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SEED PRIMING EFFECTS ON SEED QUALITY AND ANTIOXIDANT SYSTEM IN THE SEEDLINGS OF *AMARANTHUS TRICOLOR* L.

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SUMMARY

Amaranthus tricolor L. cv. 'Valentina' leaves are a promising source of dietary food supplements (DFS) and are used to prepare tea drinks that improve human health in Russia. Amaranth is a small-seeded crop, and the seed mass differs significantly, which leads to unfriendly shoots and a decrease in the potential productivity of the plant biomass. Pre-sowing treatment with growth stimulants can improve its seed quality. The study aimed to improve the seed quality, the morpho-physiological parameters, and the antioxidant properties of amaranth seedlings grown from seeds treated with plant growth stimulants at the Federal State Budgetary Scientific Institution, Federal Scientific Vegetable Center (FSBSI FSVC), Moscow, Russia. For seed treatments, water solutions of salicylic acid (SA - 138 mg/L), hydrogen peroxide (H_2O_2 - 5 mM), succinic acid (ScA - 500 mg/L), calcium chloride ($CaCl_2$ - 3000 mg/L), and gibberellic acid (GA_3 - 300 mg/L) were used. Researchers germinated the seeds first in petri dishes in natural light at a daytime temperature of 23±2°C for seven days. Determining amaranthine, chlorophyll, and carotenoid content used generally accepted methods. Pre-sowing treatment of large and small fractions of amaranth seeds with GA_3, ScA, and $CaCl_2$ improved the seed quality, whereas seed treatment of SA and H_2O_2 reduced the seed quality. Recording of antioxidants (amaranthine and carotenoids) and total chlorophyll content accumulation in seedlings grown from the treated seeds followed. The levels of amaranthine and photosynthetic pigments decreased in the seedlings of *Amaranthus tricolor* L. cv. Valentina seeds with ScA, $CaCl_2$, and GA_3 treatments, while the seedling's biomass exceeded that of control, which suggests that these chemicals act as growth regulators. The negative effect of SA on the viability of large (LF) and small (SF) amaranth seeds showed significant reductions in the morphometric indicators. It may be due to oxidative stress, enhancing amaranthine content in the cotyledonary leaves of seedlings.

Keywords: *Amaranthus tricolor* L. cv. Valentina, seeds, seedlings, growth regulators salicylic acid, hydrogen peroxide, succinic acid, calcium chloride, gibberellic acid, amaranthine, carotenoids, photosynthetic pigments

Key findings: The impacts of growth stimulants on the quality of seeds, as well as, morphometric and biochemical indicators of amaranth cv. Valentina seedlings are being reported for the first time. By treating the amaranth seeds with growth stimulants, i.e., salicylic acid (SA), hydrogen peroxide (H_2O_2), succinic acid (ScA), calcium chloride ($CaCl_2$), and gibberellic acid (GA_3), resulted in the accumulation of antioxidants (amaranthine and carotenoids) and photosynthetic pigments in the cotyledonary leaves.

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INTRODUCTION

Seed germination is the initial step in the life cycle of plants, which begins when the inactive dry seed imbibes water until the protrusion of the radicle from the seed coat (Nonogaki *et al*., 2010). The degradation of macromolecular substances, the reparation of genetic material, and the expansion of the embryo and endosperm accompany seed germination, which ultimately leads to the rupture of the seminal peel and endosperm and the appearance of a root (Han and Yang, 2015; Chen *et al*., 2021).

The germination process comprises three phases, i.e., in the first phase, the seed absorbs water and swelling occurs; in the second phase, the resumption of metabolic processes occurs; and in the third phase, the appearance of a root from the seed happens (Rajjou *et al*., 2012; Motyleva *et al*., 2021; Tetyannikov *et al*., 2022). Accelerated seed germination is crucial not only for the formation of seedlings but also valuable for enhancing the yield (Han and Yang, 2015)., A simple and inexpensive method—pre-sowing treatment of seeds with solutions of plant growth stimulants—improves seed germination and the appearance of uniform seedlings by activating physiological and metabolic processes (Johnson and Puthur, 2021).

Seed treatment promotes simultaneous germination through enzyme activation, cell regeneration, protein synthesis, and improved antioxidant defense mechanisms. Various types of seed treatments are widely used, such as, seed coating or seed soaking, by using plant growth regulators, osmolytes, and other chemicals to enhance the crop's growth with sustainability (Marthandan *et al*., 2020). Hormonal seed treatment involves the germination of seeds in an aerated aqueous medium using various substances that stimulate plant growth, such as salicylic acid (SA), gibberellin (GA), abscisic acid, and other phytohormones. Plant hormones regulate plant growth, development, reproduction, and survival, and these mechanisms involve cross-communication and signaling pathways, in which plant hormones play a vital role (De-Vleesschauwer *et al*., 2013).

Salicylic acid use affects plant growth and development, such as, seed germination, vegetative growth, flowering, yield, aging, stomata closure, thermogenesis, photosynthesis, respiration, alternative airway changes, glycolysis, and the Krebs cycle (Maruri-López *et al*., 2019). Salicylic acid reduces the effects of abiotic stresses and enhances plant resistance through various physiological manifestations, increasing the content of osmolites, polysaccharides, and antioxidant activity in wheat (Singh *et al*., 2021). Pre-sowing seeds treated with gibberellic acid improves seed germination, increases the protein, chlorophyll, and proline content, and regulates the synthesis of antioxidants under abiotic stress conditions (Ansari *et al*., 2013; Nimir *et al*., 2015). Hydrogen peroxide (H_2O_2) is an endogenous signaling substance regulating plant growth and plays a crucial role in their physiological response to adverse environmental conditions (Yao *et al*., 2021). Hydrogen peroxide treatment helps stimulate the germination of dormant seeds by breaking such state and degrading endogenous inhibitors, such as, abscisic acid (Li *et al*., 2016, 2017). Also, pre-treatment with exogenous H_2O_2 stimulates seed germination and sprout growth by increasing the mass and length of shoots and roots and reducing the average germination time (Cesur and Tabur, 2011). The use of calcium chloride ($CaCl_2$) increases the seedling survival rate and boosts the plant population and height under abiotic stress conditions (Hussain *et al*., 2016).

Amaranthus tricolor L. is a vegetable plant that is a rich source of low-molecular antioxidants, i.e., amaranthine, β-carotene, vitamin C, phenolic compounds, and flavonoids (Gins *et al*., 2017; Sarker and Oba, 2018). As a food and pharmaceutical product, antioxidants perform defensive functions, protecting the components of metabolic reactions from reactive oxygen species (ROS) (Park *et al*., 2020). In Russia, the leaves of *Amaranthus tricolor* L. cv. Valentina served as a source of biologically active substances with antioxidant activity and a tea drink that improves human health (Gins *et al*., 2017; Pivovarov *et al*., 2019).

Currently, seed quality and its improvement by various growth stimulants are acute. The pseudo-grain culture of amaranth is small-seeded, and the seed's mass and germination differ significantly. Therefore, this research work devotes to the study of the effects of pre-sowing seed treatment with various regulators that stimulate plant growth and the germination parameters of seeds to improve their quality and morphometric indicators. The study analyzed the content of antioxidants and photosynthetic pigments of *Amaranthus tricolor* L. cv. Valentina seedlings from the treated seeds. The research sought to increase the quality of seedlings and improve the morpho-physiological parameters and antioxidant properties of the seedlings grown from seeds treated with plant growth stimulants.

MATERIALS AND METHODS

The study used seeds and seedlings of the vegetable species *Amaranthus tricolor* L. cv. Valentina, with their selection taken from the Federal State Budgetary Scientific Institution, Federal Scientific Vegetable Center (FSBSI FSVC), Moscow, Russia (Motyleva *et al*., 2019). The study further experimented at the Laboratory of Physiology and Biochemistry of Plants of FSBSI FSVC.

Pre-sowing seeds treatment

The amaranth seeds got divided into two parts. i.e., with a 1000-seed index of 0.7149 g (Large) and 0.5043 g (Small). Homogeneous and complete seed types got carefully selected. In total, six batches of seeds received treatments with solutions of salicylic acid (138 mg/L), hydrogen peroxide (50 mM/L), succinic acid (500 mg/L), calcium chloride (3000 mg/L), and gibberellic acid (300 mg/L). Distilled water served as a control. The amaranth seeds got soaked in the appropriate water solutions for 4 h, then washed with distilled water, dried, and laid out on filter paper in sterilized petri dishes (90 × 90 mm). In each petridish, 50 seeds got evenly placed. The experiment employed three repetitions.

The seeds in the petri dishes received seven days of incubation at a temperature of 23±2°C. Given the period, seed germination was analyzed daily, with the number of germinated seeds being counted and recorded. The seeds were ranked as germinated when a root with a minimum length of 0.5 cm appeared (Niu *et al*., 2013). After seven days, the data on germination potential (GP), germination rate (GR), mean germination time (GT), germination index (GI), and growth strength index (GSI) of *A. tricolor* L. cv. Valentina's seedlings got jotted down.

Seed germination parameters

Measuring the germination potential (GP) included the percentage of germinated seeds out of the total seed number when the number of germinated seeds per day reached a maximum. (Yao *et al*., 2021):

,

$$GP\ (\%) = \frac{n_{3d}}{n_T} \text{x} 100,$$

where:

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

n_T = the total number of seeds in each petri dish (50).

Germination rate (GR) is an estimate of the viability of the seed population, which was calculated by dividing the number of germinated seeds on the seventh day by the total number of seeds (Zhu *et al*., 2021):

$$GR\ (\%) = \frac{n_{7d}}{n_T} \text{x} 100,$$

where:

n_{7d}= the number of germinated seeds on the seventh day after sowing, and

n_T = the total number of seeds in each petri dish (50).

The germination time (GT) was calculated to estimate the rate of germination (Refka *et al*., 2013):

$$GT = \sum \frac{(ni \text{ x } di)}{n},$$

where:
ni = the number of germinated seeds per day,
di = the incubation period, and
n = the total number of germinated seeds in a petri dish.
The seed germination index (GI) is the average germination rate of the seeds (Hongna *et al*., 2021; Li *et al*., 2021;):

$$GI = \sum \left(\frac{Gt}{Dt}\right),$$

where:
Gt = the number of seeds germinated in 24 h, and
Dt = the day corresponding to Gt.

The Viability Index (VI) was measured as per the formula proposed by Yao *et al*. (2021):

$$VI = GI \times SL$$

where:
GI = the seed germination index, and
SL = the average length of the hypocotyl.

Biochemical parameters

Amaranth seedlings were crushed and the extracted sample weight of 0.15 g got placed in double-distilled water or 96% ethanol. Then, the samples were centrifuged at 10,000 g for 15 min at 4°C. The spectrophotometric method determined the content of photosynthetic pigments. The content of chlorophyll a and b and the total content of carotenoids were calculated by the following formulas (Lichtenthaler, 1987):

$$Chl\ a\left[\frac{mg}{g}\right] = \frac{(13{,}36A_{664{,}2} - 5{,}19A_{648{,}6}) * V}{1000 * m}$$

$$Chl\ b\left[\frac{mg}{g}\right] = \frac{(27{,}43A_{648{,}6} - 8{,}12A_{664{,}2}) * V}{1000 * m}$$

$$Car^{x}\left[\frac{mg}{g}\right] = \frac{(4{,}785A_{470} + 3{,}657A_{664{,}2} - 12{,}76A_{648{,}6}) * V}{1000 * m}$$

where:
A_{470}, $A_{648.6}$, and $A_{664.2}$ = the absorption at 470 nm, 648.6 nm, and 664.2 nm, respectively, and the thickness of the cuvette was 1 cm,
V = the volume of the extract (ethanol 96%) in ml, and
m = the weight of the sample attachment in g.

The amount of amaranthine in water extracts was determined by taking into account a molar extinction coefficient of 5.66 •104 l mol^{-1} cm^{-1} and a molar weight of 726.6 g mol^{-1} (Gins *et al*., 2002).

Statistical analyses

The results were expressed as mean ±SD (standard deviation) of the three replications. The R-Studio software package was used for the data management, and analysis and presentation (Fox and Leanage, 2016). The significance level considered to assume mean differences at $P \leq 0.05$.

RESULTS

Seed treatment influence on germination and morphometric indicators

The study revealed that pre-sowing seed treatment with plant growth stimulants had an uncertain effect on the germination variables of larger and smaller seeds of *Amaranthus tricolor* L. cv. Valentina (Figure 1). The amaranth large and small seeds treated with GA and ScA increased all the studied characteristics, except for germination rate (GR) in large seeds, which performed the same as that of control. The small seeds treated with stimulants ScA, H_2O_2, and GA gave the highest germination potential (GP) as observed and exceeded the control by 62.1%, 62.1%, and 37.9%, respectively (Figure 1). The germination index (GI) in large seeds exceeded the control by 42.4% by treating them with ScA. In large and small seeds treated with GA and ScA, the vigor index (VI) increased by 59.1% and 16.0%, and 51.01% and 26.62%, respectively, compared with the control.

In smaller seeds, the germination parameters decreased by treating with growth stimulants, SA and $CaCl_2$. Their GP decreased by 6.9% compared with the control, their GR by 11.3% and 25.0%, and their GI decreased by 2.96% and 20.4%, respectively. The viability index assessed the energy of seeds in Tartary buckwheat (*Fagopyrum tataricum*) (Yao *et al*., 2021). The decrease in the seed mass also significantly reduced the germination parameters, viz., GP, GR, GI, and VI. At the same time, the VI in small seeds treated with SA, H_2O_2, and $CaCl_2$ decreased by 34.2%, 1.6%, and 34.0%, respectively, compared with the control. However, in large seeds, the seed treatment with SA reduced the VI by 1.62%.

Smaller seeds treated with growth stimulants SA, H_2O_2, and $CaCl_2$, showed a decrease in the viability index, which led to the decline in the mass of seedlings produced by these seeds. The results revealed that the five plant growth stimulants had different effects on the seed germination parameters. Thus, the treatment of large seeds with growth stimulants, viz., GA_3, ScA, H_2O_2, and $CaCl_2$, improved the seed's quality, and salicylic acid did not significantly reduce the viability of seeds. Contrarily, the germination parameters of smaller seeds received undesirable effects from SA, $CaCl_2$, and to a small extent, H_2O_2 (1.6%). In contrast, GA_3 and ScA significantly improved the quality of both types of seeds. Accordingly, the seedlings obtained from these seeds were distinguished by greater biomass and longer hypocotyl, especially in seedlings from GA_3-treated seeds, and big root sizes in seedlings from seeds treated with ScA. Further, the GA_3 and ScA enhanced the germination characteristics of large and small

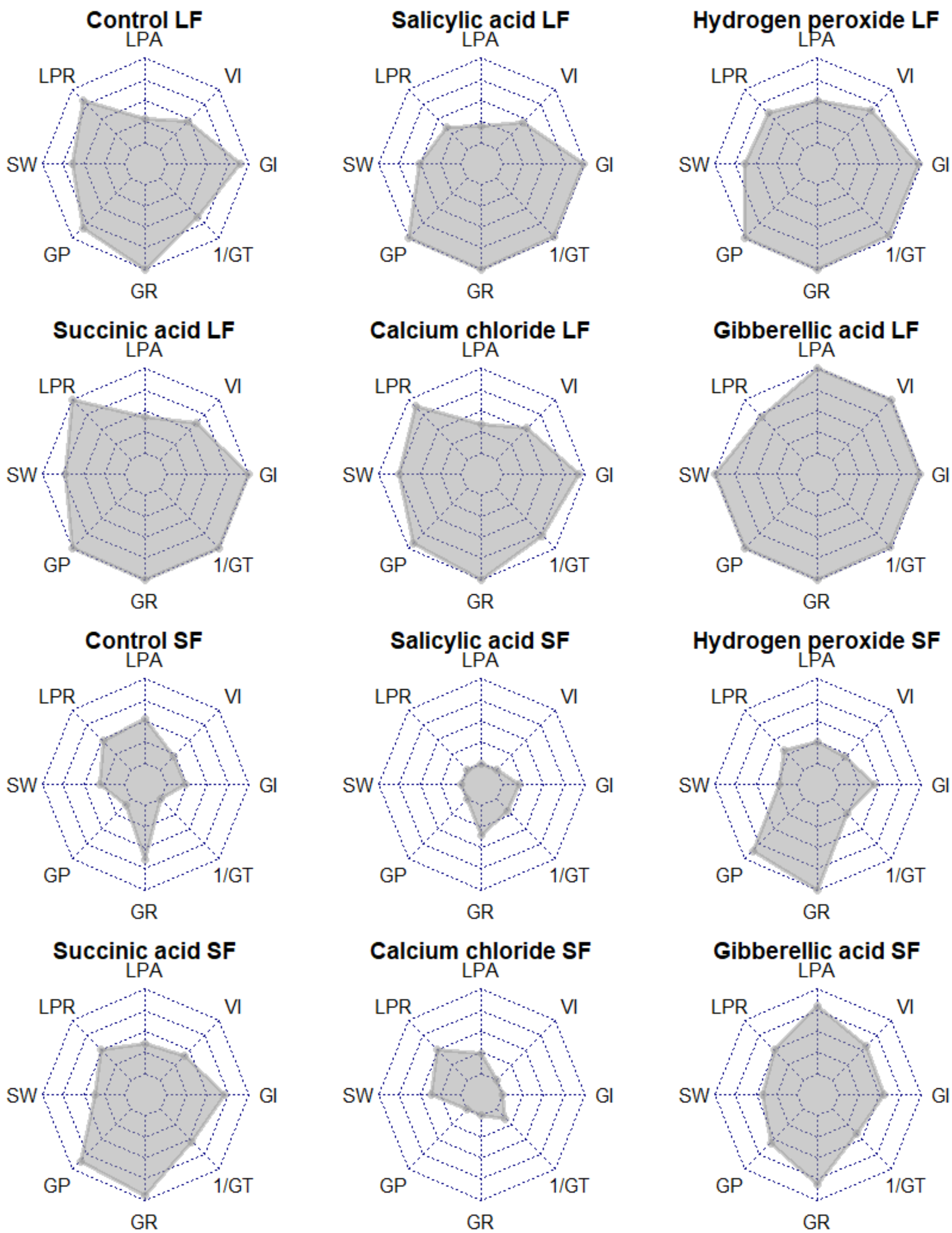

Figure 1. Influence of pre-sowing treatment on morphometric indicators (mass of seedlings, length of hypocotyl and root) and germination parameters of seeds grown from large and small fractions.

seeds and increased the biomass productivity of seedlings in the amaranth cultivar, Valentina.

The ambiguous effects of $CaCl_2$ on the germination parameters of large and small seeds and the growth of seedlings indicated varied effects on the metabolic reactions of seeds and the development of seedlings. The $CaCl_2$ reduced the germination parameters (GR, GI, and VI) of small seeds and the mass of seedlings, although it enhanced root growth in seedlings of small and large seeds (Table 1).

Table 1. Morphometric indicators of *Amaranthus tricolor* L. cv. Valentina grown from seeds treated with growth stimulants.

Treatment	Hypocotyl length (cm)		Root length (cm)		10-seedling weight (g)	
	Large fraction	Small fraction	Large fraction	Small fraction	Large fraction	Small fraction
Control (H_2O)	2.04+0.16	2.39+0.21	2.68+0.44	1.62+0.18	0.0632±0.0040	0.0418±0.0030
Salicylic acid	1.91±0.34	1.62±0.10	1.24±0.41	0.14±0.05	0.0547±0.0035	0.0227±0.0025
Hydrogen peroxide (H_2O_2)	2.35±0.34	2,00±0.38	2.02±0.77	1.15±0.53	0.0630±0.0039	0.0368±0.0016
Succinic acid	2.24±0.29	2.14±0.29	3.25±0.59	1.74±0.47	0.0685±0.0038	0.0453±0.0036
Calcium chloride ($CaCl_2$)	2.12±0.39	1.98±0.26	2.89±0.36	1.72±0.37	0.0698±0.0044	0.0452±0.0029
Gibberellic acid	3.09±0.24	2.81±0.39	2.35±0.67	1.71±0.34	0.0857±0.0054	0.0488±0.0031

Past studies revealed that the $CaCl_2$ treated seeds effectively in the root system development under optimal sowing conditions in *Brassica rapa* L. (Kwon *et al*., 2009) and other crop plants (Siddiqui *et al*., 2020).

In general, the seedling's fresh biomass and the length of hypocotyl and roots increased in the large and small seeds treated with GA_3, ScA, and $CaCl_2$, indicating the ability of these compounds to stimulate seed germination and growth traits (Table 1). Small seeds treated with SA and H_2O_2 showed reduced viability of seeds and biomass of seedlings and evidently cannot attribute to the seedling's growth.

Seed treatment effects on antioxidant contents in seedlings and cotyledonary leaves

Table 2 shows the effects of pre-sowing seed treatment on amaranthine content in the studied amaranth seedlings of large and small seeds (Table 2). Results elucidated that large seeds treated with solutions of SA and H_2O_2 showed an increased content of amaranthine by 19.23% and 4.4%, respectively, compared with the control, but it decreased with the ScA, $CaCl_2$, and GA treatments by 13.19%, 31.32%, and 56.04%, respectively. In seedlings grown from smaller seeds treated with SA and H_2O_2, the amaranthine content also increased by 40.72% and 28.51%, respectively, and decreased with the ScA, $CaCl_2$, and GA treatments by 13.57%, 15.84%, and 44.8%, respectively, compared with the control.

At the same time, seedlings obtained from smaller seeds gained distinction by a higher level of amaranthine content compared with the red pigment in seedlings from large seeds. Notably, the treatment of small seeds with SA and H_2O_2 induced the amaranthine synthesis in them in larger quantities than with the control and large seeds. The researchers further studied the red pigment accumulation in the cotyledonary leaves and hypocotyl, finding no amaranthine in the roots of the seedlings. The study found the highest amaranthine content in the cotyledonary leaves of seedlings from SA and H_2O_2-treated seeds. The remaining seed treatments with ScA, $CaCl_2$, and GA_3 did not increase the red pigment content in the cotyledonary leaves compared with the control (Table 3). The data also discovered that the bulk of the water-soluble antioxidant amaranthine was localized in the cotyledonary leaves, with a small amount was also found in the hypocotyl.

The amaranthine content in cotyledonary leaves of the SA and H_2O_2-treated seeds increased by 19.91% and 11.68%, respectively, compared with the control. Pre-sowing seeds treated with ScA, $CaCl_2$, and GA_3 solutions showed a decrease in the amaranthine content in cotyledonary leaves by 2.25%, 26.05%, and 41.77%, respectively,

Table 2. Amaranthine content in *Amaranthus tricolor* L. cv. Valentina grown from seeds treated with growth stimulants.

Pre-sowing treatment	Amaranthine (mg/g FW)		Content of amaranthine in SF vs. LF
	Large fraction	Small fraction	
Control (H_2O)	0.182±0.010	0.221±0.025	1.21
Salicylic acid	0.217±0.019	0.311±0.035	1.43
Hydrogen peroxide (H_2O_2)	0.190±0.012	0.284±0.031	1.49
Succinic acid	0.158±0.015	0.191±0.029	1.21
Calcium chloride ($CaCl_2$)	0.125±0.017	0.186±0.033	1.49
Gibberellic acid	0.080±0.009	0.122±0.015	1.53

Table 3. Amaranthine content in the seedlings of *Amaranthus tricolor* L. cv. Valentina grown from large fraction seeds, treated with various growth stimulating solutions.

Pre-sowing treatment	Amaranthine (mg/g FW)	
	Cotyledonary leaves	Hypocotyl
Control (H_2O)	0.668±0.06	0.086±0.006
Salicylic acid	0.801±0.09	0.073±0.005
Hydrogen peroxide (H_2O_2)	0.746±0.09	0.073±0.005
Succinic acid	0.653±0.08	0.076±0.005
Calcium chloride ($CaCl_2$)	0.494±0.03	0.076±0.004
Gibberellic acid	0.389±0.04	0.073±0.003

compared with the control. The amaranthine content in the hypocotyl of the seeds with ScA and $CaCl_2$ treatments decreased by 11.63% and for those treated with H_2O_2 and GA_3, the amaranthine content decreased by 15.12%, compared with the control. The seed treatment with growth stimulants affected the ratio of amaranthine in the cotyledonary leaves and hypocotyl. However, the larger amount of pigment was localized in the cotyledonary leaves of seedlings produced by the seeds treated with H_2O_2 and ScA compared with the red pigment content in the hypocotyl. The $CaCl_2$ and GA treated seeds showed a reduced ratio of amaranthine in cotyledons compared with hypocotyl, which indicated the greater amount of amaranthine in hypocotyl compared with cotyledonary leaves and control plants.

Seed treatment effects on photosynthetic pigments in cotyledonary leaves

In this section, the study investigated the total content of chlorophyll a and b, as well as, the antioxidants carotenoids in the cotyledonary leaves from large seeds of *Amaranthus tricolor* L. cv. Valentina. In said leaves, the chlorophyll a and b contents were increased by 44.19% and 18.6% after the treatment of seeds with SA and H_2O_2, respectively, whereas treatments of GA_3, ScA and $CaCl_2$ reduced chlorophyll a and b by 25.58%, 37.21%, and 67.44%, respectively (Table 3). Similar to the above findings the seeds treated with SA and H_2O_2 also significantly increased carotenoids in the cotyledonary leaves, while a reduction in their content after treatments with GA_3, ScA, and $CaCl_2$.

In cotyledonary leaves, the amount of carotenoids was boosted in the seeds treated with SA and H_2O_2 by 37.1% and 20.97%, respectively, and seeds treated with GA_3, ScA, and $CaCl_2$ reduced their values by 23.39%, 33.87%, and 66.94%, respectively (Table 4). On average, the total content of chlorophyll and carotenoids were minimal in the hypocotyl. The seed treatment of SA and H_2O_2 induced the formation of chlorophyll and carotenoids in contrast with GA_3, ScA, and $CaCl_2$, which inhibited their synthesis. The study noted that the seed treated with H_2O_2 induced a greater accumulation of photosynthetic pigments in the cotyledonary leaves than amaranthine, compared with the corresponding controls.

Table 4. Content of chlorophyll a + b and carotenoids in the cotyledonary leaves of *Amaranthus tricolor* L. cv. Valentina, grown from large fraction seeds, treated with various growth stimulating solutions.

Pre-sowing treatment	Chlorophyll a and b (mg/g FW)	Carotenoids (mg/g FW)
Control (H_20)	0.43±0.03	0.124±0.03
Salicylic acid	0.62±0.05	0.170±0.04
Hydrogen peroxide (H_2O_2)	0.51±0.04	0.15±0.04
Succinic acid	0.27±0.03	0.082±0.009
Calcium chloride ($CaCl_2$)	0.14±0.01	0.041±0.005
Pre-sowing treatment	0.32±0.04	0.095±0.010

DISCUSSION

The seed germination and initial growth are vulnerable stages dependent on environmental conditions in *Amaranthus tricolor* L. cv. Valentina and other crop plants (Ashraf *et al*., 2002; Raja *et al*., 2020; Hu *et al*., 2021; Kandel *et al*., 2021; Novák *et al*., 2021). The study suggested the pre-sowing treatment of amaranth seeds with growth stimulants to improve the seed quality, increase the biomass, and the effectiveness of seedling defense systems by activating the synthesis of low molecular weight antioxidants. However, the studied plant growth stimulants ambiguously affected these parameters, and the seeds treated with GA_3, ScA, and $CaCl_2$ improved the quality of the seedlings.

The study noticed a decrease in values of the seed germination parameters and the growth and accumulation of seedling biomass. Consequently,more visible to a greater extent during the large seeds treatment of SA and H_2O_2, whereas seeds treated with GA, ScA, and $CaCl_2$ significantly helped the seed's ability to germinate and increase the germination index, as well as, the hypocotyl and root length. Several studies reported the positive effect of pre-sowing seed treatment of GA_3 in maize (Tuna *et al*., 2008; Ghodrat and Rousta, 2012), wheat (Iftikhar *et al*., 2019), and other crop plants (Ashraf and Foolad, 2005).

The maximum increase inhypocotyl growth showed in the seeds treated with GA_3 and on the root by treating the seeds with ScA. Treatment of seeds with SA and H_2O_2 significantly reduced the viability index of seeds. Consequently, the morphometric indicators of seedlings inhibited their growth. The most significant stressful effects of SA and H_2O_2 seed treatment affected the minimal biomass build-up. The treatment of large and small seeds with GA, ScA, and $CaCl_2$ enhanced seed germination and stimulated the growth of amaranth seedlings. Past studies reported that seeds treated with growth stimulants indefinitely affected the germination parameters and morphometric indicators in *Brassica napus* L. (Zhu *et al*., 2021).

This study also observed the ambiguous effects of $CaCl_2$ on the germination parameters (GP, GR, GI, and VI) and hypocotyl length in amaranth. Notably, observations showed a similar significant decrease in germination parameters by treating seeds with $CaCl_2$, although it effectively improved the development of the root system of seedlings in crop plants (Kwon *et al*., 2009; Siddiqui *et al*., 2020). The growth stimulants SA and H_2O_2 have been reported to increase resistance to cold and facilitate seed germination in wheat (Singh *et al*., 2021) and various other crop plants (Hossain *et al*., 2015).

The red pigment amaranthine effectively neutralizes the superoxide oxygen radical, O_2^-, and performs photoprotective and antioxidant functions in amaranth tissues. In a report, an increase in the content of low-molecular antioxidants is associated with the effects of abiotic stress impact on the plant organism (Hasanuzzaman *et al*., 2018). This comparative study of seedlings of the large and small seeds treated with SA, H_2O_2, and $CaCl_2$ showed that the accumulation of amaranthine was higher by 1.43, 1.49, and 1.49 times in seedlings obtained from large seeds. Meanwhile, treating seeds with H_2O, ScA and GA_3, the content of the red pigment decreased by 1.21, 1.21, and 1.53 times.

The increase in amaranthine content in seedlings from small seeds treated with SA and H_2O2, compared with the large seed seedlings, indicates better effects of SA and H_2O_2 on the synthesis of amaranthine. It might be due to the stressful effects of these seed treatments on the metabolism associated with induction and increased red pigment content. Amaranthine accumulation in the cotyledonary leaves is 10 times greater with hypocotyl. It indicates the stress effect of SA on seed metabolism, which affects the synthesis of pigments in seedlings.

The exposure to light initiates the greening process in seedlings. After absorbing the light energy, it proceeds to autotrophy in plants (Le *et al*., 2001; Tang *et al.*, 2012). Chlorophyll plays an essential role in the collection and conversion of light energy. Likewise, it helps transfer electrons at reactive centers in plants (Eckhardt *et al*., 2004; Masuda, 2008). To ensure friendly growth during the greening process of seedlings of higher plants, the chlorophyll biosynthesis needs to be controlled (Tanaka and Tanaka, 2007; Yuan *et al*., 2017).

In studying the content of chlorophyll and carotenoids, the study found a similar amaranthine-inducing effect of SA and H_2O_2. Seeds treated with SA significantly increased the chlorophylls and carotenoids. Reports showed that pre-sowing seed treatment of SA significantly increased the total chlorophyll content compared with the control in rice seedlings and found consistent with this study (Ali *et al*., 2021). The seven-day-old seedlings accumulated the highest amount of low-molecular antioxidant amaranthine and carotenoids when processing seeds of amaranth with SA and H_2O_2, and these effects were assessed as stress. However, seed treatments of growth stimulants, GA_3, ScA, and $CaCl_2$, contributed to the increase in the mass of seedlings grown from these seeds.

CONCLUSIONS

Pre-sowing treatment of amaranth seeds cv., Valentina, with growth stimulants GA and ScA increased its seed viability by 59.1% and 16%, and 51% and 26.62% in large and small seeds, respectively. At the same time, it reduced the content of amaranthine, carotenoids, and chlorophylls in the seedlings. The seeds treated with SA and H_2O_2 induced the amaranthine synthesis and photosynthetic pigments in the seedlings. The amaranth seeds treated with ScA, $CaCl_2$, and GA_3 increased the biomass in seedlings grown from these seeds by 8.39%, 10.44%, and 35.6%, respectively. However, the treatments reduced amaranthine by 13.19%, 31.32%, and 56.04%, the total content of chlorophylls by 37.21%, 67.44%, and 25.58%, the number of carotenoids by 33.87%, 66.94, and 23.39%. The SA and H_2O_2 treatments on amaranth seeds induced increases on the following indicators: the level of amaranthine by 19.23% and 4.4%; the total content of chlorophylls by 44.19% and 18.6%; and the carotenoid content by 37.1% and 20.97%, respectively. However, these treatments reduced the growth processes, biomass, hypocotyl, and root length. The seedlings from seeds treated with SA showed the maximum amaranthine content in their cotyledonary leaves of, which were 10 times higher than in hypocotyl. On the other hand, the seeds treated with GA gave the minimum amaranthine content in their cotyledonary leaves, and were five times higher than in the hypocotyl.

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