



SEED PRIMING EFFECTS ON MORPHOLOGICAL TRAITS OF *AMARANTHUS HYPOCHONDRIACUS* UNDER OPTIMAL AND LOW TEMPERATURES

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SUMMARY

The recent research evaluated the effects of growth stimulant seed treatment on the morphological traits of *Amaranthus hypochondriacus* L. cv. 'Krepysh' grown under optimal and low positive temperature conditions. The seeds were continuously soaked for 4 h in five different solutions of growth stimulants, i.e., salicylic acid (SAA - 138 mg/L), hydrogen peroxide (H₂O₂ - 10 and 50 mmol/L), calcium chloride (CaCl₂ - 3000 mg/L), succinic acid (SUA - 500 mg/L), and control (distilled water). The stimulant-primed seeds were germinated at optimal temperature (23°C) and continued to germinate at low temperatures of 10°C (T₁₀) and 23°C (T₂₃). The results showed that seed germination rates viz., germination potential (GP), germination rate (GR), germination index (GI), viability index (VI), and seed vigor index (SVI), were significantly improved with seed quality potential compared with the control. Under low positive temperature, seeds treated with succinic acid, H₂O₂, and CaCl₂ had the most significant effects on improving seed quality and induced cold resistance in the seeds. The morphological indicators of amaranth seedlings, i.e., biomass, hypocotyl, and root length, were also significantly improved with seed treatment by growth stimulants. Priming of amaranth seeds with hydrogen peroxide and succinic acid showed a greater increase in seedlings' biomass at room (23°C) and low (10°C) temperatures. The seed treatment with SUA and SAA significantly contributed to enhancing the hypocotyl length. The amaranth roots achieved maximum length after seed treatment with SUA and CaCl₂. In general, the seed treatment effects on seedling's biomass under chilling stress were associated with the potential of inducing cold tolerance in seedlings.

Keywords: *Amaranthus hypochondriacus* L., seed soaking, germination, chilling stress, salicylic acid, hydrogen peroxide, calcium chloride, succinic acid

Key findings: With low positive temperature, the amaranth seed treatment with SUA, H₂O₂, and CaCl₂ had the most significant contribution to improve seed quality by inducing cold tolerance.

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INTRODUCTION

Extreme temperature is one of the major abiotic stresses affecting the morphology, physiology, biochemistry, and gene expression of crops (Vaseem *et al.*, 2020; Motyleva *et al.*, 2021; Tetyannikov *et al.*, 2022). In particular, the low temperature limits the timing of seed sowing and the growth of heat-loving crops, which eventually affect the genetic potential of crop plants. Areas mostly affected by return cold at the early germination stage are expanding worldwide (Muhammad *et al.*, 2021). The seed germination process has an essential economic and environmental significance since it is the initial life stage of the seed's perception of the external environment. Seed germination is a complex physiological process that begins with the absorption of water by a dry seed and ends with the interruption of the root through the seed coat (Nonogaki *et al.*, 2010; Vaseem *et al.*, 2020). The external environment and stressful effects greatly influenced germination, including low positive temperature (Muhammad *et al.*, 2021).

The most important indicators characterizing the quality of seeds are germination and germination energy, which make it possible to quickly and reproducibly differentiate any batch of seeds. In amaranth, seed germination affects the growth and final productivity of developing seedlings and plantlets. Also, to determine the quality of seed material, the concept of seed germination responsiveness is often used, which is linked with the accelerated germination and development of seedlings. In English literature, the concept of "friendliness" corresponds to the term homogeneity (uniformity), which indicates the alignment of seed material in the parameters characterizing their ability to germinate. Germination friendliness can be characterized by the term germination rate (Vaseem *et al.*, 2020; Motyleva *et al.*, 2021).

Amaranth (*Amaranthus hypochondriacus* L.) is a heat-loving pseudo-cereal plant, widely cultivated in tropical and subtropical regions of the world (Hu *et al.*, 2021; Kandel *et al.*, 2021). Amaranth is an annual crop usually sown in early spring in warm countries. Low positive temperature narrows the distribution area of this high-protein gluten-free food crop (Novák *et al.*, 2021; Tetyannikov *et al.*, 2022). However, low soil and air temperatures are some of the main factors negatively affecting the seed germination and development of seedlings in

amaranth. Low temperatures also delay early sowing and the emergence of seedlings (Ashraf and Foolad, 2005). In addition, chilling stress has a complex effect on the photosynthesis of seedlings by reducing the rate of photosynthesis, thus, inhibiting their growth and development (Zhou *et al.*, 2009). Indirectly, it also leads to a decline in the optimal ripening period, and, consequently, a decrease in the yield of crop plants (Huang *et al.*, 2013). Chilling stress significantly enhances the levels of reactive oxygen species (ROS) and lipid peroxidation with reduced oxygen (O₂) utilization (Mara *et al.*, 2016). To neutralize the excess of ROS that damages cell elements, the plant uses the enzyme and low molecular weight antioxidant (AO) system. Phenolic compounds also play a vital role in protecting the components in the plant body (Sami *et al.*, 2019).

The study aimed to evaluate the role of growth stimulants viz., salicylic acid, hydrogen peroxide (H₂O₂), calcium chloride (CaCl₂), and succinic acid in accelerating seed germination and improving the morphological, physiological, and biochemical characteristics of amaranth seedlings at low positive temperature. Seed treatment with growth stimulants significantly reduces the negative effects of cold by regulating the antioxidant potential and mechanism through increase amaranth synthesis of ascorbic acid and the total content of water- and alcohol-soluble compounds (Platonova *et al.*, 2018). Numerous studies have shown that, as a result of seed treatment with growth stimulants, the values of the seed germination parameters, i.e., germination index, viability index, seed vigor index, as well as, the shoot length of the hypocotyl, root length, seedling fresh weight, and seedling dry weight were enhanced in various crop plants (Gaba *et al.*, 2018; Cao *et al.*, 2020; Mewar *et al.*, 2020; Ahmad *et al.*, 2020; Vdovenko *et al.*, 2021).

The study devoted to the comparative analysis and evaluation of the quality of amaranth seeds treated with growth stimulants under optimal and low positive temperatures, contributing to 'friendly' shoots production and enhancing chilling stress tolerance. The study comprised of the seed germination and morpho-physiological characteristics of amaranth seedlings to understand the mechanisms of inducing resistance to abiotic chilling stress. It also aimed to identify the optimal growth stimulants and their concentrations used for pre-sowing seed treatment and to determine their impact on

germination, morpho-physiological indicators of amaranth seedlings, and induction of resistance to low positive temperature.

MATERIALS AND METHODS

Plant material

The research used the seedlings of amaranth (*Amaranthus hypochondriacus* L. cv. 'Krepysh'), where the said cultivar was registered in 2004 by the State Register of Breeding Achievements, Russia. The experiment was set up in the Laboratory of Physiology, Biochemistry, Introduction and Functional Products (Federal Scientific Center for Vegetable Growing, Russian Federation) in 2021.

Seed treatment

For amaranth (*A. hypochondriacus* L. cv. 'Krepysh') seed treatment, the following five solutions of growth stimulants were used—salicylic acid (SAA - 138 mg/L), hydrogen peroxide (H₂O₂ - 10 and 50 mmol/L), calcium chloride (CaCl₂ - 3000 mg/L), succinic acid (SUA - 500 mg/L), and control (distilled water). The amaranth seeds were separately soaked in distilled water (control) and the solutions of growth stimulants for 4 h, and then, dried. Afterward, 50 seeds were spread evenly into two layers of moistened filter paper (90 mm in diameter) in each 90 mm diameter petri dish for germination. Seeds in petri dishes were kept at a temperature of 23°C until the emergence of seedlings on the third day. In the following days, some of the petri dishes with the seeds was placed overnight in a cold chamber for 16 h in the dark (for three nights) with a temperature of 10°C and the remaining seed samples were incubated at 23°C without lighting also. The number of sprouted seeds got counted daily.

Data recorded on germination parameters

For seven days, with an interval of 24 h, the data got recorded on germination potential (GP), germination rate (GR), mean germination time (GT), germination index (GI), viability index (VI), seed vigor index (SVI), shoot length of the hypocotyl (SL), root length (RL), seedling fresh weight (SFW), and seedling dry weight (SDW).

Calculating the germination potential (GP) included the percentage of germinated seeds from the total number of seeds based on

the maximum number of germinated seeds per day (Yao *et al.*, 2021).

$$GP (\%) = n_{3d}/n_t \times 100$$

where:

n_{3d} = the number of germinated seeds on the third day after sowing, and
 n_t = the total number of seeds.

Germination rate (GR) is an estimate of the viability of a seed population, which is calculated by dividing the number of germinated seeds in seven days after sowing by the total number of seeds (Yao *et al.*, 2021).

$$GR (\%) = n_{7d}/n_t \times 100$$

where:

n_{7d} = the number of germinated seeds in seven days from sowing, and
 n_t = the total number of seeds.

Mean germination time (GT) is calculated to estimate the germination rate (Yao *et al.*, 2021).

$$GT = \sum([ni \times di]/n)$$

where:

ni = the number of seeds germinated per day i ,
 di = the germination time in days, and
 n = the total number of germinated seeds.
Germination index (GI), as statistical data, indicates the rate of average germination of seeds (Li *et al.*, 2016).

$$GI = \sum(Gt/Dt)$$

where:

Gt = the number of germinated seeds, and
Dt = the corresponding germination time.

Seed energy is the sum of the properties of seeds that determine the potential level of activity and productivity during germination and the appearance of seedlings. Seed vigor index (SVI) and viability index (VI) were used to estimate seed energy (Li *et al.*, 2016; Perry, 1978; Yao *et al.*, 2021).

$$SVI = S \times GI$$

where:

S = the average SFW for the germination period,
GI = the germination index, and

$$VI = GI \times SL$$

where:

SL = the average length of the hypocotyl during the germination period.

Analysis of the seedling's morphological indicators

Calculating the germination parameters (VI and SVI), 10 seedlings were taken from each sample, wherein the hypocotyl with the root was cut with scissors. The hypocotyl length (SL) and the root length (RL) were measured, averaged, and expressed in cm. The raw mass weight of the sprout (SFW) and its dry mass (SDW) was measured on an electric scale, expressing the average value in grams.

RESULTS

Seed treatment effects on amaranth germination traits

The amaranth seed priming with growth stimulants (SAA, SUA, H₂O₂, and CaCl₂) revealed their varied effects on germination characteristics at the temperatures of 23°C (T₂₃) and 10°C (T₁₀). The seeds began to germinate on the second day of incubation at 23°C. Compared with the control (distilled water) at T₂₃, the seeds treated with growth stimulants H₂O₂ (10 mmol/L) and CaCl₂ showed increased germination potential (GP) and germination rate (GR) by 4.2% and 2.1%, respectively. The GR refers to the germination rate and the homogeneity of sprouted seeds indicating the overall strength of the seeds. For these indicators, no difference was found between the control and SUA-treated seed groups, whereas seed treatment with H₂O₂ (50 mmol/L) and SAA reduced the GP and GR of the seeds by 4.2% compared with the control (Figure 1). Under low positive temperature conditions, seed treatment with H₂O₂ (10 mmol/L) enhanced the GP and GR of amaranth seeds by 2.1%. The seed treatment with CaCl₂ and H₂O₂ (50 mmol/L) reduced GP by 4.2%, and 2.1% in amaranth seeds. However, the amaranth seeds treated with growth stimulants SUA and SAA did not differ from the control group.

Compared with the control, under optimal conditions (T₂₃), the growth stimulants H₂O₂ (50 mmol/L), CaCl₂, and SUA shortened the mean germination time (GT) of amaranth

seeds by 9.1%, 15.6%, and 20.5%, respectively. Notably, there was no change in GT scores for seeds treated with SAA and H₂O₂ (10 mmol/L) compared with the control. Under low temperature (T₁₀) conditions, pre-sowing seed treatment with H₂O₂ (50 mmol/L), SAA, CaCl₂, and SUA compared with the control, also reduced the amaranth (*A. hypochondriacus* L. cv. 'Krepysh') seed's average GT, among which the seed treatment with SUA was more efficient. Except for H₂O₂ (10 mmol/L), other growth stimulants reduced the average GT of amaranth seeds at T₁₀ (Figure 1).

The germination index (GI) is an estimate of the time in days that are required to achieve a certain percentage of germination (Yao *et al.*, 2021). Compared with the control, the growth stimulants CaCl₂ and SUA improved the GI of amaranth seeds by 21.3% and 27.9%, respectively under normal temperature. The H₂O₂ (50 mmol/L) treatment also showed a slight upward trend, whereas seed treatment with SAA and H₂O₂ (10 mmol/L) showed a decline (Figure 1). Significantly under low temperature, the H₂O₂ (50 mmol/L), CaCl₂, and SUA improved the GI of amaranth seeds by 11.3%, 14.5%, and 26.6%, respectively. However, other treatments reduced GI in amaranth seeds compared with the control.

The viability index (VI) is a combination of the seeds germination rate and the growth of seedlings and is a good indicator of their viability (Finch-Savage and Bassel, 2016). Growth stimulants CaCl₂ and SUA increased the viability index of amaranth seeds by 8.8% and 10.2%, and 14.5% and 18.2%, respectively, under normal and low-temperature conditions. However, all other treatments reduced the seed viability index (Figure 1). Seed vigor index (SVI) is the sum of the properties of a seed that determine its potential level of activity and performance during germination (Agami and Mohamed, 2013). At optimum temperature (T₂₃), the SVI of amaranth seeds, compared with the control, increased using the growth stimulants, i.e., H₂O₂ (50 mmol/L), CaCl₂, and SUA, by 12.8%, 14.9%, and 23.8%, respectively. Under low temperature (T₁₀) conditions, compared with the control, the growth stimulators H₂O₂ (50 mmol/L), CaCl₂, and SUA enhanced amaranth seeds SVI by 13.5%, 17.1%, and 14.1%, respectively. Remarkably, SAA seed treatment reduced the SVI of amaranth seeds by 25.1% compared with the control.

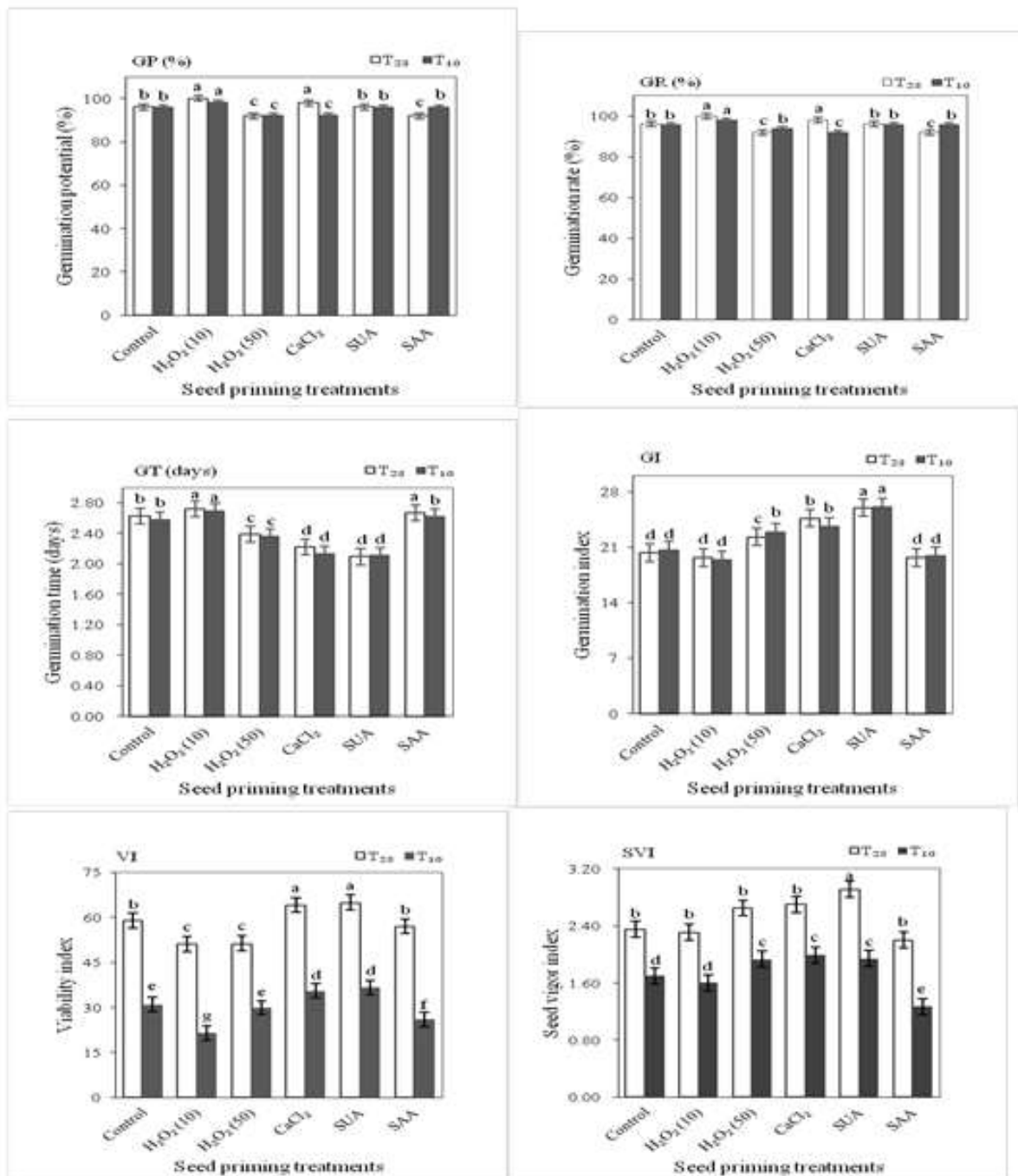


Figure 1. Effects of different seed priming treatments on germination characteristics under normal and low-temperature conditions. Averages of germination potential (GP), germination rate (GR), germination time (GT), germination index (GI), viability index (VI), and seed vigor index (SVI) are given. Treatments that do not have the same letters are significantly ($P < 0.05$) different as determined by Duncan's multiple range tests. Each point represents the mean of three replicates. The values presented are the mean \pm standard deviation (SD). Significant differences among treatments were determined using a three-way analysis of variance.

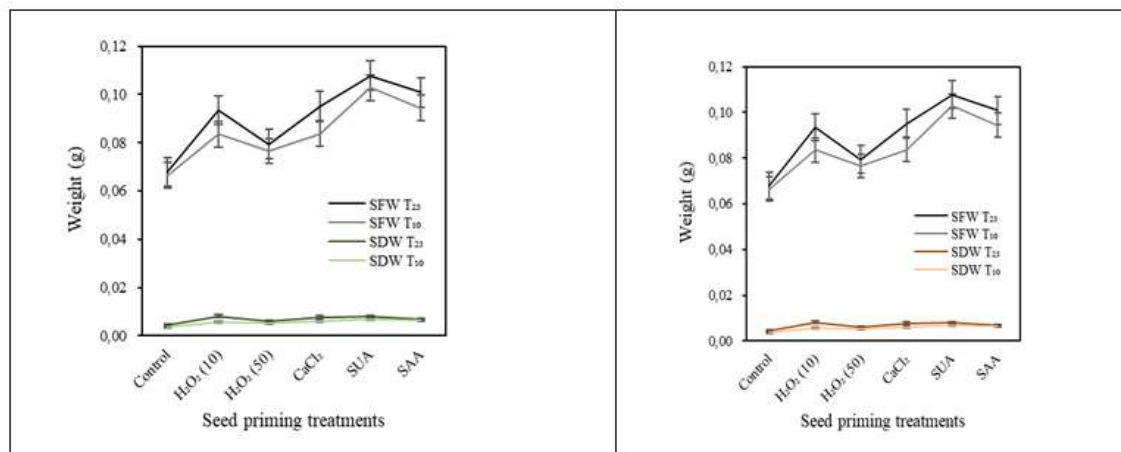


Figure 2. Effects of different seed priming treatments on plant biomass under normal and low-temperature conditions. Averages of stem fresh weight (SFW), and stem dry weight (SDW) are given. Each point represents the mean of three replicates. The values presented are the mean \pm standard deviation (SD). Significant differences among treatments were determined using a three-way analysis of variance ($P < 0.05$).

Seed treatment effects on amaranth seedlings biomass

Under normal temperature (T_{23}), the amaranth seed treatment with H_2O_2 (50 mmol/L), $CaCl_2$, SAA, SUA, and H_2O_2 (10 mmol/L) increased seedling fresh weight (SFW) by 17.3%, 40.4%, 48.7%, 59.0%, and 82.89%, respectively, compared with the control (Figure 2). Seeds incubated at low temperatures with H_2O_2 (50 and 10 mmol/L), $CaCl_2$, SAA, and SUA also increased the SFW of amaranth seedlings by 15.0%, 25.6%, 25.7%, 42.0%, and 54.6%, respectively, but to a lesser extent. In comparison with the control, the seed treatment with growth stimulants H_2O_2 (10 and 50 mmol/L), $CaCl_2$, SAA, and SUA significantly enhanced the seedling dry weight (SDW) of amaranth under normal and low temperatures. The growth stimulant SUA showed the greatest effect, with a 77.8% and 78.9% increase in SDW compared with the control, under T_{23} and T_{10} temperatures.

Seed treatment effects on amaranth morphological traits

Registering the shoot length of the hypocotyl (SL) and root length (RL) on the third, sixth, and seventh day, the growth dynamics of the hypocotyl and root of amaranth seedlings were analyzed to determine the growth trend (Figure 3). By comparing to the third day, the length of hypocotyl and root with above growth stimulants increased significantly under normal

temperature conditions. Compared with the control under these conditions, seedlings obtained from the amaranth seeds treated with H_2O_2 (10 and 50 mmol/L), $CaCl_2$, SUA, and SAA significantly increased the hypocotyl length by 16.7%, 22.2%, 27.8% 33.3%, and 50.0%; and root length by 4.2%, 8.3%, 20.8%, 37.5%, and 12.5%, respectively. Similarly, the hypocotyl and root lengths in each treatment group, including the control, were significantly enhanced under low temperature (T_{10}) conditions.

Under normal temperature conditions, on the seventh day, seedlings grown from amaranth seeds treated with H_2O_2 (10 and 50 mmol/L), $CaCl_2$, SUA, SAA, and control (H_2O), compared to the sixth day, showed an increase in hypocotyl length by 23.8%, 29.7%, 20.8%, 29.1%, 26.0%, and 19.4%, and root length by 24.0%, 23.1%, 17.2%, 36.4%, 27.8%, and 12.5%, respectively. Likewise, under low temperature (T_{10}) conditions on the seventh day compared to the sixth day, the hypocotyl and root length significantly differed for the same two traits grown at optimal temperature (T_{23}) (Figure 3). In particular, at low temperature, the seed treatment with H_2O_2 (10 and 50 mmol/L), $CaCl_2$, SUA, and SAA including control significantly increased SL in amaranth seedlings by 22.2%, 30.0%, 16.7%, 25.0%, 15.4%, and 0.5%; and RL by 23.1%, 15.4%, 13.3%, 33.3%, 24.1%, and 0.0%, respectively compared to the sixth day. Thus, it is concluded that low temperature negatively affects the growth of amaranth seedlings.

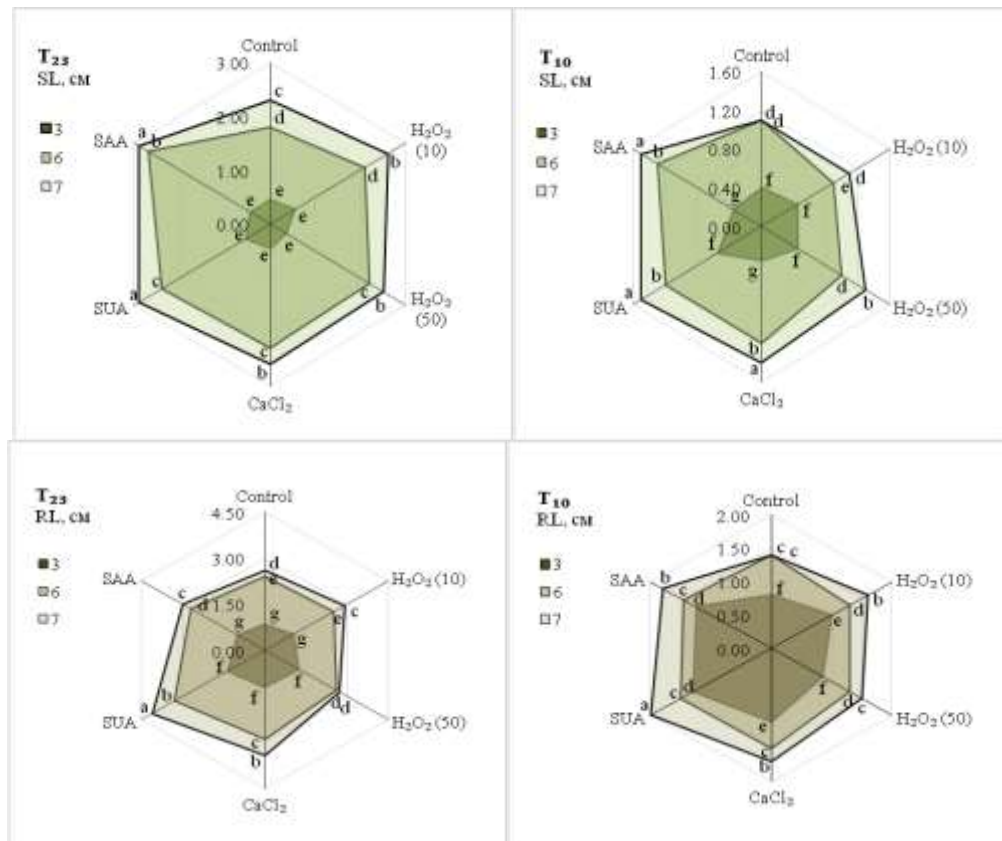


Figure 3. Effects of different seed priming treatments on plant biomass under normal and low-temperature conditions. Averages of root length (RL), and stem length (SL) on the third, sixth, and seventh day of germination are given. Treatments that do not have the same letters are significantly ($P < 0.05$) different as determined by Duncan's multiple range tests. Each point represents the mean of three replicates. The values presented are the mean \pm standard deviation (SD). Significant differences among treatments were determined using a three-way analysis of variance.

Amaranth seedlings derived from seeds treated with growth stimulants SAA and SUA increased SL significantly faster, compared with the seedlings obtained from the seeds treated with other growth stimulants including the control, at optimal and low positive temperatures. Markedly, under low-temperature conditions, on the seventh day compared to the sixth day, the amaranth seeds treated with H₂O₂ (50 mmol/L) ensured the maximum hypocotyl length, and the seeds treatment with SUA ensured the highest root length, whereas no change occurred in the control group.

DISCUSSION

The study proposes growth stimulants (SUA, SAA, CaCl₂, and H₂O₂) as a means of improving seed quality and viability of amaranth

(*Amaranthus hypochondriacus* L. cv. 'Krepysh') by strengthening resistance to low positive temperature. The study presents data on the possibility of using growth stimulants for enhancing the sowing characteristics of seeds and the protective mechanism by increasing the resistance of amaranth seedlings to low-temperature stress.

Pre-sowing treatment of seeds with various growth stimulants is considered one of the ways to increase the resistance of plants to stressful conditions due to low and high temperatures (He *et al.*, 2018; Wang *et al.*, 2018). Among them, calcium chloride under optimal temperature conditions increased the germination potential and rate, viability indices, and seed energy of amaranth seeds, while reducing the germination time. However, chilling stress due to low positive temperature, significantly reduced the germination potential of amaranth seeds (Figure 1).

Chilling stress delays the seed germination and development of seedlings in heat-loving plants, causes cell damage, and negatively affects the activity of metabolic reactions and photosynthesis (Delachiave and De, 2003; Yadav, 2010; Akram *et al.*, 2018; Wang, 2020). Past studies also reported that low temperatures significantly reduced the GP, GR, and SVI of rapeseed and expanded GT in different cultures (Li *et al.*, 2016; Ritonga and Chen, 2020).

In this study, priming of amaranth seeds with growth stimulants (SUA, CaCl₂, and H₂O₂) significantly increased their viability and reduced the average germination time at normal and low positive temperatures. However, growth stimulants had unequal effects on seed germination parameters and, consequently, on the growth and development of seedlings (Figures 1, 2, and 3). According to Jisha *et al.* (2013) and Huo *et al.* (2021), the treated seeds can quickly swell, restoring metabolism, and thereby, increasing the rate of germination in crop plants. In amaranth, seed germination and early emergence of seedlings can be damaged by returning cold, which reduces the number of plants and the friendliness of germination, contributing to a decrease in potential yield. The amaranth seeds treated with CaCl₂ and H₂O₂ (50 mmol/L) exhibited a decrease in GP and GR at a low positive temperature (T₁₀), while for seeds treated with SUA and SAA, these did not differ from the control. Under normal temperature conditions, the germination potential and germination rate of seeds treated with H₂O₂ (10 mmol/L) and CaCl₂ increased compared with the control group, whereas with SAA and H₂O₂ (50 mmol/L) decreased.

The ambiguity of CaCl₂, H₂O₂ (50 mmol/L), and SAA in the seed germination might be due to their action on different metabolic sites in germinating seeds. Nevertheless, the wet mass of seedlings and their hypocotyl with cotyledonary leaves and roots increased under optimal and low temperatures due to seed treatment, but to a varying extent. Notably, as a result of seed treatment with all the growth stimulants used in this research work, the mass of dried seedlings increased significantly under optimal and low temperatures. The maximum increase in mass of amaranth seedlings was observed with seeds exposed to the stimulants H₂O₂ (50 mmol/L), SUA, and SAA. Previous research work also revealed that cold tolerance enhanced corn seedlings by treating their seeds with H₂O₂ and salicylic acid (Guan *et al.*,

2015; Geraldo *et al.*, 2017; Cengiz *et al.*, 2020).

Importantly, seed treatment with H₂O₂, depending on the concentration and size, affected the parameters of seed germination and the morphological indicators of seedlings. Under normal temperature conditions, seeds treated with H₂O₂ (10 mmol/L) increased GP and GR and reduced the average germination time. However, the seed treatment with a higher concentration of H₂O₂ (50 mmol/L) showed a greater effect under chilling stress and increased germination index and seed energy. Although H₂O₂ is a compound that damages cell membranes, several studies have shown the ability of H₂O₂ in seed treatment with low concentrations, to increase the plant's resistance to oxidative stress caused by temperature and salinization (Sodabeh *et al.*, 2011; Cengiz *et al.*, 2020). A study reported that the seeds treatment with H₂O₂ makes a significant contribution to the regulation of growth, and enhances the plant resistance to abiotic stresses (Geraldo *et al.*, 2017).

In amaranth seedlings grown from seeds treated with salicylic and succinic acid, the contribution of the hypocotyl in the seedling mass is greater in comparison with control and other seed treatments (Figure 2). The root length and seedlings in amaranth are affected by the seed's treatment with proven growth stimulants. However, succinic acid and CaCl₂ not only increased the germination index, but also contributed to the development of the root system. In addition, Ca is an important mineral nutrient used by plants for growth and development, and has a vital role in the cell membrane to regulate gene transport (Kaczmarek *et al.*, 2017; Ahmad *et al.*, 2018).

In amaranth at a low positive temperature, seed germination and seedlings growth are the most vulnerable stage. Chilling stress can reduce the number and the homogeneity (friendliness) of the seedlings, reducing potential yield. Thus, a significant decrease in seed germination was observed when CaCl₂ was used for the seed treatment (Kaczmarek *et al.*, 2017). The seed treatment with CaCl₂ effectively improves the development of the root system under optimal sowing conditions (Ahmad *et al.*, 2018). The study findings authenticated that seed treatment with CaCl₂ and SUA can effectively influence (stimulate) the development of the root system in the amaranth seedlings (Figure 3). The H₂O₂ seed treatment has been observed to increase biomass production and accessory root formation (Gall and Rajakaruna,

2009; Xu *et al.*, 2017). Results further revealed that seed treatment with H₂O₂ (10 mmol/L) increases the seedling's biomass and the length of the hypocotyl and root system.

CONCLUSIONS

The amaranth (*Amaranthus hypochondriacus* L. cv. 'Krepysh') seeds treated with growth-stimulating compounds, viz., calcium chloride, succinic acid, salicylic acid, and hydrogen peroxide denote an improvement in the quality of seeds. Under low positive temperature, the most significant improvement in amaranth seed quality and cold tolerance was exerted by succinic acid, hydrogen peroxide, and calcium chloride. Amaranth seed treatment with growth stimulants significantly improved the morphological index of seedlings by increasing the biomass and length of the hypocotyl and roots. Under normal and low temperatures, pre-sowing treatment of amaranth seeds with hydrogen peroxide and succinic acid revealed the highest enhancement in the seedling's biomass. A significant increase in the length of the hypocotyl was made by the seed treatment with succinic acid and salicylic acid, while succinic acid and hydrogen peroxide boosted the root length up to the maximum. The seed treatment with succinic acid effectively improved the development of the root system in both optimal and low temperatures. The treatment of seed growth stimulants, especially under low temperatures, proved interesting due to the possibility of inducing cold tolerance in the seedlings.

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