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Original Research

The effect of plastoquinone derivatives on barley (*Hordeum vulgare* L.) tolerance to zinc oxide particles of different dispersion

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Abstract: The plastoquinone derivatives 10-(6'-plastoquinonyl) decyltriphenylphosphonium (SkQ1) and 10-(6'-methyl-plastoquinonyl) decyltriphenylphosphonium (SkQ3) are lipophilic cationic antioxidants which can effectively penetrate cell membranes, targeting mitochondria to provide antioxidant defense in cells. In this study, barley (*Hordeum vulgare* L.) grown with zinc oxide (ZnO) nanoparticle (NPs) stress treated with SkQ1 and SkQ3 to observe the growth rate and level of expression of oxidative stress-related genes in barley seedlings. The study reported that a concentration of 300 mgL^{-1} of ZnO NPs inhibits the growth and development of the root system, while the addition of larger ZnO NPs in a similar concentration did not affect the development of the root. With the addition of SkQ1 and SkQ3, the length and weight of roots of the plants grown treated with 300 mgL^{-1} of ZnO NPs remain at the control level, while without the antioxidant, root length and weight decrease by 14% and 12%, respectively, compared to the control indicating an increase in plant resistance under NPs induced stress conditions. In the case of 2000 mgL^{-1} of ZnO concentration, size-dependent effects were observed on the length of the roots which decreased by 41.5% and 53.8%, and the weight of the roots by 23.3% and 38.8%, respectively, compared to the control. When plants are grown with 2000 mgL^{-1} of ZnO NPs along with SkQ1 and SkQ3 treatment, an increase in root length is observed by 14.6% and 17.4%, and dry weight increases by 12.2% and 13.2%, respectively compared to the control. When a similar concentration of ZnO in the form of NPs is added, similar indicators increase by 15.5% and 14.3% in root length and 16.0% and 17.1% in root dry weight, respectively. The level of expression of antioxidant system genes in roots decreases by 10-18 times, and in leaves, it changes only slightly, which proves the effect of plastoquinone derivatives on mitochondria, which in the roots are the main suppliers of ROS.

Key Words	Oxidative stress; Abiotic stress; Metal Nanoparticles; Gene Expression
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1. INTRODUCTION

The growing need for nanotechnology may lead to the accumulation of NPs in the environment. Concerns are being raised about the impact of NPs on the ecological state of agrocenoses. (Rastogi et al. 2017). The

widespread use of metal-based NPs has revealed both the beneficial and harmful effects they have on plants (Javed et al. 2024). Stress tolerance can be controlled by the application of NPs. Due to their small size, NPs are highly reactive, making them suitable for altering various biological functions of plants (Javed et al. 2023) and promoting plant growth in a species-specific manner, even under biotic and abiotic stress conditions (Giraldo et al. 2014 Hanif et al. 2023).

However, in addition to the positive effects of MNPs on plants, these particles also have negative effects on plants (Liu et al. 2020). The induction of abiotic stress resistance by metal NPs is considered to be the main cause of nano-pollution or nanotoxicity. MNPs lead to the production of excessive reactive oxygen species (ROS) and as a result cause oxidative stress in plants, which leads to phyto-, cyto- and genotoxicity.

Zinc oxide NPs are among the most widely used in the nano-industry (Meena et al., 2021). Zinc (Zn) in the divalent state (Zn^{2+}) is the most common form found in soil and absorbed by plants (Gupta, Ram, Kumar, 2016), however, Zn^{2+} at high concentrations is highly toxic to plants (Balafrej et al. 2020). Excess Zn^{2+} in cells can cause oxidative stress and disrupt pro-oxidant and antioxidant homeostasis in plant cells (Sachdev et al., 2021). It has also been found that excess Zn has a genotoxic effect on plants, leading to genetic disorders and diminished plant health (Oladele et al. 2013, Kaur and Garg, 2021). A study has shown that a high concentration of Zn (100 mgL^{-1}) in cells causes chromosomal aberrations, including disruption of metaphase and uneven chromosome segregation. Other studies have also shown the negative effect of increased Zn concentration on plant growth parameters and crop yield (Ranjan et al. 2021; Todeschini et al. 2011). For example, under the influence of Zn^{2+}, the morphology and ultrastructure of the leaf sharply changed, and the formation of calcium oxalate crystals also increased. In addition, Zn^{2+} treatment has been shown to reduce root and shoot length, as well as leaf area (Rani et al.,2024).

One of the most frequently developed nanomaterials is ZnO NPs, which have gained popularity in industrial applications, and are also largely studied in agricultural applications inevitably being ended up in the environment due to their production in large quantities and their widespread use. Agricultural application of Zn NPs is also associated with direct environmental release and exposure and their repeated application in different cropping cycles is also allowing its accumulation and biomagnification in plants exhibiting its deleterious effects (Rajput et al. 2021). Zn NPs primarily exhibit a negative impact on the plants by triggering ROS production which ultimately affects multifacetedly the plants (Rajput et al. 2018). Studies have also confirmed the role of ZnO NPs reduce root cell viability and genomic matrix stability, as well as upregulating and downregulate microRNAs in barley seedlings (Plaksenkova et al. 2020). Further studies on exposure to bulk- and ZnO NPs at 300 and 2000 mgL^{-1} confirmed differential gene expression in barley roots, with upregulation of Mn-SOD and Fe-SOD genes associated with chloroplast protection. Cat 1 isoform predominated, contributing to increased catalase activity, suggesting activation of antioxidant defense mechanisms against oxidative stress, potentially mitigating early ontogenetic damage but leading to energy resource depletion at higher concentrations (Azarin et al. 2022).

In this regard, one of the effective methods of combating the negative impact of stress factors on plants is the regulation of ROS production (Sharma et al. 2012) through the action of natural or artificially synthesized antioxidants (Flieger et al. 2021). The pursuit and introduction of antioxidants-based treatment to stressed plants for modulating ROS-induced stress is a promising and contemporary approach. Low-molecular-weight antioxidants are best suited for such purposes as they are capable, due to their structure, of targeted penetration into cell mitochondria (Smith et al. 2011). Skulachev and his team in the year 2009 developed many such compounds such as SkQ1 (10-6'-plastoquinonyldecyltriphenyl-phosphonium) and SkQ3 (10-6'-methyl-plastoquinonyldecyltriphenyl-phosphonium) related to mitochondria-targeted antioxidants were synthesized (Skulachev

et al. 2009). Due to plastoquinone antioxidants, as the base of this class of chemical compounds, they are potentially able to be reduced in the mitochondrial respiratory chain and can exhibit unique properties, acting as multi-acting antioxidants (Antonenko et al. 2008). During the studies, substances such as SkQ1 and SkQ3 showed the greatest antioxidant activity (Samuilov et al. 2019).

To understand the effect of oxidative stress caused by pollutants is to analyze the dynamics of gene expression of the pro- and antioxidant systems of plants. In this study, we have evaluated the role of SkQ1 and SkQ3 in alleviating ZnO (bulk and nano-forms)-induced ROS stress at different concentrations and sizes as per our previous studies (Azarin et al. 2022). It can be concluded that mitochondria-targeted antioxidants SkQ1 and SkQ3 are promising protectors capable of compensating for the insufficiency of protective mechanisms under stress due to the ability of these substances to reduce the excessive amount of ROS. This hypothesis can be confirmed both by direct measurement of the ROS level in the leaves and roots of barley sprouts and by determining the transcriptional activity of the antioxidant system genes, which correlate with the rate of plant growth, expressed with the length and weight of leaves and roots.

The objective of the study was to measure the ROS level and assess the expression of antioxidant-related genes to validate the role of SkQ1 and SkQ3 in stress modulation in barley seedlings. It would help assess the insights into how plants respond to NPs' polluted environmental conditions in the presence of plastoquinone derivatives and ensure the development of application methods for enhancing agricultural crop productivity.

Barley (*H. vulgare* L) is a convenient model plant for studying the influence of various factors on resistance. Barley sprouts were used as a study's object of the impact of NPs cerium and titanium dioxide (Marchiol et al. 2016) silver (Fayez et al. 2017) selenium (Nagdalian et al. 2023) copper (Rajput V. et al. 2018) and other metals. Due to its short growth period and unpretentiousness, barley is easy to grow both in hydroponics (Wang et al, 2017) or artificial and natural substrates (Ali et al. 2004) as well as in greenhouse or phytotron conditions. Early work on enzyme isolation and characterization also meant that barley grain enzymes were one of the first plant proteins to be crystallized and with solved structures (Langridge, 2018). Currently, genes of the antioxidant system associated with enzymes that inhibit ROS are widely known for barley. That is why we chose barley as the object of our study.

It should be noted that plants, unlike other organisms, have developed a specific antioxidant defense mechanism to maintain the dynamic balance of ROS (Wang et al., 2018). The antioxidant defense system consists of two components: its own metabolites and enzymes that are coordinated in inactivating ROS and maintaining the balance between reduced and oxidized molecules in the body. Intracellular and extracellular antioxidants form complex networks that protect against oxidation and "form" stress signals (Peng et al., 2022). Thus, studying the effect of antioxidants using SkQ1 and SkQ3 as an example at the molecular level can complement the modern understanding of the mechanisms for maintaining plant redox homeostasis. Studying the reaction of the plant antioxidant system to contamination with zinc oxide nanoparticles can serve as a model to help assess the toxicity of heavy metal particles of varying degrees of dispersion. A toxicogenomic approach based on the study of the expression of genes of defense systems in various organs and tissues when exposed to heavy metal compounds is necessary for predicting possible damage to agriculture from soil pollution.

2. MATERIALS AND METHODS

2.1. SEED TREATMENT

The study included *H. vulgare L*. variety Medicum 157 OS seeds soaked in solutions of SkQ1 and SkQ3 at a concentration of 2.5 nM for 6 hours and then germinated in Petri dishes on wet filter paper for 36 hours at a temperature of 25 °C and relative humidity of 60±2.8 %. Then equally germinated seeds were transferred to water (control) and ZnO NPs solution at concentrations of 300 and 2000 mgL^{-1}. (Azarin et al., 2022; Azarin et al., 2024). The bulk form for ZnO was also selected at the same concentration. These concentrations of ZnO (bulk and nano-forms) NPs zinc oxide were not chosen randomly also but were selected based on our previous studies. (Azarin K., et al., 2022). Our previous research shows that high doses of ZnO NP (2000 mgL^{-1} and 10000 mgL^{-1}) reduce the respiration efficiency and ATP content in roots. NP ZnO also seriously changes the structure of mitochondria, reducing their number and efficiency (Azarin et al., 2024). The bulk form for ZnO was also selected at the same concentration. These concentrations of ZnO (bulk and nano-forms) were chosen based on our previous studies. (Azarin K., et al., 2022). Since ZnO NPs are insoluble in water, barley seeds were germinated on a rotary shaker (Helicon, Russia) at a speed of 115 rpm at a temperature of 25 °C and illumination of 4 ± 0.5 kLux. After 9 days, the length of roots and sprouts was measured, then the plants were placed in a drying oven for 48 hours at a temperature of 105 °C and their weight was measured.

2.2. CHARACTERIZATION OF NANOPARTICLES

Zinc oxide nanoparticles were commercially obtained from Aldrich, USA. The highest fraction of particles had a minimum Feret diameter (minFeret) of 60±4 nm and a maximum Feret diameter (maxFeret) of 132±8 nm.

The results of X-ray structural analysis showed the presence of standard peaks and high purity of the studied compound (Fig. 1). Previous measurements of the ζ-potential (22 mV – −6.5 mV) demonstrated the stability of the colloidal system of ZnO nanoparticles (Voloshina et al., 2022). Fourier transform infrared spectroscopy confirmed the presence of the ZnO functional group in the samples.

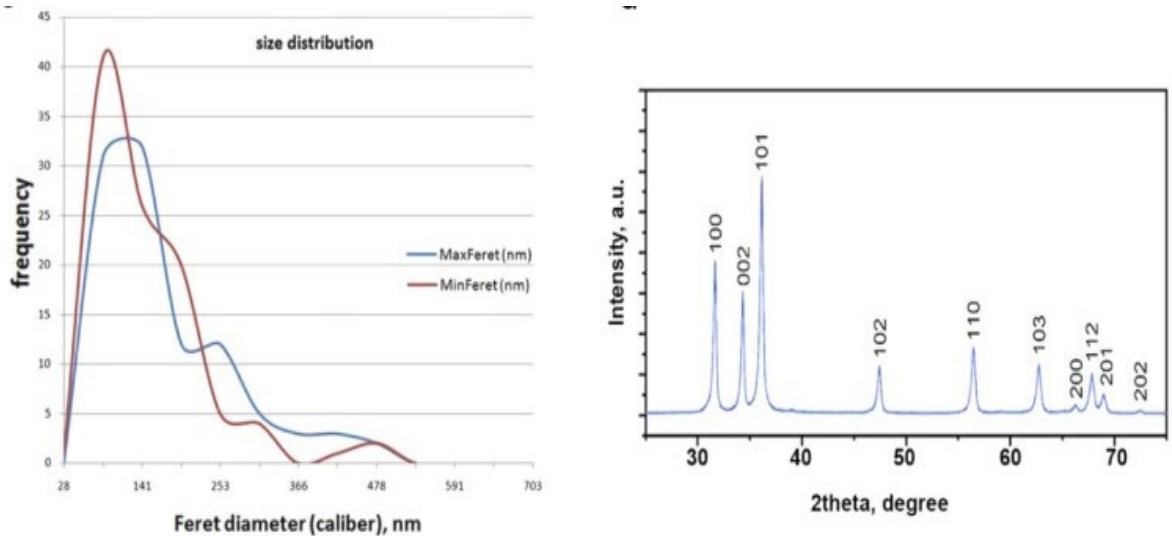

Fig. 1. Characteristics of ZnO nanoparticles: size distribution and X-ray diffraction pattern.

2.3. MEASUREMENT OF ROS LEVEL

The total ROS pool was determined using a fluorescence assay based on the formation of dichlorofluorescein from non-fluorescent dichlorofluorescein diacetate. A 0.5 g sample of fresh plant material was ground in liquid nitrogen with the addition of 2 ml of 0.2 N HClO4 and subsequent neutralization with 37 µl of 4 M KOH. Then 950 µl of 0.15 M Tris-HCl buffer (pH 7.5), 25 µl of supernatant and 25 µl of 0.5 mM dichlorofluorescein diacetate solution were added successively. The samples were stored in a thermostat at 37 ◦C for 20 min, after

which the fluorescence spectra were recorded (λex = 496 nm/ λem = 524 nm) on an RF-5301 spectrofluorimeter (Shimadzu). The ROS content was calculated in mkg g−1 FW.

2.4. RNA EXTRACTION

RNA from leaf and root tissues was isolated according to the Chomczynski method (Chomczynski and Sacchi, 1987) using a commercial ExtractRNA kit (Evrogen, Russia). RNA isolation was carried out in several steps. First, plant tissues were homogenized in a mortar with the addition of liquid nitrogen, then lysis was carried out with ExtractRNA buffer. The liquid lysate was sedimented in a centrifuge, after which chloroform was added to the supernatant and mixed in a microcentrifuge. Then the RNA was precipitated using isopropanol, incubating the mixture for 30 minutes at 4 °C to precipitate the RNA. The resulting RNA precipitate was washed with 80% ethanol and dried in open air at 37°C which was further dissolved in 60 µl of RNase-free water, stirring with a pipette tip. The RNA isolated was again treated with DNAse from the commercial kit DNAse I, RNAse-free (ThermoFisherScientific, USA). The reaction mixture contained 6 µl of RNA, 1 µl of 10× DNAse buffer, and 1.2 µl of DNase. The mixture was kept for 30 minutes at 37 °C. 1 µl of 50 mM EDTA was added and kept for 10 minutes at 65 °C, this process was performed on RNA to remove contaminating DNA, followed by inactivation of DNase enzyme.

2.5. REVERSE TRANSCRIPTION USING PCR

The reverse transcription reaction was carried out using a commercial kit MMLVRTkit (Evrogen, Russia). To 4 µl of RNA, 0.5 µl (20 µM) of primer was added and then heated for 2 min at 70 °C. The samples were then placed in the cold. A pre-prepared mixture of the following composition was added to each sample: 5x buffer for first-strand synthesis 5 µl, dNTP mixture (10 mM of each nucleotide) 2 µl, dithiothreitol (20 mM) 2 µl, MMLV reversease 1 µl. The final volume of the reaction mixture was adjusted to 25 µl with sterile RNase-free water. The content was mixed in a microcentrifuge and incubated for 1 hour at 39°C. Further heating of the mixture at 70 °C for 10 minutes stopped the reaction. The synthesized cDNA was used as a matrix for real-time PCR The polymerase chain reaction was carried out using a commercial kit in the presence of SYBR Green I (Evrogen, Russia). To carry out RT-PCR, 25 µl of the reaction mixture of the following composition was used: 5 µl of 5x buffer from the kit, 3 µl of cDNA, 1 µl of forward and reverse 10 pM primers, after which the final volume of the reaction mixture was adjusted to 25 µl with sterile water. Amplification was carried out in a CFX96 thermal cycler (Bio-Rad, USA). A thermal regime with initial denaturation at 94 °C for 3 minutes, then 36 cycles subject to the temperature-time regime: primer annealing at 58 °C for 20 sec, elongation - 30 sec at 70 °C, denaturation at 95 °C – 10 sec., final elongation – 2 minutes at 70 °C.

qRT-PCR analysis of oxidative stress-related genes in the roots and leaves in *H. vulgare.* SODA - superoxide dismutase [Mn], SODB - superoxide dismutase [Fe], CAT1 - catalase-1; CAT2 - catalase-2; GR - glutathione reductase; APX- ascorbate peroxidase. Primers used for RT-PCR (Table 1) were tested for specificity using Primer3 program and the GenBank database of NCBI.

To calculate quantitative changes in gene transcription, the $2^{-\Delta\Delta Ct}$ method was used, where Ct is the threshold value of the cycle during which fluorescence is first detected reliably above the threshold level, and 2 is the maximum PCR efficiency coefficient. b-tubulin was used as a reference gene. The resulting ΔΔCt value was used to determine changes in the expression level of the gene of interest.

Plants not treated with zinc oxide served as controls. When assessing the effect of SkQ, the expression level was calculated relative to plants treated with a similar dose of zinc oxide but without the addition of SkQ.

Table 1. Primers used for RT-PCR

Gene	NCBI Gene ID:	Sequence (5'-3') forward primer	Sequence (5'-3') reverse primer
SodA1	123428270	*TGGATGGGTGTGGCTAGCTTT*	AGTATGCATGCTCCCAGACAT
SodB	123412483	TACTACGGCCTCACGACTCC	CGGGTTGCCGTTGTTGTAGG
GR	123447388	GAGCTACGACTACGACCTCTTC	CACGTATCACGCACGTCCC
Cat1	123407150	AACTCCGCCTACTGGAAAAC	ACGTTCAGGTATGCGTTCCC
Cat2	123402406	ACCGCAACGTCGACAACTTC	TTGACGGGGAGCATCAGGTA
Apx1	123448947	GAGGTCTGGCTTTGAGGGAC	TCAGCAGAGTTTTGTCACTTGGA
β-tubulin	123444643	GGAGGCTGAGAACTGTGACTG	TCAGGGTACTCCTCCCTGATT

2.6. STATISTICAL VALIDATION

All experimental variants were carried out in three independent replicates. Data are presented as arithmetic means and their standard deviations. The reliability of the differences in the data obtained in the experimental and control groups was assessed using Student's t-test, $p < 0.05$. Statistical data processing was carried out using the Statistica 10.0 program. Statistical processing of PCR results was carried out using the statistical program R-Studio. Each PCR was carried out at least 2 times in three replicates for each gene; the nonparametric Mann-Whitney U test was used to assess the significance of differences. Data were considered reliable at $p<0.05$, where p is an indicator of statistical reliability of the data.

3. RESULTS AND DISCUSSIONS

3.1. Effect on plant morphology

The plants under ZnO NPs exposure and further treatment with SkQ1 and SkQ3 affected the length and dry weight of leaves and roots of 14-day-old barley seedlings. The findings show variations in leaf and root parameters in response to different treatments and NPs dispersions. Across treatments, the leaf length and dry weight of leaves generally remained relatively consistent compared to the control group, with leaf lengths ranging from approximately 12.1 to 13.9 cm and dry weights of leaves ranging from around 17.9 to 20.5 mg. These observations indicate the limited impact on above-ground growth parameters. However, significant changes were observed in root length and dry weight of roots, particularly in seedlings exposed to ZnO NPs with smaller dispersions (2000 nm). In these cases, root lengths ranged from approximately 6.0 to 9.2 cm, and dry weights of roots ranged from approximately 6.3 to 10.3 mg. The addition of ZnO NPs with a concentration of 300 mg L^{-1} led to a decrease in root length by 14% and weight by 12%, respectively. Similarly, when the ZnO concentration increases to 2000 mg L^{-1}, depending on the size of ZnO NPs, the length of the roots decreases by 41.5% and 53.8%, and the weight of the roots by 23.3% and 38.8%, respectively, These values represent significant reductions compared to both the control and bulk ZnO treatments, suggesting a detrimental effect of NPs size on root development.

Interestingly, the addition of SkQ1 and SkQ3 appeared to mitigate some of the negative effects of ZnO NPs exposure, as evidenced by fewer reductions in root parameters compared to NP-only treatments. For example, root lengths ranged from approximately 7.0 to 12.1 cm, and dry weights of roots ranged from around 7.5 to 10.0 mg in seedlings treated with SkQ1 and SkQ3. When treating plants grown with SkQ1 and SkQ3 with the addition of bulk ZnO at a concentration of 2000 mgL^{-1}, an increase in root length is observed by 14.6% and 17.4%, respectively, and dry weight increases by 12.2% and 13.2%, respectively compared to untreated plants. When adding a similar concentration of ZnO NPs was used, an increase of 15.5% and 14.3% in root length and 16.0% and 17.1% in root dry weight was observed respectively. Such findings indicate a potential protective role of these compounds against NP-induced stress in barley seedlings. Fig.2 presents the length and dry weight of leaves and roots of barley seedlings with the addition of ZnO in various forms and concentrations, treated with SKQ1 and SKQ1. The findings suggest an increase in growth rate under conditions of high concentrations of ZnO upon treatment with plastoquinone derivatives SkQ1 and SkQ3 may indirectly indicate the restoration of the balance between the formation and elimination of ROS under the action of the antioxidant when exposed to heavy metals.

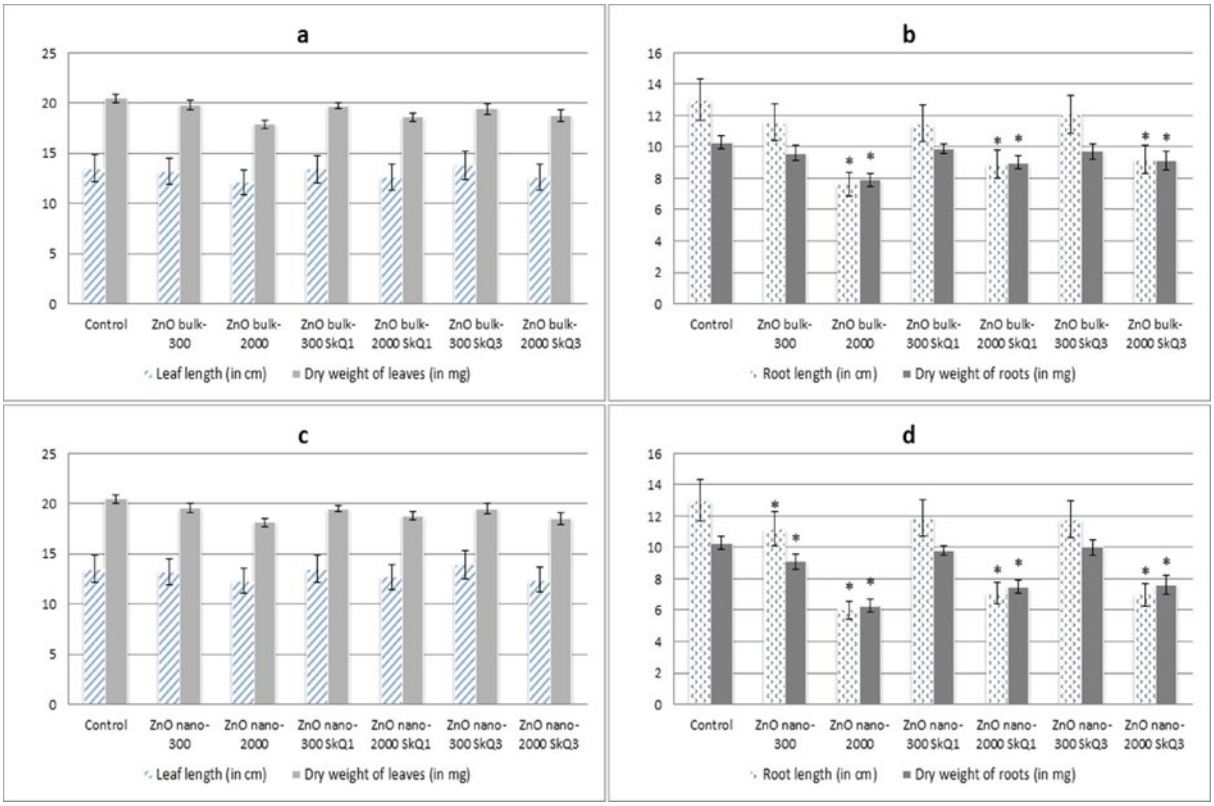

Fig. 2: Effect of treatment with SkQ1 and SKQ3 on the length and dry weight of leaves and roots of 14-day-old barley seedlings under conditions of contamination with zinc oxide particles of different dispersion. Experiments were carried out in three independent replicates.

*— Differences are significant ($p<0.05$)

Our finding is also supported by other studies which have shown that seed priming with a 2.5nM aqueous solution of SkQ3, significantly enhanced emergence and seedling growth performance, particularly under drought stress conditions (Sadoyan et al., 2021). The study has also demonstrated the mechanism by which SkQ1 improved chloroplast function by exerting a dramatic quenching effect on chlorophyll fluorescence in photosynthetic cells, likely mediated by its interaction with the manganese cluster in Photosystem II (PSII)

of chloroplasts. This interaction disables the oxygen-evolving complex (OEC), halting water splitting and generating potent quenchers providing detailed insights into plant physiology and stress response mechanisms (Ptushenko et al., 2019).

3.2. Total ROS level

Figures 3 and 4 show the total level of ROS in leaf and root cells in the control and under SkQ1 and SKQ3 treatment under bulklZnO and NPZnO at concentrations of 300 mg $L^{-1}$and 2000 mg $L^{-1.}$

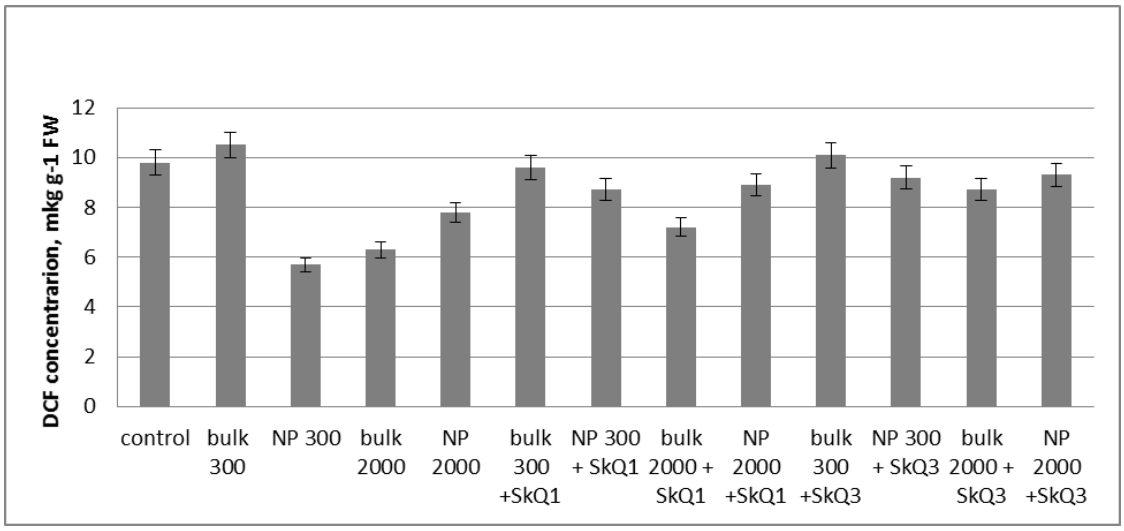

Fig. 3. Total ROS level in the leaves of barley under bulklZnO and NP ZnO treatments. Different letters on the columns refer to statistically significant differences at $P \leq 0.05$.

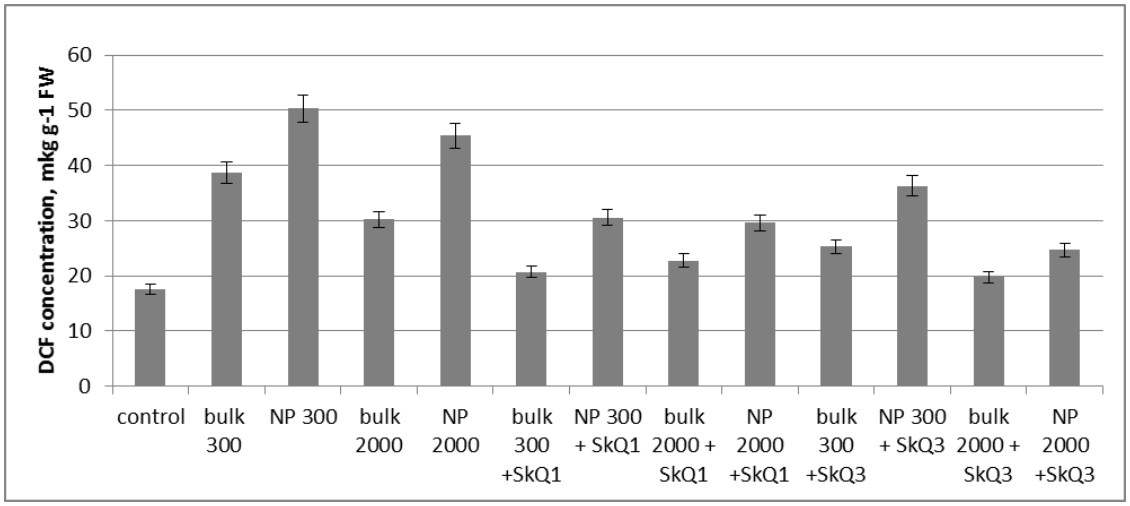

Fig. 4. Total ROS level in the root of barley under bulk ZnO and NP ZnO treatments. Different letters on the columns refer to statistically significant differences at $P \leq 0.05$.

In leaf cells, the total amount of ROS when treated with 300 mg L^{-1} ZnO in macroform remained almost unchanged compared to the control. With an increase in the ZnO concentration to 2000 mg L^{-1}, as well as with treatment with 300 mg L^{-1} ZnO nanoparticles, a decrease in the ROS concentration was recorded. The introduction of SkQ1 and SKQ3 brought all values closer to the control values (Fig. 3). An increase in the total amount of ROS in root cells was recorded when exposed to bulk-ZnO and ZnO NPs at all concentrations

studied, while at the same dosage, the nanodispersed form had a greater effect than the macrodispersed form of ZnO (Fig.4). The introduction of SkQ1 and SKQ3 leads to a decrease in the ROS concentration by 2 times compared to the samples not treated with the antioxidant.

3.3. Effect on antioxidant-related gene expression:

The levels of relative expression of ROS-related genes in the leaves and roots of barley plants varied heterogeneously. As can be seen in Figure 1 and Figure 5, the expression level of all the studied genes at a ZnO concentration of 300 mgL^{-1} decreases by 0.5-6 times in the leaves except for the *Cat1* and *Sod B* genes. However, in the roots, the expression level of the studied genes increases up to 4 times, except for the Sod genes. With increasing ZnO concentration, the transcriptional activity of oxidative stress genes sharply decreases in leaves (up to 6 times) and increases in roots (up to 7 times), with the exception of the *Cat2* gene.

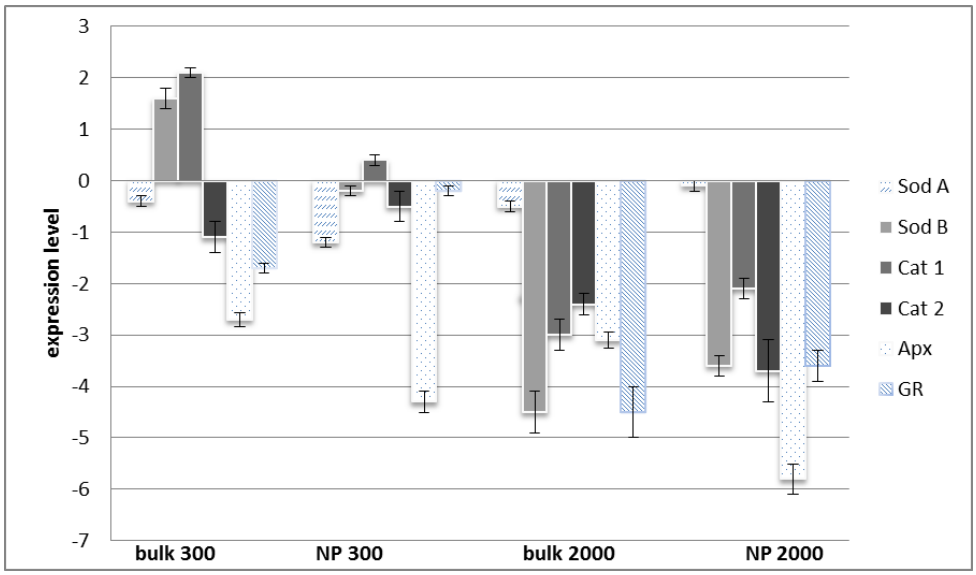

Fig. 5: Effect of bulk-ZnO and ZnO NPs on the level of expression of genes of the antioxidant system of leaf *Hordeum vulgare* L. (relative to control). Experiments were carried out in three independent replicates.

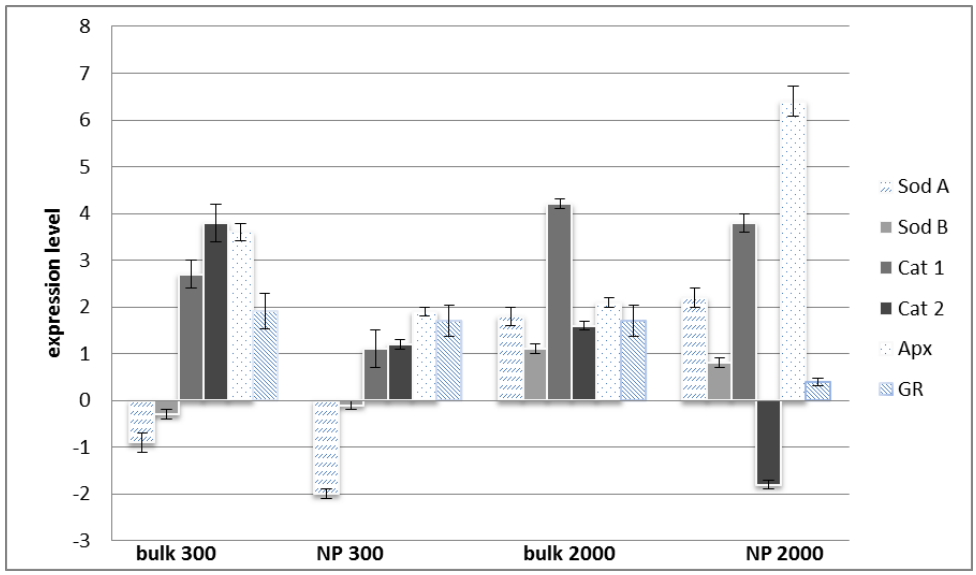

Fig. 6: . Effect of bulk-ZnO and ZnO NPs on the level of expression of genes of the antioxidant system of roots *Hordeum vulgare* L. (relative to control). Experiments were carried out in three independent replicates.

Figure 6 shows the effect of SkQ1 and SkQ3 on the level of gene expression when exposed to ZnO NPs of different dispersion at a concentration of 300 mgL^{-1} in leaves. As can be seen from the figure, the addition of antioxidants SkQ1 and SkQ3 leads to an increase in the transcriptional activity of all studied genes apart from Sod. An increase in transcriptional activity leads to an increase in the concentration of enzymes in the antioxidant system and the normalization of ROS levels.

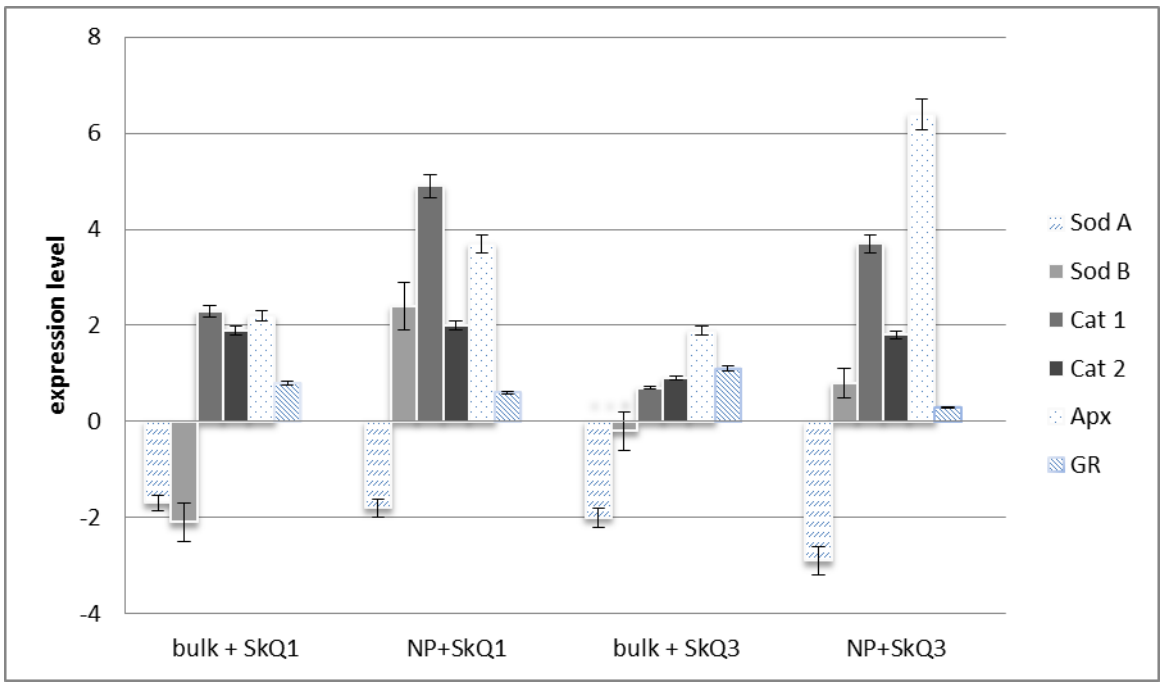

Fig 7: Effect of SkQ1 and SkQ3 on the level of gene expression of the antioxidant system of *Hordeum vulgare* L. leafs when treated with bulk-ZnO (bulk) and ZnO nps (np) at a concentration of 300 mgL^{-1} (relative to the expression level of plants not treated with SkQ). Experiments were carried out in three independent replicates.

A similar feature is observed in the leafs at a concentration of 2000 mgL^{-1} of ZnO, namely, the addition of SkQ1 and SkQ3 leads to an increase in the transcriptional activity of oxidative stress genes (figure 8).

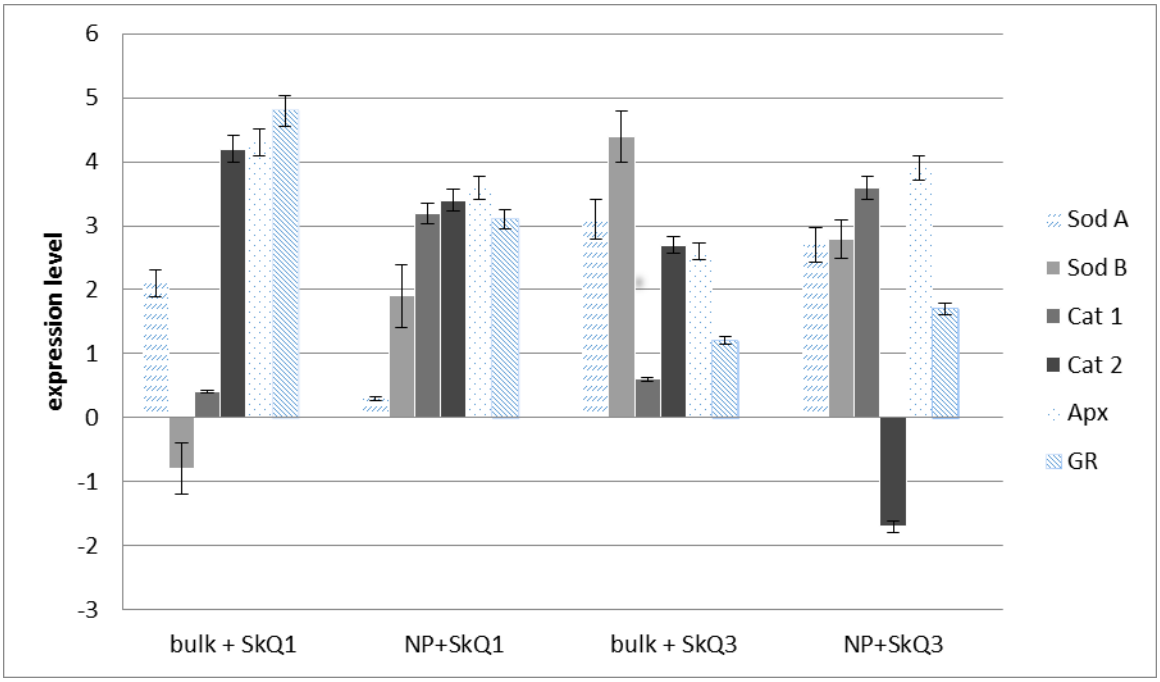

Fig 8: Effect of SkQ1 and SkQ3 on the level of gene expression of the antioxidant system of *Hordeum vulgare* L. leafs when treated with bulk-ZnO (bulk) and ZnO nps (np) at a concentration of 2000 mgL^{-1} (relative to the expression level of plants not treated with SkQ). Experiments were carried out in three independent replicates.

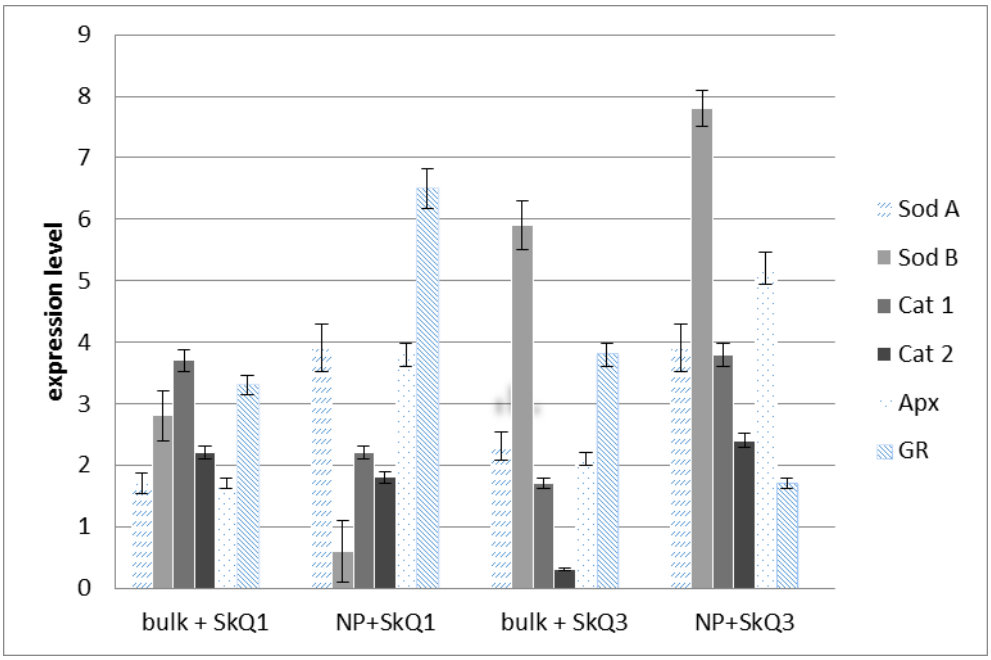

Fig: 9. Effect of SkQ1 and SkQ3 on the level of gene expression of the antioxidant system of *Hordeum vulgare* L. roots when treated with bulk-ZnO (bulk) and ZnO nps (np) at a concentration of 300 mgL^{-1} (relative to the expression level of plants not treated with SkQ). Experiments were carried out in three independent replicates.

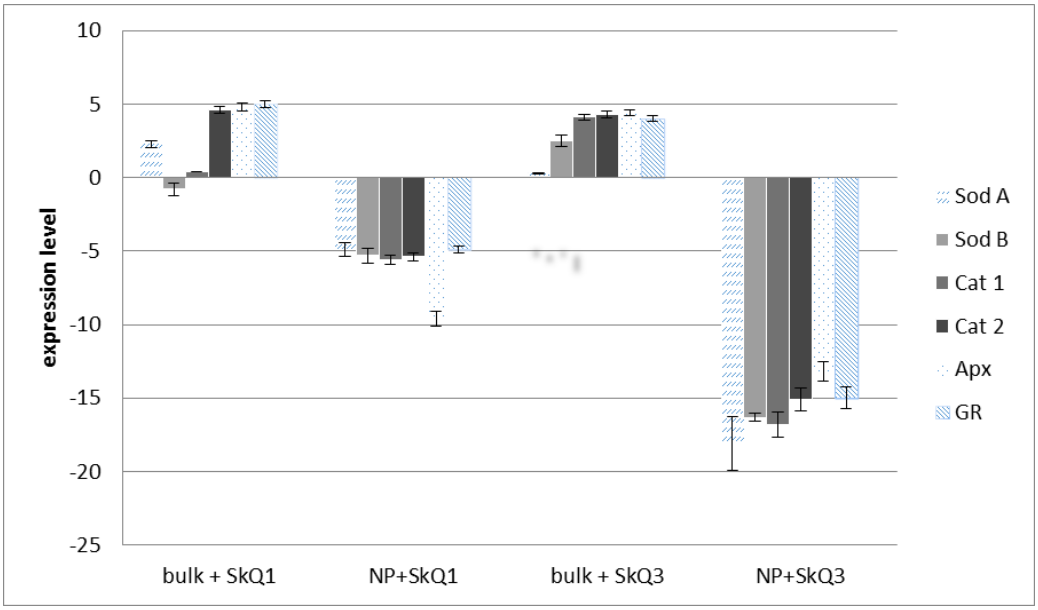

Fig.10: Effect of SkQ1 and SkQ3 on the level of gene expression of the antioxidant system of *Hordeum vulgare* L. roots when treated with bulk-ZnO and ZnO NPs at a concentration of 2000 mg L^{-1} (relative to the expression level of plants not treated with SkQ). Experiments were carried out in three independent replicates.

Figures 9 and 10 show the change in the expression level of the antioxidant-related genes when treated with SkQ1 and SkQ3 under conditions of increasing the concentration of the pollutant to 2000 mgL^{-1}. The expression level of all studied genes increases up to 8 times when treated with SkQ1 and SkQ3 when treated with ZnO at a concentration of 300 mgL^{-1}. When the concentration of ZnO increased to 2000 mgL^{-1}, the expression level of all the studied genes, except Cat 2, in the leaves increased up to 4 times, and in the roots decreased by 10-18 times. Such a sharp drop in transcriptional activity indicated a significant impact of oxidative stress on the functioning of the body. In a study using SkQ1 treatment on Arabidopsis thaliana and Triticum aestivum, it was found to extend leaf lifespan, enhance overall plant longevity, and boost resistance against oxidative stress. Moreover, SkQ1 treatments promote tillering in T. aestivum, leading to increased crop yield. By targeting mitochondrial function and reducing ROS levels, SkQ1 significantly improves plant performance, particularly under stressful conditions (Dzyubinskaya et al., 2013). Our finding also aligns with the study which has highlighted the antioxidant effect of SkQs in preventing ROS generation in mitochondria, their inhibition of non-cyclic electron transfer in chloroplasts at micromolar concentrations, and their stimulation of morphogeny in plant tissue cultures at nanomolar concentrations. SkQ1 also delayed vegetation periods and increased grain mass in wheat. However, higher concentrations of SkQs may disrupt chloroplast function (Samuilov et al., 2019). In our study, the reason for the heterogeneous changes in transcriptional activity in leaves and roots may be the fact that in the green parts of plants, chloroplasts and peroxisomes play a significant role in the generation of ROS, while the treatment of SkQ1 and SkQ3 specifically penetrated the mitochondrial matrix, and since the main suppliers of ROS in the roots are mitochondria, then the addition of an external antioxidant leads to inhibition of excessive accumulation of ROS and a decrease in the level of expression of genes of the antioxidant system.

It is interesting to note the correlation between changes in the ROS content, transcriptional activity of genes and plant growth parameters. For better visualization, the dynamics of expression of antioxidant defense genes is presented as a heat map (Figure 11).

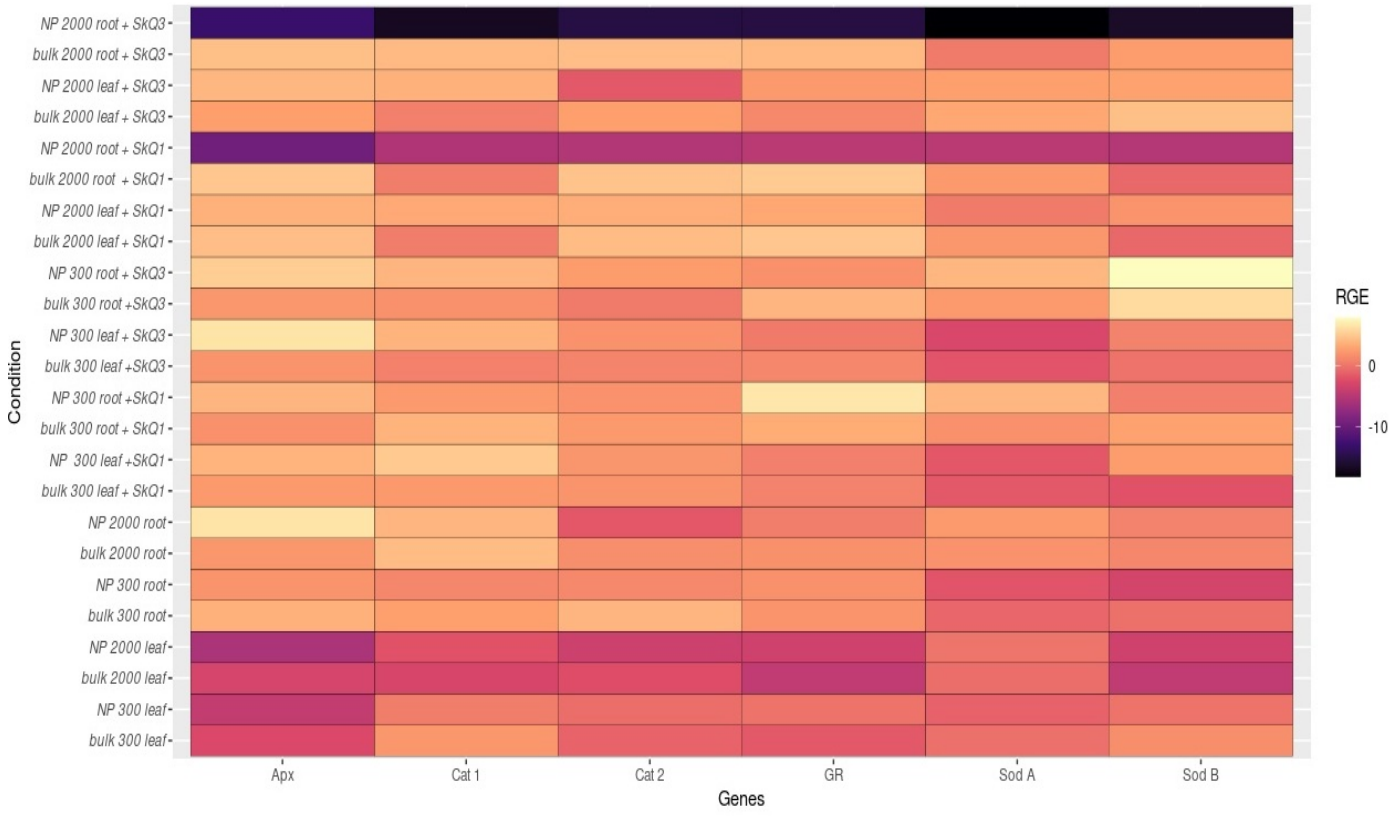

Fig. 11: Heat map of oxidative stress gene expression in leaves and roots of barley seedlings treated with bulk-ZnO and ZnO NPs under the influence of SkQ1 and SkQ3

As shown above, roots respond to zinc oxide to a greater extent than leaves. This response is especially pronounced when treated with ZnO NPs at a concentration of 2000 mg L^{-1}. While the addition of SkQ1 and SkQ3 reduces the total ROS content in the roots by 2 times, the transcriptional activity of the antioxidant system genes is significantly reduced, and the length and weight of the roots of plants treated with SkQ1 and SkQ3 increase compared to untreated ones. Thus, all experimental data indicate an increase in plant resistance to stress factors when using these antioxidants.

In this study, Zn^{2+} ions were detected in the solutions after ultracentrifugation (30,000 rpm) and filtration, indicating the solubility of ZnO regardless of its size (bulk or nano). Interestingly, the Zn concentration in the solutions in which plants were grown was higher when bulk ZnO was added, and greater Zn accumulation in barley plants occurred when nano-ZnO was added. These data, as well as the fact that a large increase in zinc was observed in the root but not in the shoot with increasing ZnO concentration, may indicate significant adhesion of ZnO to the root surface, with nano-ZnO clearly having higher adhesion than bulk ZnO (Azarin et al. 2022).

Regarding the ecological aspect of the possible influence of SkQ on agrocenoses, the existing studies on the influence of these compounds on animals in vivo experiments did not reveal any negative effects in nanomolar concentrations. On the contrary, a geroprotective effect was demonstrated for SkQ, manifested for a wide range of living organisms. An increase in the lifespan of such a diverse range of organisms as the crustacean *Cerio-daphnia*, the insect *Drosophila melanogacter*, the fish *Nothobranchius furzeri*, mammals: mice, mole voles and hamsters was noted (Anisimov et al., 2011). When SkQ derivatives were administered to laboratory animals, it was found that in the experimental group, in contrast to the control group, a number of physiological signs of aging were not observed or were slightly expressed. Such a wide range of recorded effects indicates a systemic

effect of SkQ group compounds (Shipounova et al., 2010). antioxidant properties also have a pronounced antibacterial effect.

In micromolar doses, quinone derivatives based on decyltriphenylphosphonium (SkQ1, SkQ3) can have an antimicrobial and cytotoxic effect, but with a decrease in the concentration of SkQ, such effects are not observed (Nazarov, et al., 2024).

5. CONCLUSIONS

The study investigated the effects of ZnO NPs on barley seedlings, focusing on root and leaf growth as well as the transcriptional activity of antioxidant-related genes. The results showed that ZnO NPs at a concentration of 300 mg L^{-1} inhibited root growth and development, but did not significantly affect leaf growth. However, the addition of SkQ1 and SkQ3 solutions helped to mitigate the negative effects of ZnO NPs on root growth, demonstrating increased plant resistance to NP-induced stress. Moreover, roots showed a more obvious response to ZnO exposure, showing increased expression levels of antioxidant-related genes, while leaves suffered from decreased transcriptional activity. In particular, the expression of such genes was significantly reduced in roots treated with SkQ1 and SkQ3 solutions when exposed to higher ZnO concentrations, indirectly indicating a decrease in the production of antioxidant system enzymes. The results of the study highlight the potential of plastoquinone derivatives SkQ1 and SkQ3 in modulating the growth rate and transcriptional activity of oxidative stress genes in barley seedlings exposed to ZnO NPs, suggesting their application in strategies to alleviate ZnO NPs-induced stress in plants.

As a generalization, the scheme of the influence of plastoquinone derivatives on the transcriptional activity of the genes of the antioxidant system of barley seedlings is presented in figure 11.

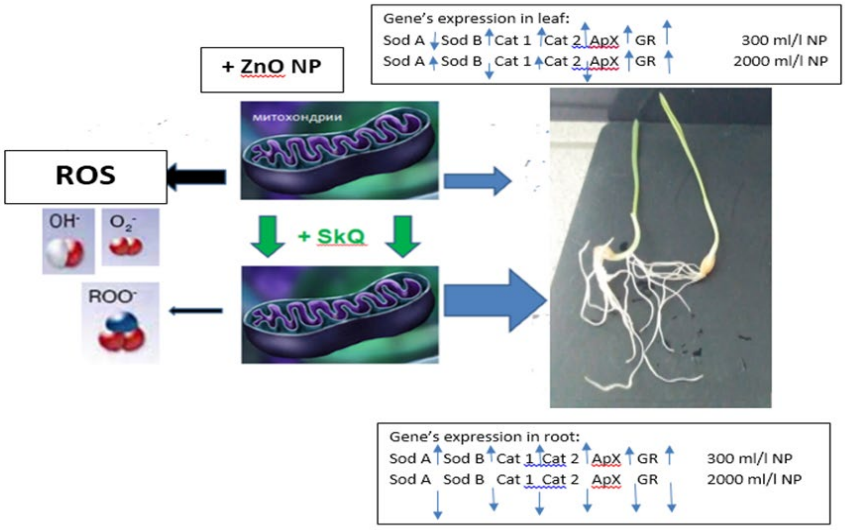

Fig.12: Scheme of the influence of SkQ on the transcriptional activity of genes of the antioxidant system of barley seedlings

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