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TRANSCRIPTIONAL PROFILING OF CATALASE GENES IN JUGLONE-TREATED SEEDS OF MAIZE (*ZEA MAYS* L.) AND WHEAT (*TRITICUM AESTIVUM* L.)

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The major aim of the present study was to investigate the influence of juglone (JU; 5-hydroxy-1,4-naphthoquinone) treatments on the expression level of *Cat1*, *Cat2* and *Cat3* genes, encoding the respective catalase isozymes in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) seeds. In parallel, germination efficiency, catalase (CAT) activity and hydrogen peroxide (H_2O_2) content in juglone-exposed cereal seeds were assessed. Juglone applications significantly stimulated abundance of three target catalase transcripts as well as induced CAT activity and generation of H_2O_2 in both maize and wheat kernels. Furthermore, germination process of juglone-affected maize seeds was more severe suppressed than in case of wheat kernels. The role of juglone in triggering the oxidative stress as well as antioxidative responses in seeds of the studied model cereal species are discussed.

Keywords: Juglone – catalase – hydrogen peroxide – seeds – maize – wheat

INTRODUCTION

Juglone (JU; 5-hydroxy-1,4-naphthoquinone) is a considerably toxic allelocompound released by tissues of many members of Juglandaceae family (e.g., *Juglans californica* Watson, *Juglans cathayensis* Dode, *Juglans cinerea* L., *Juglans major* L., *Juglans nigra* L., *Juglans regia* L., *Juglans mandshruica* Maxim) [13, 26, 32]. It has been reported that juglone treatments decreased fresh and dry mass, induced severe morphological changes and strongly inhibited several physiological processes (e.g., seed germination, root elongation, shoot growth) within a wide array of acceptor plants [8, 10, 28, 33]. In this context, juglone may be considered as a potential bioherbicide used in weed control in different crop systems [9, 15]. Additionally, antimicrobial, antiviral, insecticidal, nematicidal and molluscicidal activities of juglone have also been reported [5, 17]. For example, field tests confirmed specific antibacterial impact of juglone against *Erwinia amylovora*, the fire blight pathogen [18]. According to Fischer et al. [18], juglone may be used as naturally occurring bactericidal agent in controlling prevalence of the fire blight on apple and pear trees. In

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addition, it has been reported that hyphae of arbuscular mycorrhizal fungi may accelerate transport of juglone in soil matrix, thus leading to a significant widening of the area affected by this compound in the field [1].

Despite well-documented morphological alternations of juglone-susceptible plants, the mode of action of this compound in the plant tissues were not studied in details. In addition, there is no available information regarding the molecular background of the allelopathic influence of the juglone on the seeds of acceptor plants. Previous experiments [33] elucidated that 4-day juglone treatment markedly upregulated the expression of glutathione transferase 1 (*Gst1*) gene in maize (*Zea mays* L.) seedlings, but prolonged (8 days) exposure to the tested allelochemical was associated with repressed transcriptional responses of the target gene, compared to the untreated plants. The scale of alternations in expression of *Gst1* gene as well as suppression of coleoptiles and primary shoots length were dependent on concentrations of the naphthoquinone. It has been assumed that allelopathic impact of juglone may induce oxidative stress within maize seedlings. The purpose of the present survey was aimed at assessing the effect of juglone treatments (0.01, 0.1 and 1 mM) on transcriptional reprogramming of *Cat1*, *Cat2* and *Cat3* genes, encoding the isozymes of catalase in seeds of maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.). The tested JU concentrations were chosen based on our previous study [35]. Moreover, JU-affected modulations in catalase (CAT) activity and hydrogen peroxide (H_2O_2) content in the kernels of the cereals as well as seed germination efficiency were also investigated.

MATERIALS AND METHODS

Plant materials

Seeds of maize (*Zea mays* L., cv. Złota Karłowa) and wheat (*Triticum aestivum* L., cv. Nawra) were purchased from the grain company PNOS S.A. (Ożarów Mazowiecki, Poland) and the Plant Breeding Strzelce Ltd., Co. – IHAR-PIB Group (Kończewice, Poland), respectively.

Experimental design

Effects of three concentrations of juglone (0.01, 0.1 and 1 mM, dissolved in methanol-deionized water solution; 4%, v/v) on seed germination efficiency, transcriptional responses of catalase genes (i.e., *Cat1*, *Cat2*, *Cat3*), catalase activity and H_2O_2 content in the kernels of maize and wheat were evaluated. Surface sterilization and seed germination biotests were conducted as described previously [33]. Control kernels were treated with methanol-deionized water solution (4%, v/v). The experiments were conducted in an environmental chamber under controlled conditions (22 °C ± 2 °C/16 ± 2 °C (day/night), 16L: 8D photoperiod, 65 ± 5% relative humidity). Assessment of the expression of the catalase genes, CAT activity and H_2O_2 content in

the examined cereal seeds were determined after 1, 2 and 3 days of the biotests, whereas the number of germinated seeds was recorded after 8 days of JU exposure, in accordance with the Polish Norm: PN-R-65950 [31]. Three independent experiments were performed, and each round of the biotests included 100 seeds of a given plant species per tested juglone concentrations.

Determination of hydrogen peroxide content and catalase activity

Hydrogen peroxide generation in kernels of the tested cereals was measured following the procedure described by Sytykiewicz [34]. The content of H_2O_2 in the seeds was expressed in micromoles per gram of fresh weight (f.w.).

Catalase activity in maize and wheat kernels was assayed according to the Beers and Sizer method [7], with few modifications. The procedure was based on the measurement of the decomposition of H_2O_2 in the reaction mixture. Seeds (0.2 g) were homogenized in a mortar with 5 cm^3 Tris-NaOH buffer (50 mM, pH 8.0), containing 0.5 mM EDTA (ethylenediaminetetraacetic acid), 2% (w/v) PVP (polyvinylpyrrolidone) and 0.5% (v/v) Triton X-100. Subsequently, the mixture was centrifuged at 10,000 × *g* for 10 min (at 4 °C). The reaction mixture comprised 1.2 cm^3 K-phosphate buffer (100 mM, pH 7.0), 0.2 cm^3 200 mM H_2O_2 and 0.1 cm^3 of the supernatant. After thorough vortexing, the initial absorbance at $\lambda = 240$ nm was measured. Then, the probes were incubated for 3 min and the decrease of the absorbance at the same wavelength was recorded. The blank consisted of 1.4 cm^3 K-phosphate buffer and 0.1 cm^3 of the enzyme extract. The amount of cleaved H_2O_2 has been calculated using a molar absorption coefficient ($\varepsilon = 43.6\ M^{-1} \times cm^{-1}$). Catalase activity was expressed as millimoles of decomposed H_2O_2 per minute per milligram of protein.

Quantification of relative expression of the catalase genes

Total RNA was isolated with the application of a Spectrum Plant Total RNA Kit (Sigma-Aldrich, Poland) and the synthesis of cDNA was achieved using a High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Life Technologies, Poland), according to the protocols of the manufacturers. DNA amplification was carried out with the application of StepOne Plus Real-Time PCR System and StepOnePlus Software v2.3 (Applied Biosystems, USA). Quantification of three target catalase genes (i.e., *Cat1*, *Cat2* and *Cat3*) in maize seeds was performed using the procedure of Sytykiewicz [34]. Transcriptional responses of *Cat1* and *Cat2* genes (GenBank IDs: 542369 and 542230, respectively) in the maize seeds was performed using TaqMan Gene Expression Assays (Zm04059154_m1 and Zm04058713_g1, respectively), provided by Life Technologies (Poland). Abundance of wheat *Cat1* transcript (GenBank accession no. D86327.1; ID: 543190) was determined with the application of a TaqMan Gene Expression Assay (Ta04089449_g1), purchased from Life Technologies (Poland). Transcriptional responses of *Cat2* and *Cat3* genes in

Table 1

Sequences of primers and TaqMan fluorescent probes used for real-time qRT-PCR quantification of *Cat3* and *GAPDH* genes in maize seeds and *Cat2*, *Cat3* and *GAPDH* genes in wheat kernels

Genes	Accession no. (GenBank®)	Sequences of primers and probes
Cat3 (maize)	M33103.1	F: CAGGAGGAGCAGTACGACTTC R: GCGGAGCGGCAACAG P: 5'-FAM-TCCGGCCACGTCTTG-NFQ-3'
GAPDH (maize)	X07156.1	F: AAGCCGGTCACCGTCTTT R: CATCTTTGCTTGGGGCAGA P: 5'-FAM-CTTCACTGACAAGGACAAGGCTGCT-NFQ-3'
Cat2 (wheat)	JF808020.1	F: GTTTAGAGAAAAAGGTATAAGAAACGCACAA R: TCGGCGGTCATTGATTATAGGATTT P: 5'-FAM-CCAGCCCACTCGCCTG-NFQ-3'
Cat3 (wheat)	HQ860268.1	F: CGTCGGCGGCAAGAATC R: GCGTCGATGGAGTCGTAGAG P: 5'-FAM-CAGCCACGCCACCCAG-NFQ-3'
GAPDH (wheat)	AF251217.1	F: GGTTTGGCATTGTTGAGGGT R: CTCTCCAGTCCTTGCTGGAA P: 5-TGCCATGACTGCAACCCAGAAGACT-NFQ-3'

Custom TaqMan Gene Expression Assays for maize *Cat3* and *GAPDH* genes as well as wheat *Cat2*, *Cat3* and *GAPDH* genes, containing specific primers and fluorescent probes were designed and provided by Life Technologies, Poland (www.thermofisher.com). GAPDH – cytosolic glyceraldehyde-3-phosphate dehydrogenase gene; F – forward primer; R – reverse primer; P – TaqMan fluorescent probe; FAM – 6-carboxyfluorescein; NFQ – non-fluorescent quencher.

wheat kernels as well as *Cat3* gene in maize seeds were measured using Custom TaqMan Gene Expression Assays (Life Technologies, Poland) (Table 1). Maize and wheat *GAPDH* genes, encoding the respective glyceraldehyde-3-phosphate dehydrogenases, were used as the internal reference (Table 1). Relative expression of catalase genes was presented as *n*-fold changes (±SD) in the transcript abundance in JU-treated seeds, relative to the untreated control.

Statistical analysis

All data are presented as the mean (±SD) derived from at least three independent experiments. The results were analysed using STATISTICA 10 software (StatSoft, Poland). Factorial analysis of variance with subsequent Tukey's test were applied in order to assess the significance of studied variables (i.e., plant species, juglone concentrations and exposure time) and the interactions on expression of the catalase genes, CAT activity and hydrogen peroxide content. Furthermore, the Pearson's correlation coefficient (r) was calculated to estimate the interdependence between tested juglone concentrations and number of germinated seeds of maize and wheat.

RESULTS

Influence of juglone exposure on seed germination efficiency of the examined cereals

The impact of juglone applications (0.01, 0.1 and 1 mM) on the germination process of maize and wheat seeds was assessed after 8 days of the biotests. Three examined juglone treatments caused a significant inhibition of germination process of both maize and wheat kernels; however, more severe suppression occurred in maize seeds (15–38% reduction), compared to wheat (6–25% decrement) (Fig. 1). The highest number of seeds of both plant species germinated under exposure to 0.01 mM concentration of the examined naphthoquinone, whereas the greatest inhibition of the recorded parameter was observed at 1 mM JU, in relation to untreated control. It was found a strong negative correlation between examined juglone concentrations and percentage of the geminated seeds of maize ($r = –0.958$; $P < 0.05$) and wheat ($r = –0.889$; $P < 0.05$).

Impact of juglone treatments on expression of the catalase genes in juglone-treated seeds of the studied model plants

The effect of three tested juglone applications on transcriptional responses of *Cat1*, *Cat2* and *Cat3* genes encoding catalase isozymes in maize and wheat kernels was evaluated (Fig. 2; Table 2). All the tested concentrations of JU led to increased abundance of the studied catalase transcripts, compared to the respective controls. In most cases, the highest elevations in expression of *Cat* genes in maize and wheat seeds was stimulated by 0.1 or 1 mM naphthoquinone applications. Furthermore, the significant impact ($P < 0.05$) of investigated JU concentrations and duration of

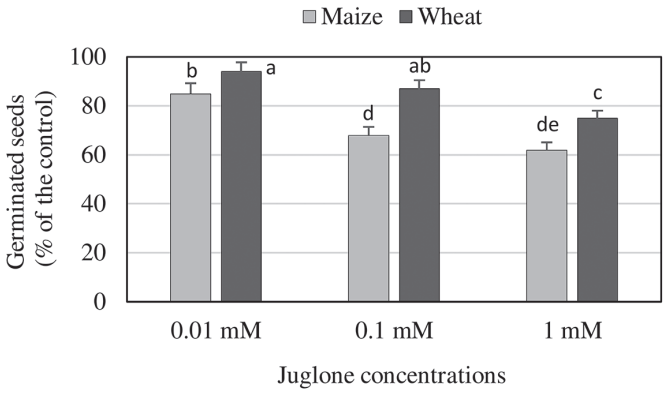

Fig. 1. Effect of juglone treatments on germination of maize and wheat seeds. Different letters above bars indicate significant differences between the mean values (Tukey's test; $P < 0.01$). Results were presented as percent of geminated seeds of tested cereal species, in relation to the untreated controls

exposure period on expression of all the three catalase genes in the examined kernels was confirmed. Transcriptional activity of the target catalase genes in the studied seeds, increased synchronously with the exposure time to all the investigated JU concentrations, reaching the highest values in the final phase of the experiment after (3 days). Conversely, maize seeds exposed to the lowest JU concentration (0.01 mM) after 2 days of the biotests, responded the greatest increases in the amount of *Cat3* mRNA.

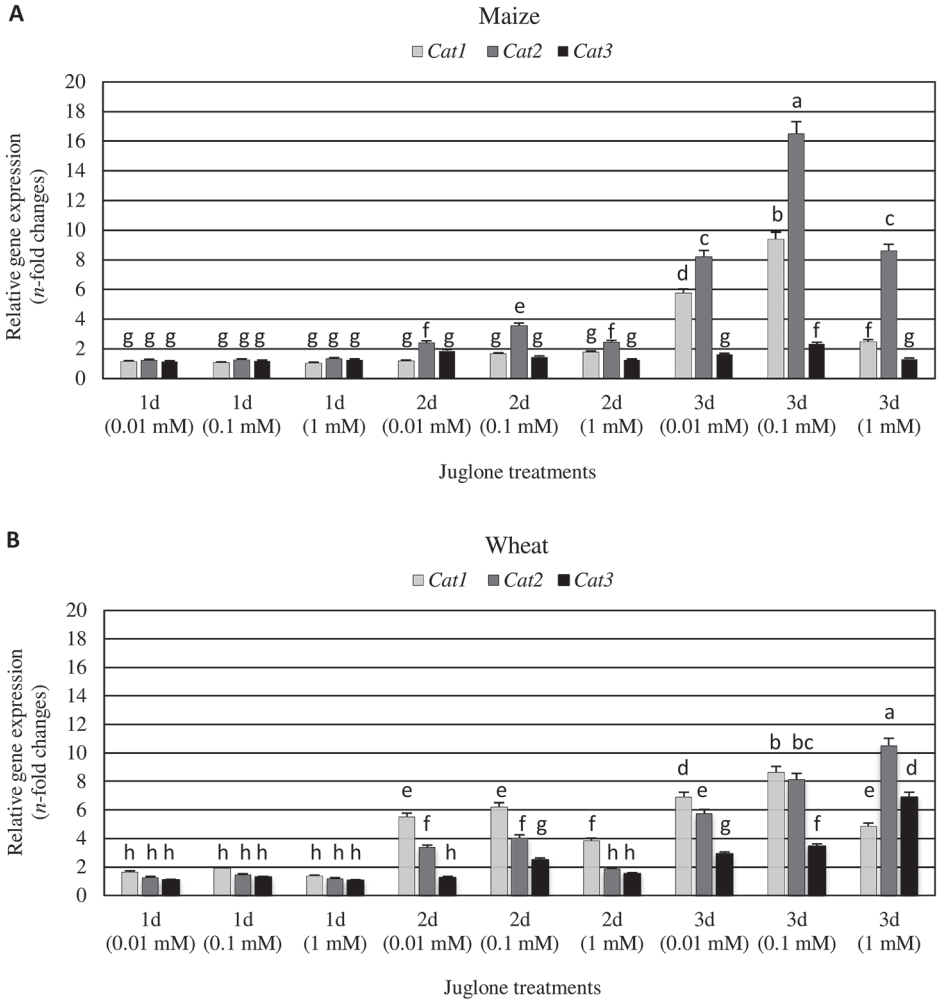

Fig. 2. Transcriptional reconfigurations of catalase genes (*Cat1*, *Cat2* and *Cat3*) in juglone-exposed seeds of *Z. mays* (A) and *T. aestivum* (B). Results are presented as *n*-fold increases (mean ± SD) in mRNA level in JU-treated seeds, compared to the non-stressed controls. 1d, 2d, 3d – 1, 2 and 3 days of the biotests, respectively. Different letters above bars indicate significant differences between the mean values (Tukey's test; $P < 0.01$)

Table 2

Results of three-factorial ANOVA analysis of the tested parameters (plant species, juglone concentrations, exposure time) and interactions on expression of the target catalase genes (*Cat1*, *Cat2* and *Cat3*), activity of catalase (CAT) and the content of hydrogen peroxide (H_2O_2) in the seeds of maize and wheat

Examined indicators	*Cat1*	*Cat2*	*Cat3*	CAT	H_2O_2
Plant species (P)	$F_{1, 64} = 196.4$ (**)	$F_{1, 64} = 247.4$ (**)	$F_{1, 64} = 92.4$ (*)	$F_{1, 64} = 774.0$ (***)	$F_{1, 64} = 588.1$ (***)
Juglone concentrations (J)	$F_{3, 64} = 657.2$ (***)	$F_{3, 64} = 1{,}332.5$ (***)	$F_{3, 64} = 192.8$ (**)	$F_{3, 64} = 825.0$ (***)	$F_{3, 64} = 734.0$ (***)
Exposure time (E)	$F_{3, 64} = 454.5$ (***)	$F_{3, 64} = 958.0$ (***)	$F_{3, 64} = 154.5$ (**)	$F_{3, 64} = 639.3$ (***)	$F_{3, 64} = 1{,}069.7$ (***)
P×J	$F_{3, 64} = 39.3$ (*)	$F_{3, 64} = 48.6$ (*)	$F_{3, 64} = 11.2$ (*)	$F_{3, 64} = 275.4$ (***)	$F_{3, 64} = 460.3$ (***)
J×E	$F_{9, 64} = 12.7$ (*)	$F_{9, 64} = 32.9$ (*)	$F_{9, 64} = 48.9$ (*)	$F_{9, 64} = 193.2$ (**)	$F_{9, 64} = 157.2$ (**)
P×E	$F_{3, 64} = 68.1$ (*)	$F_{3, 64} = 180.2$ (**)	$F_{3, 64} = 23.4$ (*)	$F_{3, 64} = 112.6$ (*)	$F_{3, 64} = 90.5$ (*)
P×J×E	$F_{9, 64} = 11.5$ (*)	$F_{9, 64} = 49.0$ (*)	$F_{9, 64} = 1.2$ (ns)	$F_{9, 64} = 67.4$ (*)	$F_{9, 64} = 83.4$ (*)

*$P<0.05$; **$P<0.01$; ***$P<0.001$; ns – non-significant. Variability source: (i) plant species – maize, wheat; (ii) juglone concentrations – 0 (untreated), 0.01, 0.1 and 1 mM; (iii) exposure time – 0, 1, 2, 3 days.

Wheat seeds treated with juglone for 1 day, responded a higher upregulation of *Cat* genes (5–89% increases) than maize kernels (3–15% elevations); however, the alternations in the abundance of the transcripts were insignificant. Dissimilar patterns of transcriptional responses of the tested catalase genes in maize and wheat kernels was identified after 2 and 3 days of JU exposure. After 2-day exposure to the xenobiotic, maize seeds responded only with slight enhancement in *Cat1* and *Cat3* transcripts abundance (up to 1.8-fold increase), but the level of *Cat2* mRNA considerably increased (up to 4-fold elevation), composed to the control. On the other hand, JU-stressed wheat kernels at 2 days characterized with predominant increment in *Cat1* gene expression level (1.3-6.2-fold increase), transcriptional activity of *Cat2* gene was moderately stimulated (1.9-4.1-fold elevation), whereas the lowest increment in the expression level was recorded in case of *Cat3* gene (up to 2.5-fold). Prolonged naphthoquinone treatments (3 days) markedly upregulated the expression of *Cat1* and *Cat2* in maize seeds (2.7-9.4-fold and 8.2-16.5-fold increases, respectively), but the increment in *Cat3* mRNA was marginal (up to 1.9-fold). Interestingly, wheat kernels after 3-day JU exposure responded with further increase in biogenesis of all the tested catalase transcripts (4.7-8.6-, 5.8-10.5- and 2.6-6.9-fold increments in expression of *Cat1*, *Cat2* and *Cat3* genes, respectively).

Effect of juglone exposure on CAT activity in maize and wheat kernels

Juglone treatments (0.01, 0.1 and 1 mM) were shown to evoke significant modulations of CAT activity in the seeds of maize and wheat (Fig. 3, Table 2). The considerably higher increments in the level of CAT activity were noted in JU-exposed wheat grains, componed to that observed on maize (12–140% and 2–96% increases, respectively). Importantly, the enhancement of CAT activity occurred in a time- and juglone dose-dependent manner, and the higher activity of the analysed biocatalyst was detected after 3 days of 1 mM JU treatment. In addition, all the three JU applications stimulated earlier the increases of CAT activity in wheat seeds (up to 25% increase at day 1), in comparison with maize grains, in which no significant changes in the quantified parameter at day 1 of the biotests were recorded.

Hydrogen peroxide content in juglone-stressed maize and wheat seeds

It was showed that all the investigated juglone treatments (0.01, 0.1 and 1 mM) triggered the H_2O_2 content in the seeds of both maize and wheat (10–152% and 5–87% increase, respectively), compared to the controls (Fig. 4; Table 2). In general, parallel with increasing JU concentrations, higher increments in level of the quantified reactive oxygen species (ROS) were observed in the tested kernels. The only exception

was noted in the group of maize seeds treated with the highest concentration of the studied naphthoquinone (1 mM) for 3 days, in which 22% elevation in H_2O_2 content was recorded, whereas two other JU applications (0.01 and 0.1 mM) induced 28% and 60% increases, respectively. Wheat kernels displayed more intensive H_2O_2 formation in response to 0.01 mM (1 and 2 days of exposure), 0.1 mM (1 day) and 1 mM

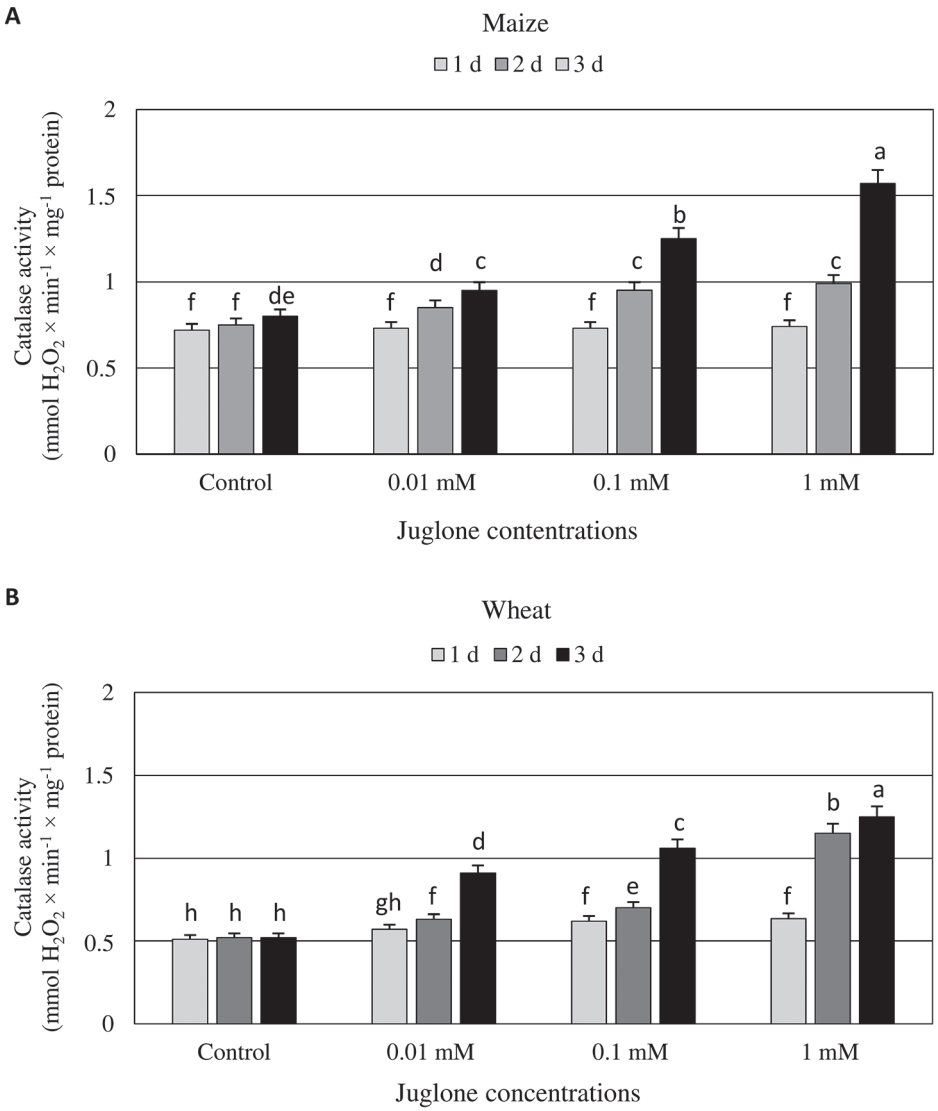

Fig. 3. Juglone-affected modulations of catalase activity (mean ± SD; mmol decomposed $H_2O_2 \times min^{-1} \times mg^{-1}$ protein) in the kernels of tested cereal plants. A – maize; B – wheat; 1d, 2d, 3d – 1, 2 and 3 days of the biotests, respectively. Different letters above bars indicate significant differences between the mean values (Tukey's test; $P < 0.01$)

JU (3 days); administrations than maize. Conversely, other juglone treatments evoked higher increase of H_2O_2 content in *Z. mays* seeds. It should be underlined that both maize and wheat kernels reached the highest production rate of H_2O_2 after 2 days of JU exposure (34–152% and 62–87%), compared to the untreated control.

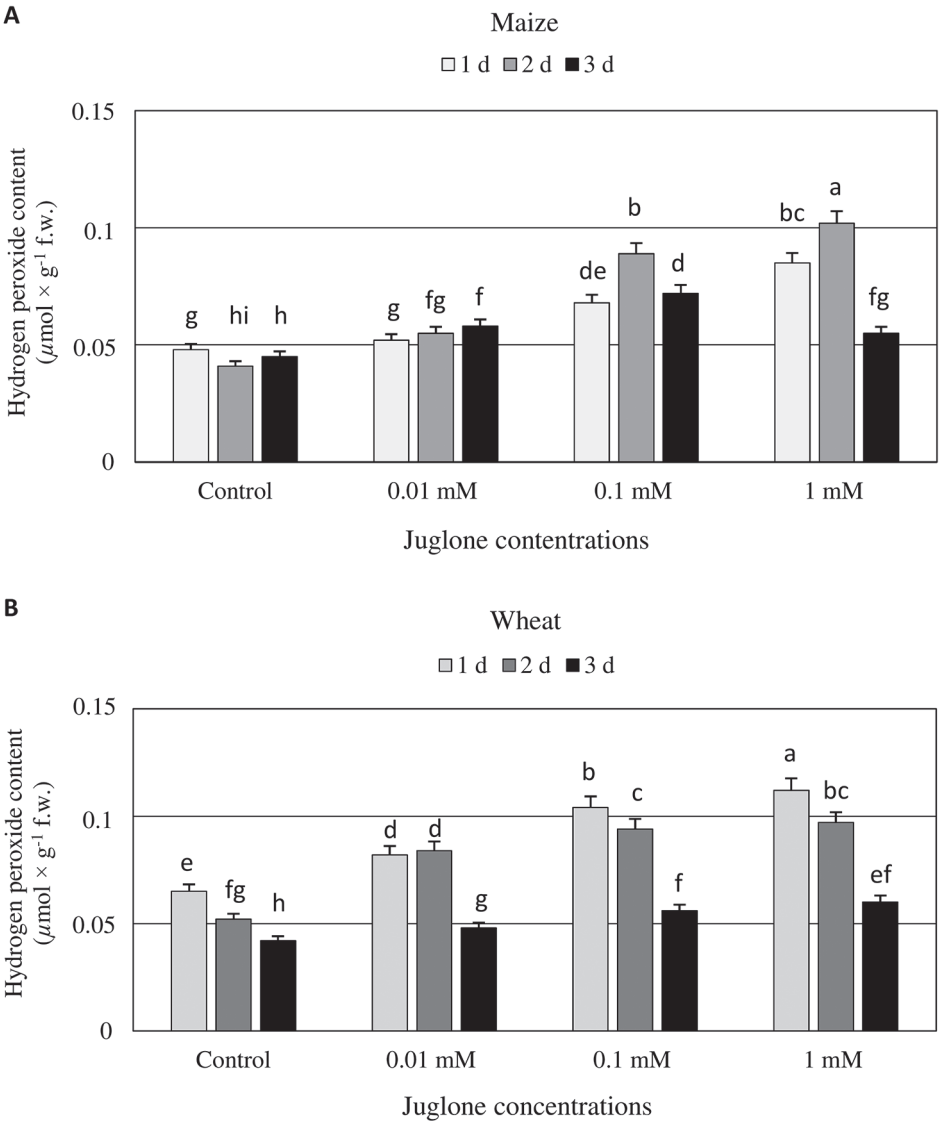

Fig. 4. Impact of juglone exposure on the content of hydrogen peroxide (mean ± SD; μmol × g^{-1} fresh weight) in the seeds of maize (A) and wheat (B). 1d, 2d, 3d – 1, 2 and 3 days of the biotests, respectively. Different letters above bars indicate significant differences between the mean values (Tukey's test; $P < 0.01$)

DISCUSSION

Catalase (CAT; EC 1.11.1.6) comprises a crucial part of the antioxidative enzymatic system in cereal plants. The biological role of this biocatalyst is associated with the turnover of H_2O_2 to water and molecular oxygen [2]. It has been provened that the activity of catalase in plant tissues was significantly altered by a multitude of exogenous factors, including high or low temperature, salinity, UV, heavy metals, pathogens, parasites and phytophagous arthropods [3, 4, 12, 16, 34]. In the present work, it has been demonstrated that juglone exposure markedly inhibited germination process of the tested cereal species (maize and wheat), compared to the untreated controls. Similarly, Matok [28] reported repressed germination of wheat kernels exposed to this allelocompound, and the inhibition degree was concentration-dependent. In addition, Khoshvaghti and Lotfi [24] ascertained that aquaous extract derived from fresh walnut leaves inhibited the germination of wheat seeds by about 50%. Moreover, Sytykiewicz [33] demonstrated that JU treatments (0.1 and 10 μM) led to 6% and 22% decreases in the germination of maize kernels, respectively, in comparison with the control. Several reports demonstrated that juglone may be involved in ROS-associated programmed cell death, disruption of mitochondrial respiratory chain, damages in plasma membrane and endoplasmic reticulum, repression of photosynthesis and transpiration processes in tissues of certain plant species [6, 11, 26, 29]. According to Babula et al. [6], juglone reduced the mitotic index, increased the number of cells at prophase and declined meristematic activity in lettuce seedling roots.

The present study also proved the significant impact of JU exposure on the enhancement of H_2O_2 content in both maize and wheat seeds. Also, the highest increment in the level of the quantified ROS form was recorded after 2 days of the biotests. The obtained results indicat that JU applications triggered oxidative stress in the examined kernels. It has been postulated that overproduction of H_2O_2 in the seeds of cereal plants under adverse environmental stimuli may suppress germination and elongation processes [30]. Mabuchi et al. [27] confirmed that exogenous treatments with H_2O_2 caused a strong activation of MYB30 transcription factor in the root tips of *Arabidopsis thaliana* L. This transcription factor is a significant regulator of several genes involved in the H_2O_2-dependent inhibition of cell elongation and transport of long-chain fatty acids. H_2O_2 is one of the most significant ROS forms in cereal seeds, that participates in regulation of numerous physiological processes (e.g., seed development and germination), ROS-dependent activation of ion channels, perception, transduction and integration of environmental signals, triggering expression of H_2O_2-responsive genes and stimulation of defense mechanisms under stress conditions [14, 21, 22, 36]. However, the exact role of H_2O_2 in plant tissues still remains to be elucidated [21]. Among the different ROS forms, H_2O_2 molecules are characterized by higher stability, lower reactiveness with intracellular components, and a capability of diffusing across plasma membranes [23, 25]. It has been reported that elevated amounts of H_2O_2 may oxidize the cysteine residues in catalytic centers of plant enzymes, thus profoundly modifying their molecular structure, conformational

stability and activity [20]. On the other hand, plants may enhance the turnover of oxidatively damaged proteins under stress conditions [19]. H_2O_2 content of plant cells is largely dependent on the intensity of prooxidative reactions as well as efficiency and coordination of multitude antioxidative enzymes (e.g., peroxidases) and non-enzymatic ROS scavengers (e.g., glutathione, ascorbate, tocopherols, phenolic compounds) [36].

Recently, Sytykiewicz et al. [35] demonstrated that JU applications evoked a prompt overproduction of superoxide anion radicals, with consecutive enhancement in activity of superoxide dismutase (SOD), in seeds of four susceptible plant species: corn cockle (*Agrostemma githago* L.), corn poppy (*Papaver rhoeas* L.), spring oat (*Avena sativa* L., cv. Maczo) and spring wheat (*Triticum aestivum* L., cv. Nawra). In another study [29], it has been ascertained a profound overexpression of three target genes (*Sod3*, *Sod4*, *Sod4A*), encoding SOD isoforms in JU-stressed maize scutella tissue. It indicated the pivotal role of SOD enzyme in diminishing JU-triggered oxidative stress in the tested seeds through the dismutation reaction of highly reactive superoxide anion radicals to less toxic hydrogen peroxide and molecular oxygen.

It is the first molecular survey unvaveling the effect of JU treatments on transcriptional responses of genes encoding catalase isoforms (*Cat1*, *Cat2*, *Cat3*) in seeds of the studied cereal species. In general, the gradual upregulation of the analysed *Cat* genes in JU-exposed seeds of maize and wheat was found. The largest increments of the studied catalase transcripts in the kernels of both investigated cereal species were recorded after 3 days of JU exposure. At the biochemical level, substantial increments in CAT activity in JU-treated seeds of both examined cereals, has also been proved in comparison to the controls. Mylona et al. [29] demonstrated that juglone applications differentially regulated the expression of all the three catalase genes (*Cat1*, *Cat2*, *Cat3*) encoding the respective CAT isoforms in scutella tissue of maize (inbred line W64A). The specific expression patterns of the catalase genes depended on the juglone dose applied, exposure time and plant developmental stage. Furthermore, Chi et al. [11] demonstrated a significant upregulation of 827 genes in JU-treated rice roots. Most of these transcripts were involved in the biosynthesis of primary metabolites, detoxification of allelochemicals, calcium regulation and signal transduction. Additionally, 268 juglone-responsive genes associated with developing oxidative stress or antioxidative responses in rice tissues (e.g., glutathione reductase, glutathione transferases, glutathione peroxidase, class III peroxidase, rice glutaredoxin, thioredoxin) were identified. On the other hand, JU exposure downregulated 142 genes, in most cases linked to secondary metabolism [11]. Additionally, Sytykiewicz [33] showed that JU-treated coleoptiles and primary roots of maize seedlings responded circumstantial increases in expression of *Gst1* gene, encoding glutathione transferase.

Summarizing, the inhibitory effect of JU application on germination process of both cereal model species (maize and wheat) was demonstrated. Moreover, the increased H_2O_2 content in the tested seeds indicated a JU-triggered oxidative stress. At molecular level, the upregulation of three studied genes (*Cat1*, *Cat2*, *Cat3*) encod-

ing catalase isoenzymes in maize and wheat seeds treated with the naphthoquinone was proved. The pivotal role of *Cat2* gene in the maize seeds as well as *Cat1* and *Cat2* genes in the wheat kernels in overcoming the excessive production of H_2O_2 was also untaveled. In addition, JU treatments resulted in significant enhancement in CAT activity in the kernels of both cereals. The elevated H_2O_2 content parallel with the upregulation of the target *cat* genes and CAT activity, indicate that the production rate of this ROS form is probably higher than the efficiency of H_2O_2-scavenging systems in the seeds of both maize and wheat, treated with the different concentrations of JU for 1–3 days. In order to gain a better insight into molecular level of the defensive responses in JU-stressed seeds of the tested cereals, further large-scale transcriptomic analysis of mRNA as well as regulatory microRNAs are needed.

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