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PROPERTIES OF CREZACIN AS A GROWTH STIMULANT OF VEGETABLE AMARANTH (*Amaranthus* L.)

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Abstract

Crezacin, tris(2-oxyethyl)ammonium ortho-cresoxy acetate-based adaptogen of humans and animals, is applied in Russia as a stimulant of growth and productivity of crops (wheat, oats, spinach, potatoes, etc.). In other countries, crezacin is not used for these purposes. There is no information about its use in the cultivation of food amaranth (*Amaranthus* L.), a source of high-quality protein and other useful substances. In this paper, we first report data on the effect of pre-sowing treatment with crezacin on seed germination, development and biometric parameters of amaranth plants during ontogenesis, and on their productivity and nutritive value. Our findings indicate the ability of crezacin to increase nitrate reductase activity, to influence the nitrite nitrogen content in the early stages of vegetation, to increase the electron transport rate ATP synthesis. The aim of the work was to assess the effect of different crezacin concentrations on seed germination, seedling quality, growth parameters, and activity of photosynthesis and nitrogen assimilation apparatus. Seeds of *Amaranthus caudatus* L. (sample K173) and *Amaranthus cruenthus* L. (sample K185) were soaked for 1 day in crezacin solutions (test) or in distilled water (control), and used in the experiments after air-drying at room temperature. In experiment 1, 10^{-10} до 10^{-5} M aqueous crezacin was applied to seeds then allowed for germination on wet filter paper in Petri dishes for 72 h at 24 °C. The proportion of germinated seeds was calculated. In experiment 2, we studied the effect of crezacin on the growth and physiological and biochemical parameters of plants. Seeds were treated with 10^{-7} M crezacin, germinated, and calibrated seedlings were transplanted into sand- filled container. Biometric parameters were measured every 15 days until harvest (120 days), productivity was estimated by the green mass increase. Chlorophyll concentration was assessed in the leaves of 45-day-old plants. The photochemical activity of isolated chloroplasts was evaluated by the rate of electron transport and photophosphorylation. From day 15 to day 45, the activity of nitrate reductase, the concentrations of $N\text{-}NO_2$ and total protein were measured in the leaves. The net photosynthesis (NP) for the period from day 45 to day 60 was calculated by A.A. Nichiporovich's method. Experiment 1 revealed a change in seed germination depending on the concentration of the preparation in both studied samples. A 10^{-8} concentration increased seed germination capacity by 10 % compared to control ($P = 0.95$), 10^{-7} M had maximum stimulating effect (by 25 %), and at 10^{-5} M the germination rate decreased by 22 %. Other concentrations had no significant effect. In experiment 2, in both varieties during latent growth stage the seedlings from the treated seeds were twice as high as the control, and the length of the main root was 1.6 times as much as in control. During later stages, the green mass of plants in the experiment exceeded the control 1.3-2.0-fold depending on the phase of ontogenesis. The treatments did not affect the height of plants. The NF value in leaves after treatment exceeded the control by 26 % ($P = 0.95$). At the same time, the chlorophyll content in the leaves did not change, and the electron transport rate in chloroplasts increased by more than 30 % while photophosphorylation by 60 %. The nitrate reductase activity in leaves on day 45 increased by almost 60 %, the total protein level by 20 %, and nitrite nitrogen amount by 16 % ($P = 0.95$). These findings indicate the stimulating effect of crezacin on amaranth seeds, plant growth, photosynthesis and protein synthesis, which leads to an increase in the productivity and nutritional value of plants.

Keywords: tris(2-oxyethyl)ammonium ortho-cresoxy acetate, crezacin, amaranth, seed germination, plant growth regulation, photophosphorylation, electron transport, protein content,

In the 1970s, a group of scientists led by M.G. Voronkova synthesized the biologically active chemical compound tris(2-oxyethyl)ammonium ortho-cresoxy acetate, or crezacin. A highly purified crezacin called trekrezan was originally intended for use in medicine as an adaptogen and immunostimulant, as well as in animal husbandry and veterinary medicine [1]. Later it was found that it has a stimulating effect not only on animal organisms, but also on plants, and undergoes natural degradation in the soil with the formation of water and carbon dioxide [2].

Currently, crezacin (triethanolammonium salt of ortho-cresol oxyacetic acid) and as part of Krezatsin, Energia-M, KPP, TAB, Mival preparations is registered in the State catalog of pesticides and agrochemicals approved for use in the Russian Federation [3] as a growth stimulant of many crops (wheat, corn, oats, cabbage, spinach, potatoes, etc.). Methods of crezacin application and its effects (increasing seed germination, enhancing growth processes, increasing yields, improving product quality, and increasing resistance to adverse environmental factors) are described in detail. However, we did not find information on the effect of crezacin on the light-dependent processes of photosynthesis, as well as on individual components of the nitrogen assimilation system and protein synthesis in plants. There are also no data on the use of crezacin in the cultivation of plants of the genus *Amaranthus* L. either in Russian or in foreign literature.

Members of the genus *Amaranth* (more than 100 species) are unique in their properties. For millennia, they have been used on the continents of South America, Asia, and Africa as food, medicine, feed, and decorative crops [4, 5]. Though all parts of plants are edible [6], they are subdivided into pseudo-grain and leaf (vegetable) forms [7, 8]. The high nutritional and medicinal value of amaranth has been scientifically substantiated by numerous modern studies of the chemical composition of the organs and tissues of these plants. All parts of amaranth plants are characterized by high protein content [4-7], and seeds (grain) surpass even legumes in its quantity and quality [9-11]. Amaranth protein enriched in lysine and other essential amino acids [10, 11] is close to animal protein in nutritional value and surpasses it in digestibility [10-12].

In addition, representatives of the genus serve as a rich source of mineral elements — iron, copper, zinc, selenium, phosphorus, calcium [12, 13]. They contain an increased levels of vitamins C [11, 15], A, E, group B [11-15] and other useful compounds such as flavonoids, anthocyanins, carotenoids, rutin [8-11], squalene [16] and antioxidants possessing antitumor, antibacterial and anti-inflammatory properties [6, 14, 17]. The use of amaranth extracts, leaves, seeds, oil or meal as a part of dishes and as a medicine helps in the prevention and treatment of diseases of the cardiovascular system [18] and the digestive system, diabetes mellitus, and obesity [13-17]. Amaranth plants are used for the preparation of baby food and diet food [14, 17].

Due to its beneficial properties, this southern culture is gaining popularity in many countries of the world [4, 5, 19], however, its introduction can cause difficulties both because of environmental conditions [19, 20] and as a result of the physiological peculiarities [4, 5, 7-10]. Amaranth has very small seeds that germinate unevenly, and small shoots with thin stems, which, after 5-7 days after germination, enter a state of hidden growth for 2-3 weeks. During this period, only the root system is actively developing, and the aerial part stops growing. Such seedlings suffer greatly from wind, lack of moisture and light, are easily clogged by weeds and die [7, 21]. In the countries of Europe and Central Russia, the development of amaranth plants is slowed down [19-21], since the condi-

tions of higher insolation and temperature are optimal for this southern culture.

It seems necessary to improve the quality of sowing material, strengthen the habit of seedlings by accelerating development at the stage of latent growth and increasing resistance to environmental factors, as well as increasing the productivity of adult plants and imptoving their nutritional value. For this, presowing seed treatment with various stimulants is widely used [20, 21].

In the context of the latest European rules on limiting the use of pesticides [22], environmentally friendly stimulants that improve the growth and adaptive qualities of plants are especially attractive. In previous works, we showed high efficiency of the use of a number of preparations for growing *Amaranthus caudatus* (cultivar K173) and *A. cruenthus* (cultivar K185), i.e. 2-(4-hydroxy) phenyl ethanol, an exometabolite of the purple bacterium with cytokinin activity [23], gibbersib, a fungal-based gibberellin preparation [24], and para-aminobenzoic acid, a component of folates [25].

Many papers describe the properties of crezacin, which are especially important for the culture of amaranth. Crezacin was found to increase the germination capacity of oat seeds [26], the weight and height of potato plants [27], the net productivity of winter wheat photosynthesis [28], the total protein content in spring wheat grain [29], and the amount of chlorophyll in leaves of *Isatis tinctoria* L. [30]. A special advantage of crezacin in cultivation of leaf amaranth may be its ability to cause significant accumulation of green mass, shown on potato and spinach plants [31, 27].

In this study, we first obtained data on the effect of presowing treatment with crezacin on seed germination, development, biometric parameters of food amaranth plants depending on the ontogenesis phase, productivity and nutrition value. The previously undescribed properties of crezacin have been disclosed, namely the ability to increase nitrate reductase activity, to influence the nitrite content in early vegetation, and to increase the electron transport rate in the chain of their transfer and ATP synthesis.

The aim of the work was to assess the effectiveness of different crezacin concentrations on seed germination, seedling quality, growth parameters of food amaranth plants, activity of photosynthesis and nitrogen assimilation apparatus.

Materials and methods. Seeds of amaranth *Amaranthus caudatus* L. (variety K173) and *Amaranthus cruenthus* L. (variety K185) with a 70% germination rate were provided by the All-Russian Institute for Selection and Seed Production of Vegetable Crops (Moscow Region). Seeds were treated by soaking for 1 day in distilled water (control) or in crezacin (a crystalline powder with an active substance content of 95%, Flora-Si LLC, Russia) solutions of different concentrations (test). Then they were dried at room temperature in a weak stream of air and used in experiments.

In determining the effect of crezacin on seed germination (experiment 1), aqueous solutions 10^{-10} до 10^{-5} M were used for soaking. The percentage of sprouted seeds was calculated after germination on wet filter paper in Petri dishes for 72 h at 24 °C.

To assess the effect of crezacin on the growth and physiological and biochemical parameters of plants (experiment 2), a 10^{-7} M solution was used for seed treatment; after germination, calibrated seedlings were transplanted into sand cuvettes, 3 cuvettes per variant, 10 seedlings per cuvette. The cuvettes were placed in a temperature chamber for plant growth LCC-1000MP Daihan Labtech (Daihan Labtech Co., Ltd, South Korea) at 150 W/m^2 illumination, 24 °C, and 14-hour photoperiod.

Watering was carried out once a day with Knopp nutrient medium. Bi-

ometric indicators were recorded every 15 days until harvesting (day 120), productivity was evaluated by the increase in green mass. The content of chlorophyll was determined in the leaves of 45-day-old plants [32]. The photochemical activity of isolated chloroplasts [33] was evaluated by the rate of electron transport [34] and photophosphorylation [35]. From day 15 to day 45, the activity of nitrate reductase (NR), the content of nitrite nitrogen [36], and total protein [37] were measured in the leaves. The net productivity of photosynthesis (NPP) from day 45 to day 60 was calculated by Nichiporovich's method [38].

The article presents the results of one typical experiment out of five. Biometric parameters were measured in 30 plants, biochemical analyzes were performed in three repetitions.

Statistical processing was performed in Microsoft Excel. The tables and figures show the arithmetic means (M) and standard errors of the means (±SEM). The significance of differences was evaluated by Student's t-test at $P = 0.95$.

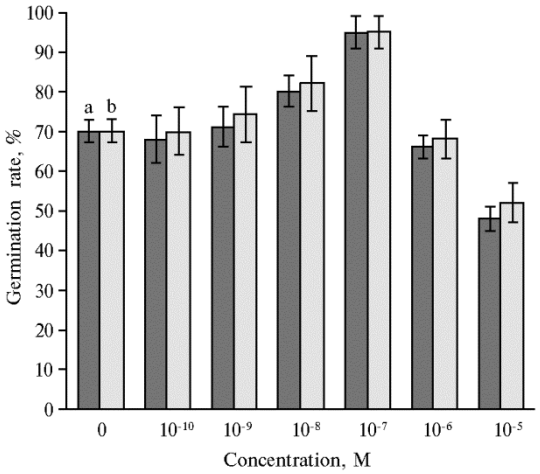

Fig. 1. Seed germination in amaranth *Amaranthus caudatus* L. (variety K173) (a) **and *Amaranthus cruenthus* L. (variety K185) (b) upon treatment with different concentrations of crezacin** (aqueous solutions, lab test).

Results. Upon presowing treatment of amaranth seeds with aqueous solutions of crezacin, the germination rate depended on the concentration of the preparation, and the effect turned out to be the same for both studied variety samples (Fig. 1). At a concentration of 10^{-8} M, the germination rate significantly ($P = 0.95$) increased by 10% compared to the control; at 10^{-7}, the maximum stimulating effect occurred, i.e. a 25% increase in germination for each variety specimen ($P = 0.95$), whereas at 10^{-5} M the indicator fell by 22% ($P = 0.95$). At other concentrations, the effect was insignificant.

In experiment 2, 10^{-7} M crezacin was used, since this concentration was optimal for seed germination. The weight of the aerial parts of the plants of both varieties throughout life exceeded the control by more than 20% (Fig. 2), and the effect of the preparation was associated with the phases of ontogenesis. Thus, 15- and 30-day-old seedlings, which are at the stage of latent growth, exceeded the control by 60% and 100% in weight, respectively ($P = 0.95$) (see Fig. 2). At this, an increase in the length of the main root of 15-day old seedlings averaged 60% in both cultivars ($P = 0.95$).

During active vegetation (days 45-60), green mass accumulation decreased compared to the previous phase, but remained on average 45% higher than in the control. The weight of leaves on day 60 was 46% higher compared to the control for K173, and 52% higher for K185 ($P = 0.95$). On day 90, the aerial biomass accumulation decreased, but nevertheless significantly ($P = 0.95$) exceeded the control by 27%. On day 120, the aboveground biomass of plants grown from crezacin-treated seeds again almost doubled the control.

The presowing treatment of seeds with crezacin did not affect the growth of amaranth plants in height throughout their life, with the exception of day 75 when the test plants lagged behind the control by almost 20% ($P = 0.95$).

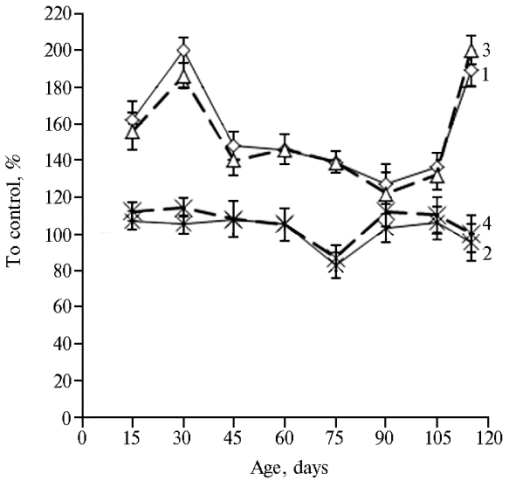

Fig. 2. The increase in weight (1, 3) **and height** (2, 4) **in growing plants of amaranth *Amaranthus caudatus* L. (cultivar K173)** (1, 2) **and *Amaranthus cruenthus* L. (cultivar K185)** (3, 4) **upon seed treatment with 10^{-7} M crezacin** (water solution, lab test**).**

Investigation of the light-dependent reaction in photosynthesis in 45-day-old K173 plants revealed an increase in the performance of the electron transport chain of isolated chloroplasts and photosynthetic phosphorylation (Table 1). The chlorophyll content in the leaves did not change significantly. The leaf NPP calculated over the period from day 45 to day 60, increased by 26±6% (P = 0.95) compared to the control.

The effect of seed treatment with crezacin on elements of the nitrogen assimilation system we assessed in K173 amaranth plants in early growing period (days 15-45). All parameters showed changes with a degree which was determined ontogenetically (Table 2). The activity of nitrate reductase increased 1.5 times only on day 45, the total protein content in leaves exceeded the control by 20-30% on average, and the nitrite N level fluctuated throughout this entire period of development (P = 0.95)

1. Chlorophyll content, electron transport and photophosphorylation in chloroplasts from leaves of 45-day-old amaranth *Amaranthanthus caudatus* L. (cultivar K173) plants upon seed treatment with 10^{-7} M crezacin (*M*±SEM, water solution, lab test)

Variant	Chlorophyll		Rate			
			electron transport		photophosphorylation	
	mg/g dry weight	to control, %	μmol $K_3[Fe(CN)_6]$	to control, %	μmol ATP	to control, %
Control	9.9±0.2	100.0±2.0	110.4±7.7	100.0±7.0	112.0±4.3	100.0±3.8
Test	10.5±0.9	106.1±9.1	148.9±7.0	134.9±6.1	184.5±9.4	164.7±4.5

N o t e. The electron transport rate is calculated per 1 mg chlorophyll per 1 h.

2. Nitrogen assimilation parameers (to control, %) **in leaves of amaranth *Amaranthanthus caudatus* L. (cultivar K173) plants upon seed treatment with 10^{-7} M crezacin** (*M*±SEM, water solution, lab test)

Plant age, days	Nitrate reductase activity	$N\text{-}NO_2$	Total protein
15	109±5	135±2	132±9
30	102±6	106±4	129±4
45	158±7	116±4	120±5

Thus, we established the dependence of the germination capacity of amaranth seeds on the dose of crezacin applied a wide range of low concentrations, 10^{-10}-10^{-5} M. In plants, such a dose-dependent response to a chemical agent is characteristic of the phytohormones [39]. The phytohormones exhibit biological activity in extremely low doses and act as stimulants in a narrow range of concentrations, being inhibitors when exceeding the range. Due to this property of crezacin, which we established for the first time, its hormone-like properties can be assumed. Note that some authors compare the manifestation of crezacin activity with the action of auxins and gibberellins [2, 28]. However, the hypothesis

about certain elicitoring properties of many small molecules with biological activity to which crezacin can be attributed seems to be more substantiated [40]. We supported this suggestion earlier in the study of the effects of p-aminobenzoic acid and 4-hydroxyphenethyl alcohol [27, 29].

We did not find the effect of crezacin on the growth of amaranth plants in height, although this property was described [30, 35], with the exception of day 75 (panicle formation). However, we confirmed another property of crezacin, established earlier, i.e. the ability to enhance the accumulation of green mass [30, 35]. In this case, the effect of the crezacin was also associated with the phases of ontogenesis (see Fig. 2). The strongest treatment effect (a 1.5-2.0-fold increase in weight) occurred at the stage of latent growth (days 15-30). Since in this period the growth of the aerial part of seedlings in the control practically ceases, it can be argued that due to the crezacin application the stop did not occur. During active vegetation (days 45-60), the positive effect of the crezacin visually decreased almost by half compared to the previous phase, but this actually happened due to a sharp increase in seedling growth in the control after they emerged from the hidden growth phase, whereas the effect of crezacin remained significant. Since the beginning of the generative phase (day 75), with initiation and formation of panicles, the energy and plastic resources of plants were mainly spent on the development of generative organs. As a result, during these periods a noticeable lag of plants from the control in height was observed, but there was no decrease in the green mass accumulation. With the onset of the heading stage (day 90), the aerial biomass further decreased. On day 120, the weight of the aerial parts of plants grown from treated seeds was almost 2 times higher than the control, mainly due to panicles with ripened seeds but not the green biomass.

Crezacin applied to seeds resulted in an increase in the aerial biomass at all stages of plant growth compared to control whereas plants remained constant in height or slightly retarded in stem elongation, which led to a positive overall effect, i.e. the habit of the plants became stronger than in the control. Due to this, plant resistance to mechanical damage and low humidity increased, which is especially important for seedlings at the stage of hidden growth

It is especially worth to note the crezacin as a stimulant of root growth which occurs due to an increase in the length of the main root. Despite the fact that during latent growth period, the root system development is quite active even without external stimulation and only the aboveground part practically stops growing, the stimulating effect of crezacin during the critical period for seedlings contributes to their stronger rooting, fixing in the soil and, therefore, better survival.

It was reported that under the action of crezacin the content of chlorophyll increases [27, 30]. We did not find such a change in amaranth leaves. In studying the effects of crezacin on the light-dependent reactions of photosynthesis, we first found an increase in the electron transport rate and the photophosphorylation rate in isolated chloroplasts. As a result, the total energy pool of cells increases, which ensures an increase in the NPP of amaranth leaves on day 45 to day 60 (see Table 1). A similar effect has been described for other plants [28].

It is known that crezacin can affect the metabolism of nitrogen compounds [29] and causes an increase in the total protein content [28, 30]. As a result of the crezacin application to seeds, we found changes in the activity of nitrate reductase, nitrite nitrogen $N\text{-}NO_2$ content and total proteins during stage of early vegetation of amaranth plants (days 15-45) compared to the control. There is no obvious interdependence in these changes, however, some studies

indicate that these parameters are not always in a direct relationship [41]. We can definitely say that under the influence of crezacin, the amount of leaf protein useful for humans is significantly higher, which, consequently, improves the nutritional value of the culture. The observed fluctuations in the nitrite nitrogen content were possibly due to its different use in synthetic processes during definite phases of this stage of ontogenesis.

So, soaking seeds of *Amaranthus caudatus* L. (variety K173) and *Amaranthus cruenthus* L. (variety K185) in 10^{-7} M aqueous solution of crezacin significantly increases seed germination capacity and significantly affects properties of the resultant plants, primarily seedling quality. Their shoots at the stage of latent growth significantly exceed the control in weight, not exceeding the control in height. Due to this, the plants possess a stronger habit, which can contribute to resistance to damaging environmental factors. An additional beneficial effect is a significant lengthening of the main root, due to which the seedlings are rooting better and become more resistant to wind, lack of moisture, and weeds. During active vegetation, productivity of test plants increased due to an increase in the biomass of the edible aboveground part. We did not reveal species-specific changes in seed germination and growth parameters of resultant plants in response to crezacin application. The nutritional value of K173 variety plants is due to an increase in leaf protein. It is established that the described effects are due to stimulation of the photochemical activity of chloroplasts, accompanied by an increase in the photosynthetic phosphorylation rate and the energy pool of cells. Crezacin can be recommended for leaf amaranth cultivation to improve seed quality, productivity, nutrition value, and to facilitate the crop introduction in the middle latitudes of Europe and Central Russia.

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