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The effect of hypothermia on the state of the prooxidant-antioxidant system of plants

Mariia Bobrova *
Olena Holodaieva **
Svitlana Koval ***
Olha Tsviakh ****
Olena Kucher *****

ABSTRACT

Aim of the research: to identify changes in the value of indicators of the state of the prooxidant-antioxidant system (PAS) in the tissues of edible parts of agricultural plants under the influence of temperature changes. Methodology: Quantitative determination of indicators of the state of PAS was performed on tissue samples of edible parts of the following plants: *Solánum lycopérsicum L.*, *Cucumis sativus L.*, *Capsicum annuum L.*, *Solanum melongena L.*, *Solanum tuberosum L.*, *Allium sativum L.*, *Allium cepa L.*, *Daucus carota L.*, *Beta vulgaris L.*, *Cucurbita pepo var. Giraumontia L.* The concentration of superoxide anion radical ($\bullet O_2^-$), TBA-active products, superoxide dismutase (SOD) activity, catalase, the concentration of ascorbic acid (AA), glutathione (GSH) were determined. The **results** of the research show that hypothermia activates both parts of the PAS, however, cooling is accompanied by more powerful both low molecular weight and enzymatic antioxidant (AO) protection. The research of AO can be divided according to the degree of reduction of the protective value in hypothermia in the following series: SOD, catalase, GSH, AA. The most resistant in terms of changes in PAS to hypothermia is *Solanum tuberosum L.*, *Allium sativum L.*, *Beta vulgaris L.*; the least resistant is *Capsicum annuum L.*, and *Solánum lycopérsicum L.* The generative organs of plants are less resistant to hypothermia than the vegetative ones. Practical consequences. As a result of the conducted biochemical analysis, it is established which method of storage of plant products is more effective in terms of preservation of AO activity: cooling or freezing.

KEYWORDS: biochemistry; metabolism; enzymes; vitamins; cooling, freezing.

* Senior Lecturer of the Department of Biology and Methods of Teaching of the Volodymyr Vynnychenko Central Ukrainian State Pedagogical University, Ukraine. E-mail: mails@kspu.kr.ua; kazna4eeva@gmail.com ORCID ID: http://orcid.org/0000-0001-7703-651X.

** Associate Professor of the Department of General and Biological chemistry #2 Donetsk national medical university, Ukraine. E-mail: contact@dsmu.edu.ua; elena.gologaeva@gmail.com ORCID ID: http://orcid.org/0000-0002-4922-7033.

*** Senior Lecturer of the Department of the Fundamental Disciplines of the International European University, Ukraine. E-mail: admissions@ieu.com.ua; kovalsyu@gmail.com ORCID ID: http://orcid.org/0000-0002-4907-177X.

**** Senior Lecturer of the Department of Chemistry of the V.O. Sukhomlynskyi Mykolaiv National University, Ukraine. E-mail: office@mdu.edu.ua; tsvyakho@gmail.com ORCID ID: http://orcid.org/0000-0002-1119-2170.

***** Senior Lecturer of the Department of Chemistry of the V.O. Sukhomlynskyi Mykolaiv National University, Ukraine. E-mail: office@mdu.edu.ua; hrizantema84.84@gmail.com. ORCID ID: http://orcid.org/0000-0002-9963-6855.

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El efecto de la hipotermia sobre el estado del sistema prooxidante-antioxidante de las plantas

RESUMEN

Objetivo de la investigación: identificar cambios en el valor de los indicadores del estado del sistema prooxidante-antioxidante (PAS) en los tejidos de partes comestibles de plantas agrícolas, bajo la influencia de cambios de temperatura. Metodología: la determinación cuantitativa de indicadores del estado de PAS se realizó en muestras de tejido de partes comestibles de las siguientes plantas: Solánum lycopérsicum L., Cucumis sativus L., Capsicum annuum L., Solanum melongena L., Solanum tuberosum L., Allium sativum L ., Allium cepa L., Daucus carota L., Beta vulgaris L., Cucurbita pepo var. Giraumontia L. Se determinó la concentración de radical anión superóxido (● O2-), productos activos TBA, actividad superóxido dismutasa (SOD), catalasa, concentración de ácido ascórbico (AA), glutatión (GSH). Los resultados de la investigación muestran que la hipotermia activa ambas partes del PAS. Sin embargo, el enfriamiento va acompañado de una protección antioxidante enzimática (AO) y de bajo peso molecular más potente. La investigación de AO se puede dividir según el grado de reducción del valor protector en hipotermia en las siguientes series: SOD, catalasa, GSH, AA. El más resistente en términos de cambios en PAS a hipotermia es Solanum tuberosum L., Allium sativum L., Beta vulgaris L .; el menos resistente es Capsicum annuum L. y Solánum lycopérsicum L. Los órganos generativos de las plantas son menos resistentes a la hipotermia que los vegetativos. Consecuencias prácticas. Como resultado del análisis bioquímico realizado, se establece qué método de almacenamiento de productos vegetales es más efectivo en términos de conservación de la actividad AO: enfriamiento o congelación.

PALABRAS CLAVE: bioquímica; metabolismo; enzimas; vitaminas; enfriamiento; congelación.

Introduction

The influence of temperature on the vital activity of plant organisms is one of the key problems of adaptive physiology. The significance of the problems of cold and frost resistance of plants is because 64% of the land area of the plant indicates the detrimental effect of low temperatures. The connection with global climate change on the planet, the urgency of the problem is growing, as caused by anthropogenic factors, the total loss is provided by increasing instability of weather and climatic conditions, while different temperature differences over relatively short periods of time (Kolupayev & Trunova, 1992; Kolupaev & Karpets, 2010; Kolupaev, 2001; Estela Urbina et al. 2020). A lot of work is devoted to the

study of temperature changes in the viscosity of membranes and cytosol, the activity of metabolic processes. However, the biochemical basis of the effect of temperature on plant homeostasis is investigated in fragments. Listening that the action of any stressors is reflected in the state of the PAS understudy, the problem determines the increased relevance. Since plants are the main source of AO to humans, the study of changes in their number under the action of different storage temperatures is of great practical importance.

Aim of the research: to identify changes in the value of indicators of the state of the PAS in the tissues of edible parts of agricultural plants under the influence of temperature changes. To achieve this aim, the following tasks were identified:

1. To investigate the effect of temperature changes on the content of enzymatic and low molecular weight antioxidants in plant tissues.

2. To identify the effect of temperature changes on the content of prooxidants in plant tissues.

3. To determine which of the antioxidants is more resistant to temperature changes.

4. To compare the resistance of plants to hypothermia in terms of changes in prooxidant and antioxidant activity in their tissues

5. To compare which method of storage of plant products is more effective in terms of preserving antioxidant activity: cooling or freezing.

1. Literature review

The first experimental results linking the state of PAS with temperature changes were obtained in the '80-'90s. XX century. The influence of temperature as a stress factor is described in numerous works by Kolupaeva Y.E., who reveals both the mechanisms of temperature influence on physiological processes of the plant organism and the peculiarities of metabolism under temperature stress (Kolupayev & Trunova, 1992), the formation of temperature adaptations (Kolupaev & Karpets, 2010), and even molecular-cellular level of stress reactions of plants (Kolupaev, 2001). Peculiarities of plant perception of the cold signal are revealed in the works of Himalov F.R., Chemeris A.V., Vakhitova V.A (Himalov et al., 2004). Cellular mechanisms of adaptation to adverse environmental factors are given in the works of Kordyum E.L., Sytnyk K.M., Baranenko V.V. (Kordyum et al., 2003). The role of proteins in low-temperature stress is given in the work of Kolesnichenko A.V., and

Voynikova V.K. (Kolesnichenko & Voinikov, 2003). Kolupaev Yu.Ye., also describes the importance of prooxidants, namely the reactive forms of oxygen (ROS) in the adaptation of plants to stressors (Kolupaev & Karpets, 2009; Kolupaev, 2007). Lukatkina A.S., combines oxidative stress with cold damage to plants (Lukatkina, 2002).

The relationship between temperature stressors and reactive oxygen species has been revealed in several works by foreign scientists. Thus, Bhattacharjee S., studied ROS and oxidative explosion in stress signal transduction (Bhattacharjee, 2005), which was also the subject of research by Apel K. and Hirt N., (Apel & Hirt, 2004), while Dat J.F., Vandenabeele S., and Vranova E., considered ROS under stress resistance (Dat et al., 2000). The Biochemical School under the direction of Nikolas Smirnoff has significant work on the biochemistry of ROS and AO in plant tissues (Smirnoff, 2005), a number of works by Scandalios J.G. devoted to problems in the same direction (Scandalios, 2002; Scandalios, 2005). In the works of Voynikov V.K., an increase in the amount of H_2O_2 and TBA-active products (TBA_{ap}) in plant tissues under the action of negative temperatures has been described (Voynikov, 2013). Piotrovsky M.S., Shevyreva T.A., Zhestkova I.M., Trofimova M.S., emphasize that the most sensitive to low-temperature stress are the processes of respiration and photosynthesis, as hypothermia causes primarily a change in the viscosity of cell membranes and dysfunction of electron transport chains with the formation of ROS (Piotrovskii et al., 2011). Common in these two works is the idea of the relationship of NADPH with hypothermic products of ROS. Awasthi R., Bhandari K., and Nayyar H., prove the formation of ROS by low-temperature activation of NADPH oxidase (Awasthi et al., 2015).

The role of the AO system in hypothermia is noted in (Berwal & Ram, 2018; Foyer & Noctor, 2005; Gill & Tuteja, 2010; Hasanuzzaman et al., 2017; Hasanuzzaman et al., 2019; Pacheco et al., 2018; Shao et al., 2008; Suzuki et al., 2012; Szalai et al., 2009), analyzing which we can conclude that almost all known enzymatic AO is involved in protecting plants from hypothermia and adaptation to it. The first link in AO protection is SOD. It is proved that the action of low temperatures not only changes the activity of SOD, but also changes the expression of genes responsible for its synthesis. For example, the increase in SOD activity has been experimentally proven in wheat (Kolupaev & Karpets, 2019; Dyachenko et al., 2007; Major et al., 2011), oats (Awasthi et al., 2015), strawberries (Luo et al., 2011), cucumber

(Ignatenko et al., 2016), potatoes, but some plants reduce the activity of SOD at low temperatures, or increase the activity of SOD in the post-stress period (Kolupaev & Karpets, 2019). The closest connection with SOD is catalase, the level of activity and gene expression of which is also enhanced by hypothermia. The work of a number of scientists experimentally proved the enhancement of catalase activity in oats and wheat, potatoes, echinacea, chrysanthemums (Kolupaev & Karpets, 2019; Janda et al., 2007). The most common low molecular weight AO is ascorbic acid (AA) (Bobrova et al., 2020). The action of low temperatures ambiguously affects the content of AA in plant tissues. For example, a number of scientists experimentally prove an increase in the content of AA in the tissues of rye (Galiba et al., 2013), barley (Huang & Guo, 2005), chickpeas (Kumar et al., 2011). However, the works of Luo Y., Tang H., and Zhang Y., show a lower content of AA in cold-resistant varieties of strawberries, compared with unstable, which indicates the species and even variety-specific role of AA. A number of scientists describe the role of AA not only during an injury but also in post-stress regeneration (Radyuk et al., 2009). The same scientists experimentally confirmed the ambiguous role of reduced glutathione in hypothermia. Because GSH has the highest correlation with AA among low molecular weight antioxidants, there is a need for further experimental studies.

2. Research methodology

Quantitative determination of PAS status was performed on tissue samples of edible parts of the following plants: *Solánum lycopérsicum L.*, *Cucumis sativus L.*, *Capsicum annuum L.*, *Solanum melongena L.*, *Solanum tuberosum L.*, *Allium sativum L.*, *Allium cepa L.*, *Daucus carota L.*, *Beta vulgaris L.*, *Cucurbita pepo var. Giraumontia L.* Exposure of the control group was carried out at 18^0C, the first experimental group was in conditions of 4^0C, the second experimental group underwent rapid freezing to -20^0C. The duration of exposure of each group is 1 month. Each experimental group included 10 samples of 10 plants of each species, so the experiment analyzed 1800 samples

Evaluation of the level and sources of ROS *generation* was performed by spectrophotometric NBT test. For analysis, 0.1 g of tissue was homogenized with glass sand in 0.9 cm^3 of phosphate buffer (pH = 7.4, composition per 1 dm^3 of a solution – 5.37 g of $KH_2PO_4 \cdot 12\ H_2O$, 8.5 g of NaCl, 1.5 g NaOH). 0.05 cm^3 of homogenate was taken, 0.05 cm^3 of buffer solution

was added (to determine the total main unstimulated activity). The samples were shaken for 2 min, added to each of 0.05 cm^3 NBT, stirred, incubated in a thermostat at 24^0C. After 30 minutes 2 cm^3 of solvent (dimethyl sulfoxide-chloroform in a volume ratio of 2:1) was added, shaken for 1 minute, and centrifuged for 5 minutes at 1500 rpm. From the obtained centrifugal, a colored supernatant was taken and photometered against the appropriate control at 540 nm on a microphotoelectrocolorimeter in a 1 cm^3 cuvette 0.5 cm thick.

To prepare the reagent control, the following solutions were poured into three tubes: 0.05 cm^3 of a buffer, 0.1 cm^3 of water, 0.05 cm^3 of NBT and were incubated min in a thermostat at 24^0C and eluted color. To build a standard calibration graph in test tubes typed 0.01, 0.02, 0.05, 0.07, 0.1, 0.2 cm^3 NBT (w = 0.2%), 0.1 cm^3 KOH (C (KOH) = 1 mol /dm^3) and 0.1 cm^3 of AK solution (18 mg /10 cm^3), stirred and incubated for 10 min at 24 ^{0}C. The color of 2 cm^3 of the solvent was eluted, the extinction (E) of each sample was determined and a calibration graph was plotted. According to the schedule, superoxide production was found in nmol per sample (n nmol ●O_2^-) and translated into nmol per g of tissue per second of incubation.

Assessment of the level of free radical peroxidation (FRPO) was carried out by the concentration of TBA-active products (TBA_{ap}). Analysis of the level of TBA_{ap} was carried out in the following sequence: 0.5 g of tissue was homogenized in 4.5 cm^3 of buffer solution (pH = 7.4, preparation: 1.9 g of tris-(oxy)-methylaminomethane was placed in a volumetric flask per 1 l with 0,5 l of distilled water, added 50 cm^3 of a solution of HCl (C (HCl) = 0.1 mol/dm^3), 1.4 g of ascorbic acid, 32 mg of $FeSO_4{\cdot}7H_2O$ in the specified order, after dissolving the previous component, added water below the mark; the finished solution was left for a day to adjust the pH, as evidenced by the change in its color from blue-violet to yellow). To determine the basic level of TBA_{ap} (TBA_{ap0}) to 2 cm^3 of the selected homogenate was immediately added a solution of trichloroacetic acid (w = 30%) and centrifuged for 30 minutes at 3000 rpm. To 2 cm^3 of centrifugate was added 3 cm^3 of thiobarbituric acid solution (w = 0.338%, extempore preparation, incubation in a water bath at 80^0 C until the reagent dissolved, and another 50 min in a boiling water bath) followed by photometry of the formed trimethine complex at 540 nm against the control, which did not contain homogenate (control composition for reagents: 1.2 cm^3 of buffer solution, 0.7 cm^3 of trichloroacetic acid, 0.1 cm^3 of water and 3 cm^3 of TBA reagent). To initiate an increase in the level of TBA_{ap} ($TBA_{ap1,5}$), the sample was pre-incubated for 90 minutes (1.5 hours, therefore $MDA_{1,5}$) in prooxidant iron-ascorbate buffer,

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shaking every 20 minutes. Further analysis was performed similarly to the determination of TBA_{ap0}. The calculations were carried out according to the formula:

$C = E \cdot 240.4$

where C is the concentration of TBA_{ap} in μmol/kg;

E – extinction;

240.4 – coefficient taking into account molar extinction and dilution.

The magnitude of the increase in the level of TBA_{ap}, which is inversely proportional to the antioxidant supply of tissue, was calculated according to the formula:

$\Delta\ TBA_{ap} = |\ TBA_{ap1,5} - TBA_{ap0}\ |\ /\ TBA_{ap0} \cdot 100\%$

where $\Delta\ TBA_{ap}$ – increase in the level of TBA_{ap}, expressed as a percentage;

TBA_{ap0}, $TBA_{ap1,5}$ – basic and stimulated levels of TBA_{ap} in μmol/kg, respectively.

The level of antioxidant protection was assessed by the activity of enzymatic and the concentration of non-enzymatic antioxidants. To determine the activity of SOD 0.5 g of tissue was homogenized in 0.5 cm^3 of water after 10 minutes added 2 cm^3 of pigment precipitant (ethanol chloroform in a volume ratio of 5: 3), stirred with a glass rod, and kept at -4^0 C day. Then stirred and centrifuged at 3000 rpm 15 min. Control (average for several determinations before, in the middle, and at the end of a series of experimental samples): in a cuvette with an optical path length of 1 cm scored successively 4.4 cm^3 of carbonate buffer solution (C = 0.2 mol/dm^3; pH = 10.2, for the preparation of which in 1 dm^3 of distilled water was dissolved 4.5 g of anhydrous sodium bicarbonate and 9.5 g of decahydrate sodium carbonate), 0.1 cm^3 of distilled water (to establish the optical zero) and added 0.5 cm^3 of adrenaline solution (C = 0.01 mol/dm^3) in citric acid (C = 0.01 mol /dm^3). Turn on the stopwatch, stir with a glass rod, and note the extinction every minute until it stops increasing. Instead of water, 0.1 cm^3 of the centrifuge was introduced into the experimental sample, followed by similar procedures. Temperature range 23-27^{0}C.

The calculation of SOD activity was carried out according to the formula:

$T = (E_1 - E_2) \cdot 100 / E_1$

T is the percentage of inhibition of oxidation of $\bullet O_2^-$ adrenaline to adrenochrome (%);

E_1 – average extinction control for 1 min (E/t);

E_2 – average extinction of the experiment for 1 min;

100 – the maximum percentage (%) of inhibition.

SOD activity was expressed in conventional units (OD):

OD = T / (100 – t)

where 1 OD corresponds to the inhibition of the reaction rate by 50%.

To determine the activity of catalase: 0.1 g of tissue was homogenized in 20 cm^3 of distilled water. 7 cm^3 of distilled water was taken from the flasks, then 1 cm^3 of homogenate was added to the experimental sample, and 1 cm^3 of boiled homogenate was added to the control sample, in which the enzyme was thermally destroyed. To both samples was added 2 cm^3 of hydrogen peroxide (w = 1%), stirred, and left at room temperature for 30 minutes, shaking every 10 minutes. Then 3 cm^3 of sulfuric acid solution (w = 10%) was added to both samples and titrated with potassium permanganate solution (C (1 /5$KMnO_4$) = 0.1 mol/dm^3) to a pale pink color that does not disappear within 30 seconds. The calculation of catalase activity was carried out by the formula:

$A = (V_{control} - V_{experimental}) \cdot 1.7$

A – catalase number;

Vcontrol – volume of solution $KMnO_4$ (C (1/5 $KMnO_4$) = 0,1 mol /dm^3), spent on titration of the control sample, cm^3;

Vexperimental – the volume of solution $KMnO_4$ (C (1/5 $KMnO_4$) = 0,1 mol /dm^3), spent on titration of the experimental sample, cm^3;

1.7 – amount of H_2O_2 (mg), which corresponds to 1 cm^3 of $KMnO_4$ solution (C($KMnO_4$) = 0.002 mol/dm^3).

Used the international unit of activity (μmol of substrate per unit time per unit mass of protein), which was calculated by the formula:

$A = (V_{control} - V_{experimental}) \cdot 1.7/t \cdot M(H_2O_2)$

t – incubation time of the sample (30 s);

M (H_2O_2) - molar mass of H_2O_2 (34 g/mol).

Determination of GSH concentration was performed in the following order: 0.1 g of tissue was homogenized with 2.4 cm^3 of trichloroacetic acid solution. After 10 minutes samples were centrifuged for 15 min at 3000 rpm, 0.2 cm^3 of the centrifuge was taken, 0.05 cm^3 of NaOH solution (w = 20%) and 5 cm^3 of Tris-buffer were added, for preparation at 1 dm^3 used 6.06 g of Tris-oxymethylaminomethane, 14.85 g of EDTA for binding of divalent cations and

275 ml of HCl, C (HCl) = 0.1 mol/dm^3). The pH of the sample was checked and, if necessary, the pH was adjusted to 8.0-8.1 with weak solutions of HCl or NaOH (because at pH <8 the reaction is almost non-existent, and at pH>8.1 DTNBK hydrolyzes to thionitrophenyl anion, which overestimates the analysis results). Then added 0.1 cm^3 of Elman's reagent (99 mg of DTNBK in 25 cm^3 of ethanol). Stirred and kept for 20 minutes in the dark. Photometered at 412 nm in a cuvette at 1 cm against control for reagents that did not contain homogenate. The calculation of the analysis results was performed according to the standard calibration schedule.

Determination of the concentration of AA was carried out by direct titrimetry. To do this, in a porcelain mortar 1 g of the test material was thoroughly ground with quartz sand. To the obtained homogenate was added 9 cm^3 of HCl solution (w = 2%), defended for 10 minutes and filtered. For quantification, 3 cm^3 of the filtrate was taken (test sample), added to the flasks, and titrated with a solution of 2.6-dichlorophenolindophenol (C (1/2 2.6 – DFIF) = 0.001 mol / dm^3) until a pink color appeared which persisted for 30 s. To control the reagents, 3 cm^3 of the filtrate was boiled with 3 drops of 3% H_2O_2, followed by titration. The calculation of the content of AA was carried out according to the formula:

$$C = Q \cdot (A_{exp} - A_{contr}) \cdot V_0 / (V_1 \cdot a)$$

C – AA content, mmol/kg;

Q is the amount of ascorbic acid, which corresponds to 1 cm^3 of a solution of 2.6-dichlorophenolindophenol (C (1/2 2.6-DFIF) = 0.001 mol/dm^3) (0.088 mg);

V_0 – total amount of extract, cm3;

V_1 – the volume of extract taken for titration, cm3;

a – the amount of test substance, g;

Acontr, Aexp - volume of solution of 2,6-dichlorophenolindophenol spent on titration of control and experimental sample, cm^3 (C (1/2 2.6 DFIF) = 0.001 mol/dm^3) (Bobrova et al., 2020).

The results obtained by us have undergone mathematical and statistical processing.

3. Results and discussion

The results of determining the antioxidant activity in the control group are shown in table 1, prooxidant - in table 2.

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Table 1. The results of determining the antioxidant activity in the control group of plants

Experimental plants	Enzyme antioxidants		Low molecular weight antioxidants	
	Catalase activity, mkmol/kg●min	SOD activity, OD	AA content, mmol/kg	GSH content, mmol/kg
Solanum tuberosum L.	46,8 ± 4,04	1,08 ± 0,01	0,28 ± 0,02	34,31 ± 2,78
Daucus carota L.	31,4 ± 2,65	1,25 ± 0,05	0,44 ± 0,01	41,98 ± 0,28
Allium cepa L.	41,4 ± 1,34	1,54 ± 0,06	0,86 ± 0,03	40,31 ± 3,39
Allium sativum L.	20,4 ± 2,22	1,72 ± 0,07	0,08 ± 0,01	48,53 ± 2,54
Cucurbita pepo var. Giraumontia L	127,0 ± 6,09	5,12 ± 0,08	0,13 ± 0,01	28,65 ± 3,19
Beta vulgaris L	115,8 ± 3,63	1,94 ± 0,03	0,76 ± 0,01	39,11 ± 2,34
Solanum melongena L.	152,0 ± 8,87	3,58 ± 0,06	0,83 ± 0,01	40,01 ± 1,56
Capsicum annuum L.	25,0 ± 1,64	5,47 ± 0,19	1,29 ± 0,02	36,36 ± 1,38
Solánum lycopérsicum L.	23,8 ± 3,41	3,94 ± 0,11	0,79 ± 0,03	38,05 ± 1,13
Cucumis sativus L.	135,2 ± 1,67	6,02 ± 0,14	0,09 ± 0,01	22,87 ± 3,15

Table 2. The results of determining the prooxidant activity in the control group of plants

Experimental plants	Indicators of prooxidant activity	
	NBT test (unstimulated activity), nmol ●O_2^-/g●sec	Δ TBA_{ap}, %
Solanum tuberosum L.	6,22 ± 0,04	32,11 ± 2,01
Daucus carota L.	0,083 ± 0,004	48,68 ± 3,67
Allium cepa L.	10,99±0,22	21,45 ± 2,32
Allium sativum L.	20,4 ± 1,19	18,72 ± 1,07
Cucurbita pepo var. Giraumontia L	18,0 ± 1,09	21,12 ± 0,08
Beta vulgaris L	4,81 ± 0,63	12,94 ± 0,43
Solanum melongena L.	54,02 ± 2,87	28,58 ± 1,06
Capsicum annuum L.	15,0 ± 1,64	15,47 ± 1,19
Solánum lycopérsicum L.	59,14 ± 2,41	54,12 ± 2,11
Cucumis sativus L.	22,06 ± 0,28	25,51 ± 4,04

Cucumis sativus L., is a false fruit, which is formed not only from the walls of the ovary but also from parts of the sepals and perianth. Given that people consume the fruits of *Cucumis sativus L.* when they reach consumer maturity, rather than physiological, they continue the processes of photosynthesis, active cell division, and growth, as well as seed formation, which requires a powerful system of AO protection. Since only *Cucumis sativus L.* actively

photosynthesizes among all experimental parts of plants, we experimentally observe the highest level of superoxide in its tissues, but the relatively low value of ΔTBA_{ap} is explained by the high AO potential. Thus, *Cucumis sativus L.*, has the highest SOD activity and one of the highest levels of catalase, but the concentration of low molecular weight AO is one of the lowest, which leads to the conclusion that in the tissues of *Cucumis sativus L.*, the enzymatic line of AO protection prevails. A decrease in temperature leads to a decrease in the content of AA and GSH, but the activity of enzymatic antioxidants increases (tables 3, 5). The level of prooxidant activity also increases, which is confirmed by the results of the experiment (tables 4, 6). A possible explanation for this is hypothermic inhibition of photosynthetic activity and growth processes. Because the effect of temperature is of primary importance on membrane enzymes and processes associated with membrane transport, in the tissues of *Cucumis sativus L.*, both photosynthesis (effect on ETC of chloroplasts) and growth (effect on ETC of mitochondria - the main energy suppliers during active growth) are inhibited. and differentiation). Evidence of a powerful system of antioxidant protection is a decrease in the level of ΔTBA_{ap} with an increasing generation of superoxide.

Table 3. The results of determining the antioxidant activity in the experimental group of plants, under the action of hypothermia to 4^0C (cooling)

Experimental plants	Enzyme antioxidants		Low molecular weight antioxidants	
	Catalase activity, mkmol/kg●min	SOD activity, OD	AA content, mmol/kg	GSH content, mmol/kg
Solanum tuberosum L.	95,2 ± 3,53	10,04 ± 1,00	0,26 ± 0,01	42,11 ± 1,76
Daucus carota L.	100,4 ± 3,63	1,31 ± 0,01	1,14 ± 0,01	55,28 ± 1,73
Allium cepa L.	52,2 ± 2,89	2,14 ± 0,02	0,59 ± 0,02	52,44 ± 1,42
Allium sativum L.	36,0 ± 2,62	5,08 ± 0,11	0,26 ± 0,01	68,12 ± 2,96
Cucurbita pepo var. Giraumontia L	130,0 ± 6,18	6,04 ± 0,09	0,26 ± 0,01	36,09 ± 1,08
Beta vulgaris L	137,0 ± 7,03	2,76 ± 0,08	0,81 ± 0,02	51,23 ± 2,05
Solanum melongena L.	185,0 ± 3,24	5,19 ± 0,11	0,07 ± 0,01	41,94 ± 0,79
Capsicum annuum L.	35,2 ± 2,44	6,38 ± 0,09	0,24 ± 0,01	29,01 ± 2,03
Solánum lycopérsicum L.	28,0 ± 3,11	4,62 ± 0,32	0,58 ± 0,02	30,56 ± 1,94
Cucumis sativus L.	143,0 ± 2,74	7,11 ± 0,06	0,07 ± 0,01	20,11 ± 1,08

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Table 4. The results of determining the prooxidant activity in the experimental group of plants, under the action of hypothermia to 4^{0}C (cooling)

Experimental plants	Indicators of prooxidant activity	
	NBT test (unstimulated activity), nmol ●O_2^- /g●sec	Δ TBAap, %
Solanum tuberosum L.	10,04 ± 0,98	35,76 ± 1,01
Daucus carota L.	0,098 ± 0,001	39,09 ± 1,21
Allium cepa L.	18,64 ± 1,34	29,48 ± 1,09
Allium sativum L.	22,9 ± 1,22	20,04 ± 1,00
Cucurbita pepo var. Giraumontia L	31,06 ± 2,04	25,12 ± 0,08
Beta vulgaris L	5,43 ± 0,06	17,06 ± 0,18
Solanum melongena L.	59,58 ± 1,84	32,23 ± 1,08
Capsicum annuum L.	33,1 ± 1,23	60,33 ± 3,14
Solánum lycopérsicum L.	66,14 ± 1,98	58,13 ± 2,11
Cucumis sativus L.	28,54 ± 0,16	23,12 ± 3,18

Table 5. The results of determining the antioxidant activity in the experimental group of plants, under the action of hypothermia to -20^{0}C (freezing)

Experimental plants	Enzyme antioxidants		Low molecular weight antioxidants	
	Catalase activity, mkmol/kg●min	SOD activity, OD	AA content, mmol/kg	GSH content, mmol/kg
Solanum tuberosum L.	114,6 ± 2,39	4,01 ± 0,94	0,22 ± 0,02	49,11 ± 2,31
Daucus carota L.	34,0 ± 1,94	1,39 ± 0,03	0,95 ± 0,03	51,14 ± 2,04
Allium cepa L.	49,6 ± 1,39	1,99 ± 0,19	0,78 ± 0,19	46,13 ± 1,16
Allium sativum L.	31,0 ± 3,46	1,99 ± 0,04	0,09 ± 0,01	53,25 ± 1,12
Cucurbita pepo var. Giraumontia L	128,0 ± 8,28	7,02 ± 0,12	0,16 ± 0,02	33,06 ± 0,98
Beta vulgaris L	124,0 ± 6,89	2,53 ± 0,18	0,88 ± 0,02	49,16 ± 2,14
Solanum melongena L.	171,0 ± 7,77	4,18 ± 0,12	0,07 ± 0,01	41,09 ± 0,76
Capsicum annuum L.	33,6 ± 2,68	8,38 ± 0,79	0,95 ± 0,02	31,34 ± 0,82
Solánum lycopérsicum L.	25,2 ± 2,74	4,11 ± 0,21	0,67 ± 0,02	34,09 ± 1,02
Cucumis sativus L.	137,4 ± 2,24	8,12 ± 0,04	0,08 ± 0,01	18,56 ± 1,22

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Table 6. The results of determining the prooxidant activity in the experimental group of plants, under the action of hypothermia to -20^{0}C (freezing)

Experimental plants	Indicators of prooxidant activity	
	NBT test (unstimulated activity), nmol ●O_2^- /g●sec	Δ TBAap, %
Solanum tuberosum L.	11,11 ± 1,04	35,84 ± 0,88
Daucus carota L.	0,111 ± 0,008	42,04 ± 3,03
Allium cepa L.	39,56 ± 1,99	46,67 ± 2,06
Allium sativum L.	28,1 ± 1,84	21,23 ± 0,99
Cucurbita pepo var. Giraumontia L	27,0 ± 2,09	23,12 ± 0,08
Beta vulgaris L	5,87 ± 0,22	18,14 ± 0,21
Solanum melongena L.	61,02 ± 2,75	30,52 ± 1,12
Capsicum annuum L.	46,1 ± 3,04	37,1 ± 1,12
Solánum lycopérsicum L.	69,89 ± 3,21	69,94 ± 2,11
Cucumis sativus L.	25,96 ± 0,87	20,23 ± 2,12

Similar processes are observed in the tissues of *Cucurbita pepo var. Giraumontia L.*, which belongs to the same family as *Cucumis sativus L.*, and is also used by humans in a state of the consumer rather than physiological maturity. That is, *Cucurbita pepo var. Giraumontia L.*, at the time of the study, was also photosynthesized. However, the level of superoxide and TBA_{ap} generation is lower compared to *Cucumis sativus L.*, which may be explained by both high levels of enzymatic and low molecular weight antioxidants and lower photosynthetic capacity. Cooling leads to a greater increase in superoxide generation compared to rapid freezing, but the TBA_{ap} level remains approximately the same, which can be explained by the increase in the concentration of AA and GSH together with increased enzyme AO activity, which was not observed in *Cucumis sativus L.* tissues. Therefore, in the biochemical protection of tissues *Cucurbita pepo var. Giraumontia L.*, hypothermia activates both enzymatic and non-enzymatic link AO protection.

Capsicum annuum L., has the highest SOD activity among all experimental plants of the control group, which is also characterized by the highest content of AA in tissues. At the same time, the level of superoxide and TBA_{ap} generation naturally turned out to be the lowest. However, freezing and cooling lead to the largest increase in superoxide content in 3 and 2.2 times, respectively. SOD activity increases 1.5 times during freezing and 1.2 times, catalase

activity 1.3 and 1.4 times, respectively. The concentration of AA drops sharply on cooling (5.4 times) and decreases slightly on freezing (1.4 times), remaining the largest among the plants of the second experimental group. The concentration of GSH also decreases more during cooling than during freezing. Therefore, although the level of SOD and AA in the tissues of *Capsicum annuum L.*, is the highest, the activity of enzymatic antioxidants increases with hypothermia, but the power of AO protection is not enough to compensate for the increase in the concentration of superoxide. The result is an experimentally confirmed increase in TBA_{ap} levels of 2.4 times during freezing and 3.9 times during cooling, which is one of the most contrasting changes among all experimental plants. The obtained digital data may also indicate that *Capsicum annuum L.*, has the lowest adaptability to hypothermia in terms of changes in prooxidant-antioxidant potential. Moreover, cooling has a more destructive effect on tissues than freezing.

Allium sativum L., has the highest content of GSH among experimental plants, and moderate hypothermia increases the value of this indicator by 1.4 times. This plant is also characterized by the lowest concentration of AA in the tissues, the content of which does not change during freezing, but increases by 3.2 times when cooled. The activity of enzymatic antioxidants also increases during cooling to a greater extent than during freezing (3 times for SOD and 1.8 times for catalase). The increase in prooxidant activity in hypothermia is insignificant, which may indicate that *Allium sativum L.*, is one of the most adapted among all experimental plants, and antioxidant resistance to cooling is greater than to freezing, low molecular weight antioxidant protection plays a stronger role, compared with enzymatic.

Tissues of *Solanum melongena L.*, are characterized by the highest value of catalase activity among experimental plants. Cooling leads to an increase in activity by 21.7%, freezing - by 12.5%. SOD activity increases by 16.9% during freezing and by 44.9% during cooling. There was no significant difference in the content of low molecular weight antioxidants in hypothermia. There is an assumption about the role of bioflavonoids in tissue protection of *Solanum melongena L.* The level of superoxide generation in plants of the control group is the highest and continues to increase with hypothermia, but no significant difference in ΔTBA_{ap} was found, indicating a powerful AO defense system.

The level of SOD in the tissues of *Solanum tuberosum L.*, increases 3.47 times when cooled and 3.71 times when frozen. Catalase activity increases 2.03 times when cooled and 2.45 times

when frozen. The concentration of GSH also increases by 22.7% when cooled and by 43.1% when frozen, the concentration of AA does not change significantly. Given that the level of superoxide generation increases 1.61 times when cooled and 1.79 times when frozen and a small difference ΔTBA_{ap}, it can be argued that *Solanum tuberosum L.*, is also one of the most resistant among experimental plants to hypothermia. The fact that starch accumulates in the tissues of *Solanum tuberosum L.*, also contributes to the resistance to exposure to low temperatures, which when broken down forms glucose, which plays the role of antifreeze, preventing water from freezing in the cell.

The tissues of *Daucus carota L.*, have the lowest level of superoxide production among all experimental plants. Moreover, the value of the control group is hundreds of times lower than other plants, and the value of antioxidants does not have even a tenfold predominance. This suggests the role of alternative compounds with antioxidant properties, such as carotene. It was also interesting that with a slight increase in the generation of superoxide both during cooling and freezing ΔTBA_{ap} decreases, the activity of SOD does not change significantly, the growth of catalase is negligible. However, the concentration of AA increases 2.2 times during freezing, and 2.6 times during cooling, which is not typical for any other experimental plant. The glutathione content increases by 21.8% during freezing and by 31.7% during cooling. The obtained results allow us to conclude that the non-enzymatic part of the antioxidant system is involved in the adaptation to the hypothermia of *Daucus carota L. Daucus carota L.*, more resistant to cooling than to freezing.

A feature of *Allium cepa L.*, tissues is resistance to cooling, but not to freezing, which is experimentally confirmed by a significant increase in superoxide production (1.7 times when cooled and 3.9 when frozen) and ΔTBA_{ap}. The content of AA decreases both when cooled and frozen, but other antioxidants increase their activity.

The level of superoxide and ΔTBA_{ap} generation in the tissues of *Solánum lycopérsicum L.*, is the highest among all experimental plants, both during freezing and cooling. Hypothermia leads to a decrease in the content of low molecular weight antioxidants and a slight increase in the activity of enzymatic AO. Thus, catalase increases the activity during freezing by 17%, and during cooling - by 6%, SOD - by 4% and 17%, respectively. This may indicate a low level of resistance of *Solánum lycopérsicum L.*, tissues to hypothermia, and the role of other AOs in protecting tissues from significant ROS generation. Given that the tissues of the fruit of

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Solánum lycopérsicum L. were used for analysis, a possible cause of high levels of superoxide in the control group is its role in fruit ripening, plastid interconversion, and tissue aging.

Tissues of *Beta vulgaris L.*, are characterized by a fairly high level of catalase and SOD activity. Hypothermia increases the value of both low molecular weight and enzymatic AO. The experimentally revealed feature of *Beta vulgaris L.*, tissues was the smallest difference between the studied parameters in the two experimental groups. Given the low level of Δ MDA on a small increase in superoxide production, it can be argued that *Beta vulgaris L.*, is a resistant plant to both cooling and freezing.

Conclusions

1. When the temperature decreases, enzymatic antioxidants undergo the greatest changes, which increase the value of their activity in all experimental plants. Thus, the average increase in catalase activity during cooling is 31.05%, during freezing - 18.03%; for SOD - 60.04% and 38.09% respectively. Therefore, SOD plays a primary protective role under the action of hypothermia, compared with catalase. Cooling promotes a greater increase in the activity of enzyme AOs than freezing.

2. Change in the production of low molecular weight antioxidants in hypothermia is a species-specific feature. From experimentally revealed general regularities it is possible to allocate the following: in fabrics of underground spare parts of plants the higher control level of GSH is characteristic; increase in GSH production is observed both during cooling and freezing of tissues (by 15.32% and 9.9%, respectively); cooling promotes greater GSH retention than freezing. AA reduces its content in almost all experimental plants, both during cooling (on average by 22.9%) and during freezing (on average by 12.6%). Therefore, freezing leads to less destruction of AA in the tissues than cooling.

3. The average value of increasing the generation of superoxide during cooling is 30.75%, during freezing - 49.35%, but the difference in the average value of ΔTBA_{ap} in different types of hypothermia is almost absent (increase is 22.12% during cooling and 23.73% when frozen). Given the above changes in the antioxidant level of protection, it can be argued that hypothermia activates both parts of the PAS, however, cooling is accompanied by a more powerful both low molecular weight and enzymatic AO system.

4. The researched AO can be divided according to the degree of reduction of the protective value in hypothermia in the following series: SOD, catalase, GSH, AA.

5. The most resistant in terms of changes in PAS to hypothermia are *Solanum tuberosum L., Allium sativum L., Beta vulgaris L.*; the least resistant are *Capsicum annuum L. and Solánum lycopérsicum L.* The generative organs of plants are the least resistant to hypothermia than the vegetative ones.

6. Freezing allows you to store plant products for a longer time than refrigeration, however, chilled vegetables retain more AO in the tissues.

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