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SEED TREATMENT EFFECT ON THE ANTIOXIDANT SYSTEM IN AMARANTH (*AMARANTHUS HYPOCHONDRIACUS* L.) SEEDLINGS UNDER CHILLING STRESS CONDITIONS

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SUMMARY

The concerned study aimed to evaluate the effects of various pre-sowing treatments on germination parameters and morphological, physiological, and biochemical traits of amaranth (*Amaranthus hypochondriacus* L.) under normal (25 °C±2 °C) and low (4 °C±2 °C) temperatures. The different treatments comprised H_2O (control), gibberellic acid (GA_3), hydrogen peroxide (H_2O_2), calcium chloride ($CaCl_2$), succinic acid (SuA), and salicylic acid (SA). The results showed GA_3 (300 mg/l), SuA (500 mg/l), H_2O_2 (50 mmol/l), SA (138 mg/l), and $CaCl_2$ (3000 mg/l) positively affected the amaranth seedlings' germination and growth traits. GA_3, SuA, H_2O_2, and $CaCl_2$ had a better effect on amaranth seeds by enhancing the germination rate and seed energy and shortening the germination time. The treatments with SA, GA_3, SuA, H_2O_2, and $CaCl_2$ boost the total antioxidants, chlorophyll a and b, and carotenoid contents in the amaranth seedlings at room and low temperatures. H_2O_2 and $CaCl_2$ treatments further stimulated and increased the chlorophyll a and b contents, while GA_3 and H_2O_2 increased the carotenoid content in amaranth seedlings with chilling stress, revealing a positive anti-cold effect.

Keywords: Amaranth (*A. hypochondriacus* L.), pre-sowing treatments, low-temperature stress, plant growth stimulants, antioxidant system, photosynthetic pigments

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Key findings: The results authenticated pre-sowing treatments with GA_3 (300 mg/l) and H_2O_2 (50 mmol/l) application considerably enhanced the carotenoid content in the amaranth seedlings under chilling stress conditions; they demonstrated a significant cold tolerance-enhancing effect.

INTRODUCTION

In the context of climate change, the highest temperatures often have extremely negative effects on crop production (Timlin *et al.*, 2024). The cold temperature is one of the major climate threats limiting cereal crops' production, such as inversions and late spring frosts that occur just after the germination and emergence of cereal crops like wheat (Aslam *et al.*, 2022).

Amaranth is a pseudo-cereal crop that can thrive in poor soils and extreme weather conditions, such as high temperatures (Kashivaqui *et al*., 2023). Previous research demonstrated that low temperatures during the seedling stage have a detrimental impact on growth and development and, eventually, reduced amaranth yield (Elballa and Cantliffe, 1996; Gins, 2023). However, an increase in amaranth production is crucial for addressing world hunger and food security globally (Chandra *et al.*, 2018). Therefore, accurate prediction and evaluation of extreme chilling stress conditions influencing the seed production of amaranth are imperative.

Pre-germination seed treatment is crucial for assessing amaranth's productivity response characteristics in light of climate change and for developing various adaptation measures (Busquère *et al.*, 2025). However, past studies are insufficient on the resistance development, variations in photosynthetic pigments, and antioxidant levels in amaranth plants under low-temperature stress conditions. Therefore, the study and evaluation of seed pre-treatment effects on developing resistance in amaranth plants under low-temperature stress conditions is necessary. Several compounds, such as gibberellic acid (GA_3), salicylic acid (SA), succinic acid (SuA), hydrogen peroxide (H_2O_2), and calcium chloride ($CaCl_2$) can serve as growth stimulants in amaranth seed germination and growth and development (Ali and Al-Atrakchii, 2022; Sasankan, 2022).

The SuA-based compound utilization in crop production, demonstrated through the different formulations, improved crop production and quality (Shao *et al.*, 2012; Grabovskaya *et al.*, 2020). Several studies have displayed that applying growth stimulants, such as $CaCl_2$ and H_2O_2, can enhance the active compounds in crop plants and boost their antioxidant potential. Additionally, the growth stimulants can mitigate the harm caused by salt stress, boost plant growth, and repair stress-induced damages (Barbosa *et al.*, 2023; Tosheva *et al.*, 2024). However, few studies have compared the synergistic effects of GA_3, SuA, H_2O_2, $CaCl_2$, and SA on amaranth's antioxidant system and photosynthetic pigments under low-temperature stress.

Based on the above discussion, the presented study aimed to evaluate the effects of growth stimulants, such as H_2O (the control), GA_3, H_2O_2, $CaCl_2$, SuA, and SA, on the promotion of seed germination in amaranths. Likewise, it sought the improvement of morphological, physiological, and biochemical traits of amaranth seedlings under low-temperature (4 °C ± 2 °C) stress conditions. This study provides technical references for amaranth cultivation in cold regions of Russia and similar climatic zones.

MATERIALS AND METHODS

Planting material

This study utilized the seeds of amaranth (*A. hypochondriacus* L.) cultivar Krepysh, earlier registered in Russia in the 1920s. The cultivar Krepysh seemed to be resistant to low temperatures due to its robust nature. The cotyledonary leaves of the cultivar Krepysh seedlings contain the red pigment amaranthine, in contrast to the green leaves of other amaranth cultivars.

Seed treatment

This preliminary experiment used the pre-seed treatments of five growth stimulators, i.e., gibberellic acid (GA_3–300 mg/l), hydrogen peroxide (H_2O_2–50 mmol/l), calcium chloride ($CaCl_2$–3000 mg/l), succinic acid (SuA–500 mg/l), and salicylic acid (SA–138 mg/l). The use of distilled water (H_2O) served as the control treatment. The amaranth cultivar Krepysh 50 seeds were specimens used in seed treatment with each growth stimulator. The amaranth seeds' careful selection ensued for uniformity of full-grain size to ensure consistency. Seeds soaked in varying solutions, as well as in distilled water separately, ran for six hours. Afterward, 50 seeds underwent even distribution on two layers of quantitatively moistened filter paper in each sterilized Petri dish for germination. The treatments reached three repetitions, with the number of germinated seeds recorded daily. All Petri dishes proceeded to be placed in the incubator room for seven days at two different temperatures, starting from the third day of emergence (the first three days had temperatures of 25 °C ± 2 °C): 25 °C ± 2 °C and 4 °C ± 2 °C (eight hours per day).

Germination parameters

Meeting the germination criterion was when the root length reached to half of its length in the amaranth seedlings. During this period, the data recording ensued on the germination potential (GP) and germination rate (GR) at a 24-hour interval, regularly replenishing with the same volume of distilled water. Comparatively, the germination time (GT), the germination index (GI), the viability index (VI), and the seed vigor index (SVI) succeeded in their calculations. Germination parameters' calculation was according to Feng *et al.* (2022).

Morphological indicators

After conducting the germination experiment, the 10 *A. hypochondriacus* L. cultivar Krepysh seedlings that met the germination standard became choices in each sample. Their hypocotyl and radicle trimming used scissors, with the hypocotyl length (HL) and the root length (RL) measured and expressed in centimeters (cm). Additionally, recording the fresh weight of the seedling (FWS) and the dry weight of the seedling (DWS) continued on an electric scale in grams (g).

Alcohol-soluble antioxidants' total content in seedlings

The total alcohol-soluble antioxidants (TASA) content assessment employed the methodology of Gins *et al.* (2013) and Yashin *et al.* (2010). The amaranth samples sustained grinding with a specific volume of extracting liquid (96% ethyl alcohol) on a homogenizer at the temperature of 20 °C–25 °C. Then, the homogenate underwent centrifugation at 10,000 g for 15 min at 4 °C. An aliquot of the supernatant helped determine the antioxidant content, diluting if necessary. The measurements carried out on the Tsvet-Yauza-01-AA device were in a direct current mode.

Photosynthetic pigments in seedlings

The chlorophyll and carotenoid contents' determination used spectrophotometry on an SF-46 device (Lichtenthaler, 1987). For determining the coexisting chlorophyll and carotenoids in the amaranth seedlings, each sample proceeded with continuous extraction in dark conditions for one hour, with the raw material pre-ground to a particle size of 1.0 mm. The extraction commenced using 70% ethanol in a ratio of 1:100 (starting material to extractant), followed by 96% ethanol in a ratio of 2:25. The solution's measurement was relative to 96% ethanol in the regions of maximum absorption of 442 and 667 nm optical density. Then, the total content of carotenoids (for violaxanthin) and chlorophyll (for absolute dry weight of the raw material) entailed calculation using the appropriate formula (Trineeva *et al.*, 2015).

Statistical analysis

All statistical analyses performed used Microsoft Excel 97-2003 and SPSS 26.0.

RESULTS AND DISCUSSION

Seed treatment effects

The observation of the effects of five growth stimulants (GA_3, SA, SuA, H_2O_2, and $CaCl_2$) had two temperature formulations on germination traits at T_{25} (incubator culture temperature: 25 °C ± 2 °C) and T_4 (incubator culture temperature: 4 °C ± 2 °C, eight hours per day). Observations were in the pre-sowing seed treatment of the amaranth. The use of seed germination potential (GP) sought to determine the seed viability, the accuracy of germination, and the seedling emergence sequence. The germination rate (GR) is a crucial indicator of seed quality, as frequently utilized in crop production to detect the number of required seeds of *Hymenaea courbaril* L. (Guariz *et al.*, 2021).

At T_{25} temperature treatment, all growth stimulants increased GP and GR of the amaranth seeds compared with the control group, i.e., GA_3 and SuA, showing a 6.45% improvement in GP and 5.32% and 6.38% increases in GR, respectively. This was in comparison with the control treatment (Table 1). In addition, at the temperature treatment T_4, the growth stimulant GA_3 resulted in a 4.26% increase in GP and a 3.16% increase in GR of the amaranth seeds versus the control group. However, nonsignificant differences were apparent among the H_2O_2 and $CaCl_2$ treatments and the control. Under optimal conditions (T_{25}), the growth stimulators SuA, GA_3, and $CaCl_2$ reduced the average germination time (GT) of amaranth seeds by 11.98%, 10.60%, and 11.98%, respectively, compared with the control. No remarkable variations were evident for the GT indicators in the amaranth seeds treated with SA relative to the control treatment. The pre-sowing seed treatment with the growth stimulators GA_3, H_2O_2, $CaCl_2$, and SuA reduced the average GT of the amaranth seeds under low-temperature conditions (T_4) compared with the control. Among these treatments, the growth stimulator GA_3 proved the most effective (Raj *et al*., 2021).

With optimum temperature (T_{25}), the germination index (GI) of the amaranth seeds gained enhancements of 20.06%, 19.62%, and 18.71% with the application of growth stimulators SuA, GA_3, and $CaCl_2$, respectively, versus the control (Table 1). At T_{25}, the water treatment outperformed SA treatment, although the H_2O_2 treatment also exhibited a slight increasing trend. Under the T_4 condition, the use of growth stimulators SuA, $CaCl_2$, GA_3, and H_2O_2 improved the GI of the amaranth seeds by 15.14%, 10.91%, 9.80%, and 5.24%, respectively. However, the other stimulant decreased the GI of the amaranth seeds. Under normal conditions, the growth stimulators GA_3, SuA, H_2O_2, and $CaCl_2$ enhanced the viability index (VI) of the amaranth seeds by 87.23%, 64.05%, 46.97%, and 29.05%, respectively. Under low-temperature conditions, the viability index (VI) rose by 59.82%, 45.80%, 22.05%, and 35.19% by using the same growth stimulants. However, other growth stimulators decreased the seed VI (Table 1). By using the growth stimulators SuA, GA_3, $CaCl_2$, and H_2O_2 with optimum temperature, the seed vigor index (SVI) of the amaranth seeds escalated in relation to the control by 44.69%, 43.47%, 40.61%, and 10.00%, respectively. Under low-temperature conditions, the growth stimulators SuA and GA_3 increased the SVI by 25.79% and 19.29%, respectively, compared with the control. Past studies enunciated that a higher germination index (GI) indicates better seed viability in crop plants (Yang *et al.*, 2021).

Previous studies reported the growth stimulant GA_3 increased the rate of seed germination in caper bush (*Capparis spinosa*) (Pascual *et al.*, 2009) and horse gram (*Macrotyloma uniflorum*) (Lalitha *et al.*, 2016). Grabovskaya *et al.'s* (2020) studies highlighted the role of SuA as a biostimulant in seed germination. According to some studies, pre-sowing seed treatment with growth stimulators H_2O_2, SA, and $CaCl_2$ may effectively improve the rice seedlings' survival under low-temperature stress conditions (Tahjib-ul-Arif, 2023). Their findings were greatly analogous to the presented results, that the exposure to low temperatures had a significant negative effect on the germination potential, rate, and index and overall increased the average germination time. However, the pre-sowing seed treatment

Table 1. Effects of growth stimulants on amaranth seed germination traits under optimal and low-temperature conditions.

Growth stimulants	Temperature treatments	GP (%)	GR (%)	GT (days)	GI	VI	SVI
Control	T_{25}	93±0.050^{c}	94±0.050^{c}	2.17±0.025^{b}	45.42±0.050^{cd}	94.02±0.005^{h}	4.90±0.030^{c}
	T_4	94±0.050^{bc}	95±0.050^{bc}	2.14±0.060^{b}	46.75±0.050^{c}	79.01±0.050^{j}	4.77±0.050^{c}
GA_3	T_{25}	99±0.000^{a}	99±0.000^{ab}	1.91±0.035^{c}	54.33±0.050^{a}	176.03±0.005^{a}	7.03±0.040^{a}
	T_4	98±0.050^{ab}	98±0.050^{ab}	1.96±0.080^{c}	51.33±0.050^{b}	126.27±0.030^{d}	5.69±0.020^{bc}
SA	T_{25}	96±0.050^{b}	98±0.050^{ab}	2.40±0.030^{a}	43.00±0.050^{d}	86.00±0.005^{i}	3.31±0.040^{d}
	T_4	96±0.050^{b}	97±0.050^{b}	2.27±0.000^{ab}	44.25±0.050^{cd}	66.38±0.040^{k}	3.37±0.040^{d}
SuA	T_{25}	99±0.050^{a}	100±0.050^{a}	1.94±0.035^{c}	54.53±0.050^{a}	154.24±0.005^{b}	7.09±0.035^{a}
	T_4	97±0.050^{ab}	97±0.050^{b}	1.90±0.070^{c}	53.83±0.050^{ab}	115.20±0.030^{f}	6.00±0.090^{b}
H_2O_2	T_{25}	96±0.050^{b}	96±0.050^{bc}	2.02±0.040^{bc}	49.00±0.050^{bc}	138.18±0.010^{c}	5.39±0.050^{bc}
	T_4	93±0.050^{c}	94±0.050^{c}	2.00±0.060^{bc}	49.20±0.050^{bc}	96.43±0.030^{h}	3.98±0.030^{d}
$CaCl_2$	T_{25}	95±0.050^{bc}	96±0.050^{bc}	1.91±0.010^{c}	53.92±0.050^{ab}	121.33±0.030^{e}	6.89±0.010^{ab}
	T_4	93±0.050^{c}	98±0.050^{ab}	2.06±0.020^{bc}	51.85±0.050^{b}	106.81±0.010^{g}	4.68±0.050^{c}

Note: The averages of the germination potential (GP), the germination rate (GR), the germination time (GT), the germination index (GI), the viability index (VI), and the seed vigor index (SVI) appear above. The treatments that do not have the same letters are significantly ($p < 0.05$) different, as determined by Duncan's multiple range test. Each point represents the mean of three replicates. The values presented are the mean ± standard deviation (SD). Significant differences among the treatments, as determined, used a three-way analysis of variance.

with growth stimulants H_2O_2, SA, and $CaCl_2$ enhanced these indicators in sorghum (Xing *et al.*, 2023). Therefore, the effect of pre-sowing seed treatment on germination in amaranth seeds was the same with the mechanism as described above; however, further experimental exploration is necessary.

Germination and morphological traits

Under normal temperature conditions (T_{25}), the *A. hypochondriacus* seed treatment with growth stimulants SuA, GA_3, $CaCl_2$, and H_2O_2 showed increased fresh weight of seedlings (FWS) by 20.59%, 20.04%, 18.55%, and 2.04%, respectively, compared with the control group (Figure 1). However, the seeds incubated at low temperature (T_4) for eight hours per day showed growth stimulators GA_3 and SuA raised the FWS of amaranth seedlings by 8.63% and 9.31%, respectively. Compared with the control group, the amaranth seed treatment with growth stimulants SuA, GA_3, and $CaCl_2$ significantly boosted the dry weight seedling (DWS) under normal and low-temperature conditions. These results are consistent with our previous studies (Feng *et al.,* 2022).

The growth dynamics of amaranth seedlings' hypocotyl and roots, when analyzed, recorded the hypocotyl length (HL) and root length (RL) on the third, fourth, fifth, seventh, and 11th days. The predictions about the growth trend relied on the analysis (Figure 2). The results revealed that on the third day, the amaranth seedlings obtained from the seeds treated with growth stimulants GA_3, SuA, and $CaCl_2$ showed significantly increased seedling HL (77.14%, 42.86%, and 8.57%, respectively) and RL (38.79%, 68.10%, and 3.45%, respectively) versus the control at optimum temperature (T_{25}). However, on the third day, the amaranth seed treatment with growth stimulators GA_3 and SuA increased the HL by 80.56% and 66.67%, respectively, and RL by 37.39% and 78.26%, respectively, compared with the control at T_4 conditions. Nonsignificant differences appeared between the other groups and the control treatment.

With optimum temperature, on the 11th day of germination, the amaranth seedlings grown from the seed treatment with growth stimulants GA_3, SuA, H_2O_2, and $CaCl_2$ showed a considerable increase in HL (57.14%, 38.93%, 35.00%, and 17.86%, respectively) and RL (45.35%, 79.93%, 48.70%, and

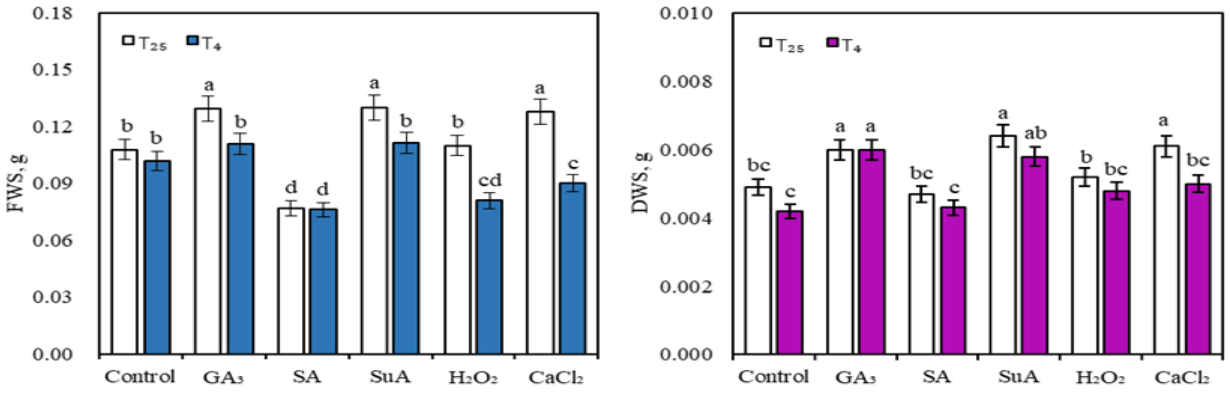

Figure 1. Effects of different growth stimulants on biomass of the amaranth seedlings under optimal and low-temperature conditions. FWS and DWS appear as average values, with each point representing the average of three replicates. The treatments that do not have the same letters are significantly ($p < 0.05$) different, as determined by the DMR test, with the values presented as mean ± standard deviation (SD).

43.12%, respectively) compared with the control treatment (Figure 2). At T_4, the traits HL and RL in amaranth seedlings significantly differed from the control, as well as amaranth seedlings grown at T_{25}. In particular, at T_4, the seed treatment with the growth stimulators GA_3, SuA, H_2O_2, and $CaCl_2$ remarkably boosted the HL (36.84%, 21.86%, 14.17%, and 21.05%, respectively) and RL (25.94%, 57.52%, 9.02%, and 24.06%, respectively) of the amaranth seedlings versus the control treatment. Furthermore, it was evident that under normal and low-temperature conditions, the HL and RL of the amaranth seedlings in seed treatment with SA were lower than those in the control group. Therefore, one can conclude that low temperature has a considerable negative effect on the growth and development of amaranth seedlings. This result received confirmation in the work of Oleti *et al*. (2024).

The amaranth seedlings grown from the seeds treated with growth stimulants GA_3 and SuA showed a significantly faster enhancement in the morphological traits HL and RL. This was in comparison with those treated with other growth stimulants, including the control, with optimal and low temperatures. On the 11th day of germination, the amaranth seedlings obtained from the seeds treated with growth stimulant SuA exhibited the longest roots, while the GA_3-treated group revealed the lengthiest hypocotyls at T_{25} and T_4. Therefore, the study suggested that growth stimulant SuA, known to promote plant growth and development, could improve the root structure, as well as the vegetative growth and physiology of entire amaranth plants. In a pot experiment, consistent with Liu and Yang (2010), this study found SuA treatment significantly promoted amaranth root growth (Figure 2), which could relate to improved root physiological traits in maize. Additionally, the SuA treatment notably inhibited the lipid peroxidation in the root sheath, increased SOD activity in the roots, and reduced MDA content. Past research demonstrated that *Passiflora spp.* exhibited larger and faster seedlings, as well as greater initial plant growth when treated with GA_3 solution (Domingues-Neto *et al*., 2024). Similarly, the sesame seeds showed an improved growth after soaking in GA_3 solution (Santos *et al.*, 2016; Hadif, 2019).

It is worth noting that treating amaranth seeds with the growth stimulator $CaCl_2$ also enhanced the HL of amaranth seedlings by 17.86% versus the control at T_{25} and by 21.05% at T_4. These results suggested the growth stimulant $CaCl_2$ (3000 mg/l) can improve the cold resistance of amaranth plant seedlings. Previous studies have also reported

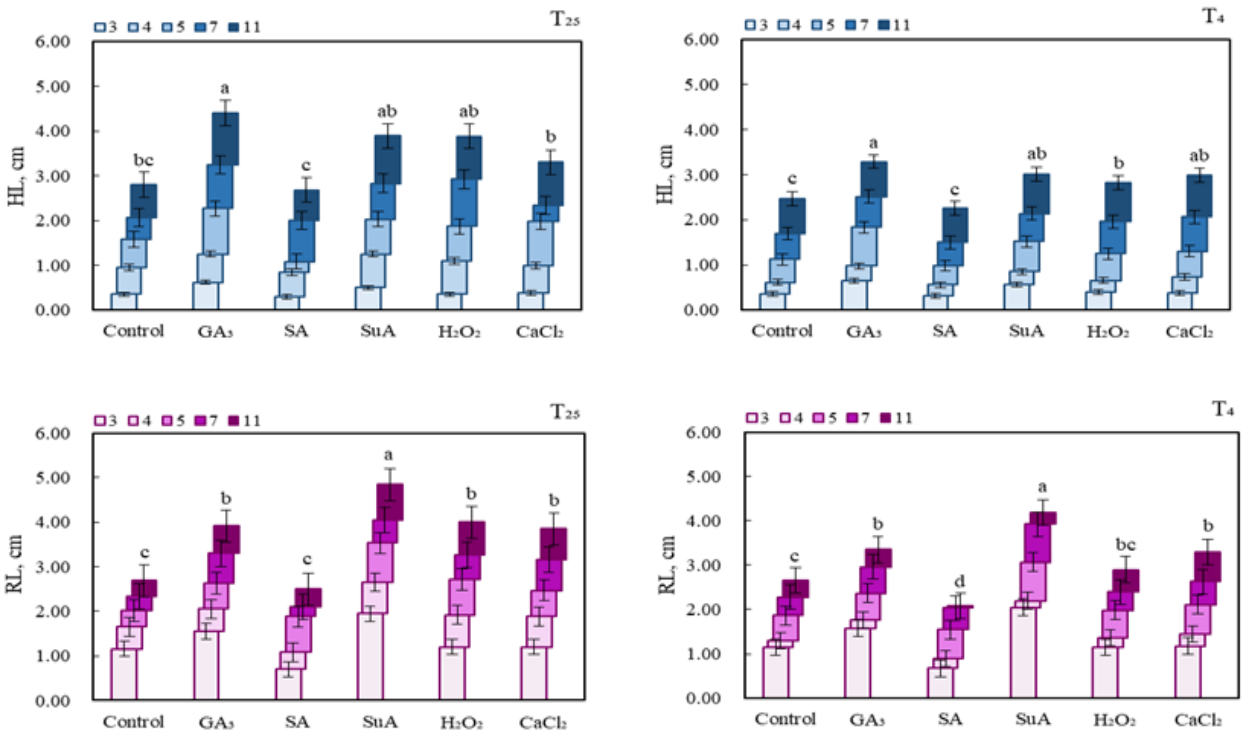

Figure 2. Dynamic effects of various growth stimulants on plant biomass of amaranth seedlings under optimal and low-temperature conditions (8 hours at 4 °C) on the 3rd, 4th, 5th, 7th, and 11th day of germination. The average values of HL and RL appear above. The treatments that do not have the same letters are significantly ($p < 0.05$) different, as determined by the DMR test. Each point represents the average of three replicates, with the values presented as mean ± standard deviation (SD).

rice seedlings tolerance to low temperature after treating the seeds with exogenous stimulants (SA, H_2O_2, and $CaCl_2$) (Tahjib-ul-Arif, 2023). Pre-sowing seed treatment of rice seeds with H_2O_2 (10 mM), SA (2 mM), and $CaCl_2$ (10 mM) emerged to be effective in reducing damage caused by low-temperature stress (Tahjib-ul-Arif, 2023).

Alcohol-soluble antioxidants in seedlings

The use of total alcohol-soluble antioxidant (TASA) to compare the potential antioxidant properties of amaranth seedlings grown from the seeds treated with different growth stimulants and temperatures (T_{25} and T_4) appears in Figure 3. The plant's physiological state can succeed in its determination by analyzing the photosynthetic pigments in chloroplasts. On the 11th day after planting in Petri dishes, the chlorophyll a (Chl. a), chlorophyll b (Chl. b), and carotenoid (Car.) content analysis ensued.

The amaranth seedlings grown from the seeds treated with gibberellic acid (GA_3), salicylic acid (SA), and hydrogen peroxide (H_2O_2) showed increased Chl. a content by 57.58%, 127.27%, and 42.42%, respectively, with optimal temperature (T_{25}) compared with the control. Meanwhile, in amaranth seedlings treated with succinic acid (SuA) at T_{25}, a 12.12% decrease resulted in the Chl. a content versus the control. However, no significant difference occurred between the seedlings obtained from seeds treated with calcium chloride ($CaCl_2$) and the control. At the same time, the amaranth seedlings treated with growth stimulants GA_3, SA, SuA, H_2O_2, and $CaCl_2$, left overnight at T_4, the Chl. a content notably rose by 79.31%, 144.83%, 31.03%,

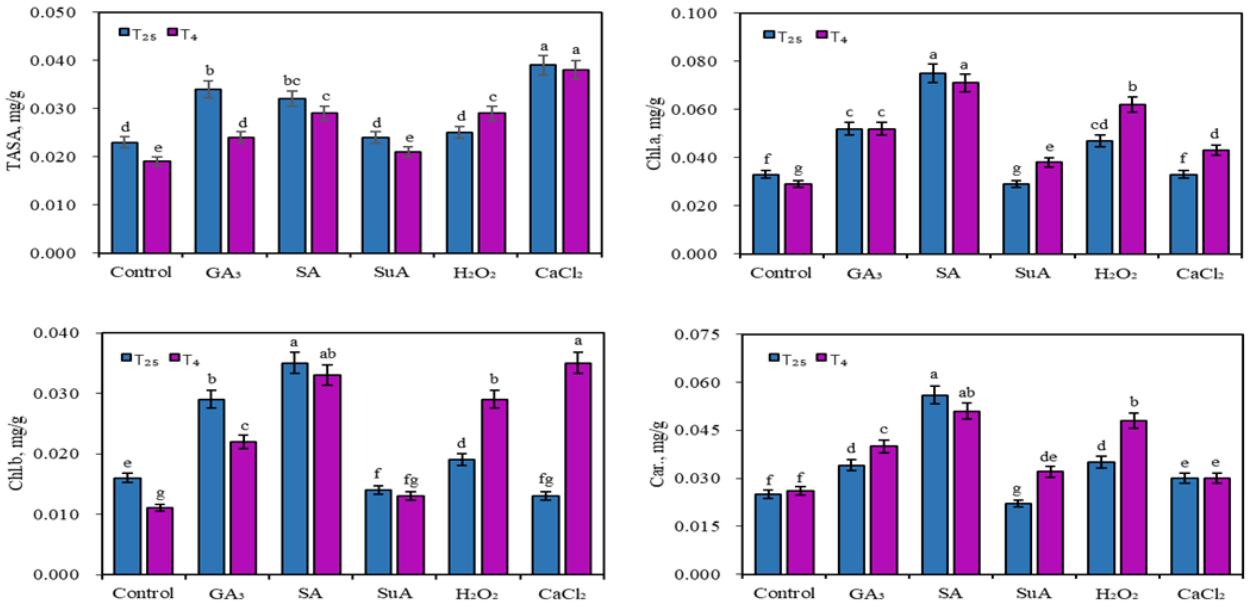

Figure 3. Influence of various growth stimulants on the biochemical compounds in the amaranth seedlings under optimal and low-temperature conditions. The average values of TASA, Chl. a, Chl. b, and Car. appear above. The treatments that do not have the same letters are significantly ($p < 0.05$) different, as determined by the DMR test. Each point represents the average of three replicates. Values presented are mean ± standard deviation (SD).

113.79%, and 48.28%, respectively, compared with the control group. Similar results emerged in experiments with beans (Tania *et al*., 2022).

In eleven-day-old amaranth seedlings, the Chl. b content was lower than that of Chl. a. However, the effect of each growth stimulant on the Chl. a and Chl. b contents were at par. In the amaranth seedlings treated with GA_3, SA, and H_2O_2 at T_{25}, the Chl. b content rose by 81.25%, 118.75%, and 18.75%, respectively, compared with the control. However, in the amaranth seedlings treated with SuA and $CaCl_2$ at T_{25}, the Chl. b content decreased by 12.50% and 18.75%, respectively, versus the control. Furthermore, in amaranth seedlings treated with GA_3, SA, SuA, H_2O_2, and $CaCl_2$ at T_4, the Chl. b content substantially enhanced by 100.00%, 200.00%, 18.18%, 163.63%, and 218.18%, respectively, compared with the control treatment. In corn seedlings under salt-stress conditions, when treated with gibberellic acid, an increase in chlorophyll content in seedlings also surfaced (Shahzad *et al*., 2021).

With optimal and low temperatures, the growth stimulants GA_3, SA, and H_2O_2 significantly increased the carotenoid (Car.) content in amaranth seedlings. The Car. content increased by 36.00%, 124.00%, and 40.00% for growth stimulators GA_3, SA, and H_2O_2, respectively, with optimum temperature (T_{25}), while at low temperature (T_4), the Car. content rose by 53.85%, 91.15%, and 84.62% for the same stimulants. The $CaCl_2$ treatment also increased the Car. content, however, to a lesser extent (20.00% at optimal temperature and 15.38% at low temperature). Meanwhile, in the amaranth seedlings, the Car. content reduced by 12.00% with SuA treatment at T_{25} but increased by 23.08% at T_4. The results further demonstrated the seed treatment with $CaCl_2$ increased the TASA content in the amaranth seedlings by two times versus the control at the optimal and low temperatures (Figure 3). The positive effect of calcium chloride treatment emerged on wheat seedlings under salt-stress conditions (Zhang *et al*., 2024).

Thus, the pre-sowing seed treatments with growth stimulators GA_3, SA, H_2O_2 and $CaCl_2$ increased the TASA and Chl. a, Chl. b and Car. contents in amaranth seedlings with optimal and low temperatures. The results demonstrated a low dose of SA (0.5 mM) can reduce cold damage to plants by improving the damage of cell plasma membrane caused partially by low temperature. The same was successful by increasing growth factors, which inhibit the leakage of K and soluble sugar into the culture medium. A past study also revealed the role of SA in inducing cold tolerance by increasing the antioxidant capacity of plants under chilling stress conditions (Pourakbar and Siavash, 2022). Furthermore, exposure to cold temperatures resulted in an increase in Car. metabolites in the amaranth seedlings treated with GA_3 and H_2O_2. Additionally, the Chl. a and Chl. b contents in the amaranth seedlings treated with H_2O_2 and $CaCl_2$ were significantly higher than at normal temperature. Therefore, a hypothesis signifies that treatments with GA_3, H_2O_2, and $CaCl_2$ could enhance plants' cold tolerance by augmenting the antioxidant content in amaranth seedlings under chilling stress conditions. Past studies revealed the exogenous hormone GA_3 and the chilling stress treatment improved plant growth and development, particularly bud germination, growth, and flowering in *Paeonia suffruticosa* (Mornya and Cheng, 2018).

Li (2014) conducted a study on the effect of $CaCl_2$ on the physiological ecology of *Nicotiana tabacum* L. seedlings under low-temperature stress, which showed a significant increase in chlorophyll content in tobacco seedlings. By treating banana seedlings with H_2O_2 and $CaCl_2$ under chilling stress conditions, they showed reduced leaf cytoplasmic leakage, increased soluble sugar content, and a slowing down of chlorophyll degradation, thereby reducing the severity of cold damage (Zhang *et al.*, 2024). The presented results revealed that seed treatment with H_2O_2 and $CaCl_2$ under low-temperature stress caused a higher content of photosynthetic pigments in the amaranth seedlings under normal temperature. Subsequent research can further explore the effects of chilling stress and H_2O_2 and $CaCl_2$ on the amaranth seedlings' growth, including possible synergies during development.

The pre-sowing seed treatments used in the amaranth seedling improve morphological indicators of pickle seedlings and stimulate seedlings to increase the number of secondary metabolites under normal and low-temperature conditions, aside from promoting antioxidant capacity and enhancing their defense capabilities. It has been evident that the plant's secondary metabolites, including carotenoids, play a protective role against environmental stress conditions (Ahmad *et al*., 2018; Albureikan, 2023; Saqlain *et al*., 2023). Therefore, additional research is necessary to clarify the mechanism by which pre-plant seed treatments enhance the germination rate, the biomass, and the secondary metabolite contents of amaranth.

CONCLUSIONS

The growth stimulators gibberellic acid (GA_3), succinic acid (SuA), salicylic acid (SA), hydrogen peroxide (H_2O_2), and calcium chloride ($CaCl_2$) revealed varied positive effects on the seed germination and growth and development of the amaranth seedlings. Under normal and cold-temperature conditions, the growth stimulant GA_3 significantly increased the amaranth seedlings' hypocotyl length, while SuA had the greatest effect on the root length. Under cold temperature, $CaCl_2$ increased amaranth seedling biomass and reduced the low-temperature stress compared with normal temperature. The results provided an important input for amaranth cultivation strategies in Russian climatic conditions.

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