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ORIGINAL PAPER

A comparison of the effects of 1,4-naphthoquinone and 2-hydroxy-1,4-naphthoquinone (lawsone) on indole-3-acetic acid (IAA)-induced growth of maize coleoptile cells

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Abstract The effects of 1,4-naphthoquinone (NQ) and 2-hydroxy-1,4-naphthoquinone (NQ-2-OH) on indole-3-acetic acid (IAA)-induced growth, medium pH changes and membrane potential (E_m) in maize (*Zea mays* L.) coleoptile cells were determined. In addition, the redox cycling properties of both naphthoquinones were also compared. The dose-response curves constructed for the effects of NQ and NQ-2-OH on endogenous and IAA-induced growth differ in shape. It was found that NQ was by 10–50% more effective in inhibiting IAA-induced growth in maize coleoptile segments than NQ-2-OH. Simultaneous measurements of growth and external medium pH indicated that NQ and NQ-2-OH reduced or eliminated proton extrusion at all of the concentrations used, excluding NQ at 1 µM. It was found that both naphthoquinones at concentrations higher than 10 µM caused the depolarisation of the membrane potential (E_m). Additionally, compared to the controls, NQ- and NQ-2-OH-exposure of coleoptile segments, at concentrations higher than 10 µM, caused an elevation of the hydrogen peroxide (H_2O_2) production and plasma membrane redox activity. The highest catalase activity was observed at 10 µM NQ and it was ca. 18-fold greater (at 4 h) than in the control medium. Moreover, it was also found that NQ and NQ-2-OH, at all concentrations studied, increased the malondialdehyde content of coleoptile segments at 4 h of the experiment. The data presented here are discussed taking into account the "acid growth hypothesis" of auxin action and the mechanisms by which naphthoquinones interact with biological systems.

Keywords Growth rate · Medium pH · Membrane potential · Naphthoquinones · Oxidative stress

Introduction

Naphthoquinones are a group of organic compounds that are widely distributed in nature. They are the products of bacterial and fungal metabolism as well as secondary metabolism in higher plants (Babula et al. 2009). As compounds with remarkable chemical and structural properties, naphthoquinones have been the focus of the attention of scientists for a long time. The biological activity of naphthoquinones is primarily based on two primary mechanisms—one is the covalent modification of biological molecules at their nucleophilic sites, such as the thiols in proteins and glutathione (GSH), in which quinones act as electrophiles, while the other mechanism consists of redox cycling, in which reactive oxygen species (ROS) are generated (reviewed in El-Najjar et al. 2011; Klotz et al. 2014; Kumagai et al. 2012). Naturally occurring naphthoquinones such as juglone (5-hydroxy-1,4-naphthoquinone), lawsone (2-hydroxy-1,4-naphthoquinone), plumbagin (2-methyl-5-hydroxy-1,4-naphthoquinone), naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) and others along with their synthetic derivatives have been studied intensively for many years in biology, medicine, agriculture and industry. Lawsone (2-hydroxy-1,4-naphthoquinone) is one of the naturally available 1,4-naphthoquinone derivatives.

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Bioassays that use plant growth are often the primary tools for determining the biological activity of natural and synthetic compounds. In this study, we chose the indole-3-acetic acid (IAA)-induced growth of maize coleoptile segments (a classical model system for studies on the elongation growth of plant cells in which the number of cells is constant and the organ grows only by elongation, see Kutschera and Wang 2016) as the main parameter in order to assess the activity of 1,4-naphthoquinone and its natural derivative lawsone (2-hydroxy-1,4-naphthoquinone). In addition, medium pH was measured simultaneously with growth and membrane potential changes were also determined. The relationships between these parameters (growth, medium pH and membrane potential) are crucial for the so-called "acid growth hypothesis" of auxin-induced growth (for a review see Hager 2003). In agreement with this hypothesis, at least in maize coleoptile cells, auxin causes the acidification of the cell wall (Rayle and Cleland 1992) and the hyperpolarisation of the membrane potential by stimulating the activity and/or amount of the plasma membrane H^+-ATPase. Acidification of the cell wall either directly lowers the yield threshold of the wall or optimises the activity of the cell wall-localised proteins that loosen the wall, whereas the hyperpolarisation of the membrane potential causes the activation of voltage-dependent, inwardly rectifying K^+ channels, the activity of which contributes to the water uptake that is necessary for cell expansion (reviewed in Kutschera 1994, 2006; Hager 2003; Kutschera and Wang 2016). In this study, the redox cycling properties of two naphthoquinones, 1,4-naphthoquinone and 2-hydroxy-1,4-naphthoquinone (estimated by H_2O_2 production, catalase activity, MDA content and hexacyanoferrate III reduction), were also compared in experiments that were performed on maize coleoptile segments incubated with or without the addition of IAA.

The main goal of our experiments was to study the mechanisms by which naphthoquinones cause changes in IAA-induced growth of maize coleoptile segments. This goal was realised by: (1) studying the effects of 1,4-naphthoquinone and 2-hydroxy-1,4-naphthoquinone (lawsone) on growth in the presence and absence of IAA and medium pH measured simultaneously with growth; (2) establishing the impact of both naphthoquinones on changes in the membrane potential in the parenchymal cells of maize coleoptile segments incubated in the presence and absence of IAA; (3) comparing the effects of 1,4-naphthoquinone and 2-hydroxy-1,4-naphthoquinone on H_2O_2 production and catalase activity in coleoptile segments; (4) examining the influence of NQ and NQ-2-OH on the plasma membrane redox activity and (5) establishing the effects of both naphthoquinones on the malondialdehyde (MDA) content of coleoptile segments incubated with or without IAA. This experimental design can provide new data on the effects of naphthoquinones on plant growth. To the best of our knowledge, the effect of 1,4-naphthoquinone and 2-hydroxy-1,4-naphthoquinone (lawsone) on IAA-induced growth of plant cells has never been studied.

Materials and methods

Plant material

The experiments were performed on ten-mm-long coleoptile segments that had been cut from 96 h etiolated maize seedlings. The maize seedlings were obtained by soaking seeds of maize (*Zea mays* L. cv. Cosmo 230) in tap water for two hours, sowing them on wet lignin in plastic boxes and growing them in the dark at 27 ± 1.0 °C in a growth chamber (Type MIR-533, Sanyo Electric Co., Japan) for 4 days (length of the coleoptile 2–3 cm). The coleoptile segments from which the first leaves had been removed were excised 3 mm below the tip and incubated in a control medium consisting of the following solutions: 1 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$. In all of the growth experiments, the initial pH of the control medium was adjusted to 5.8–6.0 with either 0.1 M NaOH or 0.1 M HCl (but see also Kutschera and Schopfer 1985a, b, wherein the effects of K versus Na-ions is described).

Chemicals

The lawsone (2-hydroxy-1,4-naphthoquinone, NQ-2-OH) was obtained from Sigma Aldrich Inc. (St. Louis, MO, USA). The concentrations of lawsone that were tested in these experiments were prepared by dissolving this allelochemical in distilled water. A 10^{-2} M stock solution was the basis for the preparation of all of the concentrations (10^{-8}–10^{-3} M). IAA (Serva, Heidelberg, Germany) was used as the potassium salt because it can be rapidly dissolved in water. IAA was used at a final concentration of 100 µM. This concentration is optimal for the elongation growth of the maize coleoptile segments, which was measured over ten hours in our elongation- and pH-measuring apparatus (Polak 2010). 1,4-Naphthoquinone (NQ) (Sigma, USA) was dissolved in ethanol and added to the incubation medium in a range of concentrations from 10^{-8}–10^{-3} M. The maximal ethanol concentration of 0.35% did not affect the growth of the coleoptile segments (data not shown). The thiobarbituric acid and trichloroacetic acid used to estimate lipid peroxidation were obtained from POCH (Polish Chemicals Reagents, Gliwice, Poland) and the butylated hydroxytoluene (BHT), which was used in the same measurements, was obtained from Sigma Aldrich. To determine catalase activity, a 10 mM H_2O_2 solution was prepared by dissolving the hydrogen peroxide (30%) from POCH in a 0.1 M K-phosphate buffer.

Growth and pH measurements

The growth experiments of coleoptile segments were carried out in an apparatus that permitted simultaneous measurements of the elongation growth and the pH of the incubation medium from the same tissue sample. A somewhat similar procedure for measuring the elongation growth of maize coleoptile segments and pH changes in their incubation medium has previously been described (Karcz and Burdach 2002; Karcz et al. 1990). Briefly, 60 coleoptile segments were arranged vertically in three narrow glass pipettes (20 segments in each), which were connected in this apparatus using a silicon hose. High-resolution measurements of the growth rate were performed using an angular position transducer (TWK Electronic, Düsseldorf, Germany), which resulted in a precise record of the growth kinetics. The coleoptile segments were incubated in an intensively aerated medium, in which the volume of the incubation medium in the elongation and pH-measuring apparatus was constant (0.3 ml/segment). The incubation medium also flowed through the lumen of the coleoptile cylinders. This feature enables the experimental solutions to be in direct contact with the interior of the segments, which significantly enhances both the elongation growth of the coleoptile segments and proton extrusion (Karcz et al. 1995). This experimental set-up also enabled coleoptile abrasion, which inhibits (c. 30%) elongation growth (Karcz et al. 1995; Lüthen et al. 1990) to be avoided (Dreyer et al. 1981). Medium circulation was driven by a peristaltic pump (1B-05A; Zalimp, Poland). The extension growth of a stack of 20 segments and the pH of the incubation medium were sampled every 3 min using a multifunctional computer meter (CX-771; Elmetron, Poland). The pH measurements were performed with a pH electrode (OSH 10–10; Metron, Poland). All of the manipulations, growth and pH measurements were carried out under dim green light at a thermostatically controlled temperature of 25 ± 0.5 °C.

Electrophysiology

The electrophysiological experiments were performed with coleoptile segments that were prepared in the same manner as for the growth experiments. A standard electrophysiological technique was used for the membrane potential measurements as was previously described (Burdach et al. 2014; Karcz and Burdach 2002). Briefly, membrane potential (E_m) was measured by recording the voltage between a 3 M KCl-filled glass micropipette that was inserted into the parenchymal cells and a reference electrode in a bathing medium of the same composition as the one used in the growth experiments. For the electrophysiological experiments, the segments were preincubated for 1 h in an intensively aerated bathing medium, after which the segments were transferred into a perfusion Plexiglas chamber mounted on a vertically placed microscope stage. Medium changes were performed using a peristaltic pump (Type Peri-Star PRO; World Precision Instruments, USA) after the stabilisation of the membrane potential (E_m). This type of peristaltic pump permits the bathing medium in the chamber to be changed (usually four times within less than 2 min) without any visible disruption of the measurements. The microelectrodes were inserted into the cells under a microscope using a micromanipulator (Hugo Sach Electronik; MarchHugstteten, Germany). The micropipettes were made from borosilicate glass capillaries (type 1B150F-3; World Precision Instruments, USA) using a vertical pipette puller (model L/M-3P-A; List-Medical, Germany).

Hydrogen peroxide detection

The hydrogen peroxide (H_2O_2) concentration in all of the tested variants was determined according to Velikova et al. (2000) (but also see Junglee et al. 2014). Briefly, coleoptile segment samples of 0.5 g were homogenised in 1.5 ml of 0.1% (w/v) tri-chloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm and 4 °C for 10 min. Subsequently, 0.5 ml of the supernatant was added to 0.5 ml of a 0.1 M K-phosphate buffer (pH 7.0) and 1 ml of 1 M KI. The absorbance of the supernatant was measured at 390 nm. The content of H_2O_2 was calculated from a standard calibration curve prepared at different of H_2O_2 concentrations (from 100 to 1000 µmol/ml) in 0.1% TCA. The H_2O_2 concentration was expressed as µmol/g fresh weight (FW).

Catalase activity

We chose the catalase activity as an indicator of oxidative stress taking into account the fact that the activity of catalase in maize coleoptiles is the main enzymatic mechanism that is responsible for the degradation of the H_2O_2 that is generated under the oxidative stress induced by juglone (5-hydroxy-1,4-naphthoquinone) and lawsone (2-hydroxy-1,4-naphthoquinone, NQ-2-OH) as was recently shown by Kurtyka et al. (2016). Catalase (CAT) activity was determined as described by Cavalcanti et al. (2004) with minor modifications. Briefly, coleoptile segment samples of 0.2 g were homogenised in 1.5 ml of a 0.1 M K-phosphate buffer (pH 7.0). The homogenate was centrifuged at 20,000 rpm and 25 °C for 20 min. Subsequently, 20 µl of the supernatant was added to 2 ml of 10 mM H_2O_2 and the decrease in absorbance was measured at 240 nm and 30 °C. Enzyme activity was calculated using the molar extinction coefficient 36×10^3/mM/m and expressed as µmol H_2O_2 oxidised/g FW/min.

Determination of MDA content

Lipid peroxidation was determined by estimating the MDA content, which was determined in terms of the concentration thiobarbituric acid-reactive substances (TBARS) as described by Hodges et al. (1999) with minor modifications. Coleoptile segments samples of 0.5 g were immediately placed in liquid nitrogen. Then, the plant tissues were homogenised in 12.5 ml 80% ethanol. A 1-ml aliquot of the appropriately diluted sample was added to a test tube with 1 ml of either (1) a -TBA solution comprised of 20% (w/v) trichloroacetic acid and 0.01% butylated hydroxytoluene or (2) a + TBA solution containing the above plus 0.65% TBA. Samples were then mixed vigorously, heated at 95 °C in a boiling water bath for 20 min, cooled, and centrifuged at 10,000 rpm and 4 °C for 10 min. Absorbances were read at 440, 532, and 600 nm. MDA equivalents were calculated in the following manner:

1. $[(Abs_{532+TBA}) - (Abs_{600+TBA}) - (Abs_{532-TBA} - Abs_{600-TBA})] = A$
2. $[(Abs_{440+TBA} - Abs_{600+TBA})\ 0.0571] = B$
3. MDA equivalents (nmol/ml) $= (A - B/157000) \times 10^6$

Redox activity

To determine the redox activity estimated as the hexacyanoferrate III (HCF III) reduction, the coleoptile segments were prepared in the same manner as for the growth experiments. These coleoptile segments were then preincubated for 1 h in distilled water and immediately transferred to 1 mM Tris–HCl (pH 6.0) containing 0.5 mM $CaCl_2$, 50 mM KCl, and either NQ or NQ-2-OH at the appropriate concentration. IAA, at a final concentration of 100 µM, was, as required, included in the incubation medium. HCF III (ferricyanide), at a final concentration of 1 mM was added to the incubation medium. The coleoptile segments were shaken at 100 rpm and the level of HCF III reduction was measured upon the addition of the coleoptile segments and every 30 min for the next 2 h. Redox activity, measured as the decay of HCF III absorption, was monitored spectrophotometrically at 420 nm, as was previously described by Federico and Giartosio (1983) and expressed in nmol of the reduced hexacyanoferrate III calculated per g of FW. Because naphthoquinones absorb the light at 420 nm, we modified the values of HCF III reduction by the ones recorded without HCF III in the incubation medium. The results are the means of three independent experiments.

Statistical analysis

Data were analysed using Statistica software for Windows (STATISTICA data analysis software system, version 12.0 http://www.statsoft.com, USA) at a significance level of 0.05. One-way ANOVA was used to test the statistical differences in the effect of both naphthoquinones on IAA-induced coleoptile growth. Afterwards, the post hoc least significant difference (LSD) test was used for further analysis ($P < 0.05$). The Student's *t*-test was used to evaluate the significance of differences between the membrane potential values that were recorded at 0 and 60 min of the measurements.

Results

The effects of 1,4-naphthoquinone (NQ) and 2-hydroxy-1,4-naphthoquinone (NQ-2-OH) on the growth of coleoptile segments

The addition of NQ or NQ-2-OH to the control medium (1 h after the start of the experiment) inhibited the endogenous growth (growth in a medium without growth substances) of maize coleoptile segments at all of the concentrations studied (see Supplementary Fig. 1 a, b in Supplementary Material). For example, at the lower concentrations (0.01, 0.1 and 1 µM), both naphthoquinones reduced endogenous growth (1193.6 ± 32 µm/cm, mean ± SE, n = 7) by ca. 40%, whereas at the highest concentration (1000 µM) by ca. 75%. Because that the addition of NQ and NQ-2-OH to the control medium induced a low growth rate in the coleoptile segments, the elongation growth of the segments is only shown (for comparison see Figs. 1, 2).

When IAA was added to the control medium (2 h after the start of the experiment), a strong increase in the growth rate was observed (Fig. 1). The kinetics of IAA-induced growth could be divided into a very rapid phase, with a maximal growth rate of ~0.12 µm/s/cm, followed by a long-lasting one that began ~30 min after the addition of the auxin. The total IAA-induced elongation growth of the maize coleoptile segments, which was calculated as the sum of the extensions measured at three-minute intervals (Fig. 1, inset), was approximately 2.5-fold greater than in the control (1193.6 ± 32 µm/cm, mean ± SE, n = 7). The data in Fig. 1 indicate that NQ, when added after one hour of the incubation of the segments in the control medium, reduced the growth of the IAA-incubated coleoptile segments at all of the concentrations studied. The first peak (rapid phase) that was observed in the kinetics of the IAA-induced growth rate was reduced in the presence of NQ concentrations higher than 10 µM. NQ-2-OH, which was added to the incubation medium at the same time protocol and concentrations as NQ, inhibited the elongation growth of maize coleoptile segments incubated in the presence of IAA, similar to NQ (Fig. 2). The first peak of the IAA-induced growth of the coleoptile segments that were treated with NQ-2-OH was inhibited only at 1000 µM, whereas

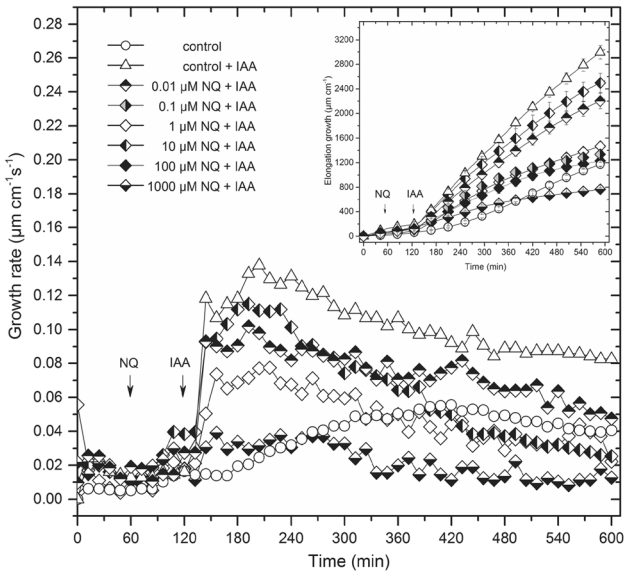

Fig. 1 Effect of 1,4-naphthoquinone (NQ) on the growth rate (μm/s/cm) of maize coleoptile segments that had been incubated in the presence of IAA (100 μM). The coleoptile segments were first preincubated (more than 1 h) in a control medium, after which NQ in a range of concentrations from 10^{-8} to 10^{-3} M was added. IAA was added to the incubation medium at 2 h. The inset on the right side shows the total elongation growth, calculated as the sum of the extensions from measurements at 3-min intervals over 10 h. For the sake of clarity, the growth rate curves at 0.1 and 100 μM (because of their similarity to NQ at 1 μM) were removed. The data presented are the means of at least seven independent experiments. Bars indicate means ± SEs

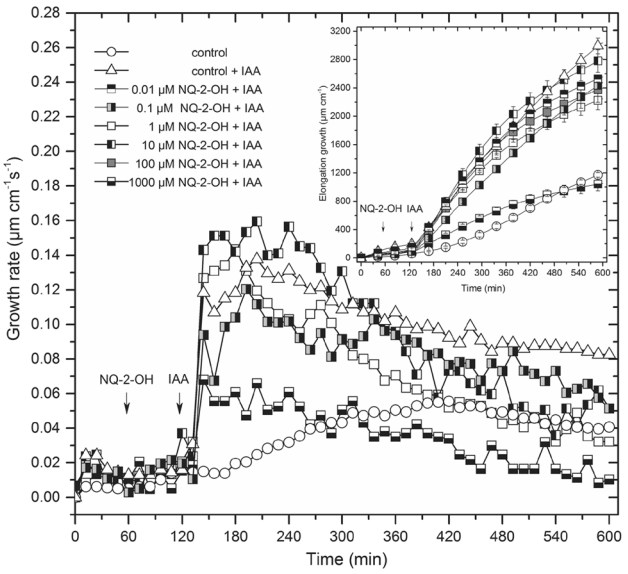

Fig. 2 Effect of 2-hydroxy-1,4-naphthoquinone (NQ-2-OH, lawsone) on the growth rate (μm/s/cm) of maize coleoptile segments incubated in the presence of IAA (100 μM). The coleoptile segments were first preincubated (more than 1 h) in a control medium, after which NQ-2-OH in a range of concentrations from 10^{-8} to 10^{-3} M was added. IAA was added to the incubation medium at 2 h. The inset on the right side shows the total elongation growth, calculated as the sum of extensions from measurements at 3-min intervals over 10 h. For the sake of clarity, the growth rate curves at 0.01 and 100 μM (because of their similarity to NQ-2-OH at 0.1 μM) were removed. The data presented are the means of at least seven independent experiments. Bars indicate means ± SEs

NQ-2-OH at 1 and 10 μM clearly stimulated it. The dose-response curves constructed for the effects of NQ and NQ-2-OH on endogenous and IAA-induced elongation growth of the maize coleoptile segments (calculated as the sum of extensions measured at three-minute intervals over eight hours between 120 and 600 min) differed in shape (Fig. 3 a). In the presence of IAA, both dose-response curves had two extremes—a maximum at 1 μM of NQ or NQ-2-OH, which had similar but statistically different values ($P < 0.05$) of IAA-induced elongation growth and a minimum at 1 and 0.1 μM for NQ and NQ-2-OH, respectively. In the case of endogenous growth, both dose-response curves were linear. At the highest concentration (1000 μM), both naphthoquinones not only eliminated IAA-induced elongation growth but also reduced endogenous growth (growth in the control medium). Taking the above into account, it can be stated that NQ-2-OH has a less toxic effect on IAA-induced elongation growth compared to NQ.

The effects of NQ and NQ-2-OH on medium pH measured simultaneously with growth

The pH of the incubation medium, which was measured simultaneously with the growth of the coleoptile segments (Supplementary Fig. 2, Figs. 4, 5), indicated that the coleoptile segments that were incubated in the control medium characteristically changed the pH of the external medium. Usually, a pH increase to 6.0–6.3 was observed within the first 2 h, followed by a slow decrease to a pH of approximately 5.6 after 10 h. When NQ or NQ-2-OH was added to the control medium (after 1 h of the preincubation in the control medium), this characteristic pattern of external medium pH was disturbed (see Supplementary Fig. 2 in Supplementary Material). Both naphthoquinones added to the control medium inhibited proton extrusion at all of the concentrations studied (see Supplementary Fig. 2 in Supplementary Material). When IAA, at a final concentration of 100 μM, was added to the incubation medium 2 h after the start of the experiment, an additional decrease in pH to ca. 4.8 was observed. However, when NQ or NQ-2-OH was added to the incubation medium (after 1 h of the preincubation of the segments in the control medium), the IAA-induced medium acidification was disturbed (Figs. 4, 5, insets). In order to express the complex pH changes in the medium much more suggestively, they are shown as $\Delta[H^+]$ per coleoptile segment ($\Delta[H^+]$/cm), where $\Delta[H^+]$ means the difference

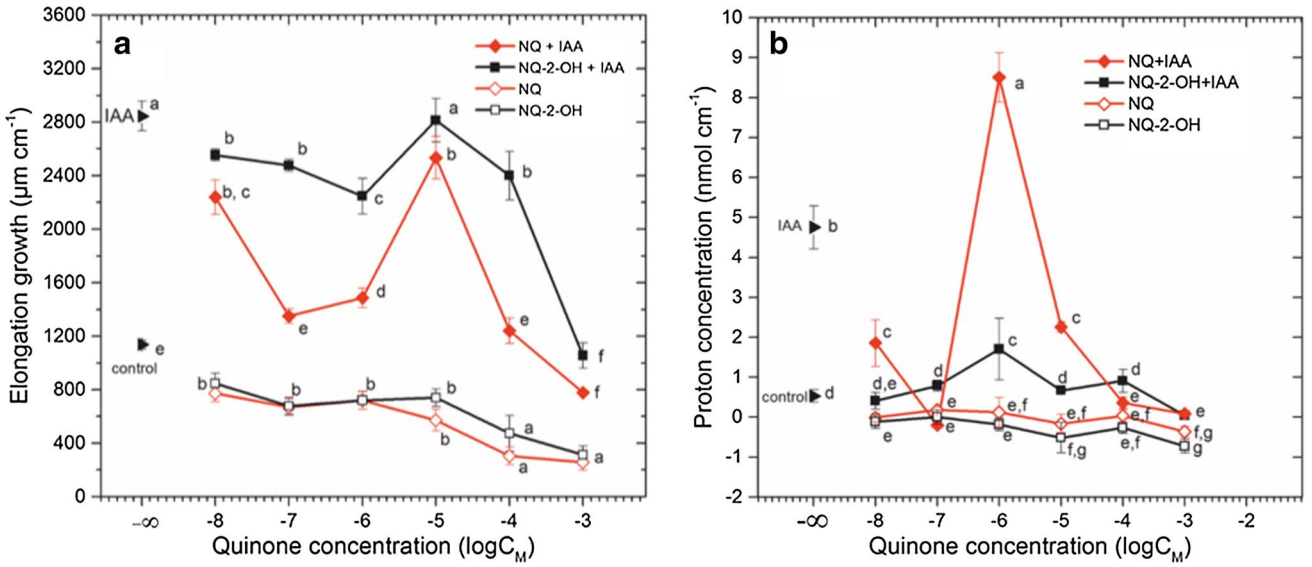

Fig. 3 Dose–response curves for the effects of NQ and NQ-2-OH on: **a** endogenous and IAA-induced growth of maize coleoptile segments. The curves were constructed taking into account the total IAA-induced growth and endogenous growth, calculated as the sum of extensions from measurements at 3-min intervals over 8 h, between 120 and 600 min. The data presented in this figure are the same as those shown in Supplementary Figure. 1, Figs. 1, 2 between 120 and 600 min of the experiment. Bars indicate mean ± SE. Mean values followed by the same letter are not significantly different from each other according to the LSD test ($P < 0.05$). **b** Proton extrusion measured in the presence and absence of IAA. The curves were constructed taking into account the differences between the H^+ concentration per coleoptile segment at 600 and 120 min. The data presented in this figure are the same as those shown in the insets in Supplementary Fig. 2b, c and in the insets on the right side in Figs. 4 and 5. Bars indicate means ± SEs. Mean values followed by the same letter are not significantly different from each other according to the LSD test ($P < 0.05$)

between the H^+ concentration ($[H^+]$) at 600 and 120 min (addition of IAA) (Supplementary Fig. 2, Figs. 4, 5). As is illustrated in Fig. 4, NQ decreased the IAA-induced proton extrusion at almost all of the concentrations used (excluding NQ at 1 μM). This effect was especially visible at higher NQ concentrations (100 and 1000 μM) and at 0.1 μM where the alkalinisation of the medium was observed. When comparing the effect of NQ-2-OH on medium pH with that which was recorded in the presence of NQ, it should be pointed out that NQ-2-OH, in contrast to NQ, lowered IAA-induced proton extrusion at all of the concentrations studied (Fig. 3b). Moreover, NQ-2-OH at 0.01, 0.1, 10 and 100 μM decreased IAA-induced proton extrusion to the same level as that observed in the control medium (0.53 ± 0.2 nmol/cm, mean ± SE, n = 7), whereas NQ at 0.1 and 100 μM, in the presence of IAA, caused medium alkalinisation. NQ at 1000 μM, similar to NQ, caused the alkalinisation of the incubation medium.

Effect of NQ and NQ-2-OH on the membrane potential (E_m) of parenchymal coleoptile cells

The mean E_m of the control parenchymal cells was − 105.6 ± 0.9 mV (mean ± SE, n = 36). The addition of NQ to the control medium at 100 and 1000 μM caused a delayed (within 60 min) depolarisation of the E_m by 12.2 and 41.0 mV, respectively (Table 1). NQ at 0.01, 1.0, and 10 μM did not change the original value of E_m significantly, whereas at 0.1 μM it caused membrane hyperpolarisation of 14.6 mV, which usually started after 20 min. The addition of IAA to the control medium alone caused the hyperpolarisation of the E_m by 14.5 mV (from − 111.7 ± 5.3 to − 126 ± 5.2 mV) after more than 60 min. When the coleoptile segments were first preincubated with NQ for 60 min, and then IAA was subsequently added, the auxin-induced membrane hyperpolarisation decreased by 4.5 and 7.0 mV at 0.01 and 0.1 μM of NQ, respectively (Table 1). NQ at higher concentrations (> 0.1 μM) not only eliminated IAA-induced E_m hyperpolarisation but also caused small membrane depolarisation in the range of 12.5 to 7.5 mV. The NQ-2-OH that was studied at the same concentrations and time protocol as NQ did not significantly change (excluding NQ-2-OH at 0.1 μM) the picture of the membrane potential changes that was observed in the presence of NQ (Tables 1, 2). NQ-2-OH at 100 μM induced a similar depolarisation of E_m as NQ, but at 1000 μM, NQ-2-OH was less effective than NQ (by ca. 10 mV) in the depolarisation of E_m. In contrast to NQ, NQ-2-OH at 1 and 10 μM, acting in the presence of IAA, caused a clear E_m hyperpolarisation within the first 40 min, which decreased to a level comparable with

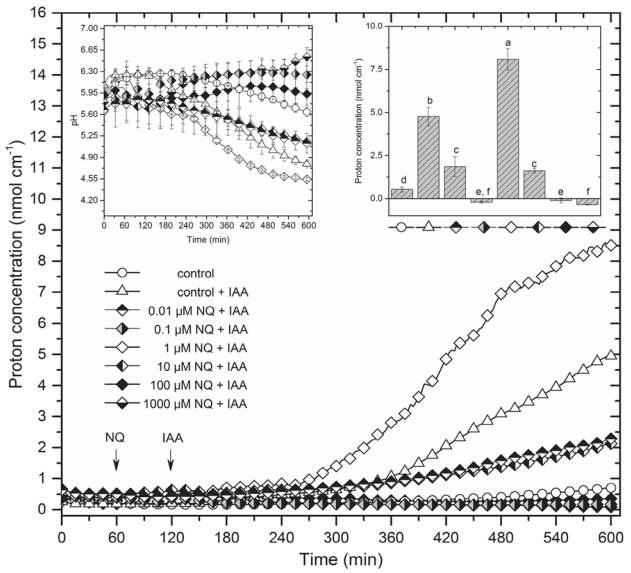

Fig. 4 Effect of 1,4-naphthoquinone (NQ) on the medium pH of maize coleoptile segments incubated in the presence of IAA (inset on the left side). The medium pH changes observed in the presence of all of the studied NQ concentrations are expressed as changes in the H^+ concentration per coleoptile segment ($[H^+]$/cm) and are shown below the figure. The inset on the right side shows the differences between the H^+ concentration per coleoptile segment at 600 and 120 min. Auxin and NQ were added to the incubation medium at the same time protocol as described for the growth experiments shown in Fig. 1. pH values are the means of at least seven independent experiments, which were performed simultaneously with growth on the same tissue sample (as described in the section "Material and methods"). Bars indicate means ± SEs. Mean values followed by the same letter are not significantly different from each other according to the LSD test ($P < 0.05$)

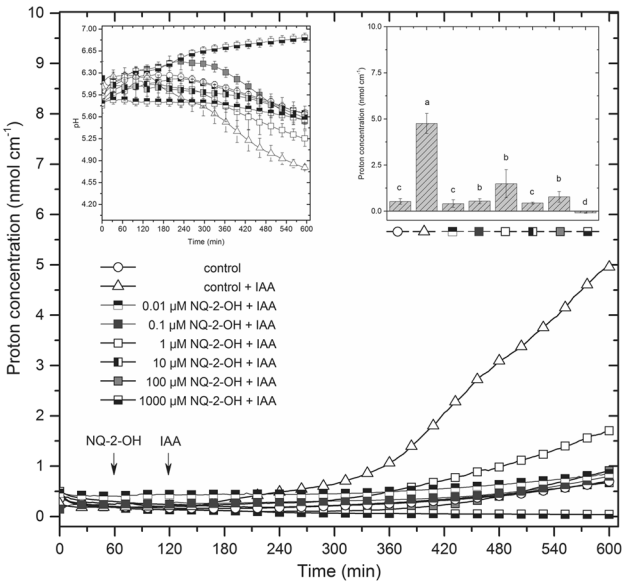

Fig. 5 Effect of 2-hydroxy-1,4-naphthoquinone (NQ-2-OH, lawsone) on the medium pH of maize coleoptile segments incubated in the presence of IAA (inset on the left side). The medium pH changes observed in the presence of all of the NQ-2-OH concentrations studied are expressed as changes in the H^+ concentration per coleoptile segment ($[H^+]$/cm) and are shown below the figure. The inset on the right side shows the differences between the H^+ concentration per coleoptile segment at 600 and 120 min. Auxin and NQ-2-OH were added to the incubation medium at the same time protocol as described for the growth experiments shown in Fig. 2. pH values are the means at least seven independent experiments, which were performed simultaneously with growth on the same tissue sample (as described in the section "Material and methods"). Bars indicate means ± SEs. Mean values followed by the same letter are not significantly different from each other according to the LSD test ($P < 0.05$)

the original value during the next 20 min. NQ-2-OH at all of the concentrations studied eliminated IAA-induced membrane hyperpolarisation.

Summing up the data obtained in the electrophysiological experiments, it should be pointed out that both naphthoquinones at concentrations higher than 10 μM caused membrane potential depolarisation.

Effect of NQ and NQ-2-OH on H_2O_2 production

The application of either NQ or NQ-2-OH (after 1 h of preincubation of the segments in the control medium) at concentrations higher than 10 μM resulted in an increase in hydrogen peroxide production after 2–3 h, which was similar for both naphthoquinones (Fig. 6). For example, treatment of maize coleoptile segments with NQ or NQ-2-OH at 1000 μM increased H_2O_2 production by 97% and 80% within 4 h as compared to the control (301.4 ± 25.8 μmol/g FW; mean ± SE, n = 5, shown here as −log ∞in Fig. 6).

At lower concentrations (< 10 μM), both naphthoquinones decreased H_2O_2 production within the first hour. Addition of IAA to the control medium alone (shown here as −log∞) did not change the H_2O_2 production significantly compared to the control. However, when IAA was applied one hour after the addition of NQ or NQ-2-OH at higher concentrations (> 10 μM), it also enhanced H_2O_2 production in the first hours of the experiments. At lower concentrations of both naphthoquinones (< 10 μM), IAA did not change the level of H_2O_2 production.

The effects of NQ and NQ-2-OH on the catalase activity

Catalase (CAT) activity in the coleoptile segments treated with NQ was significantly higher at 1 and 10 μM, while at the remaining concentrations, it was similar to that in the control medium (3.69 ± 0.35 μmol H_2O_2/min/g FW; mean ± SE, n = 4) (see Supplementary Fig. 3, inset in Supplementary Material). For example, the highest CAT activity was observed at 10 μM NQ (at 4 h) and it was ca. 18-fold greater than in the control medium (at 4 h). Treatment of the maize coleoptile segments with NQ at 1 and 10 μM and

Table 1 E_m changes in parenchymal coleoptile cells after the addition of NQ, IAA and NQ with IAA. At time 0 (A), the control medium was exchanged for a new one with the same salt composition containing NQ, IAA or NQ with IAA

Treatments	E_m (mV)				
	A 0 min	B 20 min	C 40 min	D 60 min	ΔE_m (D − A) (mV)
0.01 µM NQ	− 101.8 ± 2.7	− 98.1 ± 9.6	− 97.4 ± 3.3	− 101.9 ± 8.9	− 0.1[a]
0.1 µM NQ	− 100.3 ± 0.9	− 107.8 ± 6.6	− 113.7 ± 5.4	− 114.9 ± 9.1	− 14.6*
1 µM NQ	− 106.9 ± 4.5	− 110.9 ± 2.4	− 103.9 ± 4.9	− 104.0 ± 7.3	2.9[a]
10 µM NQ	− 106.2 ± 3.5	− 108.2 ± 11.5	− 112.6 ± 8.1	− 108.3 ± 3.2	− 2.1[a]
100 µM NQ	− 104.8 ± 1.9	− 106.2 ± 6.7	− 94.9 ± 2.3	− 92.6 ± 6.9	12.2*
1000 µM NQ	− 105.8 ± 2.3	− 70.5 ± 3.7	− 68.4 ± 6.3	− 64.8 ± 8.8	41*
100 µM IAA	− 111.7 ± 5.3	− 117.5 ± 6.7	− 124.8 ± 4.8	− 126.2 ± 5.2	− 14.5*
0.01 µM NQ + IAA	− 101.5 ± 6.7	− 107.0 ± 1.8	− 109.9 ± 5.3	− 111.5 ± 3.9	− 10*
0.1 µM NQ + IAA	− 109.2 ± 9.4	− 109.9 ± 6.7	− 114.0 ± 6.7	− 116.7 ± 4.7	− 7.5*
1 µM NQ + IAA	− 111.6 ± 9.3	− 99.7 ± 7.2	− 103.1 ± 3.4	− 99.1 ± 4.6	12.5*
10 µM NQ + IAA	− 109.5 ± 2.1	− 104.3 ± 7.2	− 106.2 ± 5.1	− 99.4 ± 1.6	10.1*
100 µM NQ + IAA	− 102.9 ± 4.6	− 98.3 ± 6.5	− 98.3 ± 5.4	− 93.1 ± 6.9	9.8*
1000 µM NQ + IAA	− 73.4 ± 4.9	− 70.7 ± 3.7	− 66.3 ± 2.9	− 65.9 ± 4.6	7.5[a]

In a case in which NQ was added together with IAA, the coleoptile segments were first preincubated for more than 1 h in the presence of NQ, whereupon E_m was measured. Data are the means of at least six independent experiments. Error indicates ± SE

*Statistically significant ($P < 0.05$)

[a]Not statistically significant

Table 2 E_m changes in parenchymal coleoptile cells after the addition of 2-hydroxy-1,4-naphthoquinone (NQ-2-OH), IAA and NQ-2-OH with IAA

Treatments	E_m (mV)				
	A 0 min	B 20 min	C 40 min	D 60 min	ΔE_m (D − A) (mV)
0.01 µM NQ-2-OH	− 106.2 ± 0.2	− 110.1 ± 5.6	− 104.3 ± 4.0	− 107.6 ± 1.9	− 1.4[ns]
0.1 µM NQ-2-OH	− 102.3 ± 3.3	− 109.9 ± 5.4	− 112.7 ± 9.5	− 104.8 ± 9.6	− 2.5[ns]
1 µM NQ-2-OH	− 108.7 ± 9.9	− 110.2 ± 6.3	− 110.4 ± 4.9	− 101.2 ± 6.3	7.5[ns]
10 µM NQ-2-OH	− 109.9 ± 1.6	− 116.4 ± 8.0	− 108.5 ± 7.5	− 104.0 ± 1.3	5.9[ns]
100 µM NQ-2-OH	− 110.1 ± 3.6	− 102.0 ± 8.5	− 105.7 ± 5.3	− 102.8 ± 6.2	7.3*
1000 µM NQ-2-OH	− 104.6 ± 3.0	− 91.7 ± 4.2	− 86.6 ± 4.9	− 74.2 ± 8.3	30.4*
100 µM IAA	− 111.7 ± 5.3	− 117.5 ± 6.7	− 124.8 ± 4.8	− 126.2 ± 5.2	− 14.5*
0.01 µM NQ-2-OH + IAA	− 106.9 ± 2.4	− 90.7 ± 10.2	− 114.6 ± 4.9	− 100.1 ± 8.3	6.8[ns]
0.1 µM NQ-2-OH + IAA	− 104.1 ± 9.0	− 116.2 ± 2.7	− 102.1 ± 9.9	− 106.2 ± 9.0	− 2.1[ns]
1 µM NQ-2-OH + IAA	− 100.2 ± 1.6	− 109.3 ± 3.9	− 116.4 ± 3.3	− 109.2 ± 9.9	− 9.0[ns]
10 µM NQ-2-OH + IAA	− 106.5 ± 4.4	− 122.5 ± 2.3	− 126.7 ± 8.6	− 104. 2 ± 9.5	2.3[ns]
100 µM NQ-2-OH + IAA	− 104.7 ± 6.2	− 108.7 ± 5.2	− 107.9 ± 12.9	− 99.4 ± 9.8	5.3[ns]
1000 µM NQ-2-OH + IAA	− 82.1 ± 4.8	− 80. 7 ± 1.1	− 78.3 ± 9.5	− 74.7 ± 8.3	7.4[ns]

At time 0 (A), the control medium was exchanged for a new one with the same salt composition containing NQ-2-OH, IAA or NQ-2-OH with IAA. In a case in which NQ-2-OH was added together with IAA, the coleoptile segments were first preincubated for > 1 h in the presence of NQ-2-OH, after which E_m was measured. Data are the means of at least six independent experiments. Error indicates ± SE

*Statistically significant ($P < 0.05$)

[ns]Not statistically significant

in combination with IAA decreased the CAT activity to a similar level as that observed in the control (see Supplementary Fig. 3 in Supplementary Material). CAT activities in the coleoptile segments incubated at 1000 µM NQ were inhibited regardless of the presence of IAA. The NQ-2-OH that was studied at the same concentrations and time protocol as NQ did not significantly change the picture of the CAT changes that were observed in the presence of NQ (excluding NQ at 1 and 10 µM) (see Supplementary Fig. 4 in Supplementary Material). NQ-2-OH at 100 and 1000 µM,

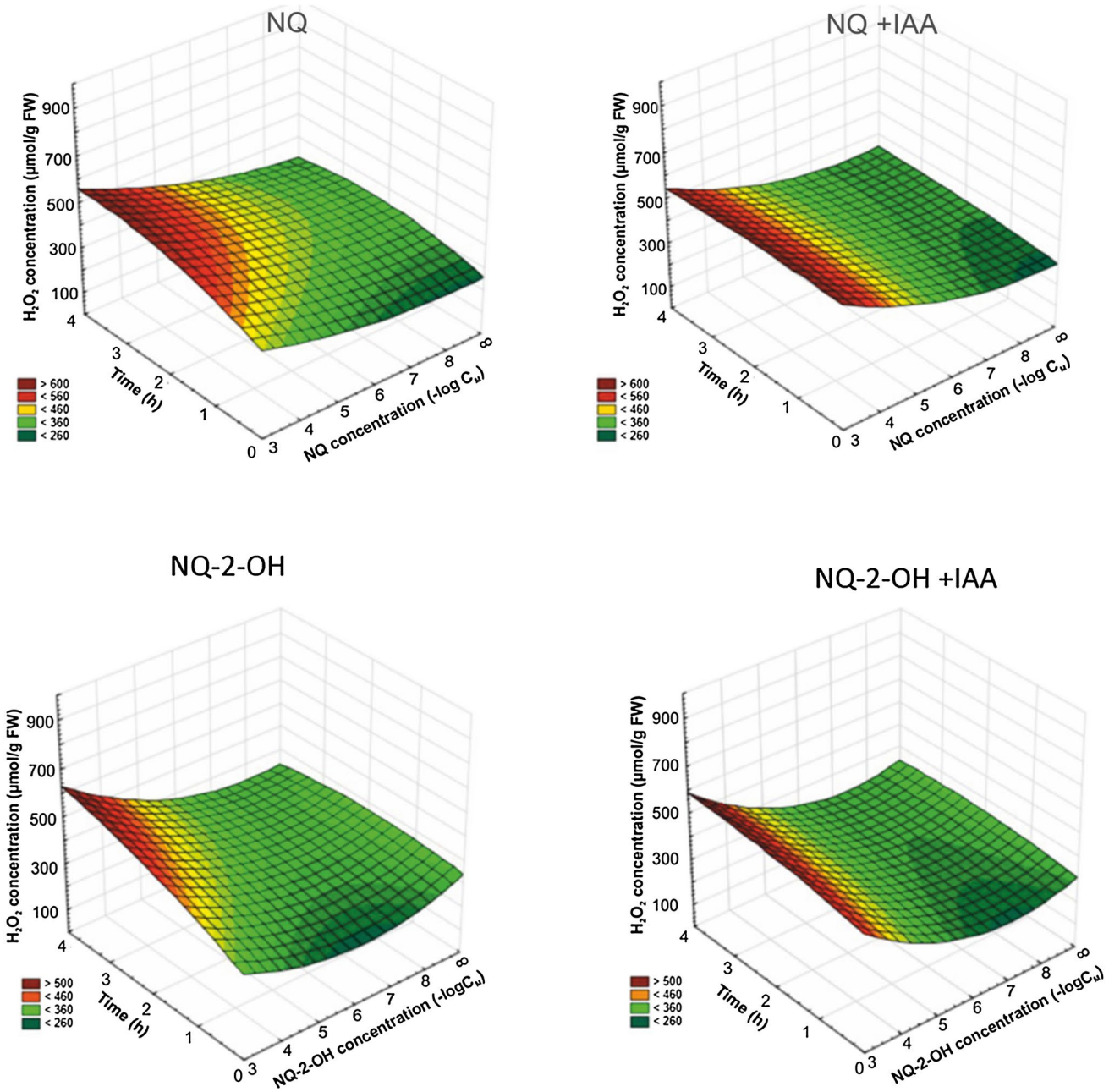

Fig. 6 Dose-response curves for the effects of NQ, NQ + IAA, NQ-2-OH and NQ-2-OH + IAA on H_2O_2 production in maize coleoptile segments as a function of time. The coleoptile segments were first preincubated for 1 h in the control medium, after which NQ or NQ-2-OH at various concentrations was added. Time "0" means H_2O_2 production after 1 h of incubation of the segments in the presence of NQ or NQ-2-OH. H_2O_2 production in the control medium (without naphthoquinones) is shown here at a concentration of naphthoquinones equal $-\log\infty$ (Fig. 6). IAA, at a final concentration of 100 µM, was applied at the same time protocol as NQ and NQ-2-OH. H_2O_2 production in the presence of IAA (without naphthoquinones) is shown here at a concentration of naphthoquinones equal $-\log\infty$ (Fig. 6). The data presented are the means of at least three independent experiments

acting in the presence of IAA, caused a clear decrease in CAT activity after 2 h (see Supplementary Fig. 4 in Supplementary Material). A significant increase in CAT activity was observed in the coleoptile segments that were treated with 1 µM NQ-2-OH added together with IAA (see Supplementary Fig. 4 in Supplementary Material).

The effects of NQ and NQ-2-OH on lipid peroxidation

Our results indicated a progressive decrease in the MDA content of maize coleoptile segments that were incubated in the control medium (medium without NQ and IAA) (see Supplementary Fig. 5 in Supplementary Material) within

4 h. We found that NQ at almost all of the concentrations studied, when added to the incubation medium, increased the MDA content of coleoptile segments compared to the control (see Supplementary Fig. 5 in Supplementary Material). When the coleoptile segments were incubated with NQ applied together with IAA, an increased level of the MDA content at higher NQ concentrations (100 and 1000 µM) was observed within the first 3 h (see Supplementary Fig. 5 in Supplementary Material). However, at 4 h the MDA content was high for all of the concentrations studied. In the case of NQ-2-OH, the MDA contents of the coleoptile segments increased, similar to NQ, at all of the concentrations studied (excluding NQ-2-OH at 1000 µM) and were higher at 1 and 100 µM (see Supplementary Fig. 6 in Supplementary Material). This higher MDA content was significantly decreased in the presence of IAA (see Supplementary Fig. 6 in Supplementary Material). The MDA content of the coleoptile segments that were treated with NQ-2-OH added together with IAA were relatively high at 4 h of the experiment (see Supplementary Fig. 6 in Supplementary Material).

The effects of NQ and NQ-2-OH on the redox activity of the coleoptile segments

Figure 7 shows the dose-response curves for the effects of NQ and NQ combined with IAA on redox activity (measured as HCF III reduction by the maize coleoptile segments) as a function of time. As can be seen in Fig. 7, NQ at concentrations higher than 1 µM increased HCF III reduction by the maize coleoptile segments within 2 h. For example, treatment of the maize coleoptile segments with NQ at 100 and 1000 µM induced two-fold and six-fold increase in HCF III reduction within two 2 h compared to the control (660 ± 16 nmol/g FW; mean ± SD, n = 3, shown here as $-\log\infty$), respectively. However, the addition of NQ at the same concentrations as mentioned above in combination with IAA induced a 1.5-fold and 2.9-fold increase in HCF III reduction within 2 h compared to the control with IAA (949 ± 10 nmol/g FW; mean ± SD, n = 3, shown here as $-\log\infty$), respectively (Fig. 7). The results in Fig. 7 and the ones shown above in brackets also indicate that IAA alone increased HCF III reduction by 44% within two hours. NQ-2-OH, which was added to the incubation medium at the same time protocol and concentrations as NQ, stimulated ferricyanide reduction by a mean 50% (1.5-fold) at moderate concentrations (0.1, 1 and 10 µM), while NQ-2-OH at 100 and 1000 µM induced a 2.4-fold and 4.6-fold increase in HCF III reduction within two hours compared to the control (Fig. 7), respectively. In the presence of IAA, NQ-2-OH only at the highest concentrations stimulated hexacyanoferrate III reduction significantly (2.2-fold) within 2 h compared to the control with IAA (Fig. 7). NQ-2-OH at 1000 µM, when added in combination with IAA, similar to NQ, decreased HCF III reduction by 30% compared to the variant without IAA.

Taking the above into account, it can be stated that NQ-2-OH was more active than NQ in terms of the lower concentrations that were needed to induce an increase in HCF III reduction by the maize coleoptile segments, although at the highest concentration, it caused a much weaker increase in HCF III reduction than NQ.

Discussion

The major aim of the studies described here was to understand the crosstalk between naphthoquinones such as 1,4-naphthoquinone (NQ) and 2-hydroxy-1,4-naphthoquinone (NQ-2-OH, lawsone) and the plant growth hormone IAA. Understanding the interrelation between the actions of auxin and naphthoquinones on plant growth and development is important because of the allelochemical properties of quinones and their toxic effect on the environment. Recently, we showed that juglone (5-hydroxy-1,4-naphthoquinone), which is a naturally occurring naphthoquinone in walnut trees (*Juglans*), inhibited both IAA-induced growth and proton extrusion in maize coleoptile cells in a concentration-dependent manner. The strong correlation between both of these magnitudes suggested that the mechanism by which juglone inhibits IAA-induced growth involves the inhibition of PM H^+-ATPase activity (Rudnicka et al. 2014). Quite recently, Kurtyka et al. (2016) published a paper in which the authors obtained that juglone and lawsone contribute to H_2O_2 generation and activity of ROS scavenging enzymes (SOD, POX and CAT) in maize coleoptile segments.

In order to compare the biological activity of 1,4-naphthoquinone (NQ) and 2-hydroxy-1,4-naphthoquinone (NQ-2-OH, lawsone), we considered three parameters that are related to auxin action—elongation growth, proton extrusion and membrane potential. The interrelation between these parameters, as was mentioned in the Introduction, constitute the basis for the "acid growth hypothesis" of auxin action, in which PM H^+-ATPase plays a key role. Moreover, the redox cycling properties of both naphthoquinones, which are measured as H_2O_2 production; HCF III reduction; MDA content and CAT activity in maize coleoptile segments were estimated. The data presented here indicate that IAA added to the control medium alone caused the stimulation of elongation growth (Fig. 1), the enhancement of proton extrusion (Figs. 3b, 4) and the hyperpolarisation of the membrane potential (Table 1). These data are in good agreement with the results obtained previously and recently by others investigators (Burdach et al. 2014; Felle et al. 1991; Karcz and Burdach 2002; Keller and Van Volkenburgh 1996; Kutschera and Schopfer 1985a, b; Kutschera 1994, 2006; Lüthen et al. 1990; Rücke et al. 1993; Siemieniuk and Karcz 2015). It

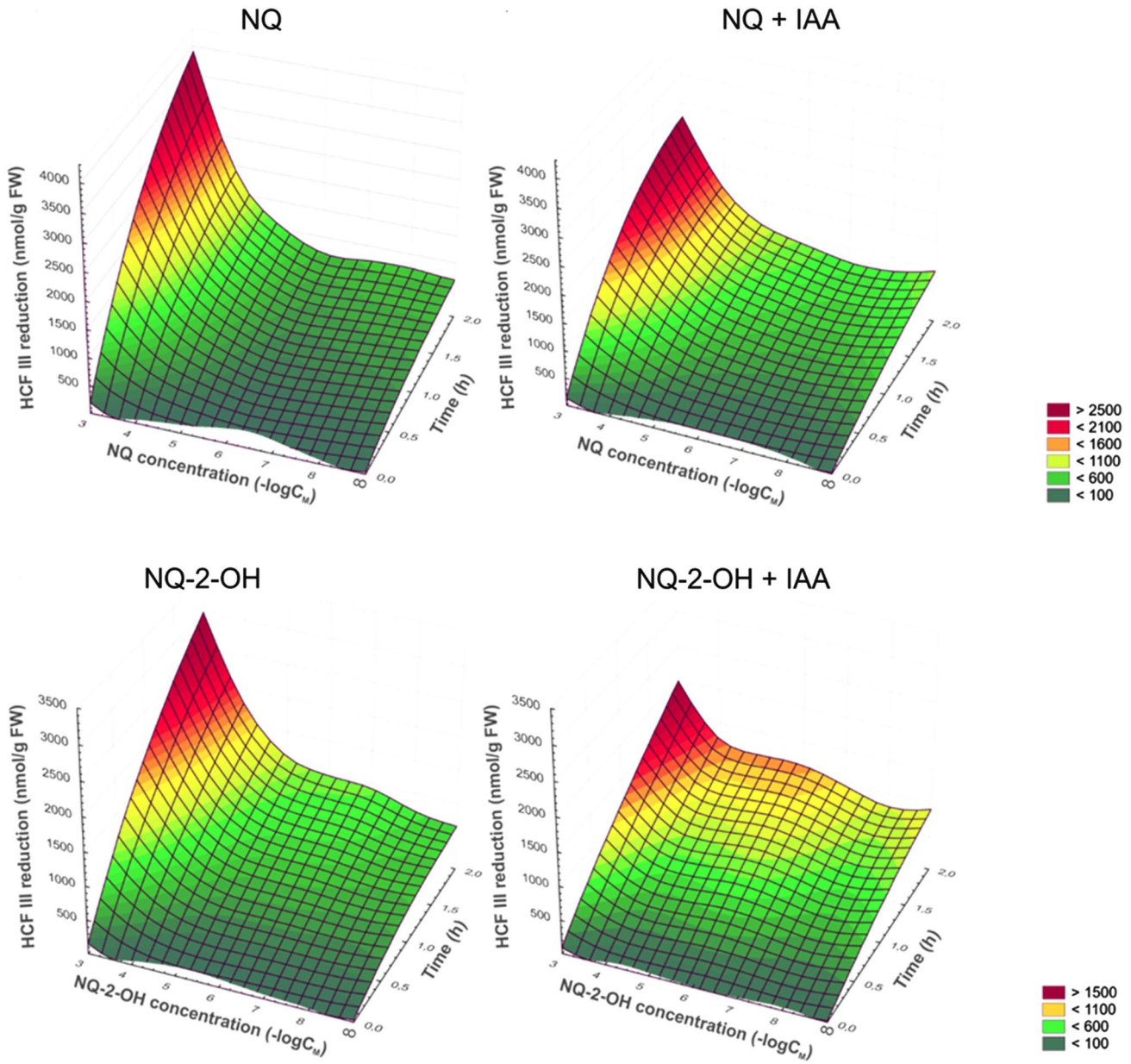

Fig. 7 Effect of NQ, NQ + IAA, NQ-2-OH and NQ-2-OH + IAA on HCF III reduction by maize coleoptile segments as a function of time. The coleoptile segments were first preincubated for 1 h in distilled water and immediately transferred to an incubation medium (see "Materials and methods") containing NQ or NQ-2-OH at the appropriate concentrations. IAA, at final concentration of 100 µM, was, as required, included in the incubation medium. The level of HCF III reduction was measured upon the addition of the coleoptile segments to the incubation medium and every 30 min for the next 2 h. The data presented are the means of three independent experiments

is also well established that the plasma membrane hyperpolarisation that was recorded in the presence of IAA is a consequence of stimulated proton extrusion through PM H^+-ATPase (Hedrich et al. 1995; Rücke et al. 1993).

When comparing the dose-response curves for both quinones, it should be pointed out that in the presence of IAA, they are similar in shape but differ in the values recorded for IAA-induced growth. However, in the absence of IAA (endogenous growth), they are linear (Fig. 3a). Medium pH, which was measured simultaneously with growth, indicated that both naphthoquinones added to the control medium inhibited or eliminated proton extrusion at all of the concentrations used (Figs. 3b, 4, 5).

The electrophysiological experiments performed with the parenchymal cells of the maize coleoptile segments showed that the addition of naphthoquinones to the control medium

caused the depolarisation of the membrane potential. Moreover, IAA-induced membrane potential hyperpolarisation was also eliminated.

Like other stress factors, allelochemicals result in the increased production of ROS, which can interfere with different cellular processes, thus causing an inhibition of growth (Bais et al. 2003; Fujita et al. 2006). Our results clearly demonstrate (Fig. 6) that both naphthoquinones at higher concentrations enhanced H_2O_2 production in maize coleoptile segments, which was accelerated in the presence of IAA. Catalase (CAT) is one of the key enzymes that decomposes H_2O_2 into water and molecular oxygen and is involved in the removal of toxic peroxides. In the present study, CAT activity in the maize coleoptile segments significantly increased at the moderate NQ concentrations, while at higher concentrations and longer times of action it was usually lower compared to the control (see Supplementary Figs. 3, 4 in Supplementary Material). Interestingly, NQ at 1 and 10 µM added in combination with IAA diminished CAT activity to a similar level as that in the control medium. This decrease in CAT activity might be explained by two facts: (1) that auxin is involved in the tolerance to oxidative stress (for a review see Tognetti et al. 2012) and (2) that auxin can modulate ROS homeostasis by inducing GTS (glutathione *S*-transferase) and FQR (flavodoxin-like quinone reductase) primary auxin-response genes expression (Laskowski et al. 2002) and regulating H_2O_2 levels (Iglesias et al. 2010). However, the decrease in CAT activity that was observed here at higher NQ and NQ-2-OH concentrations is probably due to enzyme inhibition by an elevated H_2O_2 concentration (Hema et al. 2007). In contrast to NQ, NQ-2-OH did not show such extreme changes in CAT activities, regardless of the presence of IAA (see Supplementary Fig. 4 in Supplementary Material). It has only been recently shown (Kurtyka et al. 2016) that the application of NQ-2-OH at 100 µM to medium containing maize coleoptile segments increased H_2O_2 production and CAT activity. Because different methods are used to estimate H_2O_2 production and CAT activity, a direct quantitative comparison of our data with the results presented by Kurtyka et al. (2016) is difficult. The treatments of maize coleoptile segments with NQ and NQ-2-OH, at almost all their concentrations studied, induced oxidative stress as was also evidenced by the increased MDA content compared to the controls (see Supplementary Figs. 5, 6 in Supplementary Material). Moreover, the MDA content of the maize coleoptile segments that were treated with NQ and NQ-2-OH was modified in the presence of IAA (generally was diminished), thus suggesting that exogenous auxin may play a role in lipid peroxidation.

The main strategy of the experiments concerning the plasma membrane redox activity was estimating the reduction rate of HCF III by the maize coleoptile segments. Since quinones are known to be electron transporters (e.g. ubiquinone, vitamin K), they may affect the plasma membrane redox systems (so-called "standard redox system"), which in plant cells are involved in elongation growth, proton release and plasma membrane depolarisation (reviewed in Lüthje et al. 1997). Moreover, it was also shown that auxin and fusicoccin modify the plasma membrane redox activity in maize coleoptile cells (Lüthen and Böttger 1993; Carrasco-Luna et al. 1995). Here, it was found that NQ-2-OH was more active than NQ in terms of the lowest concentrations needed to stimulate HCF III reduction by maize coleoptile cells, although at the highest concentration it produced a much weaker increase in HCF III reduction than NQ (Fig. 7). Our data on plasma membrane redox activity are in good agreement with the results obtained previously by Döring et al. (1992) and Lüthje et al. (1992) in experiments performed with maize roots that were treated with vitamin K_3 (lipophilic naphthoquinone, methyl-NQ). These authors showed that within minutes after the addition of K_3 (at 200 µM) to the medium containing maize roots, an increase of HCF III reduction rate was observed. In order to explain the mechanism of the higher reduction rate of external electron acceptors (HCF III) after pre-incubation with vitamin K_3, the authors proposed two scenarios: (1) the molecules of vitamin K_3 may act as electron acceptors at the same site as HCF III or (2) K_3 molecules that integrate into the plasma membrane may accept electrons from a site on the chain that is inaccessible for the electron acceptors in the incubation medium. Those electrons may be directly transferred to HCF III. Döring et al. (1992) and Lüthje et al. (1992) also observed that after the addition of K_3 to the medium, there was an inhibition of H^+-extrusion by the maize root cells and a depolarisation of their membrane potential. The results presented here also showed that in the presence of IAA, both naphthoquinones at 1000 µM induced a 30% lower increase in HCF III reduction by the maize coleoptile segments compared to variant without auxin. In trying to explain this finding, we assumed that auxin, which is able to change the fluidity of the lipid systems and weaken the interactions between their components (De Melo et al. 2004; Celik et al. 2006) may disturb the integration of naphthoquinones into the plasma membrane (hypothesis proposed by Lüthje et al. 1992) and subsequently decrease HCF III reduction. It should also be added that IAA alone stimulated HCF III reduction as was previously shown by Lekacz and Karcz (2006). Taking into account the above, it may be suggested that the redox cycling properties of NQ and NQ-2-OH are involved in their toxic effect on the IAA-induced growth of maize coleoptile cells.

When attempting to explain the mechanism by which naphthoquinones inhibits the IAA-induced growth of maize coleoptile segments, two mechanisms by which they interact with biological systems should be taken into account. The first is their ability to undergo a nucleophilic attack and the

second is the capacity of quinone to produce reactive oxygen species (ROS) via redox cycling (see also "Introduction"). It should also be added that some naphthoquinones such as NQ or 2-methyl-1,4-NQ undergo both redox cycling and nucleophilic substitution reactions in cell systems (Gant et al. 1988; Zaborska et al. 2007). In turn, in accordance with the "acid growth hypothesis" of auxin action, IAA-induced growth is predominantly regulated by PM H^+-ATPase, which similar to other ATPases, is sensitive to sulfhydryl reagents (Elzenga et al. 1989; Hager and Lanz 1989). Taking the above into account, it can be suggested that both naphthoquinones may inhibit the PM H^+-ATPase activity, thereby interacting directly with enzyme thiols, which leads to the generation of thioethers (arylation process). They may also inhibit PM H^+-ATPase indirectly by producing reactive oxygen species (ROS) via redox cycling. There are many reports in the literature concerning the impairment of the transport ATPases through interactions with ROS either through the direct oxidation of amino acid residues or via the peroxidation of membrane phospholipids (reviewed in Kourie 1998). It is also possible that ROS-induced cytosol Ca^{2+} elevations (reviewed in Mori and Schroeder 2004), in turn, can inhibit PM H^+-ATPase activity (Brault et al. 2004; Kinoshita et al. 1995; Polevoi et al. 1996), and depolarise plasma membrane potential (Trouverie et al. 2008). It can be also speculated that the inhibition of IAA-induced growth may also be caused by an ROS-induced K^+ efflux, which results in a dramatic K^+ loss from plant cells (reviewed in Demidchik et al. 2014). Here, the suggestion that NQ and NQ-2-OH inhibit PM H^+-ATPase is supported by four pieces of evidence: (1) the inhibition of IAA-induced proton extrusion by both naphthoquinones (excluding NQ at 1 µM, Fig. 3b); (2) the decrease and elimination of IAA-induced membrane potential hyperpolarisation by NQ and NQ-2-OH; (3) the induction of the alkalinisation of the medium and membrane depolarisation by the highest naphthoquinone concentrations and (4) the increase in H_2O_2 production, which may indirectly inhibit PM H^+-ATPase. Tian et al. (2003) found that juglone (5-hydroxy-1,4-naphthoquinone) inhibit the interaction between the IAA/AUX transcriptional repressor and SCF-TIR1 and lead to the decrease of auxin genes expression. Taking above into account it can be suggested that 1,4naphthoquinone and 2-hydroxy-1,4-naphthoquinone can similarly affect auxin nuclear signaling system. Furthermore, it can be suggested that naphthoquinones can interfere the cytoplasmic auxin signaling mechanisms consisting of ABP1 (auxin binding protein 1), TMK (transmembrane kinase) and ROPs (Rho-like GTPase) (Xu et al. 2010, 2014; Grones et al. 2015). ABP1 is membrane auxin receptor and is crucial for mediating a range of auxin fast non-transcriptional effects. ABP1 is localized in the endoplasmic reticulum (ER) however, small fraction of ABP1 is localized in the plasma membrane (Woo et al. 2002; Grones et al. 2015). The auxin-binding pocket of ABP1 consist of hydrophobic area, reacting with aromatic ring of auxin and Zinc-binding histidine cluster which is binding site for carboxylate of auxin. The changes in auxin-binding pocket sequence can cause disturbance in auxin cytoplasmic signaling system and provide to disruption in physiological processes associated with ABP1 (Woo t al. 2002). ABP1 is involved in: regulation of the membrane potential and ion fluxes; elevation in cytosolic Ca^{2+} concentration (Shishova and Lindberg 2010) and changes in the cytoskeleton (Nagawa et al. 2012), in the vesicular trafficking (Robert et al. 2010), in the cell division and expansion (Braun et al. 2008).

Considering the effects of NQ and NQ-2-OH on both IAA-induced growth and proton extrusion in the category of the "acid growth theory" of auxin action, the lack of a correlation between these two parameters was recorded. For example, NQ-2-OH at 0.01, 0.1, 10, and 100 µM inhibited IAA-induced growth by no more than 16% (Fig. 3a), whereas the same concentrations of NQ-2-OH decreased IAA-induced proton extrusion to the level of the control (Fig. 3b). The lack of correlations between IAA-induced growth and proton extrusion were also found in experiments performed with NQ (for comparison see Figs. 1, 4). One explanation for the discrepancy between both of these parameters that is found in the presence of NQ and NQ-2-OH probably results from the fact that ROS, similar to protons, cause cell wall loosening, as was postulated by Schopfer's group in experiments performed, among others, with maize coleoptile segments (Frahry and Schopfer 2001; Schopfer 2001; Schopfer et al. 2002). Those authors proposed the hypothesis in which $^{\cdot}OH$ is causally involved in the chemorheological wall-loosening reaction that is responsible for the auxin-controlled growth of maize coleoptiles. In turn, the much greater inhibitory effect of NQ, compared to that of NQ-2-OH, on the IAA-induced growth of maize coleoptile segments probably results from the fact that the substitution of the hydroxyl group at the C_2 position (lawsone) contributes to reducing the electrophilicity C_3 and also becomes a spherical obstacle (steric hindrance) in interactions with nucleophiles (Öllinger and Brunmark 1991; Klotz et al. 2014). A steric hindrance effect was previously proposed for the elucidation of the difference between the influence of 5-hydroxy-1,4-naphthoquinone (juglone) and 2-hydroxy-1,4-naphthoquinone (lawsone) on the urease activity of the jack bean (Kot et al. 2010). Taken together, the results presented in this article demonstrate that NQ was much more effective than NQ-2-OH in inhibiting the IAA-induced growth of maize coleoptile segments probably as a result of steric hindrance. Simultaneous measurements of growth and external pH indicated that NQ and NQ-2-OH decreased or eliminated IAA-induced proton extrusion at almost all of the concentrations used (excluding NQ at 1 µM), thus suggesting that this process involves the inhibition of PM

H^+-ATPase activity. This suggestion is also supported by the fact that the depolarisation of the membrane potential (E_m) and alkalinisation of the incubation medium of coleoptile cells was observed in the presence of higher NQ or NQ-2-OH concentrations. In addition, the hyperpolarisation of E_m that was induced by IAA was suppressed in the presence of both naphthoquinones. The lack of a correlation between IAA-induced growth and medium pH, which was simultaneously measured with growth (see also Kutschera and Schopfer 1985a, b; Kutschera 1994, 2006), indicates that the loosening of the cell wall probably depends on the ROS that are liberated during the action of quinones as was shown here and previously by Schopfer et al. (2002) and Kurtyka et al. (2016). In addition, the obtained results may also indicate that NQ and NQ-2-OH at higher concentrations undergo both redox cycling and nucleophilic substitution reactions in maize coleoptile cells.

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