

Original Research

Effects of Plastoquinone Derivative 10-(6'-Plastoquinonyl) Decyltriphenylphosphonium on Rice Seeds Grown under Complete Flooding Conditions

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Academic Editor: Penna Suprasanna

Special Issue: [Plant Genetics and Mutation Breeding](#)

OBM Genetics

2025, volume 9, issue 3

doi:10.21926/obm.genet.2503305

Received: February 20, 2025

Accepted: July 20, 2025

Published: July 22, 2025

Abstract

The present work aimed to study the effects of 10-(6'-plastoquinonyl) decyltriphenylphosphonium (SkQ1) on rice (*Oryza sativa*) plants germinating under flooding conditions. The influence of different concentrations of SkQ1 (mitochondria-targeted antioxidant) on resistance to complete flooding was assessed on rice seedlings of the Kuboyar variety. The total level of reactive oxygen species (ROS), H₂O₂-induced chemiluminescence, and gene expression of antioxidant enzymes (*OsSODA* (superoxide dismutase A), *OsSODB* (superoxide dismutase B), *OsCATA* (catalase A), *OsCATB* (catalase B), *OsCATC* (catalase C), *OsAPX1* (ascorbate peroxidase)), as well as indexes of germination, fresh and dry weight of roots and shoots, were determined. The plastoquinone derivative SkQ1 increased the submergence (hypoxia) tolerance of rice seedlings at nanomolar concentrations. The results



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indicated that SkQ1 protects rice seedlings by maintaining redox homeostasis. ROS content in seedlings revealed that the introduction of SkQ1 in nanomolar (10^{-9} M and 10^{-8} M for the shoot and 10^{-9} M for the root) concentrations led to a decrease in the total level of oxygen radicals compared to the control. On the other hand, a further increase in SkQ1 concentration induced a dose-dependent increase in the amount of ROS in plant tissues, which indicates its pro-oxidant activity. The 10^{-5} M SkQ1 increased the ROS content in the root by 2.8 times compared to the control, and by 5.1 times compared to normoxia. At a concentration of 10^{-5} M, SkQ1 exhibits pro-oxidant activity, expressed in the suppression of shoot growth and almost blocked root development. Thus, SkQ-type compounds are promising for use in agriculture as universal protectors and growth stimulants for sustainable development.

Keywords

SkQ1; *Oryza sativa*; flooding; hypoxia; reactive oxygen species

1. Introduction

Changes in the global environment lead to a combination of abiotic and biotic stresses that impact agricultural productivity [1]. Extreme factors have a significant impact on plant development by altering their physiology, disrupting biochemical reactions, including the synthesis of secondary metabolites, and affecting the transcriptional activity of genes [2]. Flooding is a significant environmental stressor that affects numerous artificial and natural ecosystems worldwide, disrupting fundamental plant physiological processes, including water uptake, respiration, and photosynthetic activity. Crop losses due to flooding depend on the plant species and age, soil type, and duration of flooding. Numerous crops, such as rice, are semi-aquatic plants. Deep-water rice is an ancient cultivated crop that originated in Asia and Africa among the *Oryza sativa* and *Oryza glaberrima* species [3]. The molecular mechanisms underlying the induction of the flooding response in *O. glaberrima* are similar to those found in the deep-water rice *O. sativa* [4]. Rice flood tolerance is governed by a combination of physiological adaptations like aerenchyma formation and stem elongation, and key genetic factors including *SUB1A*, *SNORKEL1*, *SNORKEL2*, *OsTPP7*, and anaerobic metabolism genes that together enable survival and recovery from flooding stress [5].

Flooding rice fields provides plants with sufficient water and nutrients; however, it can also kill plants if the water level is too high or the flooding lasts too long. Approximately 30% of the total rice-growing area, which amounts to 12–14 million hectares, is prone to flash flooding [6]. Despite the knowledge of physiological processes occurring in plants and regulation at the molecular level, the understanding of the mechanisms underlying plant responses to flooding remains poorly understood. Studies in *Arabidopsis* [7] and rice [8] have shown the presence of multiple genes associated with the flooding response, suggesting that the regulation of flood tolerance in plants is highly complex.

Genetic variation in flood response encompasses a dormancy strategy that enables plants to withstand prolonged periods of submergence, water avoidance through stem elongation, and alterations in plant morphology, anatomy, and metabolic responses [9]. Studies on the mechanism of flood survival in wild species and modern rice have provided significant insights into the

evolutionary, physiological, and molecular strategies for surviving floods and waterlogging. The dormancy strategy involves the accumulation of carbohydrates and energy during prolonged flooding, followed by the restoration of normal growth and development after the flood subsides. In contrast, the avoidance strategy is characterized by a set of anatomical and morphological traits that enable gas exchange between submerged and non-submerged plant organs through their elongation [10]. Investigations into the physiological mechanisms of this adaptation have shown that gibberellin (GA), abscisic acid (ABA), and ethylene are the major plant hormones involved in the elongation of deep-water rice [11, 12]. Physiological studies have shown a relationship between deep-water elongation and the ethylene response. With complete immersion, there is a rapid increase in intra-lacunar ethylene, while the use of an ethylene inhibitor suppresses internode elongation. Similarly, low oxygen sensitivity stimulates internode elongation. During reoxygenation after a period of oxygen starvation, ethanol remaining in the tissues is converted to acetaldehyde, which causes cell damage. In addition, under reoxygenation or oxygen starvation conditions, ROS accumulate excessively under light conditions [13].

Oxygen deficiency and low light intensity are two significant factors that limit the survival of rice plants under long-term submergence, due to the inability of plants to develop new leaves and severe damage to existing leaves [14]. In addition, submerged plants are often deprived of oxygen and good light, which leads to the formation of ROS such as hydrogen peroxide, hydroxyl radical, and superoxide anion, which, if not inhibited, can seriously damage the cellular organization, possibly leading to plant death [15]. However, rice varieties that are effective in inhibiting ROS after submergence are characterized by the ability to retain chlorophyll, maintain plant growth, regenerate new leaves, and preserve old leaves. Therefore, rice plants protect themselves from oxidative damage through two mechanisms: the presence of an antioxidant enzyme system and natural antioxidants [16]. Natural low-molecular-weight antioxidant compounds include phenols, alpha-tocopherol, ascorbate, carotenoids, and glutathione. Compared to other antioxidants, ascorbate has been more extensively studied in plants, especially regarding its biosynthesis, metabolism, and role in stress responses. This depth of knowledge makes it a focal point for understanding plant antioxidant defense under hypoxia and other stresses [17]. High levels of ascorbate content in the root system were observed under hypoxic conditions, and the concentration decreased with repeated aeration. Thus, the search for new antioxidants that reduce ROS levels remains an urgent task [18].

Numerous studies have been published proving that mitochondria are one of the prominent organelles of the cell where free radicals are produced. It is the mitochondria that, when subjected to oxidative damage, can activate signaling pathways that lead to cell death, which, in turn, leads to tissue destruction and pathological changes in the functioning of organs and systems [19]. For this reason, the task of scientific research is to find new and improve the effectiveness of existing antioxidants, ensuring their targeted accumulation in the mitochondria.

The lipophilic cation 10-(6'-plastoquinonyl) decyltriphenylphosphonium or SkQ1 is well known as a mitochondria-targeted antioxidant with broad protective properties [20]. Its activity has been confirmed in numerous studies, often performed on animals, less often on microorganisms used as model objects [21-25]. Rare experimental studies have shown the protective properties of SkQ for plants. The results of these studies indicate that cationic derivatives of plastoquinone in pico- and nanomolar concentrations prevent the formation of ROS in plant mitochondria, protect plant cells from programmed cell death, stimulate the regeneration of potato microplants in vitro culture,

improve the crop structure, and increase the productivity of spring wheat *Triticum aestivum* [26, 27].

In this work, we studied the effects of SkQ1 on rice seedlings. The herbicide-free technology of rice cultivation implies obtaining seedlings from under a layer of water, while the lack of oxygen (hypoxia), caused by the death of weeds, negatively affects the seedlings of the crop itself, which ultimately leads to a decrease in yield [28]. A common consequence of the influence of most abiotic stressors on organisms, including hypoxia, is an increase in the intracellular concentration of reactive oxygen species (ROS) [29]. Moreover, one of the latest studies, based on the data of complete transcriptome and metabolome analysis, concluded that plant resistance to oxidative stress is the main factor in resistance to flooding and anaerobiosis [30]. Thus, there is a serious reason to believe that the mitochondria-targeted antioxidant SkQ1 can increase the resistance of rice seedlings to flooding and, as a result, increase crop productivity. The purpose of this work is to study the effect of SkQ1 on rice plants germinating under flooding conditions.

2. Materials and Methods

2.1 The Aim of Research and Methodology

To assess the effect of SkQ1 on resistance to water flooding, rice seeds (*Oryza sativa*) of the Kuboyar variety, kindly provided by Prof. Kostylev P.I. from FGBNU "Agrarian Scientific Centre "Donskoy", were germinated under a 10 cm layer of water in laboratory beakers. The concentration of O₂ in the water was measured using a Clark electrode. SkQ1 was added to the water where experimental groups of plants were grown once to final concentrations of 10⁻⁹ – 10⁻⁵ M. The concentration range of SkQ1 from 10⁻⁹ M to 10⁻⁵ M was chosen based on prior experimental evidence demonstrating efficacy and safety at low nanomolar to micromolar levels, as well as pilot studies identifying optimal dosing that balances antioxidant benefits with toxicity risks. It has been estimated that SkQ1 molecules accumulate in mitochondria at concentrations up to 8 orders of magnitude higher than in the surrounding medium [31]. Experimental work on animals tested SkQ1 concentrations roughly corresponding to 10⁻⁸ M to 10⁻⁶ M (12 ng/mL to 1200 ng/mL) [32]. Thus, even low extracellular concentrations of SkQ1 can lead to an increase in its level in mitochondria, justifying the use of low nanomolar to micromolar doses in experiments.

Plants grown under flooding conditions (control) and under normal aeration conditions (water level <1 cm) without adding SkQ1 were used as a comparison. Rice was grown in a KBWF720 phytotron (Binder, Germany) under fluorescent lamps at 38 W/m² with a 14-hour light/10-hour dark cycle and a temperature of 26°C. After 7 days, the germination rate, fresh and dry weight of roots and shoots of rice seedlings were measured.

2.2 Analysis of Total Level of Reactive Oxygen Species

The total level of reactive oxygen species (ROS) was determined using a fluorescence test based on the formation of dichlorofluorescein from non-fluorescent dichlorofluorescein diacetate [33]. Briefly, the fresh plant material (0.5 g samples) was ground in a porcelain mortar in liquid nitrogen with the addition of 2 ml of 0.2 N HClO₄ and subsequent neutralization with 37-38 µl of 4 M KOH. Next, 25 µl of the supernatant and 25 µl of a 0.5 mM dichlorofluorescein diacetate solution were successively added to 950 µl of 0.15 M Tris-HCl buffer (pH 7.5) and mixed. All samples were

incubated for 20 min in a thermostat at 37°C, after which fluorescence spectra were recorded ($\lambda_1 = 496$ nm, $\lambda_{\text{obs}} = 524$ nm) on a Spectrofluorophotometer (Shimadzu RF-5301). The ROS content was calculated from the amount of dichlorofluorescein formed ($\mu\text{g g}^{-1}$ fresh weight (FW)), the concentration of which was determined using a calibration curve.

2.3 Chemiluminescence Analysis

H₂O₂-induced luminol-dependent chemiluminescence analysis was performed on an AutoLumat Plus LB 953 device (Berhold Technologies, Germany). A weighed portion of plant tissue (5 mg) was homogenized in 1 ml of Tris-HCl buffer (0.1 M, pH 7.4). The homogenate was centrifuged at 5000 g for 10 minutes. The reaction mixture containing 2 ml of 5×10^{-4} M luminol solution (5-amino-2,3-dihydro-1,4-phthalazinedione, Sigma) in Tris-HCl buffer (0.1 M, pH 7.4) and 0.2 ml of supernatant was incubated in a thermostat at 25°C for 10 min. Then 0.45 ml of H₂O₂ (0.35 M) solution was added to the cuvette with the reaction mixture, and the CL kinetics were recorded for 100 seconds with a step of 0.1 sec. When evaluating the kinetograms, such informative parameters as the maximum fast flash (I_{max}) and the light sum (S) of the CL reaction were used.

2.4 RNA Extraction, Reverse Transcription, and Gene Expression Analysis

Total RNA was isolated from leaf and root tissue using the commercial ExtractRNA kit (Eurogen, Russia) [34]. The isolated RNA was treated with DNase using the commercial DNase I, RNase-free kit (Thermo Fisher Scientific, USA). The reverse transcription reaction was performed using the commercial MMLVRT kit (Eurogen, Russia). For the reverse transcription procedure, 4 μl of the isolated RNA was mixed with 0.5 μl of specific primers (20 μM) and the mixture was heated for 2 min at 70°C. The samples were then placed on ice. Next, a pre-prepared mixture consisting of 5 $\times$ buffer, dNTP (10 mM), dithiothreitol (20 mM), and MMLV reverse transcriptase was added to each tube. The mixture was incubated for 60 minutes at 39°C. The reaction was stopped by heating the mixture at 70°C for 10 minutes. Amplification was performed using a QuantStudio 5 thermal cycler (Applied Biosystems). The thermal regime was: initial denaturation at 94°C for 3 min, then 35 cycles: 95°C (10 sec), 60°C (30 sec), 70°C (30 sec), final elongation - 2 min at 70°C. The $2^{-\Delta\Delta\text{Ct}}$ method was used to calculate the quantitative change in gene transcription. Normalization was performed using *OsActin*. For our study, we selected isoforms of the main antioxidant enzymes involved in ROS detoxification as genes of interest. The design of primers for gene sequences was carried out using the Primer3 program. The specificity of the primers was checked using the Blast tool (NCBI). The sequences of the primers are presented in Table 1.

Table 1 Primer sequences for real-time quantitative PCR.

	Gene name	Accession No.	Sequence (5'→3')
1	<i>OsSODA</i>	NM_001402510.1	5'-ATCTGGATGGGTGTGGCTAGCTTT-3' 5'-AGTACGCATGCTCCCAGACATCAA-3'
2	<i>OsSODB</i>	NM_001402741.1	5'-TCCGCCGTATAAACTTGATGCCCT-3' 5'-TGGGTTGCCGTTGTTGTATGCTTC-3'
3	<i>OsCATA</i>	NM_001401748.1	5'-CAACCGCAACGTCGACAACTTCTT-3' 5'-TTCACCGGCAGCATCAGGTAGTTT-3'

4	<i>OsCATB</i>	NM_001402963.1	5'-GCTTGCTTTCTGCCCAGCGATAAT-3' 5'-AAATAGTTTGGGCCAAGACGGTGC-3'
5	<i>OsCATC</i>	KY752529.1	5'-AGAAGGTGGTGATTGCCAAGGAGA-3' 5'-TCTCTTGATGAACCGGTCTTGCCT-3'
6	<i>OsAPX1</i>	NM_001402136.1	5'-AGGTGCCACAAGGAAAGATCTGGT-3' 5'-TCAGCAGGGCTTTGTCACTAGGAA-3'
7	<i>OsAPX2</i>	NM_001403284.1	5'-TGGGAAGATGCCACAAGGAGAGAT-3' 5'-TCCGCAGCATATTTCTCCACCAGT-3'
8	<i>OsActin</i>	NM_001418593.1	5'-ACAGGTATTGTGTTGGACTCTGG-3' 5'-AGTAACCACGCTCCGTCAGG-3'

2.5 Statistical Analysis

The data are presented as mean values with standard deviation and were estimated based on the results of 6 independent repetitions. The significance of the differences in the data obtained in the experimental and control groups was assessed using the Student's t-test. qPCR data were evaluated using the nonparametric Mann-Whitney U-test in the R-Studio program. The results were considered reliable at a significance level of $p < 0.05$.

3. Results

3.1 Analysis of Seed Germination and Biomass Accumulation of Rice Seedlings

The seed germination rates and the growth rate of the roots and shoots of rice seedlings under complete flooding and in the presence of SkQ1 are shown in Table 2. The results showed that the addition of 10^{-9} M SkQ1 increased seed germination and completely neutralized the negative impact of hypoxia on the growth rate of the above-ground part of plants. At a concentration of 10^{-8} M of the cationic plastoquinone derivative, the fresh and dry weights of rice seedlings statistically significantly exceeded those of plants grown not only under flooding but also under regular aeration. The fresh weight of roots treated with 10^{-8} M SkQ1 was 24% higher than that under normoxia and more than twice as high as the fresh weight of rice roots grown under hypoxia. However, further increasing the concentration of SkQ1 led to a decrease in the growth rate. Moreover, at a concentration of 10^{-5} M, SkQ1 almost completely inhibited the development of the root system (Table 2).

Table 2 Effect of different concentrations of SkQ1 on seed germination and biomass accumulation of rice seedlings under complete flooding conditions.

Experiment condition	Germination, %	Fresh weight, mg		Dry weight, mg	
		Shoot	Root	Shoot	Root
Control (flooding, O ₂ concentration - 9.2 mg/L)	83.5 ± 2.57	10.7 ± 0.20	4.1 ± 0.42	1.4 ± 0.03	0.8 ± 0.04

Normoxia (O ₂ concentration ~ 298.9 mg/L)	83.3 ± 1.00	15.5 ± 0.32*	6.7 ± 0.37*	1.7 ± 0.09*	0.9 ± 0.06
Flooding (O ₂ - 9.2 mg/L) + SkQ1, M					
10 ⁻⁹	92.0 ± 1.56*	14.6 ± 0.36*	5.4 ± 0.64*	1.6 ± 0.04*	0.7 ± 0.08
10 ⁻⁸	93.3 ± 1.20*	16.8 ± 0.54*	8.3 ± 0.63*	2.0 ± 0.06*	1.2 ± 0.05*
10 ⁻⁷	98.2 ± 1.70*	15.0 ± 0.30*	5.0 ± 0.83	1.5 ± 0.04	0.6 ± 0.10
10 ⁻⁶	83.0 ± 3.72	14.9 ± 0.33*	4.6 ± 0.33	1.4 ± 0.07	0.6 ± 0.04*
10 ⁻⁵	66.7 ± 1.66*	3.0 ± 0.47*	0.1 ± 0.11*	1.2 ± 0.04*	-

* Statistically significant effects (p < 0.05)

3.2 Analysis of Total Reactive Oxygen Species (ROS) Level

The ROS level in the control flooded seedlings was 53.6 µg g⁻¹ FW in shoots and 226.2 µg g⁻¹ FW in roots. The ROS content in rice seedlings under regular aeration was 33.4 µg g⁻¹ FW in shoots and 119.5 µg g⁻¹ FW in roots. The addition of SkQ1 at concentrations of 10⁻⁹ M and 10⁻⁸ M for shoots and 10⁻⁹ M for roots resulted in a decrease in the total oxygen radical level compared to the control (Figure 1). On the other hand, a further increase in SkQ1 concentration induced a dose-dependent increase in the amount of ROS in plant tissues. For example, 10⁻⁵ M SkQ1 increased the ROS content in the roots by 2.8 times compared to the control, and by 5.1 times compared to normoxia.

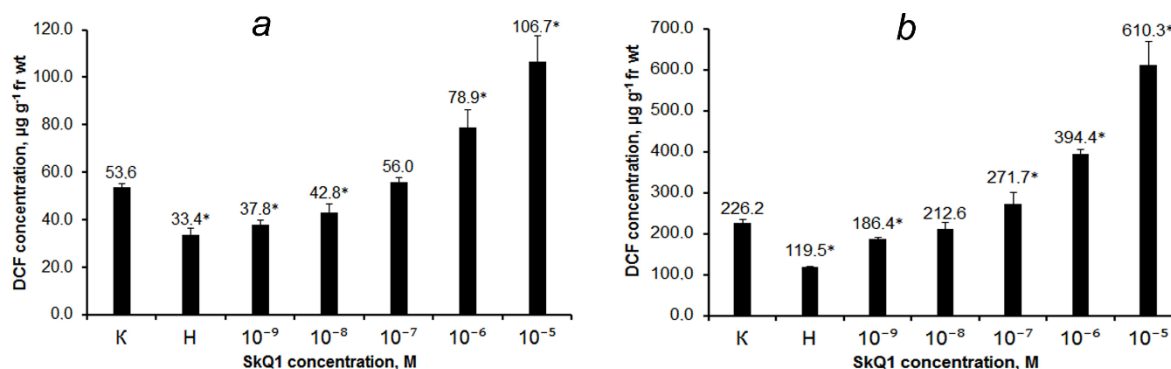


Figure 1 Effect of different concentrations of SkQ1 on the total ROS level in 7-day-old rice seedlings: a – shoots, b – roots. K – control (flooding), O₂ concentration - 9.2 mg/L. H – normoxia, O₂ concentration ~ 298.9 mg/L. * – Statistically significant effects (p < 0.05).

3.3 Chemiluminescence Analysis

Comparative data on the ability of different SkQ1 concentrations to influence the intensity of chemiluminescence in rice seedlings are presented in Figure 2. With an increase in SkQ1 concentration, both in the roots and shoots of rice, there was a decrease in the indices of H₂O₂-luminol-induced chemiluminescence (Figure 2). Thus, the maximum intensity of the fast flash and the light sum of chemiluminescence in plants grown in the presence of SkQ1 (10⁻⁵ M) for the shoot were almost four times lower than in the control group. For the root, these parameters were reduced by 7.9 and 18.8 times, respectively. At a SkQ1 concentration of 10⁻⁸ M, which showed the

most significant effect according to the results of the pot experiment, the level of the chemiluminescent signal in the seedlings was approximately 1.5 times lower compared to the control. Still, it did not statistically significantly differ from that of plants grown under normal aeration conditions (Figure 2).

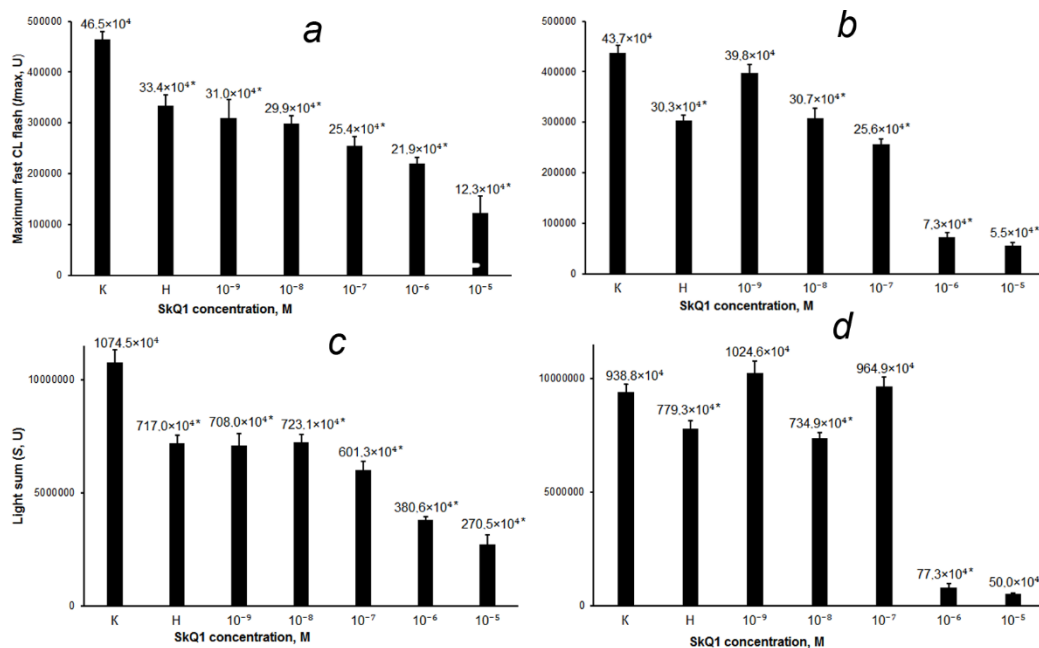


Figure 2 Effect of different concentrations of SkQ1 on the chemiluminescence parameters of 7-day-old rice seedlings: a,c – shoots, b,d – roots, K – control (flooding), O₂ concentration - 9.2 mg/L. H – normoxia, O₂ concentration ~ 298.9 mg/L. * – Statistically significant effects (p < 0.05).

3.4 Gene Expression Analysis

In shoots, the expression level of superoxide dismutase isoform genes, *OsSODA* and *OsSODB*, under normal aeration conditions was 36% lower than in the control (complete flooding), while in root tissues their activity was two times lower (Figure 3, Figure 4). The introduction of SkQ1 at concentrations of 10⁻⁹ M and 10⁻⁸ M led to a significant decrease in the transcription of *OsSODA* and *OsSODB*, both in roots and shoots. The exception was the expression level of *OsSODA* in the shoot at 10⁻⁹ M SkQ1, which did not differ from the control (Figure 3). The greatest, sixfold, decrease was observed for *OsSODB* upon the introduction of 10⁻⁸ M SkQ1.

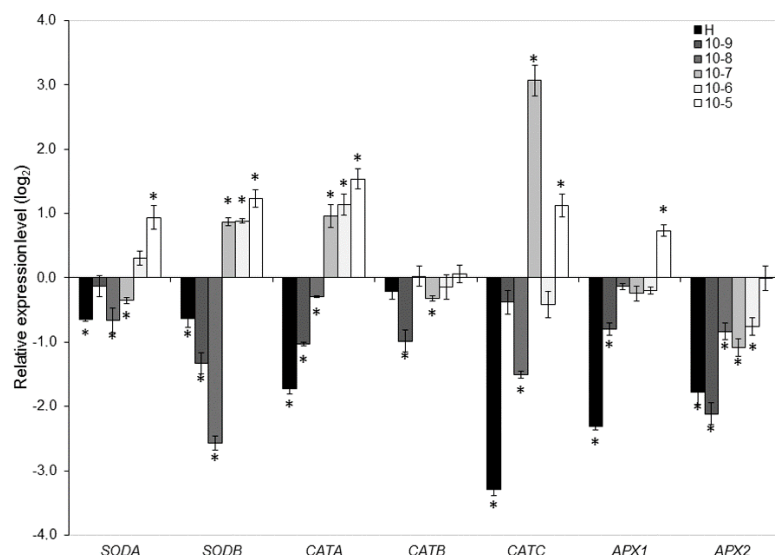


Figure 3 Effect of different concentrations of SkQ1 (10^{-9} – 10^{-5} M) on the gene expression of antioxidant enzymes in shoot of 7-day-old rice seedlings: H – normoxia, O_2 concentration ~ 298.9 mg/L. *OsSODA* (superoxide dismutase A), *OsSODB* (superoxide dismutase B), *OsCATA* (catalase A), *OsCATB* (catalase B), *OsCATC* (catalase C), *OsAPX1* (ascorbate peroxidase 1), *OsAPX2* (ascorbate peroxidase 2). The expression level of target genes in treated plants is presented relative to their expression level in the control (flooding, O_2 concentration - 9.2 mg/L). * – Statistically significant effects ($p < 0.05$).

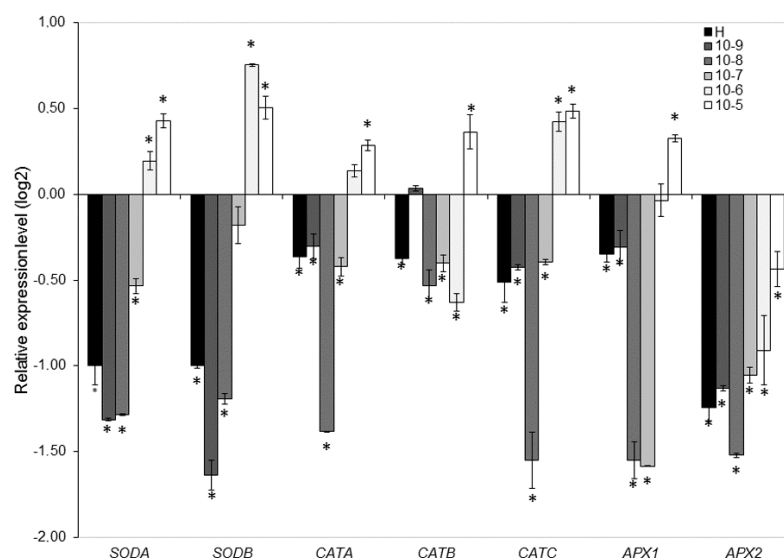


Figure 4 Effect of different concentrations of SkQ1 (10^{-9} – 10^{-5} M) on the gene expression of antioxidant enzymes in roots of 7-day-old rice seedlings: H – normoxia, O_2 concentration ~ 298.9 mg/L. *OsSODA* (superoxide dismutase A), *OsSODB* (superoxide dismutase B), *OsCATA* (catalase A), *OsCATB* (catalase B), *OsCATC* (catalase C), *OsAPX1* (ascorbate peroxidase 1), *OsAPX2* (ascorbate peroxidase 2). The expression level of target genes in treated plants is presented relative to their expression level in the control (flooding, O_2 concentration - 9.2 mg/L). * – Statistically significant effects ($p < 0.05$).

It is worth noting that under the influence of 10^{-9} M and 10^{-8} M, the transcription level of *OsSODA* and *OsSODB* was also lower in most cases than in seedlings under normal aeration conditions. When SkQ1 was added at concentrations of 10^{-6} M and 10^{-5} M, *OsSODA* and *OsSODB* were highly expressed in seedlings. Similar results were observed when analyzing the expression of the *OsCATA* and *OsCATC* genes encoding catalase isoforms. The addition of 10^{-9} M SkQ1 stabilized the transcription of *OsCATA* and *OsCATC* in the roots of flooded seedlings to levels observed under normal aeration conditions (Figure 4). The most significant decrease (more than 60%) in the activity of these genes was observed when SkQ1 was applied at a concentration of 10^{-8} M. An increase in the SkQ1 concentration to 10^{-6} M and higher caused a reliable increase in the regulation of *OsCATA* and *OsCATC*. An increase in the transcriptional activity of *OsCATA* and *OsCATC* was also observed in shoots under the influence of high doses of SkQ1. At the same time, a decrease occurred relative to the control under the influence of low concentrations of this compound.

The expression level of the *OsCATB* gene, with some exceptions, did not differ from the control values (Figure 3). In root tissues, SkQ1 at concentrations from 10^{-8} M to 10^{-6} M reduced *OsCATB* expression to the level characteristic of normal aeration conditions (Figure 4). A dosage of 10^{-5} M increased *OsCATB* activity by 30% relative to the control. A significant change in the activity of the ascorbate peroxidase isoform gene, *OsAPX1*, was detected in green tissue only under the influence of SkQ1 at concentrations of 10^{-9} M (a decrease of 42%) and 10^{-5} M (an increase of 66%) (Figure 3). It is worth noting that the level of *OsAPX1* expression under normoxia was 80% lower than under control conditions. In the root, the level of *OsAPX1* transcription under normoxia and with the introduction of 10^{-9} M SkQ1 did not differ, but was 20% lower than in the control plants (Figure 4). Application of SkQ1 at concentrations of 10^{-8} M and 10^{-7} M decreased the transcriptional activity of *OsAPX1* by more than 65%. On the contrary, a dosage of 10^{-5} M SkQ1 caused an increase in *OsAPX1* expression. At all studied concentrations, SkQ1 decreased *OsAPX2* gene transcripts (Figure 3, Figure 4). The exception was shoots under the influence of 10^{-5} M SkQ1, where the transcription level did not differ from the control. The most significant decrease in *OsAPX2* expression was shown in shoots upon application of 10^{-9} M SkQ1, and in roots upon addition of 10^{-8} M SkQ1. However, in both cases, the transcription level was similar to that under normoxia.

4. Discussion

During the assessment of the effect of various SkQ1 concentrations on rice resistance to complete flooding, it was found that this compound at a concentration of 10^{-9} M eliminated the negative impact of hypoxia on the growth of plant biomass and also increased seed germination. Moreover, at a concentration of 10^{-8} M, the values of fresh and dry weight of rice seedlings exceeded those of plants grown not only under flooded conditions, but also under regular aeration. It should be noted that the level of biomass growth is an integral characteristic reflecting the influence of environmental conditions on living systems, and the accumulation of aboveground biomass by plants is closely related to their economic productivity and varies significantly depending on the influencing factors [35]. Thus, the effect of SkQ1 at a concentration of 10^{-8} M on the germination of rice seeds underwater can be assessed as positive. At the same time, a further increase in the SkQ1 concentration led to a decrease in growth indicators. At a concentration of 10^{-5} M SkQ1, it almost completely inhibited the development of the root system. It is important to note that the difference in concentrations between the onset of stimulation and growth suppression was

four orders of magnitude. To date, there are few studies on the use of exogenous compounds to increase the resistance of rice to flooding. For example, the treatment of rice with natural antioxidants chaetoglobosin A and hydroxystyryl formamide increased rice resistance to flooding by modulating the redox status of plants [36]. Soaking the seeds in chitosan oligosaccharides stabilized rice development under flooding by altering hormonal regulation and antioxidant protection [37]. Treatment with phenolic acids reduced malondialdehyde levels and increased antioxidant enzyme activity [38]. Treatment of rice seeds with the natural antioxidant melatonin also significantly improved resistance to flooding [39]. It is worth noting that in the studies mentioned, protective compounds were used in relatively high concentrations.

The mechanism of action of SkQ1 is known to be based on effective penetration and accumulation in the mitochondria due to its lipophilicity and positive charge. In mitochondria, SkQ1 inhibits ROS formation in two ways. The first consists of the direct reduction of radicals due to the oxidation of plastoquinone in the SkQ1 molecule [40]. The second is due to a slight decrease in the transmembrane potential, which occurs because SkQ1, being a lipophilic cation, ensures the transport of fatty acid anions across the mitochondrial membrane [41]. Another essential property of SkQ1 is that after oxidation it is quickly reduced by the complex III of the electron transport chain (ETC), thereby maintaining a constant pool of reduced SkQ1 in the mitochondria. It is the property of renewal that can explain the high efficiency of SkQ1 when used at such low doses.

A study of the ROS content in rice seedlings found that the introduction of SkQ1 in nanomolar (10^{-9} M and 10^{-8} M for the shoot and 10^{-9} M for the root) concentrations led to a decrease in the total level of oxygen radicals compared to the control. In contrast, a further increase in the SkQ1 concentration induced a dose-dependent increase in the amount of ROS in plant tissues, which indicates its pro-oxidant activity. It has also been previously demonstrated that at concentrations exceeding one micromole, SkQ1 begins to act as a pro-oxidant [42]. This phenomenon likely arises from SkQ1's ability at higher concentrations to disrupt electron transport in the ETC, leading to increased electron leakage [43]. The SkQ1 redox cycle is associated with complexes II and III of the ETC, and therefore, excess SkQ1 can overload these complexes, paradoxically promoting ROS formation rather than quenching it [42]. High doses can also disturb mitochondrial membrane potential, which exacerbates oxidative stress [44]. It is worth noting that at high SkQ1 concentrations (10^{-6} and 10^{-5} M), ROS accumulation occurs much more strongly in the roots than in rice shoots. The reason for this may be the fact that the primary sources of ROS in root tissues are mitochondria, while in green parts of plants, in addition to mitochondria, chloroplasts and peroxisomes play a significant role in generating ROS [45]. In this case, the positive charge of SkQ1 ensures its targeted delivery to the negatively charged mitochondrial matrix [46]. Unlike mitochondria, in chloroplasts, almost the entire value of the proton-motive force is created by the pH gradient, and the contribution of the membrane potential is insignificant. This occurs because the transfer of H⁺ protons into the thylakoid cavity is accompanied by the transfer of Mg²⁺ in the opposite direction or Cl⁻ in the same direction, which maintains electroneutrality [47]. Moreover, it has been shown that in illuminated thylakoids, electron transfer generates $\Delta\psi$ with a plus sign inside, therefore the cationic derivative SkQ1 does not accumulate in energized chloroplasts, but on the contrary, is expelled from them [25].

Analysis of the expression level of genes encoding superoxide dismutase isoforms (*OsSODA* and *OsSODB*), catalase (*OsCATA*, *OsCATB* and *OsCATC*) and ascorbate peroxidase (*APX1* and *APX2*) demonstrated a significant effect of SkQ1 on the regulation of their transcription. It has also been

previously shown that SkQ1 modulates the expression of microRNAs involved in the regulation of genes associated with the antioxidant system [48]. A higher level of transcription of the studied genes under hypoxic conditions compared to normoxia indicates activation of the antiradical system in response to stress caused by complete flooding. Low concentrations of SkQ1 (10^{-9} M and 10^{-8} M) decreased transcription of the *OsSODA* and *OsSODB* genes in both roots and shoots of rice. Interestingly, *OsSODA* activity in leaf tissue was lower than that of *OsSODB*, while their expression patterns were similar in root tissues. *OsSODA* is known to be associated with mitochondria, while *OsSODB* is associated with plastids [49]. Superoxide generated in cells is dismutated into hydrogen peroxide by SOD [50], while catalase and ascorbate peroxidase are responsible for further peroxide detoxification [51]. Analysis of the expression of the *OsCATA*, *OsCATC*, *OsAPX1*, and *OsAPX2* genes demonstrated that the addition of SkQ1 at nanomolar concentrations, as in the case of the *OsSOD* genes, stabilized their transcription in flooded seedlings. The functional specialization of the antioxidant enzyme isoforms encoded by these genes is still poorly understood, which emphasizes the importance of the results for understanding the adaptive mechanisms of plants under stress.

It is also important to note that despite some tissue and isoform specificity, the results of transcriptional analysis indicate that at concentrations of 10^{-9} and 10^{-8} M SkQ1 generally reduces the expression level of genes encoding antioxidant enzyme isoforms in flooded seedlings to the level of their activity characteristic of normal aeration conditions or even lower. Probably, the reduction in ROS levels by SkQ1, shown above, leads to a weakening of redox signaling and, as a consequence, to a decrease in the expression of genes induced by oxidative stress. In turn, an increase in ROS content at high SkQ1 concentrations results in the hyperexpression of the corresponding genes. Such a bimodal response is characteristic of a number of antioxidants, which in high doses can exhibit pro-oxidant properties or disrupt redox homeostasis, provoking compensatory activation of antioxidant systems [52].

With increasing SkQ1 concentration in both roots and shoots of rice, there is a decrease in the indices of H_2O_2 -luminol-induced chemiluminescence. It is known that the index of H_2O_2 -luminol-induced chemiluminescence inversely correlates with the activity of the antioxidant defense system [53]. Thus, the data of chemiluminescence analysis confirm the results of transcriptional analysis, which established an increase in the expression level of antioxidant enzyme genes at high SkQ1 concentrations. Nevertheless, such activation of the antioxidant system does not enable plants to avoid oxidative stress induced by excessive SkQ1 concentrations, as evidenced by the accumulation of ROS and growth suppression. In turn, at a SkQ1 concentration of 10^{-8} M, which showed the most significant effect according to the results of the pot experiment, the level of the chemiluminescent signal in the seedlings was approximately 1.5 times lower than the control. Still, it did not statistically significantly differ from that of plants grown under normal aeration conditions.

5. Conclusion

The mitochondria-targeted antioxidant SkQ1 is an effective protector, exhibiting the property of compensating for the insufficiency of protective mechanisms during complete flooding (hypoxia) conditions at the initial stages of rice plant growth. Increased resistance occurs, primarily due to the ability of SkQ1 to maintain redox homeostasis. At nanomolar concentrations, SkQ1 modulated antioxidant enzyme gene (*OsSODA*, *OsSODB*, *OsCATA*, *OsCATB*, *OsCATC*, *OsAPX1*, *OsAPX2*) expression, suppressed H_2O_2 -induced luminol-dependent chemiluminescence, and reduced total

ROS levels. According to the data collected, compounds belonging to the SkQ series are promising for use in agriculture as universal growth enhancers. Future research should focus on the development and optimization of SkQ1 field application protocols for rice cultivation, with an emphasis on dosage accuracy, treatment timing, and integration with agricultural methods.

Acknowledgments

The research was financially supported by the Ministry of Science and Higher Education of the Russian Federation within the framework of the state task in the field of scientific activity (№ FENW-2023-0008).

Author Contributions

Nadezhda G. Duplii - obtained experimental data. Kirill V. Azarin, Nadezhda G. Duplii, Alexander V. Usatov, Andrey A. Plotnikov, Ritu Rani and Vishnu D. Rajput were equally involved in the conception and writing of the manuscript, and are also responsible for plagiarism, self-plagiarism and other ethical transgressions.

Competing Interests

The authors declare no conflict of interest.

Data Availability Statement

Data will be made available on request.

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