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A NEW NATURAL STIMULATOR 4-HYDROXYPHENETHYL ALCOHOL EFFECTS ON AMARANTH SEEDS GERMINATION AND PLANT PRODUCTIVITY

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S u m m a r y

The results of comparative studying effects of 4-hydroxyphenethyl alcohol — exometabolite of purple bacterium Rhodospirillum rubrum and synthetic cytokinin 6-benzylaminopurine on seed germination, growth, productivity and development of amaranth vegetable form (Amaranthus caudatus L.) and also on the set of their physiological-biochemical parameters are presented. It was established, that seeds treatment with solutions of investigated substances (10-6 M) led to similar positive effects in all studied indices in the plants growing from these seeds. The authors suggest to use the 4-hydroxyphenethyl alcohol as the growth cytokinin-like natural stimulator for improvement of seed grain and plant productivity.

Keywords: 4-hydroxyphenethyl alcohol, cytokinins, amaranth, seeds germination, ontogenesis, productivity, protein content.

Amaranth (*Amaranthus caudatus* L.) is very popular in Europe as a valuable food culture. Introducing this plant in Russia is complicated by a number of reasons, one of which is a specific ontogenesis: nearly a month after germination, young amaranth plants start the phase of so-called latent growth when aboveground phytomass almost doesn't grow while the rapid development of a root system (1). This period is critical to young plants because they are very sensitive to environmental factors at this time and can be easily suppressed by weeds. To prevent the death of sprouts, it is necessary to promote their faster development and earlier start of active growth.

This problem can be solved by using the appropriate growth stimulators, particularly, 4-hydroxyphenethyl alcohol (HPEA). In earlier research, the authors have derived this substance from the culture medium of *Rhodospirillum rubrum* (2). Previously, a specific cytokinin-like action was found in HPEA: it stimulates the synthesis of amaranthine in isolated amaranth seedlings. Later this fact was confirmed by results of bioassays on fragments of various plants (3). These data suggest an assumption that HPEA performs cytokinin-like properties on a whole plant as well.

Cytokinins perform various effects in plants; they regulate seed germination and ripening (4), stimulate plant growth and development, increase total protein content and activity of a photosynthetic apparatus; along with it, cytokinins specifically increase the activity of nitrate reductase (NR) and help to eliminate adverse effects of stress (5). Stimulatory action of cytokinins improves plant productivity. Should these properties be found in HPEA, it can be used to improve seed quality, viability of plants at critical developmental stages and increase agricultural productivity.

The purpose of this study was revealing the cytokinin-like stimulatory action of 4-hydroxyphenethyl alcohol on seeds and plants of *Amaranthus caudatus* L. in ontogeny and a comparison of those with effects of a synthetic cytokinin 6-benzylaminopurine (6-BAP).

Technique. The experiment was performed on seeds of the vegetable *Amaranthus caudatus* L. (cultivar K-173) obtained from the All-Russia Institute of Vegetable Plant Selection and Seed Growing (Moscow province). Bioactive substances: natural HPEA isolated from the culture medium of non-sulfur photosynthetic bacteria *Rhodospirillum rubrum* (2) and the synthetic cytokinin 6-BAP ("Sigma", USA).

Pre-sowing treatment of the seeds differed by variants of the experiment. In the first variant (germination rate testing), seeds (initial germination rate 70%) were soaked for 24 h in aqueous solutions of HPEA and 6-BAP (concentration range from 10^{-9} to 10^{-4} M, test) or in distilled water (control) and then dried in a weak stream of air at room temperature. In the second variant (determining indicators of plant growth, physiological and biochemical processes) seeds (initial germination rate 70%) were treated with 10^{-6} M solutions of HPEA and 6-BAP (test) or distilled water (control) similarly to the first variant and then grown to adult plants. In the third variant (studying the effects of growth stimulators against the background of artificial aging), seeds (initial germination rate 90%) were soaked in water as described above (control) or subjected to artificial aging (AA, test) for 4 days at 41 °C and relative air humidity 100% (6). A part of the "aged" seeds were treated with 10^{-6} M HPEA solution.

Germination rate was established as proportion of seeds germinated on moist filter paper (Petri dishes, 72 h at 24 °C). To grow plants, 10 calibrated seedlings were planted in vessels with sand (3 vessels in each variant of the experiment) and grown using Knopp's nutrition medium at 24 °C, 14-hour photoperiod and illumination 150 Vtšm^{-2}.

Biometric indicators of plants were recorded every 15 days up to harvest (115th day), productivity was evaluated upon the growth of biomass. Daily chlorophyll content was measured in suspensions of isolated chloroplasts (7) derived from leaves of 45 plants (8); photochemical activity of those was assessed by the rate of electron transfer (9) and intensity of phosphorylation (10). Along with it, NR activity (11) and total protein content in leaf tissue were determined (12). Net photosynthetic productivity of leaves (NP) for the period from the 45th to 60th days was calculated according to A. Nichiporovich (13).

The data presented in this article were obtained in one typical experiment out of five ones. Biometric parameters were accounted in 30 plants. Biochemical assays were performed 3-fold. Statistical processing of data was conducted using Student's t-test for the first threshold of 0,95.

Results. Initial germination of amaranth seeds amounted to 70%. Seed dressing with HPEA and 6-BAP solutions caused the strongest simulation of seed germination (28%) and its inhibition (on average by, respectively, 20% and 12%) at the contents of, resp.,

10^{-6} M and 10^{-4} M (Fig. 1). 6-BAP solution with the content of 10^{-7} M didn't change germination rate of seeds, while 10^{-7} M HPEA reliably increased it by 13%. Other concentrations of these substances were found to be ineffective.

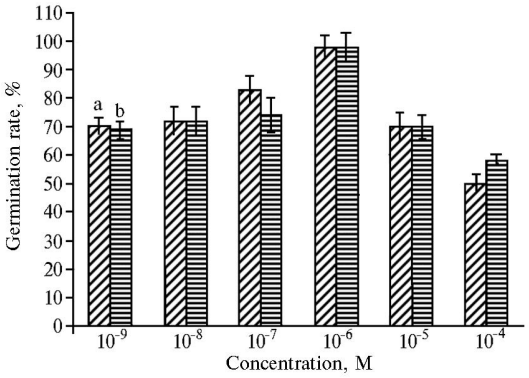

Fig. 1. Germination rate of seeds of *Amaranthus caudatus* L. (cultivar K-173) **treated with 4-hydroxyphenethyl alcohol** (HPEA, a) **and 6-benzylaminopurine** (6-BAP, b)**.**

Plants grown from the treated seeds significantly exceeded control individuals by height and biomass during a critical period (15^{th} – 30^{th} days) (Table 1). Along with it, seed treatment stimulated root growth in 15-days-old seedlings: both HPEA and 6-BAP provided elongation of the main root up to, resp., 70% and 50% compared with control plants.

Stimulatory effect of these substances on plant height and weight of aboveground parts persisted in ontogeny except the 75^{th} day (Table 1). In this period individuals started to develop generative organs; plant height almost didn't grow while the increase in weight of aboveground parts.

1. Dynamics of growth indicators (relative to control, %) **in *Amaranthus caudatus* L.** (cultivar K-173) **plants grown from seeds treated with 4-hydroxyphenethyl alcohol** (HPEA) **and 6-benzylaminopurine** (6-BAP) ($\bar{X}\pm x$)

Growth stimulator	Age of plants, days							
	15	30	45	60	75	90	105	115
	Plant height							
HPEA	154±4	167±14	185±12	132±10	119±12	148±15	133±6	122±7
6-BAP	122±4	141±15	144±10	129±8	100±7	140±9	133±5	122±4
	Weight of aboveground parts							
HPEA	252±6	237±12	209±12	186±9	162±10	180±9	152±18	254±22
6-BAP	229±8	190±9	175±10	166±10	159±12	168±10	174±18	250±21

Plants grown from the treated seeds on the 45^{th} day (active vegetation) exceeded control by physiological and biochemical properties of leaves: they manifested significantly higher levels of chlorophyll content and the rate of electron transfer with simultaneous accumulation of ATP (Table 2), as well as NR activity and total protein content. These data indicate intense utilization of nitrogen in plants whose seeds were pre-treated with HPEA and 6-BAP.

2. Physiological and biochemical characteristics (relative to control, %) **in leaves of *Amaranthus caudatus* L.** (cultivar K-173) **45-day-old plants grown from seeds treated with 4-hydroxyphenethyl alcohol** (HPEA) **and 6-benzylaminopurine** (6-BAP) ($\bar{X}\pm x$)

Growth stimulator	Chlorophyll content	Rate of electron transfer	ATP content	Nitrate reductase activity	Total protein content
HPEA	151±12	140±14	190±15	200±16	140±14
6-BAP	146±15	135±14	186±13	185±18	138±14

The increased photosynthetic efficiency of nitrogen utilization in experimental plants provided the growth of net photosynthetic productivity (Fig. 2).

Long-term storage of seeds (over the permitted period) or the influence of adverse factors during storage significantly worsens germination (14). Plants grown from such inferior seeds are often stunted and develop lower productivity than those obtained from high-quality seeds (15). Cytokinins are known to increase germination rate of seeds stored for a long time and eliminate the effects of stress in plants (4, 5).

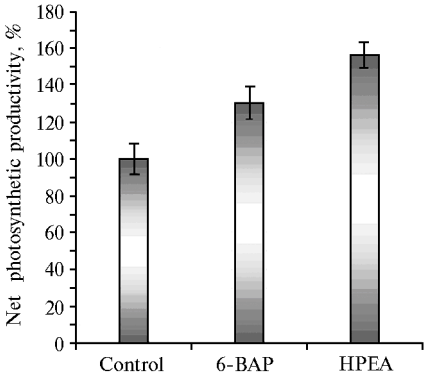

Fig. 2. Net photosynthetic productivity in leaves of *Amaranthus caudatus L.* (cultivar K-173) **plants grown from seeds treated with 4-hydroxyphenethyl alcohol** (HPEA) **and 6-benzylaminopurine** (6BAP) **for the period from the 45^{th} to the 60^{th} days of vegetation.**

AA contributed to 27% loss of germination of amaranth seeds, which defect was completely restored by subsequent treatment with HPEA. 45-days-old plants grown from seeds exposed to AA manifested significantly worse growth indicators compared with control and this tendency persisted in future. Pre-sowing seed dressing with HPEA completely eliminated the effects of stress and slightly improved all investigated parameters of plants relative to control (intact seeds).

Seed treatment with HPEA positively affected the length of panicle and weight of seeds per plant; these plants demonstrated weight of 1000 seeds much higher than in control (Table 3). These indicators determine initial growth of seedlings, their quality and viability, and they ultimately affect productivity of a crop, so revealing the latter effect of HPEA was very important for amaranth whose seeds are very small in size and lightweight.

3. Age-related changes of biometric parameters (relative to control, %) **of *Amaranthus caudatus* L.** (cultivar K-173) **plants grown from seeds subjected to artificial aging** (AA) **and subsequent treatment with 4-hydroxyphenethyl alcohol** (HPEA) ($\bar{X}\pm\bar{x}$)

Parameter	Age of plants, days			
	45th		115th	
	AA	AA + HPEA	AA	AA + HPEA
Plant height	64±5	131±11	73±3	104±5
Weight of a plant	76±7	131±10	61±7	110±5
Weight of leaves per plant	63±8	116±9	63±5	114±8
Leaf area	72±9	118±11	73±9	113±9
Length of a panicle			62±9	111±10
Weight of seeds per plant			85±7	117±7
Weight of 1000 seeds			72±6	129±8

HPEA can be considered as environmentally safe substance because it is used at micromolar concentrations and it is a product of natural origin - exometabolite of purple non-sulfur photosynthetic bacteria which normally inhabit soil and water bodies (2, 16).

Thus, specific cytokinin-like effects were established in 4-hydroxyphenethyl alcohol (GFES) in respect to all studied biometric, physiological and biochemical parameters of *Amaranthus caudatus*. Similar effects are performed by the well-known synthetic adenine-type cytokinin 6-benzylaminopurine (6-BAP), whose activity in some cases was inferior to that of HPES. The authors suggest HPEA as a promising stimulator of plant growth suitable for use in modern agriculture.

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