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MODULATION OF LOW-MOLECULAR-WEIGHT ANTIOXIDANTS IN *AMARANTHUS TRICOLOR* LEAVES EXPOSED TO COLD STRESS DURING THE RIPENING STAGE

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SUMMARY

Amaranth is an indispensable C_4 agricultural crop with considerably reduced productivity under hypothermia loading. During seed ripening, chilling stress (1 °C–2 °C) can harm the photosynthetic organs in the plants and reduce the efficiency of low-molecular-weight defense systems. The studies on the content of low-molecular-weight antioxidants commenced in the leaves of the Amaranth cultivar Valentina cultivated in an open field in the post-stress period (after a chilling stress). After chilling at 2 °C in the post-stress period, older leaves of the main shoot displayed partial damage, while young leaves of the lateral shoots visually maintained a native appearance. The ascorbic acid (AA) content showed significant variations in the leaves. The content of possessing antioxidant properties revealed red-colored amaranthine decreased during this period, i.e., 1.5–1.9 times in young leaves, 3.5 times in leaves damaged by cold (DC), and non-damaged by cold (NDC) leaves showed a 1.1-times decrease. The decline in photosynthetic pigment content varied from 14% for carotenoids (Cars) to 60% for chlorophylls *a* (Chl *a*) and *b* (Chl *b*) in NDC leaves. The water and ethanol-soluble antioxidant contents improved with repeated cold stress (2 °C) in young leaves. The same pattern was also evident for the ascorbic acid and amaranthine content enhancement (20%–25% and 30%, respectively). The formation of hypothermia-induced tolerance in the leaves of different ages in autumn, which are the prime producers of low-molecular-weight antioxidants, signified a close relationship to the functioning of hydrophilic and hydrophobic antioxidants.

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Keywords: *Amaranthus tricolor* L., red-colored Valentina cultivar, cold stress, low-molecular-weight antioxidants, amaranthine, ascorbic acid

Key findings: New data have been notable from the amaranthine pigment contributing to increasing the resistance of amaranth plants to chilling stress. Variations in the low-molecular-weight antioxidants' content and functional activity, which have become traditionally resistance actors, were also evident.

INTRODUCTION

The latest research on amaranth correlates with the determination of its nutritional value, including antioxidant characteristics under standard conditions in the absence of stress conditions, which makes its stress resistance appealing and relevant to study (Karamac *et al*., 2019; Sarker and Oba, 2019; *Sarker et al*., 2020; Gins *et al*., 2021). Among the climatic factors, low, warm temperatures have the most considerable damaging effects on cold-sensitive C_4 plants. Under cold stress, the accumulation of reactive oxygen species (ROS) causes oxidative damage to biomolecules and cellular structures, commonly a strain, often associated with the formation of protective reactions to hypothermia (Suzuki and Mittler, 2006).

Low, humid temperatures with long-term adverse effects have caused several physiological deviations and damage to the leaves of cold-sensitive C_4 plants. However, the degree of damage depends upon the temperature value, the duration of impact, the plant species, and the physiological state of the plant organs during their exposure to cold. It is vital to recover the physio-biochemical parameters of plants with temperature increase before irreparable damage occurs (Suzuki and Mittler, 2006; Shibaeva *et al*., 2018).

The plant's physiological recovery often takes place under natural conditions, and in high-altitude areas, a warm day comes after a cold night (Wang, 1993). The fluctuation of low and high temperatures during a short period prevents cold damage and reduces the rate of photosynthesis and transpiration in the sprouts of cold susceptible C_4 plants (Koscielniak and Biesaga-Koscielniak, 2000; Skrudlik *et al*., 2000). Amaranth is an essential vegetable and pseudo-cereal culture; however, it belongs to cold-sensitive plants, with its productivity markedly reduced by low temperatures. Plant growth even stops at a temperature below 8 °C. The introduced amaranth cultivars can produce moderate seed yields with the highest leaf biomass in Central Russia. However, early autumn with short-term temperatures (1 °C–2 °C) may cause plant damage leading to death.

The cold-sensitive C_4 crops can bear damage by ROS even at a slight decrease in temperature (Shibaeva *et al*., 2018), indicating the principal role of oxidative stress in damage incidences in these plant species (Wang, 1993). Under hypothermia, antioxidants produce a defense mechanism by removing the excess ROS and supporting the cell redox balance with the involvement of low-molecular components and enzymes with an antioxidant function (Gins, 2022; Gins *et al*., 2022). In hypothermia, the process of plant adaptation occurs with the participation of biologically active compounds, such as ascorbic acid, carotenoids, phenolic compounds, and amaranthine (Radyuk *et al*., 2009; Gins *et al*., 1998).

The ascorbic acid, amaranthine, and carotenoids accumulate massive amounts in amaranth leaves and perform protective functions in the tissues. Ascorbic acid directly reacts with superoxide anion radicals, molecular singlet oxygen, and hydroxyl radicals, also involved in the regeneration of zeaxanthin and tocopherol molecules (Radyuk *et al*., 2009). The red-colored amaranthine pigment belongs to the group of betalain pigments. Previously, it was noticeable that amaranthine inhibits free-radical reactions with stress-protective properties. It also hinders the peroxide oxidation of lipids, participates in the detoxification of superoxide anion radicals, and chelates iron ions (Gins *et al*., 1998).

Carotenoids are effective quenchers of singlet oxygen that prevent the photo destruction of chlorophyll (Radyuk *et al*., 2009).

The pertinent research studied the variations in the ascorbic acid, amaranthine, water- and ethanol-soluble antioxidants, and photosynthetic pigments of amaranth leaves of different ages at the seed maturation phase in the post-stress period (2 °C) with night/day temperatures of 4 °C–7 °C/8 °C–13 °C, respectively.

MATERIALS AND METHODS

Study site and experimental protocol

The object of the study was the mixed-age fresh plant leaves of the *Amaranthus tricolor* L. (cv. Valentina) selected by the Federal Scientific Vegetable Center and grown on sod-podzolic clay loam soil in the experimental fields of Moscow region (55° 39.51' N, 37° 12.23' E). The experiment setup was a randomized complete block design with three replicates (Dzhos *et al*., 2024). The agrochemical characteristics of the tilth-top (0–20 cm) soil layer before planting the seedlings were as follows: content of humus (1.62%), reaction of the medium pH_{Kcl} (6.1), hydrolytic acidity (1.32 mg-eq/100 g of soil), amount of absorbed substrates (19.2 mg-eq/100 g of soil), degree of substrate saturation (93.6%), and on average, the contents of P, K, and N were 472, 167, and 9 mg/kg, respectively. In the maturation phase, the growing amaranth plants sustained twice exposure to night cold temperatures (2 °C) in autumn on September 30 and October 21. In the period after two cold snaps (post-stress period—October 2, 4, and 28), the daily temperature changed within the 8 °C–14 °C range, and at night, from 4 °C to 7 °C.

In the post-stress period, the fully developed leaves of the main shoot were the center of biochemical analysis - 1) non-damaged leaves (NDC), 2) damaged by cold leaves (DC), 3) young leaves of lateral shoots: (3a) young small (YS) leaves, which were in the active growth phase, the sheet plate reached half the final area size; and (3b) young big (YB) reached 2/3 of the final size. After the first cold drop, all the young leaves of the amaranth lateral shoots remained visually almost unchanged, while some leaves of the primary shoot showed partial damage by the cold. Thus, the upper half of the damaged leaf exhibited complete dehydration, acquiring a gray-brown color, and the lower half of the leaf maintained a red-purple color and was comparable to the NDC leaves of the amaranth main shoot. As a result, several prime shoot leaves displayed partial damage after the first cold drop and fell off after repeated cold exposure.

Determination of the relative water content

For the relative water content (RWC) determination, a sample of weighed leaves placed in distilled water remained at room temperature for complete saturation (de Ollas *et al*., 2019). Then, drying the leaves with filter paper and weighing them help determine the amount of absorbed water after a 24-hour saturation. Acquiring the dry weight of the leaves resulted after drying the samples in a thermostat at 85 °C for 48 h.

Calculating the RWC used the formula:

$$RWC\ (\%) = 100(\text{fresh weight} - \text{dry weight}) / (\text{saturated weight} - \text{dry weight})$$

Ascorbic acid

Ascorbic acid measurement utilized the Tillman's reagent (Dzhos *et al*., 2023). The sample extraction used 3% meta-phosphoric acid, with the volume adjusted to 100 mL with another 3% meta-phosphoric acid. An aliquot of 10 mL of the extract continued titration with Tillman's reagent (2, 6-dichlorophenol indophenol) until a pale pink endpoint was visible, which persisted for 15–20 s. The expression of the results was as mg g^{-1} FW.

Amaranthine

The amaranthine extraction with water followed, measuring the optical density of the supernatant at 536 nm using a

spectrophotometer. The amaranthine content calculation used the formula (Cai *et al*., 1998):

$$\text{Amaranthine} = A_{536}\,(MW)V_a(DF)/\varepsilon LWa$$

Where: A_{536} is the absorbance at 536 nm (λ_{max}); V_a is the total extract volume; DF is the dilution factor; L is the path length of the cuvette; and W_a is the fresh weight of the sample. The molecular weight (MW) and molar extinction coefficient (ε) of amaranthine are 726.6 and 5.66 × 10^4 $M^{-1}cm^{-1}$, respectively (Gins *et al*., 2017). The results' expression was in mg g^{-1} FW.

Chlorophylls and carotenoids

All the pigments' extraction consisted of ethanol. The absorbance measurement was at 470, 648.6, and 664.1 nm. The content of chlorophylls *a* and *b* and carotenoids identification used the equations described by Lichtenthaler (1987) and Lichtenthaler and Buschmann (2001), with the results expressed as mg g^{-1} FW.

Total antioxidant content

The total antioxidant content derivation used the amperometric method (Gins *et al*., 2020), adapted to determine both the ethanol and water-soluble fractions. The sample's homogenization continued in the ethanol solution (50 mL), using these to produce extracts from the plant tissue. Then, the homogenate bore centrifugation within 15 min at 5000 rpm at 4 °C. A supernatant aliquot acquired aided in measuring the total antioxidant content. The measurements ran on the device "Tsvet-Yauza-01-AA" in the constant current mode, with the results expressed as mg gallic acid equivalents (mg GAE g^{-1} FW) (Mamedov *et al*., 2017).

Statistical analysis

The descriptive statistical analysis used OriginPro 9.0. The reported data represent the means of at least three replicates ± standard errors. The data underwent analysis of variance (ANOVA), followed by Duncan's Multiple Range Test at $P \leq 0.05$ using SPSS for Windows.

RESULTS

Phenological analysis, ascorbic acid, and amaranthine

The relative water content (RWC) derivation characterized the water status of the plant. After the action of hypothermia on the first day, it decreased from 97.6% ± 0.4% to 83.5% ± 0.5% in non-damaged cold (NDC) old leaves, and in young leaves, from 98.5% ± 0.5% to 86.4% ± 0.5% and from 98.8% ± 0.6% to 86.2% ± 0.5% (young small (YS) and young big (YB) leaves, respectively). Thus, in the post-stress period, the RWC in old intact leaves decreased by 14.1% ± 0.6%, and in the young leaves decreased as YS (12.1% ± 0.7%) and YB (12.6% ± 0.8%).

The amaranth leaves of different ages—NDC and damaged cold (DC) old and young leaves (YS and YB) in the post-stress period (2 °C at night)—significantly differed for the ascorbic acid content (Figure 1). Thus, in all the non-damaged old and young leaves, the levels of ascorbic acid were close to each other, unlike the DC leaves, where the ascorbic acid content significantly lowered. On the second day, the level of ascorbic acid declined in the young leaves of the lateral shoots (YS and YB), while in the old leaves, it rose. On the fourth day after cold stress, a decrease in the ascorbic acid concentration resulted in all the main and lateral shoots, with a significant reduction of 2.7 and five times observed in NDC and DC leaves, respectively.

A repeated cold drop (2 °C) caused a further dwindle in the ascorbic acid content in YS leaves, while an increase in the ascorbic acid level compared with the previous measurement was evident in YB (Figure 1). Further analysis of the ascorbic acid content conducted in the post-stress period revealed its increase in levels in YS and YB leaves at 52% and 38%, respectively, compared with the ascorbic acid value with repeated cold stress reflecting an increase in the resistance of lateral shoots young leaves to suboptimal night

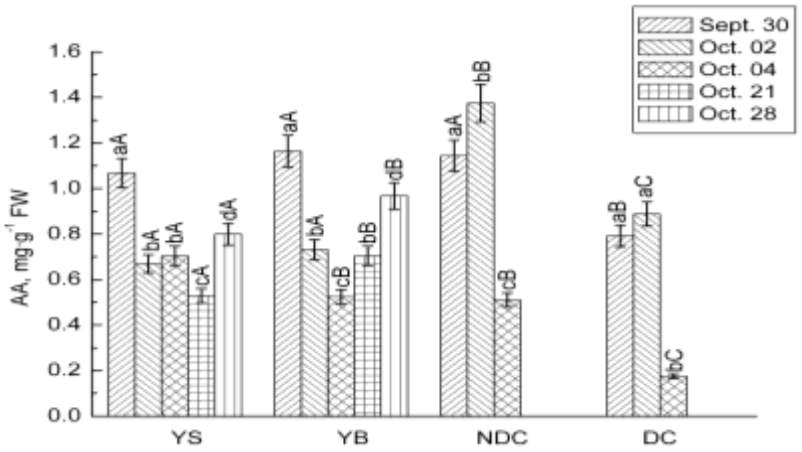

Figure 1. Changes in the ascorbic acid content in the post-stress period after the first cold drop on September 30 and after the repeated cold stress on October 21 to 2 °C (means with one or more identical letters in common do not differ significantly from each other; lowercase letters refer to the comparison between different dates and capital letters refer to the comparison between plant leaves for the same date). YS-young small; YB-young big; NDC-non-damaged by cold; and DC-damaged by cold leaves.

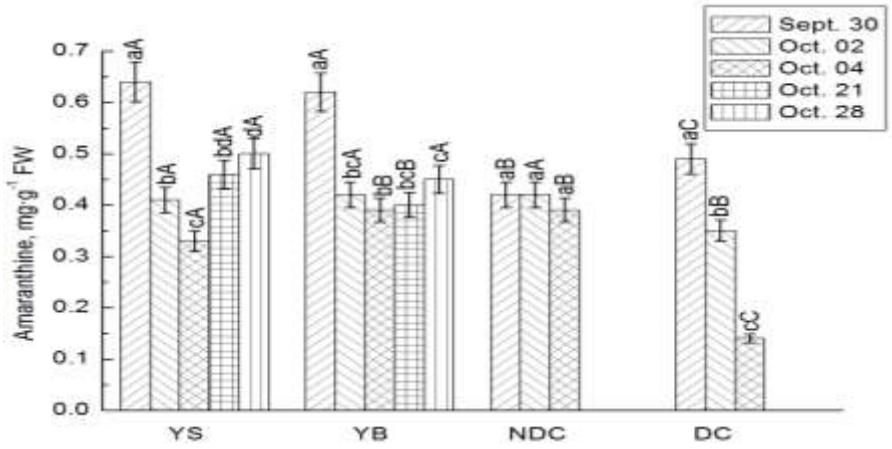

Figure 2. Changes in the amaranthine content in the post-stress period after the first cold drop on September 30 and after the repeated cold stress on October 21 to 2 °C (means with one or more identical letters in common do not differ significantly from each other; lowercase letters refer to the comparison between different dates, and capital letters refer to the comparison between plant leaves for the same date). YS-young small; YB-young big; NDC-non-damaged by cold; and DC-damaged by cold leaves.

temperatures. However, the prime shoot leaves had fallen by this period.

In red-colored amaranth, the purple-red pigment amaranthine (betacyanin from the betalain pigment group) - an important antioxidant, emerged in all organs of the plants. In young leaves of lateral shoots, the highest content of amaranthine in the post-stress period indicated a gradual decrease, and four days later, the concentration decreased 1.9–1.6 times (Figure 2). Only an inclination to decrease amaranthine content in old NDC

leaves was apparent during this period, while in DC leaves, the antioxidant concentration was 3.5 times lower. After the repeated temperature drop, in the young leaves of the lateral shoots, the increase in amaranthine content was prominent, which indicated the existence of de-novo pigment synthesis, the vital role of amaranthine in plant protection, and its ability to participate in plant cold hardening.

By studying the dynamics of ascorbic acid and the amaranthine content in amaranth leaves of different ages, similar patterns of ascorbic acid and amaranthine levels in young leaves of lateral shoots resulted in a decrease in the content of these antioxidants after the first temperature drop and a tendency to increase after the repeated cold stress (Figures 1 and 2).

Antioxidant pool and their behavior during stress

In the water extracts of old NDC, DC, and YS leaves of amaranth on the second day of the post-stress period, the maximum total content of hydrophilic antioxidants increased, amounting to 1.52 and 1.27 mg GAE g^{-1} FW, which decreased twice in two days (Figure 3). However, a permanent decrease of that value was visible in YB leaves. After the repeated cold drop (October 21), the enhancement of the total content of hydrophilic antioxidants in young leaves (YS and YB) reached 11% and 33%, respectively. In the NDC leaves on the fourth day, the level of ethanol-soluble antioxidants decreased by 1.9 times, while in young and old DC leaves, the total content of antioxidants was virtually unchanged (Figure 4). The total antioxidant content (TAC) increased twice in YS and YB leaves after the repeated cold drop (Figures 3 and 4).

Photosynthetic pigments

During the post-stress period after the first cold drop, the characterization of the comparable content of carotenoids ensued in young leaves (YS and YB) of amaranth (Figure 5). The carotenoid content was 1.3 times higher than the appropriate value for DC leaves in NDC leaves. On the following day of the post-stress period, a minimal decrease in the content of carotenoids was evident in young leaves of amaranth, contrary to the 1.9 times decrease of the carotenoid level in DC leaves. However, the repeated cold drop caused a decline, followed by increased carotenoid contents in YS and YB leaves.

After the first cold phase, chlorophyll *a* showed a 1.5–1.7 times decrease in the non-damaged-by-cold old and young amaranth leaves, while in the damaged leaves, it decreased by 2.1 times (Figure 6). After the repeated cold stress, the inclination of chlorophyll *a* to increase manifested only in YB leaves. A significant decrease in chlorophyll *b* content appeared on the second day of the post-stress period—typical for chlorophyll-containing pigments functioning in non-damaged-by-cold mixed-age leaves. The chlorophyll *b* content showed a 2.7–2.8 times decrease in young leaves, a 2.7 times decline in old non-damaged leaves, and a 3.6 times decrease in DC leaves (Figure 7). Similarly, the chlorophyll *b* content decreased more than chlorophyll *a*. The repeated cold drop revealed a tendency to enhance the chlorophyll *a* content in YB leaves of amaranth. The repeated low-temperature oxidative stress (2 °C) indicated an increase in all studied antioxidants in young leaves; however, it also accelerated the damage in the NDC and DC leaves of the main shoots**,** which caused them to fall.

DISCUSSION

Among heat-loving plants of tropical origin, the amaranth C_4 plant is one of the unique plants showing induced resistance to hypothermia due to its widespread habitat from the tropics to Northern latitudes. By introducing the amaranth in the Non-chernozem Belt of Russia, its growth, development, and productivity were imperfect due to low temperatures. During cold summers, the period from the seed sowing to harvesting may last until mid-October. In autumn, the low, warm daytime temperatures (7 °C–13 °C) alternate with periodic night cold (2 °C), causing oxidative stress in amaranth

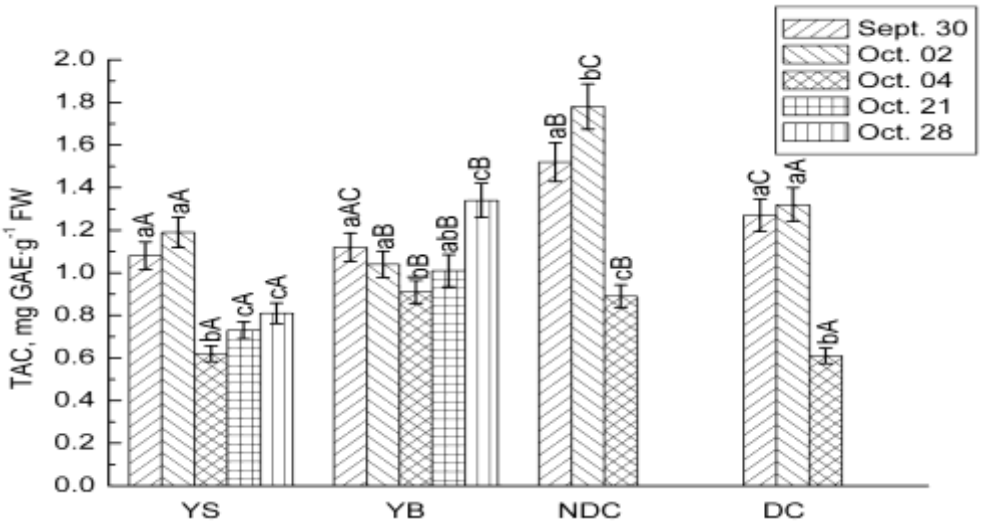

Figure 3. Changes in the hydrophilic AOs content in the post-stress period after the first cold drop on September 30 and after the repeated cold stress on October 21 to 2 °C (means with one or more identical letters in common do not differ significantly from each other; lowercase letters refer to the comparison between different dates and capital letters refer to the comparison between plant leaves for the same date), YS-young small; YB-young big; NDC-non-damaged by cold; and DC-damaged by cold leaves.

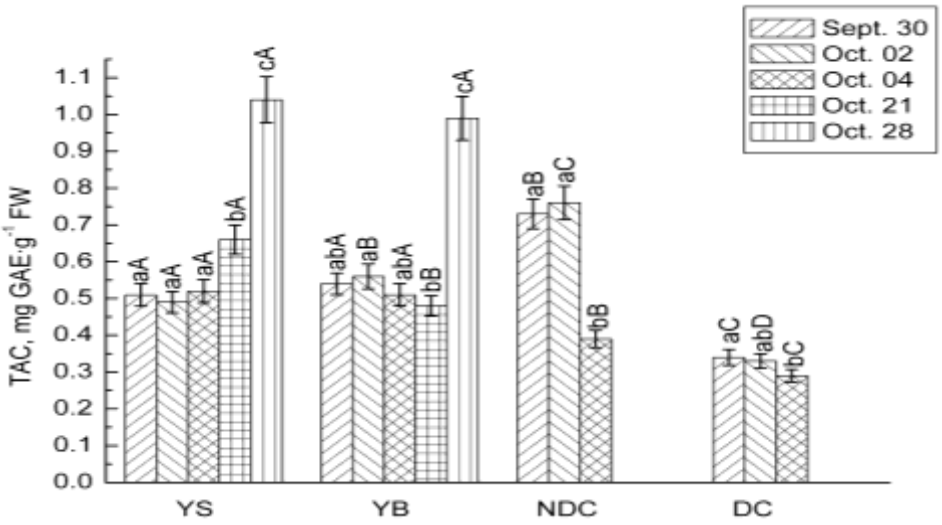

Figure 4. Changes in the hydrophobic AOs content in the post-stress period after the first cold drop on September 30 and after the repeated cold stress on October 21 to 2 °C (means with one or more identical letters in common do not differ significantly from each other; lowercase letters refer to the comparison between different dates and capital letters refer to the comparison between plant leaves for the same date). YS-young small; YB-young big; NDC-non-damaged by cold; and DC-damaged by cold leaves.

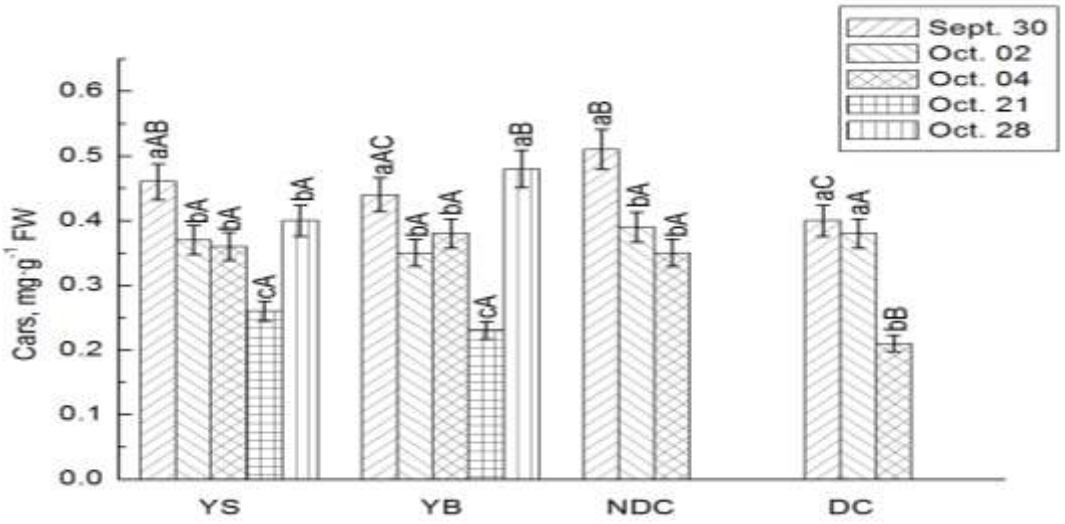

Figure 5. Changes in the carotenoid content in the post-stress period after the first cold drop on September 30 and after the repeated cold stress on October 21 to 2 °C (means with one or more identical letters in common do not differ significantly from each other; lowercase letters refer to the comparison between different dates and capital letters refer to the comparison between plant leaves for the same date). YS-young small; YB-young big; NDC-non-damaged by cold; and DC-damaged by cold leaves.

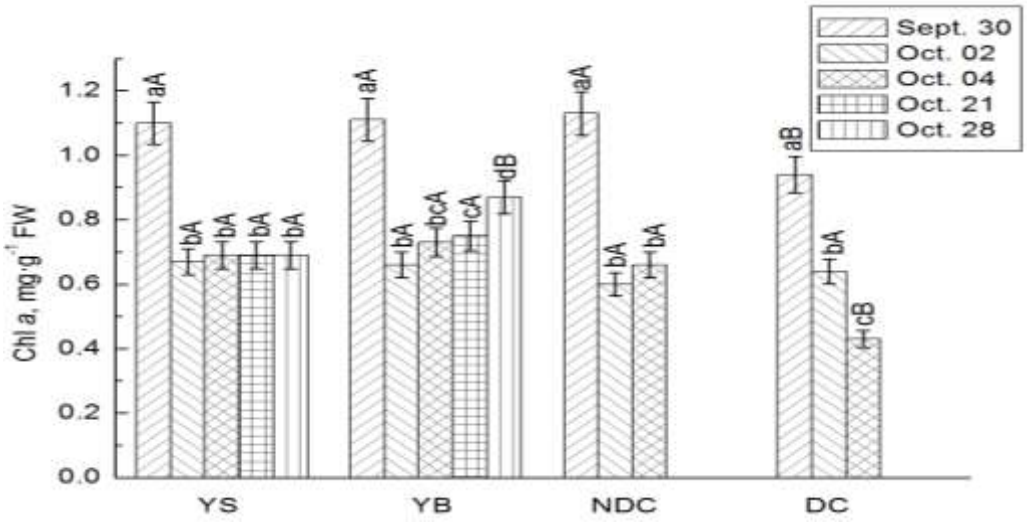

Figure 6. The change in the chlorophyll *a* content in the post-stress period after the first cold drop on September 30 and after the repeated cold stress on October 21 to 2 °C (means with one or more identical letters in common do not differ significantly from each other; lowercase letters refer to the comparison between different dates and capital letters refer to the comparison between plant leaves for the same date). YS-young small; YB-young big; NDC-non-damaged by cold; and DC-damaged by cold leaves.

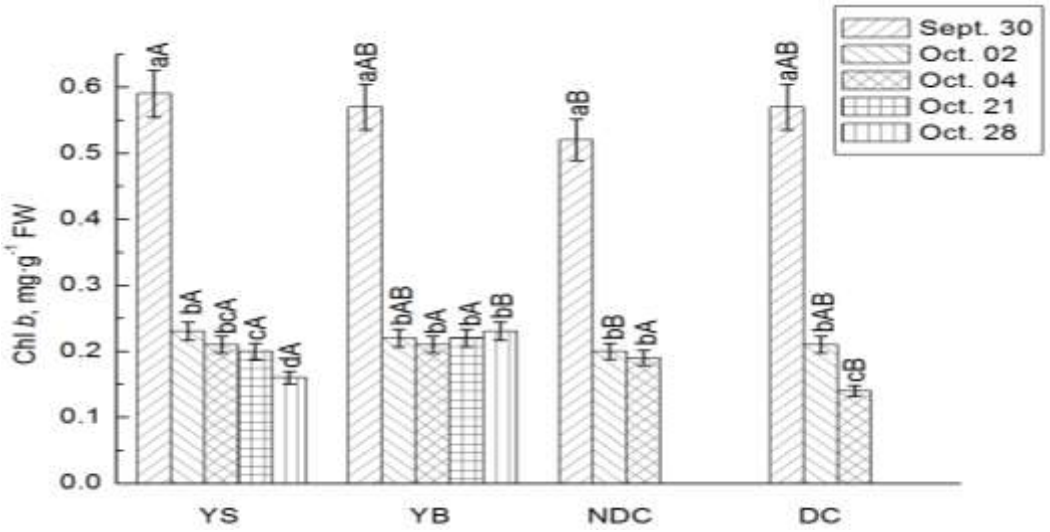

Figure 7. The change in the chlorophyll *b* content in the post-stress period after the first cold drop on September 30 and after the repeated cold stress on October 21 to 2 °C (means with one or more identical letters in common do not differ significantly from each other; lowercase letters refer to the comparison between different dates and capital letters refer to the comparison between plant leaves for the same date). YS-young small; YB-young big; NDC-non-damaged by cold; and DC-damaged by cold leaves.

leaves, disrupting the complete seed maturation process.

The centuries-old history of amaranth cultivation in almost all countries worldwide, with broadly differing climatic conditions, resulted in an active multi-component low-molecular-weight antioxidant protection system capable of rapidly neutralizing ROS in photosynthetic leaves. In this case, the detoxification of singlet oxygen, hydroxyl radical, and superoxide anion-radical molecules occurs with the involvement of ascorbic acid, amaranthine, carotenoids, and antioxidants of phenolic nature**,** which are highly essential components of redox reactions. Ascorbic acid also evidently contributed to the renaissance of the biological components, i.e., phenols, tocopherol, and zeaxanthin (Foyer *et al*., 1994).

In addition to these well-known low-molecular antioxidants**,** which are characteristic of higher plants, the amaranth leaves synthesize a specific red-purple pigment with antioxidant activity—amaranthine—that can participate in the safety of cell components, including chloroplasts and increasing the functional activity of the photosynthetic machinery (Ptushenko *et al*., 2002; Pivovarov *et al*., 2019). During the stability and adaptation study of the amaranth plants under low, humid temperatures in late September–October, it caused damage to the main shoot old leaves with a fully decorated sheet plate, appearing to be the plant's coldest organs.

The results showed that the water status of the upper part of the DC leaf affected by hypothermia decreased dramatically, completing dehydration and irreparable damage and drying of the leaf's upper part, while the underlying part maintained a native appearance. The dehydration of leaf cells under low-temperature stress may refer to cellular structure damage, the disturbance of membrane function, and water release from the cell into extracellular space (Uemura and Steponkus, 1999; Mashaghi *et al*., 2012). The physiologically active lower part of the leaf can link with the acclimation zone and the leaf upper part to the cold damage, although the boundaries of temperature zones can shift, based on the physiological and biochemical circumstances of leaf tissues.

Under cold conditions, biochemical disturbance occurrence can have partial healing if the return to normal temperature takes place before irreversible damage happens. Under repeated cold stress, this reversibility was apparent in non-damaged leaves by studying the dynamics of the antioxidant contents, i.e., ascorbic acid, carotenoids, amaranthine, and the total antioxidant content (Figures 1 to 5). Thus, in the young leaves after the post-stress period, the level of ascorbic acid, amaranthine, carotenoids, and TAC rose, which could also confirm an enhancement in the active oxygen species and serve as an adaptive response to oxidative stress. Low temperatures can enhance the antioxidant defenses, activate the biosynthetic pathways of secondary metabolites, and induce the accumulation of phenolic antioxidant compounds in *Brassica oleracea* (Lee and Oh, 2015; Soengas *et al*., 2018). An assumption was that alternating daytime (8 °C–14 °C) and night (4 °C–7 °C) temperatures induced the resistance in young amaranth leaves. Under these conditions, the functioning of antioxidant protection mechanisms reached activation, which included the additional synthesis of antioxidants, regeneration of oxidized antioxidants, and modification of their structure and properties.

In the plant's protective system, the formation of the antioxidant potential arises from the plant's organ antioxidant systems, and the efficiency of their functioning will determine the resistance of the plant to hypothermia in autumn (Gins *et al*., 2019). Stress-resistant genotypes have an increased capacity of the antioxidant pool compared with sensitive genotypes (Shalata *et al*., 2001; Awasthi *et al*., 2015). In rice, higher activities of antioxidant enzymes (CAT, SOD, and APX) and higher AsA content were notable, which possibly could provide cold tolerance (Huang and Guo, 2005). Plants with high levels of multiple antioxidants compared with one/two antioxidants showed more resistance (Sharma *et al*., 2012). The total antioxidant content is a sensitive indicator of oxidative stress and the accompanying process of membrane lipid degradation (Baikov *et al*., 2012).

In the post-stress period, the efficiency assessment of the antioxidant protection system depended on the total content of hydrophilic and hydrophobic antioxidants, which form a pool of low-molecular antioxidants. The level of water-soluble antioxidants decreased sharply in old NDC and DC leaves, and in YS (almost twice) compared with YB leaves, where the total content of hydrophilic and hydrophobic antioxidants revealed a slight decrease during the studied period (from September 30 to October 4). The old non-damaged leaves of the main shoot initially possessed a larger TAC, suggesting that those leaves might have a higher diversity of antioxidants with immense quantities. With the repeated action of oxidative stress, the amount of TAC increased in the water and ethanol extracts of lateral shoots in young leaves. It indicated that the leaf's resistance to the damaging effects of low, warm temperatures (2 °C) correlated with the ability of a hydrophilic and hydrophobic antioxidant pool to maintain the antioxidant content at an ever-high level at the end of the stress conditions. The post-stress period might be associated with the partial restoration of biochemical damages and the synthesis of low-molecular compounds involved in the cell protective mechanisms.

In low, humid temperatures of autumn, the strategy of the amaranth, a heat-loving plant, focused at the efficiency of young leaves' functioning to protect photosynthesis capability to maintain the leaves and inflorescences growth and produce seeds. The formation of resistance in the photosynthetic active leaves, as the main producers of low-molecular antioxidants under hypothermia conditions displayed close association with the increased efficiency of hydrophilic and hydrophobic antioxidants, i.e., their ability to deactivate reactive oxygen species and regenerate oxidized antioxidants.

In the post-stress period, the total content of cytoplasmic water-soluble antioxidants surpassed the amount of hydrophobic antioxidants in the young amaranth leaves. It further validates that the initiation of oxidative processes was more active in the cytosol. After repeated cold

stress, the number of ethanol-soluble antioxidants increased significantly**,** which may indicate an enhancement in the hydrolytic processes of the cell structural components. It serves as a precondition for a significant accumulation of metabolites providing a complex of the cell metabolism adaptive adjustment**,** increasing the plant stability. The cold acclimation of the amaranth leaves between the first and second cold drops induced an increase in the content of low-molecular-weight antioxidants in young leaves (Figures 1–6). The higher resistance of amaranth young leaves lateral shoots may have a connection with an increased pool of amaranthine, ascorbic acid, carotenoids, and other low-molecular metabolites with antioxidant properties.

An increased ability to synthesize and accumulate ascorbic acid and amaranthine and the total content of water and ethanol-soluble antioxidants in young leaves compared with old leaves indicate different adaptations in the leaves contributing to the survival of plants exposed to hypothermia. The unique ability of the leaves to form antioxidant defense systems via low-molecular-weight metabolites participating in the cell oxidative–restorative reactions can succeed due to an increase in reducing sugars, amino acids, and organic acids (Gins *et al*., 2019; Morelli *et al*., 2003). The temperature-dependent modulation and the modification of metabolites of different chemical natures allow the tissues to obtain an additional number of osmoprotectants and antioxidants.

CONCLUSIONS

The comparative analysis of the temporal sequence of variation in antioxidants and photosynthetic pigments in mixed-age leaves served as a means of developing the mechanisms of the plant leaf adaptations to hypothermia. The contributions of the individual low-molecular-weight metabolites with antioxidant activity to the cell protection system at various stages of hypothermia varied. At the initial stage of the post-stress period, water-soluble antioxidants formed a high level of protection, which gradually reduced with the constant effect of lowered temperatures. During the same period, the young leaves hardened, and after repeated exposure to cold stress, a significant increase in the total content of water-soluble and especially, ethanol-soluble antioxidants was evident. It ultimately led to an increase in the indicator that is their quotient, i.e., $TAC_{ethanol\text{-}soluble}/TAC_{water\text{-}soluble}$, possibly due to partial hydrolysis of cellular structures and the release of the related hydrophobic antioxidants. The adaptability of the amaranth plants to hypothermia implied an association with increased resistance of young leaves, which formed by the restructuring of photosynthetic and metabolic reactions due to the synthesis of cold-resistant compounds with antioxidant properties.

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