Original Research

Effect of 4-Hydroxyphenethyl Alcohol on Growth and Adaptive Potential of Barley Plants under Optimal and Soil Flooding Conditions

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Abstract

Barley (*Hordeum vulgare* L. cv. "Rudzik") seeds were soaked in aqueous 10^{-6} M 4-hydroxyphenethyl alcohol (4-HPEA) to estimate its influence on seed germination, growth of seedlings, and their adaptive potential under soil flooding conditions. The adaptive potential of the plants was estimated by the concentration of thiobarbituric acid reactive substances (TBARs) and the activity of guaiacol-dependent peroxidase (GPX). It was shown that the 4-HPEA had a stimulatory action on seed germination, shoot and root growth, and biomass production, expressed to a greater extent in the early stage of plant development. Pre-sowing soaking of barley seeds in aqueous 4-HPEA solution increased plant tolerance to the effect of soil flooding, too.

Introduction

An increase in the adaptation of plants to unfavourable factors under changing environmental conditions is an important aim of investigations by plant physiologists and ecologists. Soil flooding, like soil drought, salinity, and other negative stress factors, often takes place in nature and results in a decrease of crop production. One of the essential negative consequences of soil flooding is oxygen deficiency in the tissues of submerged plant organs. Oxygen deficiency affects the intensity and direction of a number of physiological and biochemical reactions and induces oxidative stress in plant cells [1-3]. Under oxygen deficiency

conditions experienced by plant roots during the period of soil flooding, a generalized adaptive response takes place in leaves in normal aerobic conditions [3, 4]. The root hypoxia results in an increase of the value of stomatal diffusive resistance, controlling the tissue turgor, and the rate of CO_2 fixation [5]. Protection of plants from oxidative destruction is associated with active functioning of low molecular antioxidants (carotenoids, ascorbic acid, glutathione, etc.), as well as antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol-dependent peroxidase (GPX), and others, detoxifying the reactive oxygen species (ROS) [6-9]. Besides APX, GPX is also involved in scavenging of soluble hydroperoxides in plants [10]. In our earlier experiments, soil flooding induced SOD and APX in plant leaves [11-13], but GPX activity did not increase significantly [13]. The dependence of membrane

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lipid peroxidation intensity on the activity of antioxidant enzymes has been shown repeatedly in roots and leaves of cultural plants (barley, maize, pea, wheat) under flooding conditions [4, 5, 13-15]. Physiological biochemical reactions of damaging and adaptation of the investigated plants were found to depend on both the dose of acting stress factors and the evolution-formed tolerance of these cultures to soil flooding and oxygen deficiency in the root zone.

Nowadays, in connection with the growing ecological effort, the search for and development of new technologies for increasing the adaptive potential of cultural plants is actual. One such approach may be the treatment of seeds with physiologically active substances, for example with natural or synthetic cytokinins [16-18]. The beginning of investigations on substances of cytokinin nature is connected with the name of D. Letham [19], who isolated zeatin from unripe maize corns and decoded its structure.

At present, there are enough data confirming that these phytohormones play a role in the regulation of seed development, dormancy, and germination [20-24]. Taking into consideration that seeds in deep dormancy usually contain indeterminable small amounts of cytokinins [21, 24], the use of exogenous substances with cytokinin activity to promote an increase of germinating energy and growth acceleration in the initial stage of development should take special significance for regions with critical climatic and ecological conditions.

4-hydroxyphenethyl alcohol (4-HPEA) is a phenolic compound isolated from the phototrophic bacterium *Rhodospirillum rubrum* and characterized as a non-purine cytokinin-like substance [25]. It has been shown in a few studies that 4-HPEA stimulates plant growth in the juvenile phase [26].

The aim of the presented work was to investigate the influence of 4-HPEA on the germination energy of barley seeds (*Hordeum vulgare* L. cv. "Rudzik"), as well as on further growth of seedlings, their biomass production and adaptive potential under optimal soil watering and flooding. Intensity of the oxidative processes in the plants was estimated by the concentration of TBARs, and tolerance to H_2O_2 was estimated by GPX activity.

Materials and Methods

Plants and Soil Materials

Hordeum vulgare L. cv. "Rudzik" plants were used in the experiments. The plants were grown in 1 dm^3 plastic pots filled with soil material taken from the Ap horizon of a brown loess soil (Orthic Luvisol) of a cultivated field in Felin (Lublin region, Poland). Soil pH measured with a pH meter and glass electrode [33], was 7.3 in distilled water (pH 7.1 in 0.01 M KCl at soil to water or solution ratios = 1:2.5) and the soil contained 1.69% C_{org}, 29% of 1-0.1 mm fraction, 67% of 0.1-0.002 mm fraction, and 4% of clay. Each pot had 0.75 kg of soil (dry weight) packed to the bulk density of 1.35 Mg m^{-3}.

Seed Treatment

Barley seeds were soaked in distilled water, (-4-HPEA)-treatment, or in aqueous 10^{-6} M 4-HPEA, (+4-HPEA)-treatment. In the preliminary experiments as well as in the earlier work [15] we selected 4-HPEA concentrations that are more effective both for seed germination and seedling growth. As for liquid, saturation of the seeds reached 65-67% (usually after 22 h) and were germinated as described earlier [11] on a moist filter paper at 25±2ºC. Energy of germination was estimated by the percentage of the full sprouted seeds 72 h after beginning germination. Three independent biological replications (100 seeds per replication) were made for each treatment (-4-HPEA) and (+4-HPEA), respectively. Each value represents the mean±SD.

Plant Growth

Equally well germinated (-4-HPEA) and (+4-HPEA) seeds were sown in soil-filled pots (6 seedlings per pot and 120 pots total). Air temperature in the growth chamber was kept at 23±2ºC during the day and 16±2ºC during the night. The light period was 12 hours; light intensity was 950 µmol photons m^{-2} s^{-1}. Relative air humidity was 45±5% during the day and 70±5% during the night. All the seedlings were maintained under optimal conditions of aeration and water availability during the first 5 days of growth. Then, on the 5th day of growth, the pots were divided into four groups:
1. (-4-HPEA) – seeds soaked in distilled water, seedlings grown under optimal soil watering.
2. (- 4-HPEA; +flooding) – seeds soaked in distilled water, seedlings grown under soil flooding.
3. (+4-HPEA) – seeds soaked in aqueous 10^{-6} M 4-HPEA, seedlings grown under optimal soil watering.
4. (+4-HPEA; +flooding) – seeds soaked in aqueous 10^{-6} M 4-HPEA, seedlings grown under soil flooding.

In this way, half of the pots with (-4-HPEA) and (+4-HPEA) plants were maintained at optimal soil watering; the other pots were flooded with a 1-cm of water layer that stagnated on the soil surface for 7 days.

Plant Measurements

The intensity of growth processes was evaluated both by elongation of shoots and roots and by increase of their fresh biomass (fresh weight, FW) on the 3rd, 5th, 6th, 9th and 12th days after the beginning of the experiment; the last 3 points (6th, 9th, and 12th) corresponded to the 1st, 4th, and 7th day from the beginning of soil flooding. TBAR concentration and GPX activity were determined in plant leaves on the 1st, 4th, and 7th days from the beginning of the soil flooding. The TBAR concentration was measured in crude homogenates of the first leaves of seedlings. GPX activity was examined in enzyme extracts of the same leaves. All results were calculated per one g of FW.

To prepare crude homogenates and enzyme extracts the middle parts from the first leaves (0.5 g) were homogenized manually with a piston in a mortar with cooled 30 mM K/Na phosphate buffer of pH 7.4 containing 0.1 mM of EDTA and 2% of PVP (M.m=25,000). The homogenate was filtered through a nylon cloth and its samples were used for assessment of TBAR concentration. A portion of this homogenate was centrifuged at 3,000 g for 10 min, and the supernatant was used for determination of GPX activity.

TBAR Measurements

The intensity of lipid peroxidation in the leaves was assessed by the method of Uchiyama and Mihara [27] on the basis of TBAR content. 3 cm³ of 1% phosphoric acid, one cm³ of 0.6% aqueous solution of TBA, and 0.1 cm³ of aqueous solution of $FeSO_4 \times 7H_2O$ (2.8 mg cm⁻³) were added to 0.3 cm³ of the crude homogenate. The reaction mixture was heated in a water bath during one hour. After cooling, 4 cm³ of butanol-1 were added, mixed vigorously and centrifuged at 3,000 g for 10 min. Absorbance of the samples was measured at 532 and 600 nm using a Shimadzu UV-VIS 160A spectrophotometer (Kyoto, Japan). Concentration of TBAR products was calculated using coefficient of extinction equal to 1.56×10^{-5} M⁻¹ cm⁻¹.

GPX Activity Measurements

The activity of GPX was measured spectrophotometrically [28] on the basis of guaiacol oxidation; 26.6 mM⁻¹ cm⁻¹ coefficient of extinction for tetraguaiacol at 470 nm was used for calculations.

Soil Measurements

Redox potential (Eh) of the soil samples was measured potentiometrically using four Pt electrodes, a saturated calomel electrode as reference, and a laboratory pH meter (Radiometer, Copenhagen) [29]. The electrodes were placed at a soil depth of 2 cm. The measurements were taken after stabilization of the readings, which usually did not exceed 5 minutes.

Statistical Analyses

All of the biometric parameters (growth and biomass production) were determined in 15-17 independent biological replications of each experimental treatment. The biochemical characteristics (enzyme activity and TBAR concentration) were determined in three independent biological replications of each experimental treatment. The data of root and shoot lengths, enzyme activity and TBAR concentration are reported as means ± standard deviation. Analysis of variance by the least significant difference test (95% LSD-test) was performed to determine whether a significant difference existed ($p < 0.05$) between the means of treatments. All statistical analyses were performed using Statgraphics Plus 5.1 software.

Results

At the beginning of the experiment, the soil pH values ranged from 7.4 to 7.8. There were no significant changes in this parameter during the whole experiment. The initial values of soil redox potential (Eh) ranged from 562 mV to 584 mV. Under optimal soil watering no distinct differences in Eh values were observed during the experiment. After one day of the experiment in the soil with (-4-HPEA;+flooding) and (+4-HPEA;+flooding) plants, the Eh values decreased to 375 and 393 mV, respectively.

Seed Germination

The percentage of full sprouted seeds pre-soaked with water was 64±5% 72 h after the beginning of germination, but in the case of pre-soaking in aqueous 10^{-6} M 4-HPEA the percentage of full sprouted seeds was 85±7%. This result showed that 4-HPEA stimulated seed germination by about 20%.

Plant Growth

The investigation of growth processes on the basis of shoot and root elongation showed that on the 3rd day of growth the average length of the (-4-HPEA)-barley seedling shoots was about 4.6 cm, and then reached 18.2 cm on the 12th days of growth under optimal soil moisture conditions (Fig. 1a). The average length of the (+4-HPEA)-

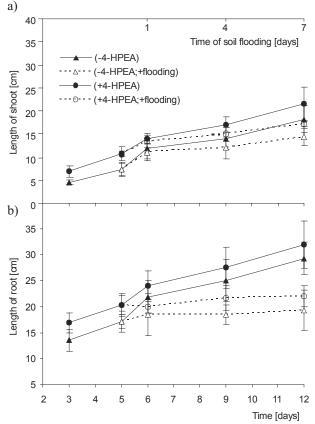Fig. 1. The effects of pre-sowing treatment of *Hordeum vulgare* seeds with 10^{-6} M 4-hydroxyphenethyl alcohol (4-HPEA) on the shoot (a) and root (b) length of seedlings grown under optimal soil watering or flooding conditions.

Table 1. Statistical significance of differences in shoot and root length among seedlings germinated from the seeds treated (+4-HPEA) and non treated (-4-HPEA) by 4-HPEA and grown under optimal soil conditions (-flooding) and under soil flooding (+flooding) starting on the 5ᵗʰ day of growth (calculations were made on the basis of 95% LSD method).

Compared treatments	Time [days]				
	3	5	6	9	12
	Time of soil flooding [days]				
	-	-	1	4	7
	Difference				
	Shoot length				
(+4-HPEA) / (-4-HPEA)	2.3*	3.36*	2.17*	2.67*	3.29*
(+4-HPEA) / (+4-HPEA+flooding)	-	-	0.57	1.85*	4.33*
(-4-HPEA) / (-4-HPEA+flooding)	-	-	0.81	2.06*	3.71*
(+4-HPEA+flooding) / (-4-HPEA+flooding)	-	-	2.41*	2.88*	2.67*
(+4-HPEA) / (-4-HPEA+flooding)	-	-	2.98*	4.73*	7.0*
(+4-HPEA+ flooding) / (-4-HPEA)	-	-	1.6*	0.82	-1.04
	Root length				
(+4-HPEA) / (-4-HPEA)	3.4*	3.17*	2.19*	2.5*	2.65*
(+4-HPEA) / (+4-HPEA+flooding)	-	-	3.97*	5.86*	9.93*
(-4-HPEA) / (-4-HPEA+flooding)	-	-	3.34*	6.59*	10.0*
(+4-HPEA+flooding) / (-4-HPEA+flooding)	-	-	1.56	3.23*	2.72*
(+4-HPEA) / (-4-HPEA+flooding)	-	-	5.53*	9.09*	12.65*
(+4-HPEA+ flooding) / (-4-HPEA)	-	-	-1.79	-3.36*	-7.28*

*denotes a statistically significant difference.

seedling shoots was about 6.9 and 21.5 cm on the 3ʳᵈ and 12ᵗʰ days of growth, respectively. The data set in Fig. 1a demonstrate more intensive growth of the (+4-HPEA)-seedling shoots, especially during the first 3-5 days of growth, when the difference between the treatments reached 50%. A significant increase in shoot length of the (+4-HPEA)-plants, in comparison with (-4-HPEA)-ones, was observed during the whole period of the experiment (Table 1). The shoot length of (+4-HPEA;+flooding)-plants, one day after the beginning of the overwatering, was significantly greater than the shoot length of (-4-HPEA;+flooding)-plants. This positive influence, though not significant, lasted until 4 days after the beginning of soil flooding (Table 1). It means that soaking of barley seeds with aqueous 4-HPEA solution led to a stronger adaptive response in the leaves under oxygen deficiency conditions, even compared with well aerated soil. Soaking of barley seeds in the 4-HPEA solution induced root growth, too, but to a lesser degree than growth of the shoots. The length of roots in 3- and 5-day (+4-HPEA)-plants exceeded significantly the length of roots in the (-4-HPEA)-seedlings, by 25 and 19%, respectively (Table 1, Fig. 1b).

Soil hypoxia resulted in a tendency to inhibit shoot elongation and in a reliable inhibition (by 15-17%) of the root elongation both in the (-4-HPEA;+flooding) and (+4-HPEA;+flooding)-plants, after one day of soil flooding action (Fig. 1a,b). During ulterior development of the hypoxic stress, the elongation of shoots decreased reliably by 14-20% in all of the plants, but differences between the (-4-HPEA;+flooding) and (+4-HPEA;+flooding)-plants were maintained (Fig. 1a). The effect of soil hypoxia on the root elongation was more pronounced. At the end of the flooding experiment the roots in the (-4-HPEA;+flooding) and in (+4-HPEA;+flooding)-seedlings were significantly shorter (by 34 and 31%) than in (-4-HPEA) and in (+4-HPEA) seedlings, respectively (Fig. 1b). Soaking of barley seeds with 4-HPEA induced stimulation of shoot and root elongation under flooding compared with non-treated by 4-HPEA flooded plants (Table 1).

The examination of biomass production showed that shoot biomass of the (-4-HPEA) and (+4-HPEA)-seedlings averaged 0.054 and 0.079 g, respectively, on the 3ʳᵈ day of growing, and reached 0.174 g (-4-HPEA) and 0.189 (+4-HPEA) on the 12ᵗʰ day of growth (Fig. 2a). The data presented in Fig. 2a show that the effect of 4-HPEA on biomass accumulation was more significant during the first 3-5 days of growth, when the differences between the variants reached 46 and 41%, respectively. The promoting effect of 4-HPEA on root biomass was similar to the phytohormonal effect on shoot biomass accumulation (Fig. 2a,b).

Table 2. Statistical significance of differences in TBAR concentrations and GPX activity among seedlings germinated from the seeds treated (+4-HPEA) and non treated (-4-HPEA) by 4-HPEA and grown under optimal soil conditions (-flooding) and under soil flooding (+flooding) starting on the 5th day of growth (calculations were made on the basis of 95% LSD method)

Compared treatments	Time [days]				
	3	5	6	9	12
	Time of soil flooding [days]				
	-	-	1	4	7
	Difference				
	TBAR concentration				
(+4-HPEA) / (-4-HPEA)	-	-	21.0*	9.0	-9.0
(+4-HPEA) / (+4-HPEA+flooding)	-	-	24.0*	23.0*	-14.0
(+4-HPEA) / (-4-HPEA+flooding)	-	-	30.0*	-30.0*	-7.0
(-4-HPEA) / (+4-HPEA+ flooding)	-	-	3.0	14.0	-5.0
(-4-HPEA) / (-4-HPEA+flooding)	-	-	9.0	-39.0*	2.0
(+4-HPEA+flooding) / (-4-HPEA+flooding)	-	-	6.0	-53.0*	7.0
	GPX activity				
(+4-HPEA) / (-4-HPEA)	-	-	6.13*	6.45*	0.84
(+4-HPEA) / (+4-HPEA+flooding)	-	-	2.24*	3.27*	-4.23*
(+4-HPEA) / (-4-HPEA+flooding)	-	-	5.76*	5.64*	-3.57*
(-4-HPEA) / (+4-HPEA+ flooding)	-	-	-3.89*	-3.18*	-5.07*
(-4-HPEA) / (-4-HPEA+flooding)	-	-	-0.37	-0.81	-4.41*
(+4-HPEA+flooding) / (-4-HPEA+ flooding)	-	-	3.52*	2.37*	0.66

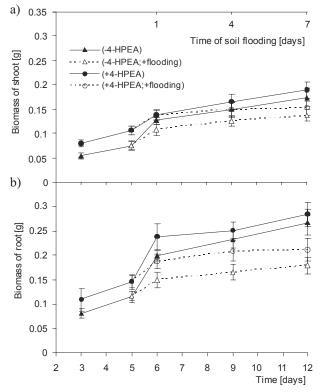*denotes a statistically significant difference.

Fig. 2. The effects of pre-sowing treatment of *Hordeum vulgare* seeds with 10^{-6} M 4-hydroxyphenethyl alcohol (4-HPEA) on the shoot (a) and root (b) biomass production of seedlings grown under optimal soil watering or flooding conditions.

Under optimal growth conditions, the root biomass of the (+4-HPEA) seedlings exceeded the (-4-HPEA) value of this parameter on the 3rd and 5th days of growth by 35 and 26%, respectively. Then the differences between the variants declined and by the end of the experiment were about 6-8% (Fig. 2b).

Under soil flooding conditions the growth of the shoot and root biomass decreased both in the (-4-HPEA;+flooding) and (+4-HPEA;+flooding) seedlings (Fig. 2a,b). After 7 days of stress factor effects, the shoot biomass of the (-4-HPEA;+flooding) and (+4-HPEA;+flooding) seedlings declined by 20%; the root biomass of the same seedlings declined by 33 and 26%, respectively, compared to the (-4-HPEA) and (+4-HPEA) ones grown with optimal soil watering (Fig. 2a,b). It is significant that under flooding conditions the seedlings grown from seeds treated with 4-HPEA kept the advantage in biomass accumulation, in comparison with untreated plants.

Lipid Peroxidation Intensity

The TBAR concentration (Fig. 3) in the first leaves of 6-day seedlings of the (-4-HPEA) and (+4-HPEA) plants averaged 111 and 132 nmol g^{-1} FW, respectively, and varied significantly (Table 2). During the next 6 days no significant decrease of the TBAR concentration was observed in the leaves of the (-4-HPEA) and (+4-HPEA) plants. As a

result, the TBAR concentration in the leaves of seedlings of 9-12 days of age, in both of the variants, was practically the same (Fig. 3). The opposite effect on the TBAR concentration was observed in the (-4-HPEA; +flooding) and (+4-HPEA;+flooding) seedlings under soil hypoxia. An increase followed by a decrease of the TBAR concentration took place in the (-4-HPEA;+flooding) plants, and a significant decrease of the TBARs concentration with subsequent increase to the level of the control variant was noted in the plants (+4-HPEA;+flooding) (Table 2, Fig. 3).

GPX Activity

GPX activity in the leaves of the (-4-HPEA) seedlings under optimal soil watering was monotone decreased during plant growth, from 7.55 μmol tetraquaiacol g^{-1} FW min^{-1} in 6-day seedlings to 3.48 μmol tetraquaiacol g^{-1} FW min^{-1} in 12-day seedlings (Fig. 4). GPX activity in the leaves of 6- and 9-day (+4-HPEA) seedlings was significantly higher than in the (-4-HPEA) plants of the same age, by 81 and
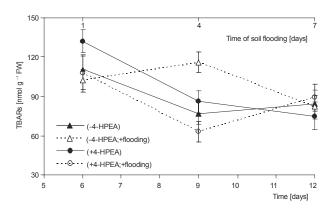
130%, but after that it decreased and until the 12th day of growth was about the same value as the (-4-HPEA) variant (Table 2, Fig. 4). During the first 4 days of soil hypoxia action, the GPX activity in the leaves of the (-4-HPEA;+flooding) plants did not practically differ from the (-4-HPEA) variant (Fig. 4). In contrast, the GPX activity in the leaves of (+4-HPEA;+flooding) plants, during the first 4 days of hypoxic stress, was lower than in (+4-HPEA) plants but significantly higher than in the (-4-HPEA) and (-4-HPEA;+flooding) plants (Table 2, Fig. 4). On the 7th day of hypoxic stress there were no reliable differences between the levels of GPX activities in the (-4-HPEA;+flooding) and (+4-HPEA;+flooding) plants. Moreover, the activity of this enzyme in all flooded plants was twice as high as that in the plants non-stressed by flooding (Fig. 4).

Discussion

Analysis of Eh was performed and showed that soil redox potential decrease was affected by soil flooding. Włodarczyk and Kotowska [30] also observed a decrease of redox potential after the first day since the moment of soil flooding. The effect of natural and synthetic cytokinins on seed germination has been shown in numerous works [22, 23, 31, and others]. It is known that soaking of seeds in water with growth regulator solutions with antigibberellin and cytokinin activity before sowing increases the energy and rate of cauliflower seed germination and the phenophasis replacement [31]. It has also been found that cytokinins have a unique capability to eliminate different inhibitory effects on the seeds. The germination of seeds could be blocked by abscisic acid or by various phenolic compounds. Treatment of seeds with cytokinins removes or reduces the inhibitory effect of such compounds [23]. It has been proposed that the regulation of dormancy and germination of seeds is realized through the balance of their stimulators and inhibitors [22]. The mechanism of withdrawal of seeds from the dormancy state is connected with activation by cytokinins of the RNA synthesis [22].

Our results showed that pre-treatment of seeds with 4-HPEA stimulated their germination by about 20%. Besides germination, shoot and root growth induction and their biomass production were shown in the (+4-HPEA)-barley seedlings. These effects of 4-HPEA on the seedlings were significant at the earliest phase of plant development, with subsequent decrease at the end of the growth experiment. Moreover, the growth of (+4-HPEA)-seedlings was not suppressed by soil flooding to as significant a degree as in the (-4-HPEA)-plants. This result indicates that 4-HPEA plays a role in plant tolerance process.

There is little data in the literature about the cytokinin regulatory role in the formation of plant tolerance to unfavourable factors [16, 17, 32] in comparison with a lot of data about cytokinin effect on seed germination and seedling growth. In particular, the cytokinin influence on plant tolerance to drought has been shown on maize, horse bean, and wheat plants [17]. It also has been established that kinetin diminished the effect of a higher dose of zinc

Fig. 3. The effects of pre-sowing treatment of *Hordeum vulgare* seeds with 10^{-6} M 4-hydroxyphenethyl alcohol (4-HPEA) on the concentrations of thiobarbituric acid reactive substances (TBARs) in the leaves of seedlings grown under optimal soil watering or flooding conditions. FW (fresh weight).
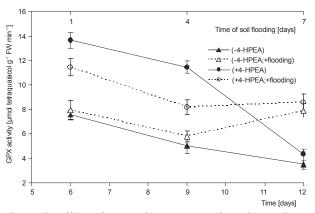

Fig. 4. The effects of pre-sowing treatment of *Hordeum vulgare* seeds with 10^{-6} M 4-hydroxyphenethyl alcohol (4-HPEA) on the quaiacol peroxidase (GPX) activity in the leaves of seedlings grown under optimal soil watering or flooding conditions. FW (fresh weight).

on pea plant growth [16]. The cytokinin action on stress tolerance is associated with the induction of "stress" protein biosynthesis [18], activation of leaf growth, stimulation of chloroplast differentiation, and mesophyll cell growth and division [32]. The effect of a cytokinin-like substance on the intensity of lipid peroxidation process and activity of antioxidant enzyme in the aspect of estimation of the adaptive potential of the plants was investigated by us for the first time.

Lipid peroxidation is known to be a parameter of stress response indicator in living organisms [14]. Usually, the intensity of lipid peroxidation is estimated by measuring TBAR concentration [29]. In our experiments, TBAR concentration increased in (-4-HPEA)-barley seedlings after 4 days flooding. This increase indicates intensification of the oxidative destruction processes induced by flooding conditions. The (+4-HPEA)-seedlings did not show any increase in the concentration of TBARs but, in contrast, they exhibited a decreasing trend in TBAR concentration during flooding. We suppose that seed pre-soaking with 4-HPEA provides activation of antioxidant system in plants. Measurements of GPX activity supported this assumption. GPX activity was increased significantly in the first leaves of (+4-HPEA)-plants. This increase was the biggest in the first stage of seedling growth, both under optimal and flood conditions.

Thus, pre-soaking with 4-HPEA stimulates germination of barley seeds, growth, and development of seedlings, and their stress tolerance. The adaptive potential in the 4-HPEA-treated plants is higher than in the non-treated plants. Treatment of plant seeds with 4-HPEA could be used to increase plant tolerance to unfavourable factors.

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