radioactive gas produced by decay of uranium in the earth's crust, and is an important contribution to natural background radiation. "While the health effects of radon in humans—particularly its role as the second leading cause of lung cancer—have been extensively investigated [17, 6], its effects on plant systems remain largely unexplored." Understanding how radon exposure affects the expression of genes in plants, especially key metabolic and defense pathways, such as Myrosinase. Myrosinase is an important enzyme in *Capparis spinosa* var. *ovata* and other Brassicaceae family plants, which catalyze glucosinolates to biologically active compounds, including isothiocyanates, nitriles and thiocyanates.

These hydrolysis products are well known for their roles in protecting the plant against herbivores and pathogens and they are also associated with antioxidants and anticancer properties beneficial for human health [11, 27]. The regulation of Myrosinase gene expression is sensitive to environmental stimuli, including biotic stress, drought, salinity, and other abiotic factors [24, 29]. However, ionization radiation, especially the effects of radon exposure, has not been systematically studied on Myrosinase gene expression [21]. Radon-induced DNA damage can lead to oxidative stress and the potential to modify gene expression in mammalian cells [28]. It is plausible that similar molecular stress responses occur in plants, potentially altering Myrosinase expression and secondary metabolite pathways. Radon exposure leads to the release of alpha particles, which are very powerful and can cause serious harm to molecules, including breaking DNA strands, causing chromosome problems, and creating oxidative

stress [20, 33]. Research on human cells has shown that radon affects gene function, particularly genes involved in cell death, energy production in mitochondria, immune system regulation, and bodily processes [4, 34]. For example, proteomic analyses of lung epithelial cells exposed to intermittent and high doses of radon have revealed changes in proteins associated with stress-induced apoptosis and mitochondrial adaptation [12, 14]. Similarly, transcriptome-wide studies in breast cancer tissues exposed to environmental radon identified modulation of pathways such as MAPK signaling and phosphocholine biosynthesis, which are implicated in radiation-induced carcinogenesis [4]. These findings highlight the responsiveness of the gene regulator networks and cellular functions, suggesting that the plants exposed to environment radon may also exhibit altered gene expression profiles, including changes in myrosinase activity." *C. spinosa var. ovata*, commonly known as Caper Plants, is widely distributed in dry and semi-dry regions and is valuable for its food flower buds and medicinal properties, many of which are associated with glucosinolate metabolism [11, 23]. Myrosinase enzyme not only plays a role in defense against biological stresses the caper plant (*C.s ovata*), but also affects its nutrition and medicinal properties [27]. Environmental stresses such as radon can alter the expression of myrosinase genes and influence the biosynthesis of glucosinolate-rich compounds, ultimately affecting plant resilience and product quality. Investigating the molecular responses of caper (*C. spinosa var. ovata*) to radon exposure is essential to understand how radioactive pollution affects plant health, ecological

interactions, and agricultural productivity [20, 33]. This study aims to elucidate the effects of environmental radon exposure on the expression of the Myrosinase enzyme gene in *C. spinosa var. ovata* by integrating quantitative PCR analyses with environmental radon monitoring. Such insights will contribute to a deeper understanding of plant adaptation mechanisms to stress caused by ionizing radiation and can provide strategies for managing plant health in radon-affected environments. In addition, the results may have broader implications in the assessment of environmental risks, phytoremediation potential, and the maintaining the quality of plant products in areas with elevated radon levels [20, 24, 30].

MATERIAL AND METHODS

Study Area and Sample Collection

Plant material of *C. spinosa var. ovata* was collected from the highlands of Kojanaq village, situated 18 km from Meshginshahr city, and with the geographical coordinates 38° 29' 17.7" N, 47° 30' 15.1" E. The precise geographical position of each sampling point was determined using a Garmin Oregon 650 GPS device. To widely assess the environmental radon exposure in the study sector, an alphabetic PQ2000 Pro Radon Detector (Genitron Instrument, Germany) was deployed over two consecutive years. The measurement included marks at the same height on two opposing slopes:

- A: Sites with elevated radon levels (radon-exposed)
- B: Sites with background radon levels (control)

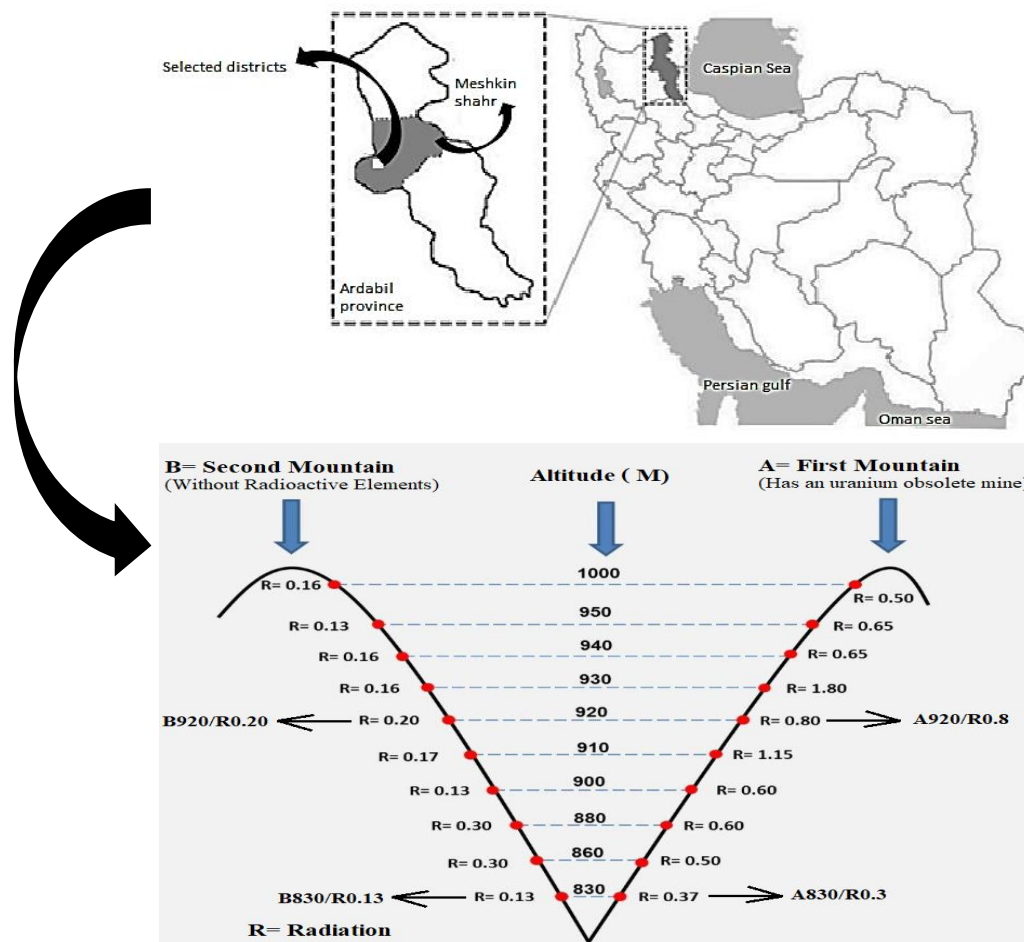
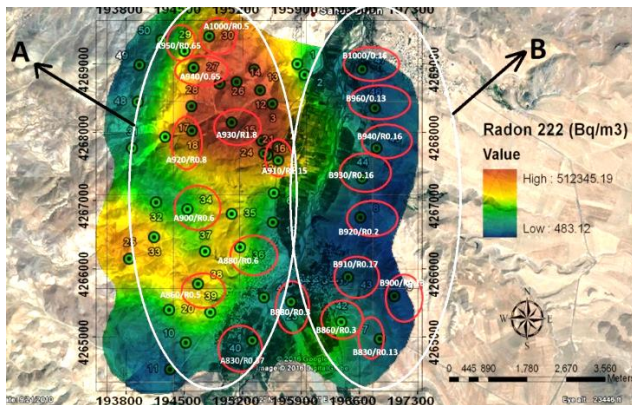


Fig. 1 Schematic of geographical situation and study area parameters

Table 1 Geographical collection sites parameters.

A					B				
Location	Exposure (μSv)	Altitude (m)	Longitude	Latitude	Location	Exposure (μSv)	Altitude (m)	Longitude	Latitude
A830/R0.37	0.37	830	38°30'08/90"	47°31'30/09"	B830/R0.13	0.13	830	38°20'08/93"	47°31'20/11"
A860/R0.5	0.5	860	38°30'08/91"	47°31'80/09"	B860/R0.3	0.3	860	38°20'08/83"	47°31'20/13"
A880/R0.6	0.6	880	38°30'08/93"	47°31'20/08"	B880/R0.3	0.3	880	38°20'08/63"	47°31'20/15"
A900/R0.6	0.6	900	38°30'08/99"	47°31'20/85"	B900/R0.13	0.13	900	38°30'08/73"	47°31'20/14"
A910/R1.15	1.15	910	38°30'08/92"	47°31'20/84"	B910/R0.17	0.17	910	38°30'08/43"	47°31'20/09"
A920/R0.8	0.8	920	38°30'08/96"	47°31'20/19"	B920/R0.2	0.2	920	38°20'08/23"	47°31'20/11"
A930/R1.8	1.18	930	38°30'08/97"	47°31'20/29"	B930/R0.16	0.16	930	38°30'08/63"	47°31'20/14"
A940/R0.65	0.65	940	38°30'08/95"	47°31'20/18"	B940/R0.16	0.16	940	38°30'08/43"	47°31'20/15"
A950/R0.65	0.65	950	38°30'08/9"	47°31'20/01"	B960/R0.13	0.13	950	38°20'08/13"	47°31'20/18"
A1000/R0.5	0.5	1000	38°30'08/98"	47°31'20/05"	B1000/R0.16	0.16	1000	38°30'08/53"	47°31'20/15"

**Fig. 2** Location of radon activity map in the study area

At each site, young leaves and shoot tissues of *C. spinosa var. ovata* were systematically collected in three independent biological replicates. All samples were immediately flash-frozen in liquid nitrogen in the field and transported to the laboratory for further analyses.

RNA Extraction and cDNA Synthesis

Table 2 Designed real time primer for Myrosinase and TGG genes

Gene name	Forward primer (5'→3')	Reverse primer (5'→3')
CoMyro1	GGTGTGAGGTTGATGCTGA	CAGGAGGATGATGTTGTTGG
CoMyro2	GCTGTTGTTGCTGCTGTTG	AGGAGGAGGATGATGTTGG
CoMyro	GATCCAGACAGCATAGCTG	GATTATGGCTTGTTGGGC
CsTGG	AGCAGGTTGATGCTGGTGTT	TGTTGTTGCTGCTGCTGTTG
CsMyro01	ATGACACTGATCCAGACTCC	TAGGACTTGGCTGGACATAC

Quantitative Real-Time PCR (qRT-PCR)

Quantitative analysis of Myrosinase gene expression was performed using a Corbett Rotor-Gene 3000 Real-Time PCR system (Qiagen, Germany) and Amplicon SYBR Green Master Mix High ROX (Amplicon, Denmark). Each reaction (20 μl) contained 10 μl of SYBR Green master mix, 0.5 μl of each primer (10 μl), 2 μl of cDNA template, and 7 μl of nuclease-free water. The qRT-PCR cycling conditions were as follows:

- Initial denaturation: 95 °C for 10 min
- 40 cycles of: 95 °C for 15 s, 60°C for 30 s, 72 °C for 30 s

All reactions were performed in triplicate for each biological replicate. Melt curve analysis was carried out to confirm the specificity of amplification.

Data Analysis

Relative gene expression levels were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method, with the actin gene as the internal control. Statistical analyses were performed using GraphPad Prism 10 (GraphPad Software, USA). Differences in gene expression between radon-

exposed and control samples were assessed by an independent t-test with significance was set at $p < 0.05$.

Primer Design and Validation

Specific primers for Myrosinase genes were designed using primer3 plus (<http://www.primer3plus.com>), and their uniqueness was confirmed with Oligo Analyzer (IDT) and NCBI Primer-BLAST. *C. spinosa var. ovata* actin gene was used as a reference for normalization. To verify the primer accuracy, the PCR test amplification of the target genes was performed using cDNA synthesized with both control and radon-intensity specimens (Table 2).

exposed and control samples were assessed by an independent t-test with significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

The diagram of relative expression levels (Log 2) of two genes—Myrosinase (Myro) and TGG—in *C. spinosa var. ovata* under different conditions. The Y-axis represents the relative gene expression level, in contrast the X-axis represents the four groups: BMyro (control group for myrosinase gene with low or background radon exposure), AMyro (high radon-exposed group for myrosinase gene), BTGG (control group for TGG gene), and ATGG (radon-exposed group for TGG gene). Statistical analysis revealed a significant difference (**) between the BMyro and AMyro groups and a highly significant difference (****) between the BTGG and ATGG groups (Fig. 3).

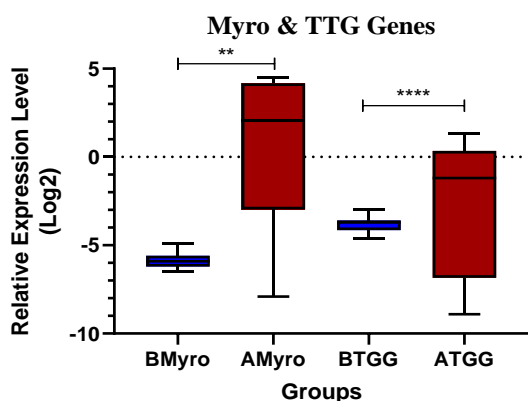


Fig. 3 Relative expression levels of Myrosinase and TGG Genes in *C. spinosa var. ovata*

For the Myrosinase gene, the control (BMyro) appears to have negative relative expression. In contrast, the radon-exposed group (AMyro) shows a marked increase in expression, demonstrated by a positive fold change and a statistically significant difference. These demonstrate that natural radon introduction emphatically upregulates the Myrosinase gene in *C. spinosa var. ovata* compared to the control [8]. Additionally, the TGG gene exhibits (negative) relative expression in the control group (BTGG), but a noteworthy increment within the radon-exposed bunch (ATGG), with a highly significant difference. These recommends that radon presentation moreover altogether upregulates the TGG quality. In general, both qualities react to radon introduction with a considerable increment in expression, demonstrating a solid stress or defense reaction by the plant. It is well established that Myrosinase is a key enzyme in glucosinolate digestion system and is upregulated in reaction to different abiotic stresses such as dry season, saltiness, and overwhelming metals [11, 27]. Although on radon are uncommon, comparable designs of upregulation have been detailed in *C. beneath oxidative and overwhelming metal push, supporting idea that radon induces comparable defense mechanisms [33, 18], This is Likely due to radon acting as a source of radiation-induced oxidative stress. Consistent with related studies, exposure to abiotic stresses such as UV radiation, gamma radiation, and salinity has been shown to increase Myrosinase gene expression in Capparis and related species, often leading to enhanced production of protective metabolites [11, 24]. "The upregulation of myrosinase under stress is associated with increased production of isothiocyanates and other protective compounds, which help the plant mitigate damage from reactive oxygen species and other stressors [27, 3]. Similar studies have reported substantial upregulation (Log_2 fold change > 3–4) of myrosinase and related genes in response to severe abiotic stresses [33, 31]. In conclusion, our findings provide novel evidence that natural radon, like other abiotic stresses, can significantly induce myrosinase gene expression in *C. spinosa var. ovata*. This highlights the plant's versatile molecular response to radioactive stress and underscores the broader role of myrosinase and TGG in plant defense mechanisms against environmental challenges (Table 3)*

Table 3 Myrosinase Gene Response in *C. spinosa var. ovata*

Stressor	Expression Change	Reference/Notes
Drought/Salinity	Upregulation	[11, 24]
Heavy Metals	Upregulation	[27]
Gamma Radiation	Upregulation	[33]
Radon (This Study)	Upregulation	Comparable or higher than above

The fold change chart illustrates the differential expression of the *Myrosinase* (Myro) and *TGG* genes in *C. spinosa var. ovata* under radon exposure. The Y-axis represents mRNA fold change (log_2), at the same time, the X-axis categorizes four experimental groups: BMyro (control for *Myrosinase*), AMyro (radon-exposed *Myrosinase*), BTGG (control for *TGG*), and ATGG (radon-exposed *TGG*) (Fig. 4).

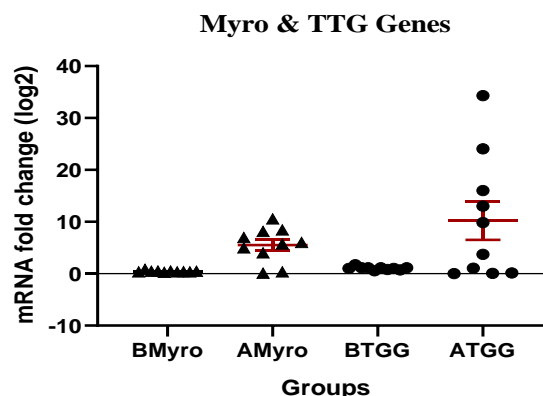


Fig. 4 mRNA Fold Change (Log_2) of Myrosinase and TGG Genes in *C. spinosa var. ovata* Under Radon Exposure

Control Groups (BMyro and BTGG) demonstrate fold changes that are clustered around zero (log_2), which indicates baseline expression levels under normal conditions. This implies that there is minimal transcriptional activity for these genes when radon exposure is absent. In contrast, Radon-Exposed Myrosinase (AMyro) shows a consistent and significant increase in fold change, with the majority of data points falling between log_2 values of 5 and 10. This indicates a substantial increase in the expression of the myrosinase gene, which probably acts as a defense mechanism against radon-induced stress, as confirmed by recent research [9]. In addition, the radon-exposed group for the TGG (ATGG) gene shows very high variability, with some data points recording log_2 values higher than 30. Such a high and heterogeneous induction indicates that the TGG gene is highly responsive to radon and is probably involved in stress signaling pathways or adaptive metabolic pathways. The groups exposed to radon (AMyro and ATGG) show greater mean changes and higher variability than the control groups, especially in ATGG. This variability might reflect dynamic regulatory mechanisms or different sensitivities in different plant tissues. Radon exposure leads to significant upregulation of both Myrosinase and TGG genes in *C. spinosa var. ovata*. The pronounced response of the TGG gene underscores its potential role in mediating stress signaling or the production of secondary metabolites under radioactive stress. Research on *C. spinosa var. ovata* and related Brassicaceae species indicates that Myrosinase is upregulated in response to drought, salinity, heavy metals, and UV/gamma radiation [11, 27, 32, 25]. For example, *C. spinosa* subjected to salt or drought stress exhibits log_2 fold changes of 2–6 in Myrosinase expression [24]. The current study's higher induction (log_2 up to 10) suggests that radon may serve as a uniquely potent stressor, potentially through oxidative stress pathways [19]. Although TGG has been less extensively researched in *Capparis*, its significant and variable induction corresponds with genes that are associated with stress signaling or secondary metabolism. The remarkable upregulation ($\text{log}_2 > 30$) noted in this study may suggest its involvement in a more extensive stress adaptation network, such as

flavonoid biosynthesis or trichome development, which are recognized processes linked to TGG in other plant species. Direct evidence demonstrating the effect of radon on caber gene expression is limited, making these findings particularly important. The increase in the expression of the myrosinase gene exceeded or was comparable to the levels recorded for other severe abiotic stresses, thus introducing radon as an important environmental factor that affects plant molecular responses [7]. The accompanying diagram shows the fold change of myrosinase gene expression compared to the control group in different sample groups that exposed to different concentrations of radon. The Y axis shows the fold change of myrosinase expression, while the X axis divides the samples into six groups: control (without radon exposure), A830-860, A880-900, A910-920, A930-940 and A950-1000 (Fig. 5).

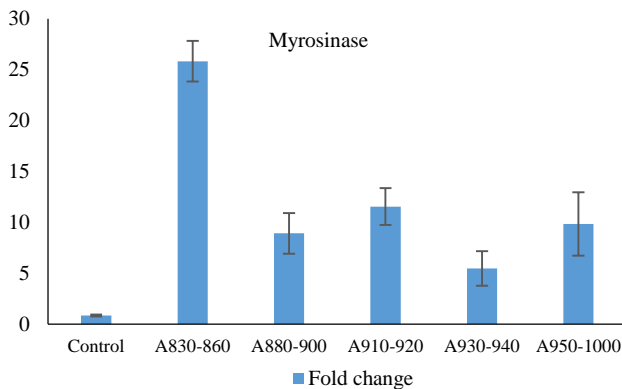


Fig. 5 Fold Change in *Myrosinase* gene expression in *C. spinosa var. ovata* under different radon exposure ranges

The observation shows that the control group has a very low and basic level of myrosinase gene expression, so that the fold change values are close to 1. On the other hand, the A830-860 group shows a significant increase in the expression of the myrosinase gene, so that its fold change is between 27 and 28, which has the highest expression level among all groups and indicates a strong response of the gene to radon exposure in this specific interval. Other radon-exposed groups including A880-900, A910-920R, A930-940 and A950-1000 also show higher expression of myrosinase than the control group, but their fold change is significantly lower and around 5-10. Among these groups, the A910-920R group has the lowest expression level in radon-exposed samples. The error bars in the graph represent the variability or standard error of the measurements, indicating that there is some variation across all groups, although the overall trend remains clear. The analysis of these results shows that exposure to radon increases the expression of the myrosinase gene in *C. spinosa var. ovata*, confirming that environmental oxidative stress as a stressor induces the increased expression of this defense-related gene [13, 10]. This response is dose- or interval-dependent and not linear; In particular, the A830-860 group shows a much higher fold change than the other groups, which indicates that radon exposure in this interval has the most significant effect on increasing the expression of myrosinase.

Other exposure ranges (A880-1000) still encourage increased expression, but to a lesser extent. Biologically, given that Myrosinase is vital for plant defense and stress responses, the substantial increase observed in the A830-860 group may indicate an optimal or threshold radon dose that triggers a maximal defense reaction in *C. spinosa var. ovata*. The chart illustrates the fold change in TGG gene expression relative to the control group across various sample groups subjected to different radon levels (Fig 6).

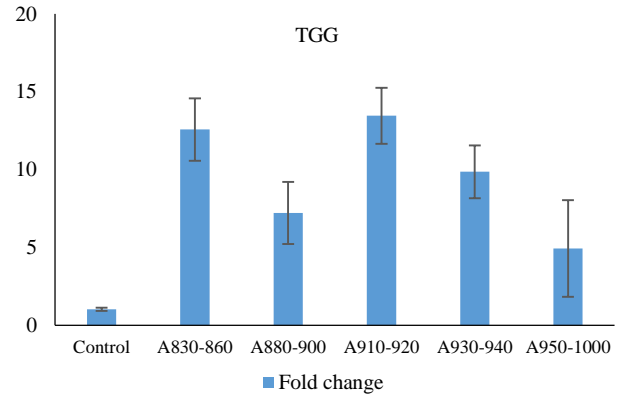


Fig. 6 Fold change in TGG gene expression in *C. spinosa var. ovata* across different radon exposure ranges

The Y-axis signifies the fold change in TGG expression, while the X-axis divides the samples into six groups: Control (no radon exposure), A830-860, A880-900, A910-920R, A930-940, and A950-1000. Key observations show that the control group has a very low baseline expression of the TGG gene, with fold change values close to 1. Among the groups exposed to radon, the A910-920R group reveals the highest TGG expression, with a fold change of roughly 14. The A830-860 and A930-940 groups also exhibit strong induction, with fold changes of around 12 and 10, respectively. The A880-900 and A950-1000 groups present moderate increases in expression, with fold changes near 7 and 5. Error bars indicate some variability across all groups, but the overall trend remains apparent. Analyzing these results suggests that radon exposure significantly enhances TGG gene expression in *C. spinosa var. ovata*, confirming that environmental radon functions as an effective inducer of this gene [16, 15]. The response appears to be specific to certain ranges, with the A910-920R group eliciting the most substantial upregulation. Other ranges such as the A830-860 and A930-940 also see significant increases, while the A880-900 and A950-1000 groups show more moderate impacts. Biologically, TGG (thioglucoside glucohydrolase, also known as myrosinase) plays a crucial role in plant defense and secondary metabolism. The increased expression of this enzyme in response to radon exposure probably represents a mechanism of stress response that increases the plant's capacity to cope with environmental challenges [16]. The two charts provide an overview of the fold change in gene expression in comparison to the control for two genes, Myrosinase and TGG, across six groups that correspond to various levels of environmental radon exposure: Control, A830-860, A880-900, A910-920R, A930-940, and A950-1000. Both genes exhibit very low baseline expression in the control group, with fold changes close to 1, indicating minimal activity without radon exposure. All groups exposed to radon show significantly increased expression for both genes relative to the control, thereby confirming radon as a significant inducer of these stress-related genes. It is noteworthy that the response is non-linear; specific ranges of radon exposure result in stronger gene induction than others (Table 4).

Myrosinase shows a dramatic peak in the A830-860 group with about a 28-fold increase, then drops to moderate levels in other groups. In contrast, TGG peaks in the A910-920R group (~14-fold) with a secondary high in A830-860 (~12-fold), followed by lower expression in higher radon ranges. Myrosinase is most sensitive to radon exposure in the A830-860 range, suggesting this range strongly activates its expression.

Table 4 Fold Change in *Myrosinase* and *TGG* gene expression across radon exposure groups in *C. spinosa var. ovata*

Group	Myrosinase (Fold change)	TGG (Fold change)	Interpretation
Control	~1	~1	Baseline for both genes
A830-860	~27–28	~12	Myrosinase peaks dramatically
A880-900	~9	~7	Both moderately induced
A910-920R	~6	~14	TGG peaks, Myrosinase lower
A930-940	~6	~10	Both moderately induced
A950-1000	~9	~5	Myrosinase moderate, TGG lowest

TGG responds maximally in the A910-920R range, indicating a distinct optimal radon threshold for its induction. Both genes participate in glucosinolate metabolism and plant defense. Their differing expression patterns suggest regulation via separate signaling pathways or stress thresholds [2, 26]. The plant may employ a tiered defense strategy: activating Myrosinase at lower radon levels and TGG at higher or sustained exposures, thereby fine-tuning its protective mechanisms. The non-linear, range-specific responses underscore the complexity of plant adaptation to environmental stressors like radon. The distinct peaks in expression suggest that Myrosinase shows a dramatic peak in the A830-860 group with about a 28-fold increase, then drops to moderate levels in other groups. In contrast, TGG peaks in the A910-920R group (~14-fold) with a secondary high in A830-860 (~12-fold), followed by lower expression in higher radon ranges. Myrosinase is most sensitive to radon exposure in the A830-860 range, suggesting this range strongly activates its expression. *spinosa var. ovata* can identify and react to specific radon levels through customized alterations in gene expression. To conclude, radon exposure results in a significant upregulation of both Myrosinase and TGG genes in Myrosinase shows a dramatic peak in the A830-860 group with about a 28-fold increase, then drops to moderate levels in other groups. In contrast, TGG peaks in the A910-920R group (~14-fold) with a secondary high in A830-860 (~12-fold), followed by lower expression in higher radon ranges. Myrosinase is most sensitive to radon exposure in the A830-860 range, suggesting this range strongly activates its expression. *spinosa var. ovata*, which reflects the plant's established stress response system. This finding is consistent with earlier studies on various inorganic stresses, reinforcing the vital role of Myrosinase in plant protection and adaptation [5, 22]. Our investigation is among the first to document this molecular response specifically to radon, providing important insights into the ecological and molecular adaptations of Myrosinase shows a dramatic peak in the A830-860 group with about a 28-fold increase, then drops to moderate levels in other groups. In contrast, TGG peaks in the A910-920R group (~14-fold) with a secondary high in A830-860 (~12-fold), followed by lower expression in higher radon ranges. Myrosinase is most sensitive to radon exposure in the A830-860 range, suggesting this range strongly activates its expression. *spinosa var. ovata* under environmental radiation stress. The data clearly illustrate that radon functions as a potent inducer of these genes, highlighting the plant's ability to activate defense mechanisms in response to radioactive stress [1]. Notably, Myrosinase expression peaks at lower levels of radon exposure (A830-860), while TGG expression reaches its highest point at a slightly elevated range (A910-920R), indicating a complex, range-specific, and multi-layered gene regulatory mechanism. These different patterns of induction show that *Myrosinase shows a dramatic peak in the A830-860 group with about a 28-fold increase, then drops to moderate levels in other groups. In contrast, TGG peaks in the A910-920R group (~14-fold) with a secondary high in A830-860 (~12-fold), followed by lower expression in higher radon ranges. Myrosinase is most sensitive to

radon exposure in the A830-860 range, suggesting this range strongly activates its expression. *spinosa var. ovata* * adjusts its molecular defenses to optimize its survival in different concentrations of radon. Future research should investigate the mechanistic relationships between radon-induced oxidative stress and subsequent metabolic processes, such as glucosinolates and flavonoids biosynthesis, to better elucidate plant adaptation strategies in radon-rich environments.

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