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The value of the prooxidant-antioxidant system in ensuring the immunity of plants

Mariia Bobrova *
Olena Holodaieva **
Hanna Arkushyna ***
Olena Larycheva ****
Olha Tsviakh *****

ABSTRACT

Aim of the research: to identify changes in the state of the prooxidant-antioxidant system in the organs of different plant varieties, depending on their level of resistance to disease. The **subject** of the research is the role of individual components of the prooxidant-antioxidant system in ensuring plant resistance to disease. **Methodology**. Quantitative determination of PAS status was performed on onion tissue samples taken from the following varieties: "Globus" (high-resistant variety), "Rainbow" (medium-resistant variety) and "Donetsk Golden" (low-resistant variety). For biochemical analysis, tissues from the top of the leaf, the middle of the leaf, the scales of the onion-turnip, stem, flower, roots, and seeds were used. The concentration of superoxide anion radical, TBA-active products, superoxide dismutase activity, catalase, GSH-peroxidase, the concentration of ascorbic acid, glutathione, cytochrome oxidase activity were determined. The **results** of the research show that in the tissues of photosynthetic vegetative organs of onions, there is an increase in both parts of the prooxidant-antioxidant system; in tissues that are not capable of photoproduction, there is an advantage of the antioxidant link in accordance with the increased resistance of the variety to disease. Initiation of germination processes enhances the activity of both parts of the prooxidant-antioxidant system and is highest in flower cells. Onion seed tissues, which are at rest, have the advantage of a prooxidant link and an increase in the concentration of low molecular weight antioxidants. **Practical consequences**. As a result of the correlation analysis of the studied indicators, the presence of a close relationship between the concentration of TBA-active products and the activity of cytochrome oxidase and superoxide dismutase, ascorbate with glutathione was established.

KEYWORDS: ascorbic acid, catalase, superoxide anion radical, superoxide dismutase, cytochrome oxidase.

* PhD in Biology, Senior Lecturer of the Department of Biology and Methods of Teaching of the Volodymyr Vynnychenko Central Ukrainian State Pedagogical University, Ukraine. ORCID ID: 0000-0001-7703-651X.

** PhD in Chemistry, Associate Professor of the Department of General and Biological chemistry #2 Donetsk national medical university, Ukraine. ORCID ID: 0000-0002-4922-7033

*** PhD in Biology, Associate Professor of the Department of Biology and Methods of Teaching of the Volodymyr Vynnychenko Central Ukrainian State Pedagogical University, Ukraine. ORCID ID: 0000-0002-5261-7315

****PhD in Biology, Associate Professor of the Department of Pharmacy, Pharmacology, Medical, Bioorganic and Biological Chemistry of the Petro Mohyla Black Sea National University, Ukraine. ORCID ID: 0000-0001-7399-3339

*****PhD in Biology, Associate Professor of the Department of Chemistry of the V.O. Sukhomlynskyi Mykolaiv National University, Ukraine. ORCID ID: 0000-0002-1119-2170

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El valor del sistema prooxidante-antioxidante para garantizar la inmunidad de las plantas

RESUMEN

Objetivo de la investigación: identificar cambios en el estado del sistema prooxidante-antioxidante en los órganos de diferentes variedades de plantas, dependiendo de su nivel de resistencia a las enfermedades. El tema de la investigación es el papel de los componentes individuales del sistema prooxidante-antioxidante para garantizar la resistencia de las plantas a las enfermedades. **Metodología**. La determinación cuantitativa del estado de PAS se realizó en muestras de tejido de cebolla tomadas de las siguientes variedades: "Globus" (variedad de alta resistencia), "Rainbow" (variedad de resistencia media) y "Donetsk Golden" (variedad de baja resistencia). Para el análisis bioquímico, se utilizaron tejidos de la parte superior de la hoja, la mitad de la hoja, las escamas del nabo, el tallo, la flor, las raíces y las semillas. Se determinó la concentración de radicales anión superóxido, productos activos TBA, actividad superóxido dismutasa, catalasa, GSH-peroxidasa, la concentración de ácido ascórbico, glutatión, actividad citocromo oxidasa. **Los resultados** de la investigación muestran que en los tejidos de los órganos vegetativos fotosintéticos de las cebollas, hay un aumento en ambas partes del sistema prooxidante-antioxidante. En los tejidos que no son capaces de fotoproducción, existe una ventaja del enlace antioxidante de acuerdo con el aumento de la resistencia de la variedad a la enfermedad. El inicio de los procesos de germinación mejora la actividad de ambas partes del sistema prooxidante-antioxidante y es más alto en las células de las flores. Los tejidos de semillas de cebolla, que están en reposo, tienen la ventaja de un enlace prooxidante y un aumento en la concentración de antioxidantes de bajo peso molecular. **Consecuencias prácticas**: Como resultado del análisis de correlación de los indicadores estudiados, se estableció la presencia de una estrecha relación entre la concentración de productos activos TBA y la actividad de citocromo oxidasa y superóxido dismutasa, se estableció ascorbato con glutatión.

PALABRAS CLAVE: ácido ascórbico, catalasa, radical anión superóxido, superóxido dismutasa, citocromo oxidasa.

Introduction

Changes in the values of the state of the prooxidant-antioxidant system (PAS) accompanies all physiological and pathological processes, so it is the object of study of clinical medicine and gerontology (Kasote et al., 2015; Marrocco et al., 2017; Pacheco et al., 2018; Ye, 2013). The prospect of using and modifying certain components of antioxidant protection (AOP) to increase the body's immune defenses opens new areas of research in the

field of immunology, breeding, biotechnology, and genetic engineering and draws scientists' attention to the problem of PAS (Kohen & Nyska, 2002; Kostyuk, 2004). A feature of PAS of plants is the enhancement of prooxidant activity due to photosynthesis and generation of reactive oxygen species (ROS) by plastids, peroxisomes, apoplast, cytosol (Merzlyak, 1999; Polesskaja, 2007). The connection between the immunity of plants and their adaptation to the conditions of existence in terms of changes in the values of PAS indicators; the role of individual components of PAS in ensuring plant resistance to diseases, biochemical and molecular mechanisms of this resistance; features of the distribution of components of PAS in various organs of an organism of plants has not been studied. Recently, in an unfavorable environmental situation, increased attention to the quantitative content of antioxidants, ROS, and products of free radical peroxidation (FRPO) of biopolymers that enter our body with foods of plant origin. It is important to study the dynamics of their change with increasing duration of storage of edible vegetative parts of plants (Baiano et al., 2015; Xu et al., 2017; Song et al., 2010).

Aim of the research: to identify changes in the state of the prooxidant-antioxidant system in the organs of different plant varieties, depending on their level of resistance to disease. To achieve this aim, the following **tasks** were identified:

1. To identify the state of the components of PAS in photosynthetic and non-photosynthetic vegetative organs of onions.
2. To identify the state of the components of the PAS of the generative organs of onions.
3. To establish the relationship between the indicators of the state of PAS among themselves and with the level of resistance of plant varieties to disease.

1. Literature review

The formation of ROS in the process of life of plant and animal organisms was first established by M. Shenbein. In the formation of ideas about the participation of ROS and free radicals in biochemical processes of great importance were: the theory of Bach-Engler peroxidation, the theory of chain free radical oxidation, developed by M.M. Semenov, as well as a study by American scientist I. Fridovich, who proved the formation of oxygen radicals in enzymatic reactions and discovered the ability to inactivate some ROS with enzymes (Merzlyak, 1999). According to the works of O.P. Dmitrieva and Zh.M. Kravchuk, ROS in

plant cells are by-products of normal metabolism due to leaks in electron transport chains (ETC) in chloroplasts, mitochondria, as well as in those compartments where there are enzymes of redox reactions. In a normally functioning cell, there is a certain balance between activation and deactivation of Oxygen, so the number of its active forms remains at a safe level, but damage to plant tissues under stress, usually leads to activation of Oxygen, while disturbing the balance between ROS formation and destruction (Dmytriyev & Kravchuk, 2005). The generation of ROS by the plant cell occurs in response to environmental stressors of abiotic origin (Apel & Hirt, 2004; Foyer & Noctor, 2005; Gill & Tuteja, 2010; Pacheco et al., 2018; Kohen & Nyska, 2002). In the works of I.V. Maksimov, I.A. Tarchevsky, A.A.Averyanov noted an increase in total AFO production by plants during the invasion of pathogens (bacteria, fungi, mycoplasmas) and described the mechanisms of hypersensitivity reactions (Maksimov, 2006; Aver'yanov, 1991; Tarchevskiy, 2002). O.P. Dmitriev and Zh.M. Kravchuk in his work "Oxygen Active Forms and Plant Immunity" note the importance of ROS in the formation of acquired systemic resistance of plants to pathogens as signal intermediates for the activation of genes of enzymes involved in the synthesis of AO and phytoalexins (Dmytriyev & Kravchuk, 2005). At the present stage, the British School of Biochemistry, headed by Dr. Nicholas Smirnoff, is developing the problem of ROS and AOS of plant organisms, one of the most powerful complex works of which is "Antioxidants and reactive oxygen species in plants" (Smirnoff, 2005). In general, the question of the importance of ROS and AO in the plant cell is not unambiguous and exhaustive and requires careful study and systematization.

According to the works of O.G.Polesskaja is most important for plant cells singlet oxygen, superoxidation ion radical, hydrogen peroxide, and hydroxyl radical (Polesskaja, 2007). The system of antioxidant protection (AOP) of plants includes prevention of formation of ROS, their inactivation, break of a chain of reactions of FRPO, inactivation of products of FRPO, repair of damages (Halliwell, 2006). According to the literature, the most important enzymatic AOs present in all plant cells and organs are SOD, catalase, a number of peroxidases, ascorbate-glutathione cycle enzymes and cytochrome oxidase (Pacheco et al., 2018; Kostyuk, 2004; Shao, 2008; Smirnoff, 2005; Xu et al., 2017). Many authors (Pacheco et al., 2018; Kostyuk, 2004; Shao, 2008; Smirnoff, 2005; Xu et al., 2017) agree that the most important non-enzymatic low molecular weight antioxidants of the plant organism are AA,

α-tocopherol, reduced GSH, carotenoids, flavonoids, and isoprene. Estimation of the FRPO level of biopolymers of membranes is carried out on the value of an indicator of the activity of cytochrome oxidase.

The study of the role of ROS in anti-infective protection of animals, oxidative explosion processes, mechanisms of aging and apoptosis has opened up prospects for the search for analogs in the plant world. This area is the subject of research A.A. Averyanov, according to whose work in a normally functioning plant cell there is a balance between activation and deactivation of oxygen, so the number of its active forms remains at a safe level (Aver'yanov, 1991). However, structural and functional disorders of plant tissues, as a rule, lead to the activation of oxygen. The normal balance between the formation and disposal of ROS can be disturbed in a variety of pathological conditions of plants (Dmytriyev & Kravchuk, 2005). Since the activation of oxygen is one of the first responses of the plant cell, ROS may play an important role in inhibiting the development of pathogens. Thus, when carrying out a hypersensitivity reaction, phenols are released from vacuoles and their enzymatic oxidation. And since this process is accompanied by the generation of activated oxygen in toxic concentrations, it is possible that it is the cause of death of both infected host cells and invading pathogens (D'yakov et al., 2002). In I.A.Tarchevsky's laboratory, it is established that pathogenic microorganisms induce in a plant cell a cascade of protective reactions long before stability or susceptibility is shown in full. This is achieved by the functioning of signaling systems, the main of which are in the plant body: calcium, lipoxygenase, NADPH-oxidase (superoxide synthase), NO-synthase, adenylate cyclase, phosphoinositol, and MAP-kinase (Tarchevskiy, 2002). ROSs play a key role in the functioning of the first four of them. This information, and the ever-increasing number of publications on the involvement of ROS in other important physiological processes (metabolism and synthesis of phytohormones, regulation of photosynthetic reactions and mitochondrial oxidation, apoptosis, aging), require a more detailed, qualitatively new approach to studying the biological role of ROS and AO plant life (Foyer & Noctor, 2005; Pacheco et al., 2018).

2. Research methodology

Quantitative determination of PAS status was performed on onion tissue samples taken from the following varieties: "Globus" (high-resistant variety - 9th class of disease resistance), "Rainbow" (medium-resistant variety - 7th class of disease resistance) and "Donetsk Golden" (low-resistant variety - 5th class of resistance to diseases). For biochemical analysis, tissues from the top of the leaf, the middle of the leaf, the scales of the onion-turnip, stem, flower, roots, and seeds were used. The tops of the onion leaf were cut at a distance of up to 0.5 cm from the point of growth, the middle of the leaf was selected geometrically, the analysis of onion-turnip scales was carried out on its cross-section, onion roots were cut at a distance of 0.1 cm from the tillering node. The studied parts of the plants were in the growth stage. The stem was selected at the stage of the flowering of the plant. Tissues of cross-section of the middle of the stem were used for analysis. Onion flowers were selected at the stage of flowering, flowers without peduncles were used for analysis. Onion seeds were analyzed at dormant sites. In parallel, the seeds were examined during the initiation of germination, which was carried out by the previous 12-hour soaking in clean stagnant water. Each experimental group included 10 samples, so the experiment analyzed 2880 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 in 3 tubes: 0.05 cm^3 of buffer solution was added to I (to determine the total main unstimulated activity); in AI was added 0.05 cm^3 of NaF solution (w = 0.01%, stimulation of Ca^{2+} messenger system); in III – 0.05 cm^3 of yeast solution (w = 1%, stimulation of oxidative explosion), in IV – 0.05 cm^3 of NADH solution (w = 3%, stimulation of mitochondrial generation), in V – 0.05 cm^3 of NADPH solution (w = 3%, stimulation of microsomal generation). 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 (for test tubes I– III) and after 10 minutes. (for IV–V tubes), 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, 0.05 cm^3 of water, and 0.05 cm^3 of NBT. Added: in I – 0.05 cm^3 of water; in II – 0.05 cm^3 of NaF solution (w = 0.01%); in II – 0.05 cm^3 of yeast solution (w = 1%), in IV– 0.05 cm^3 of NADH solution (w = 3%), in V – 0.05 cm^3 of NADPH solution (w = 3%) were incubated min, for test tubes I–III, 10 min. for test tubes IV–V) in a thermostat at 24^{0}C 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 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, 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 ●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 ($C(1/5KMnO_4) = 0.1$ 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;

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

$V_{experimental}$ – 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).

To determine the activity of GSH-peroxidase, 0.1 cm^3 of tissue was homogenized in 1.9 cm^3 of water. In the experimental and control tubes were collected 0.1 cm^3 of sodium azide solution ($C(Na_3N) = 0.01$ mol/dm^3), 0.25 cm^3 of a solution of reduced glutathione ($C(GSH) = 0.004$ mol/dm^3; on phosphate buffer, pH = 7.05), 0.05 cm^3 of homogenate and 1.8 cm^3 of water. Incubate for 5 minutes at 37 ^0C. 0.25 cm^3 of H_2O_2 solution was added to the experimental sample (0.04 cm^3 of H_2O_2 (w = 33%) was dissolved in 100 cm^3 of water), and the same amount of water was added to the control sample. Incubate for 10 minutes at 37^0 C, add 0.75 cm^3 of trichloroacetic acid (w = 30%), stirred, centrifuged at 3500 rpm for 15 minutes. The reduced

glutathione was determined in the supernatant photometrically at a wavelength of 412 nm (cuvette per 1 cm). The calculation was carried out according to the formula:

$$A = (C_{control} - C_{experimental}) \cdot K$$

A – activity of glutathione peroxidase (OD, μmol/cm^3·min or μmol/g·min);

$C_{control}$ and $C_{experimental}$ – concentrations of reduced glutathione before and after incubation (in control and experimental samples);

K is the coefficient that takes into account the dilution and incubation time.

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 – volume of extract taken for titration, cm3;

a – the amount of test substance, g;

A_{contr}, A_{exp} - 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).

Evaluation of the effects of PAS changes was performed by changes in cytochrome oxidase activity.

Procedure: 0.5 g of tissue on ice was thoroughly homogenized with 4.5 cm^3 of phosphate buffer solution (pH 7.6). 1 cm^3 of homogenate was collected in the test tube, and 1 cm^3 of diluted buffer solution in the control tube. Extempore quickly prepared the reaction mixture by merging 0.25 cm^3 of α-naphthol (w = 0.1%; 50 mg of α-naphthol was dissolved in 50 cm^3 of ethanol (w = 22%)), 0.35 cm^3 of a solution of N,N-dimethyl- para-phenylenediamine hydrochloride (w = 0.1%; 5 mg of reagent was dissolved in 5 cm^3 of distilled water), 0.25 cm^3 of dilute buffer solution, 0.15 cm^3 of cytochrome c solution (w = 0,02%). Preincubated the mixture for 2 minutes at 37^0C.

Added to the control and test sample 1 cm^3 of the reaction mixture, stirred, incubated under the same conditions for 5 minutes. 10 cm^3 of an ethereal alcohol mixture (diethyl ether and ethanol in a volume ratio of 9:1) were added, shaken, and placed in the cold (4^0C, 30 min), shaking periodically.

Adjust the volume of the essential alcohol extract to 10 cm^3 and photometer at 540 nm against the control. The calculations were carried out according to the formula:

$$A = E_{exp} \cdot 10 / E_{st} \cdot 5 = 2\ E_{exp} / E_{st}\ ,$$

A – cytochrome oxidase activity in indophenolic units per gram of tissue per minute;

E_{exp} – extinction of the test sample;

E_{st} – extinction of the standard, calculated from the calibration graph at a dose of 100 mg / cm3 of α-naphthol (1 cm^3 in the mixture; it is possible to equate to a conventional unit proportional to the amount of indophenol);

10 – breeding; 5 – incubation time.

Standard solutions for plotting the calibration graph (α-naphthol – 100 μg / cm^3, n-phenylenediamine – 150 mg / cm^3 and potassium dichromate – 210 mg / cm^3) were taken in

the first series of 0.1 cm^3, in the second – 0.2 cm^3 and so on to portions of 1.2 cm^3; incubated for 5 min, extracted with 10 cm^3 of the ether-alcohol mixture and photometered.

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

3. Results and discussion

Analysis of the distribution of PAS components in different organs of onion. As a result of quantitative analysis of the prooxidant activity of onion tissues by spectrophotometric NBT test revealed an increase in the basic production of superoxide in photosynthetic (leaf, stem) and generative (flower, seed) cuttings. A possible explanation for the obtained distribution is that the most reliable ROS generator of a plant cell is chloroplasts. Photosynthetic products $\bullet O_2^-$ is carried out by ETC thylakoids of photosystem I, where electrons are intercepted from 4Fe-4S clusters and from reduced ferredoxin. In photosystem II $\bullet O_2^-$ is formed at Q_A and Q_B sites. In addition, the source of $\bullet O_2^-$ is O_2^1, which is formed by chlorophyll (Polesskaja, 2007). The fact of the predominance of the basic level of generation of $\bullet O_2^-$ by the tissues of the top of the onion leaf of all three experimental varieties in comparison with the tissues of the middle of the leaf (Fig. 1) is noteworthy.

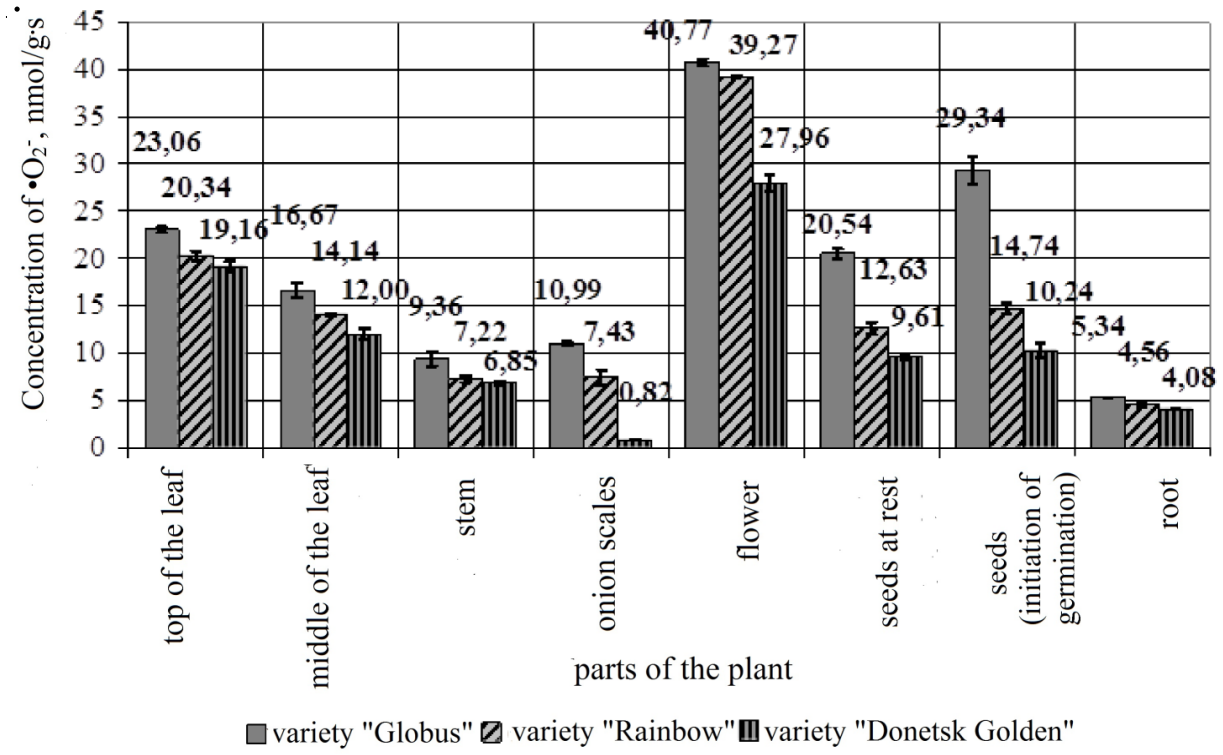

Fig. 1. Comparison of the concentration of superoxide in the tissues of onions (basic level of generation)

A possible explanation for this phenomenon is that the leaves of onions grow at the base, so the ROS of the cells of the top of the coot accompanies the aging process. Thus, ROS are involved in the synthesis of enzymes that destroy chlorophyll and cell membranes, activate lipoxygenase, which induces lipid peroxidation of thylakoid membranes. There is also evidence that high levels of ROS are important for blocking cell division. Thus, the tissues of the apex of the onion leaf are equipped with a group of cells at rest, with a minimum frequency of division. The content of AA in these cells is also low because all the ascorbate is oxidized by ascorbate oxidase, the activity of which, on the contrary, is quite high. Low levels of AA, GSH-peroxidase, and cytochrome oxidase activity in this area lead to an increase in the level of ROS, which causes a low frequency of cell division. The role of ROS cells of the apex of the leaf in the chain of the signaling cascade of the growth reaction through the activation of Ca^{2+} channels and, as a consequence, the determination of the direction of growth of the organ is possible. The opposite is the explanation of the reduced generation of superoxide by the cells of the middle and base of the leaf, providing growth of the organ. It is known that active cell division processes occur in the tissues of the meristem of the base of the onion leaf, while the cells of the middle leaf, in addition to photosynthesis, provide organ growth by stretching, which is carried out by ROS involved in processes aimed at weakening the links between cell wall polymers. Thus, OH● cuts the polymers of cell walls, promoting their stretching during growth. The formation of OH● in the apoplast is strictly controlled by enzyme systems, in particular, peroxidases, which are able in the presence of added NADH to induce reactions leading to the formation of ROS, with: oxidation of phenolic compounds by peroxide forms NAD-radical that spontaneously reduces O_2 to $●O_2^-$, which in turn quickly turns into H_2O_2 either with the participation of SOD or due to the oxidation of another NADH (Polesskaja, 2007).

It should be noted that due to the small pool of NADH and NADPH in the apoplast, the concentration of OH formed is insignificant (up to 1 mM), which significantly reduces its contribution to growth processes by stretching. Closing cells of the stomata of the leaves contain chloroplasts, so they are characterized by the enhanced generation of ROS. ROSs are involved in conducting the signal that occurs when ABA acts on the closing cells of the stomata. Thus, under the action of ABA closure of the stomata is preceded by the accumulation in the closing cells of H_2O_2 formed in the apoplast. It is known that in the

closing cells of the stomata the expression of genes encoding the synthesis of NADPH oxidase is higher compared to other leaf cells, and is able to increase under the action of ABA. Formed with the participation of NADPH-oxidase $\bullet O_2^-$ under the action of SOD turns into H_2O_2, which is included in a number of signal reactions, the end result of which is the closure of the stomata. Thus, according to a number of scientists (Kolupaev, 2007; Maksimov, 2006; Polesskaja, 2007), H_2O_2 induces an increase in the cytosolic concentration of Ca^{2+} and hence the activation of potential-dependent Ca^{2+} channels in the plasmalemma of closing cells. ROS regeneration occurs in the apoplast with the participation of NADPH oxidase and SOD. The relationship between the level of ROS and the state of Ca^{2+} channels is carried out through signaling pathways: Ca^{2+} channels can be regulated through phosphorylation, and ROS triggers individual signaling reactions that lead to the activation of the corresponding protein kinases or protein phosphatases. In addition to H_2O_2, $\bullet O_2^-$ and $OH\bullet$ are also included in the signaling processes as ROS.

The study found that the onion stem also has a slightly enhanced generation of $\bullet O_2^-$, but the lowest of all photosynthetic organs. A possible explanation for this is the ability of stem cells to photoproduct, the presence of the respiratory system, but its main transient function.

It is established that the highest generation of both basic and stimulated levels of superoxide is carried out by flower cells of all experimental varieties of onions (Fig. 2, Fig. 3).

This distribution may be explained by the participation of ROS in the reception of specific areas of the pollen grain membrane by the pistil receptacle during pollination, pathogen recognition with the subsequent triggering of signaling processes to include protective reactions, especially activated at the time of pollination and fertilization.

An increase in the level of ROS is also observed during pollen germination. It is a known fact that the addition of AO, in particular, ascorbic acid inhibits the process of pollen germination. Onion pistil also performs photosynthesis, equipped with stomata, contains polyploid cells, participates in the protective reaction of hypersensitivity, hormonal regulation of growth processes in the cells of the germ. According to one version of the formation of parcarp gynoecium, onion occurs by apoptotic destruction of the inner shells of carpels with the formation of a cavity. This process is initiated by mitochondrial and microsomal generation $\bullet O_2^-$.

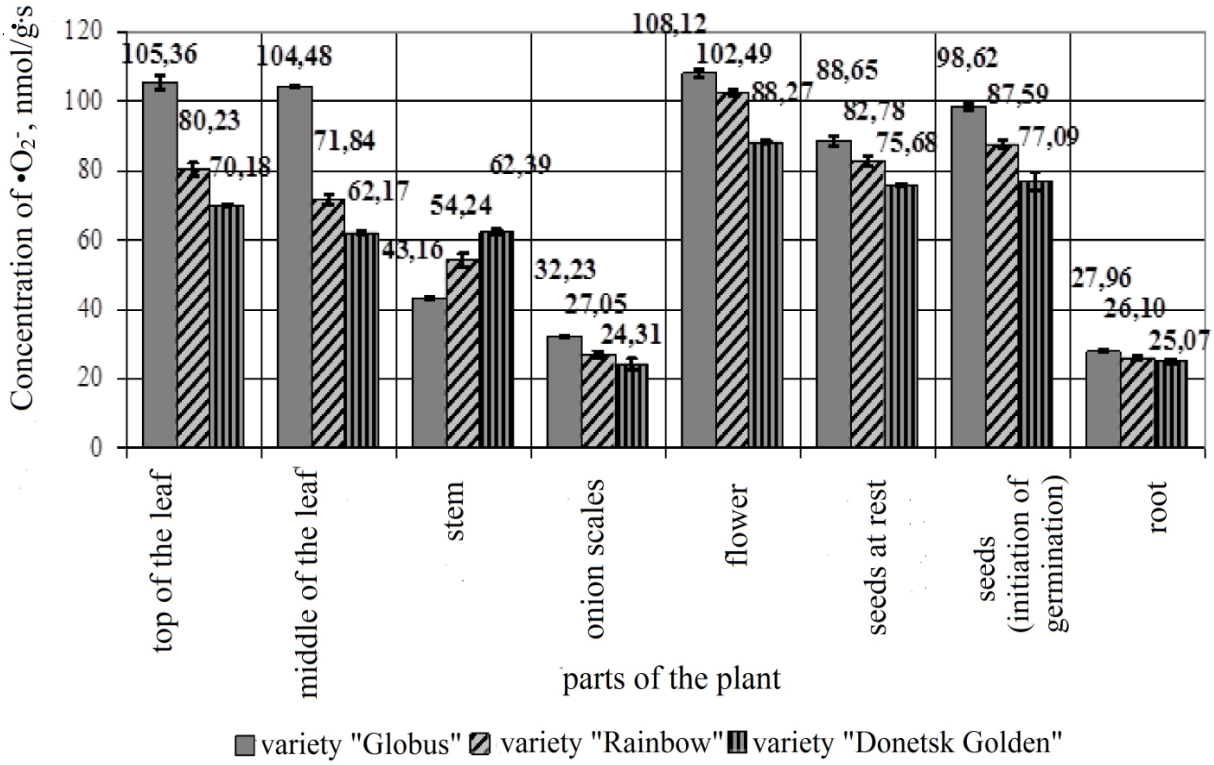

Fig. 2. Comparison of superoxide concentration in onion tissues (NADP·H stimulation).

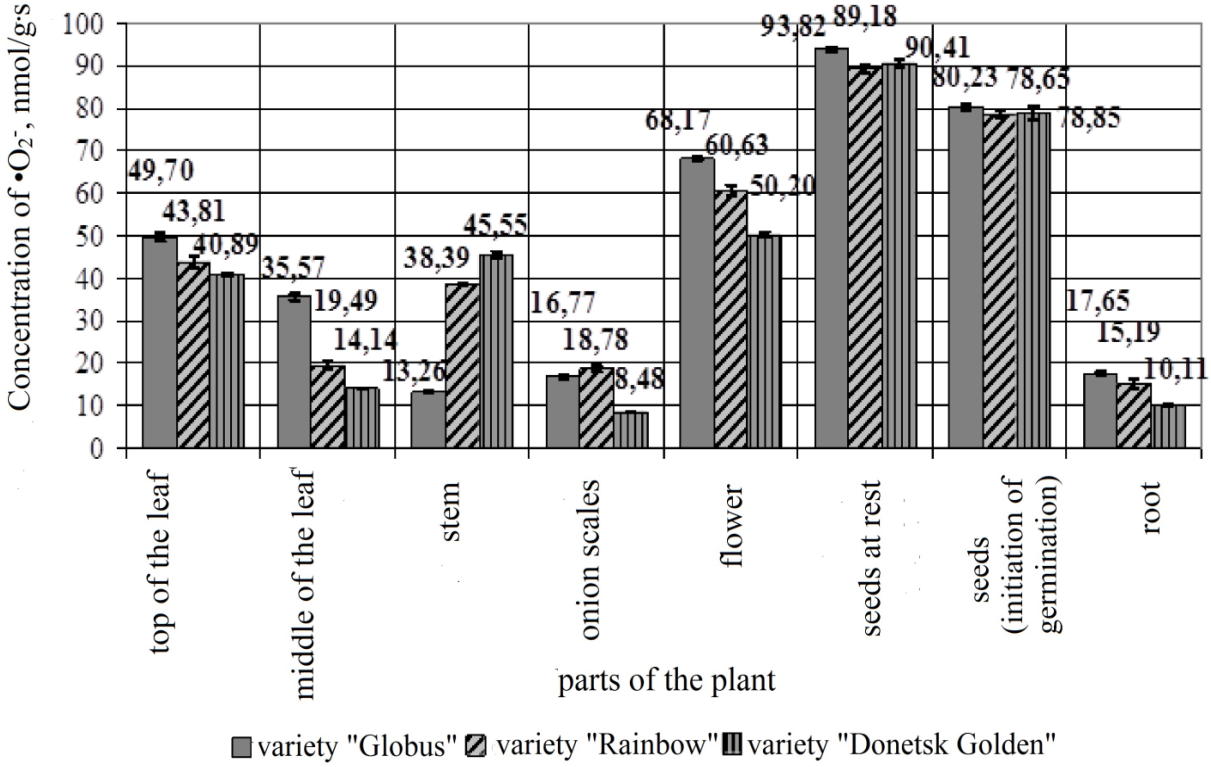

Fig. 3. Comparison of the concentration of superoxide in onion tissues (stimulation of NAD·H).

It was found that onion scales have a slightly reduced level of generation $\bullet O_2^-$, which may be due to their inability to photosynthesis and perform the main storage function. However, the tissues of the scales are capable of the formation of the protective flavonoid pigment quercetin, which is a highly toxic chemical barrier to parasite damage and accumulates in the dead cells of the outer covering scales.

It was experimentally found that the root of the onion has one of the lowest basic levels of superoxide generation, but is characterized by a significant increase in all types of stimulation. A possible explanation for this phenomenon is the increase in the level of ROS during the growth of root hairs, reception when interacting with the soil microflora, participation in the gravitropic reaction, and polar growth. Thus, upon receipt of gravistimulus, the pressure of amyloplasts on the membrane activates Ca^{2+} channels, indirectly leads to the redistribution of auxin transporters in cell membranes, followed by its concentration on the underside of the root. With the orientation of the plant horizontally, there is a rapid increase in the level of ROS in the apical part of the root, which includes the root cover, meristem, and part of the stretching zone (Tsebrzhinskiy, 1992).

ROSs also control the growth of root hairs, which is associated with the activation of Ca^{2+} channels when Ca^{2+} enters the apical part of the cell and its active pumping from the opposite side with the participation of Ca^{2+}-ATPase. Thus, with increasing cytosolic concentration of Ca^{2+}, there is an increase in the level of ROS in the apex as a result of the activation of NADPH oxidase. ROS in the apex of the rhizoderm cells activates Ca^{2+}channels, resulting in a growing cell, a gradient of Ca^{2+} ions, which provides polar growth of root hairs. Root meristems are also equipped with a group of dormant cells with a minimum division rate maintained by a high concentration of ROS, resulting in a decrease in the level of AOS of root cells, including catalase, SOD, AA, GSH, and GSH peroxidase.

The analysis revealed the enhanced generation of superoxide in onion seeds of all studied varieties, which may be explained by the fact that the seeds belong to the generative organ formed directly from the pistil of the flower. It should be noted that the seed cells are at rest, so the level of $\bullet O_2^-$ in them is lower compared to the flower.

Attention is drawn to the experimentally established fact of the growth of $\bullet O_2^-$ generation at the initiation of seed germination, which confirms the participation of ROS in the start of germination processes. It should be noted that the detected increase in the level

of $\bullet O_2^-$ is observed both when stimulated with NAD●H and under the action of NADP●H, NaF, and yeast in all experimental varieties of onions. The results of the analysis show that the largest increase in $\bullet O_2^-$ is observed when stimulated with a solution of NAD●H and NADP●H, which means that the largest contribution to increasing the concentration of $\bullet O_2^-$ when starting seed germination processes are mitochondria and microsomes, and for onion seed tissues "Globus" is dominated by microsomal generation, and for "Donetsk Golden" – mitochondrial. When comparing the increase in the level of $\bullet O_2^-$ under the action of NaF and yeast, it was found that stimulation by yeast enhances the generation of $\bullet O_2^-$ to a greater extent than the Ca^{2+}-messenger system.

Analyzing the results of the study of the level of AOP in the tissues of different organs of onions revealed the similarity of a number of decreases in the activity of SOD to a number of decreases in the level of generation $\bullet O_2^-$ (Fig. 4). A possible explanation for the established distribution is that the first powerful line of protection against the harmful effects of $\bullet O_2^-$ is SOD, which utilizes $\bullet O_2^-$ by decomposing it into H_2O_2 and triplet O_2. It was also found that the activity of SOD in the tissues of the onion flower variety "Globus" and "Rainbow" ranks third after the top and middle of the leaf (II for the tissues of the onion "Donetsk Golden"), while in a number of decreasing generation $\bullet O_2^-$ flower index is in the first place.

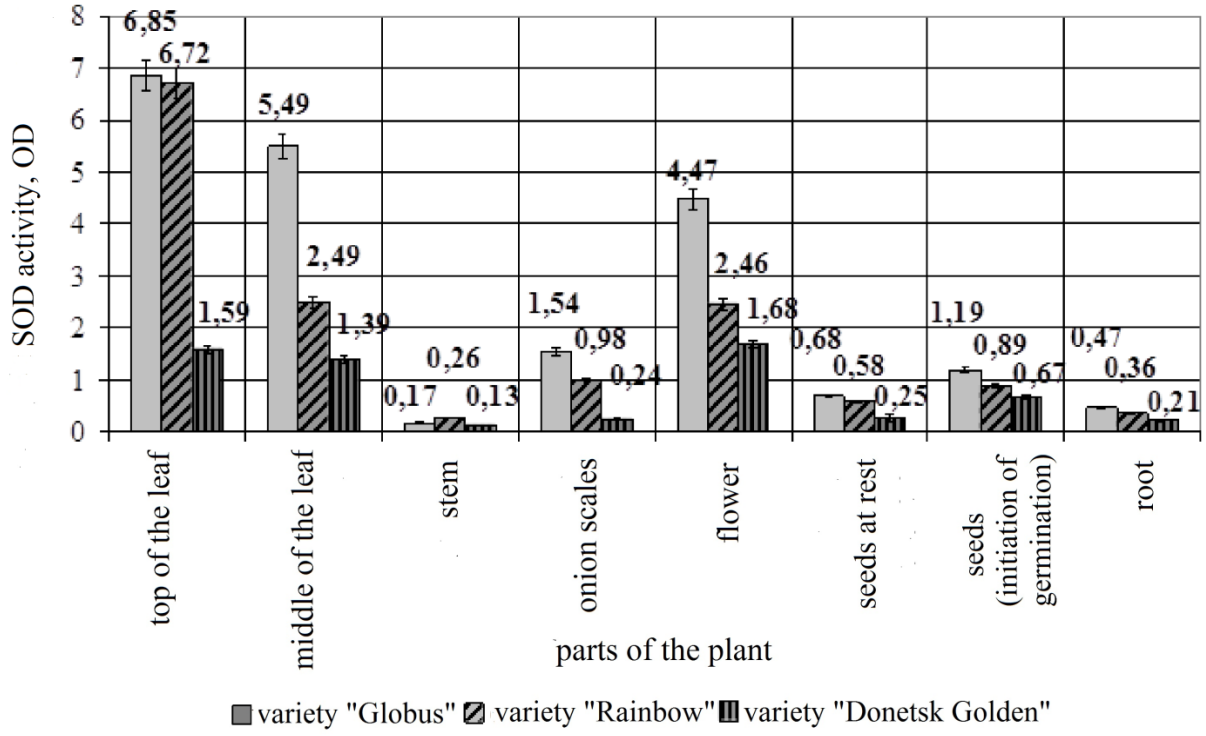

Fig. 4. Comparison of SOD activity in onion tissues

A similar discrepancy is observed in relation to seed tissues when activating germination, which takes the second place after the flower in terms of concentration $\bullet O_2^-$ and V place in a number of SOD, as well as the opposite correspondence of the arrow and root. A possible explanation for this phenomenon is the presence of another competing method of recycling $\bullet O_2^-$, which is carried out through the operation of the Hallivel-Asada cycle. The general pattern of both ways of utilization $\bullet O_2^-$ is the formation of H_2O_2 as the final product. It is also known that the utilization of H_2O_2 is possible with the participation of catalase, AA, GSH, and GSH peroxidase. Thus, as a result of the research, it was found that the top activity of SOD has the top of the onion leaf of the variety "Globus", "Veselka" and "Donetska Zolotista" which naturally has the highest activity of catalase (Fig. 5). Quite a high level of H_2O_2, formed as a result of utilization $\bullet O_2^-$ in the cells of the flower and the middle of the leaf neutralizes AA, the concentration of which is highest in the cells of these tissues.

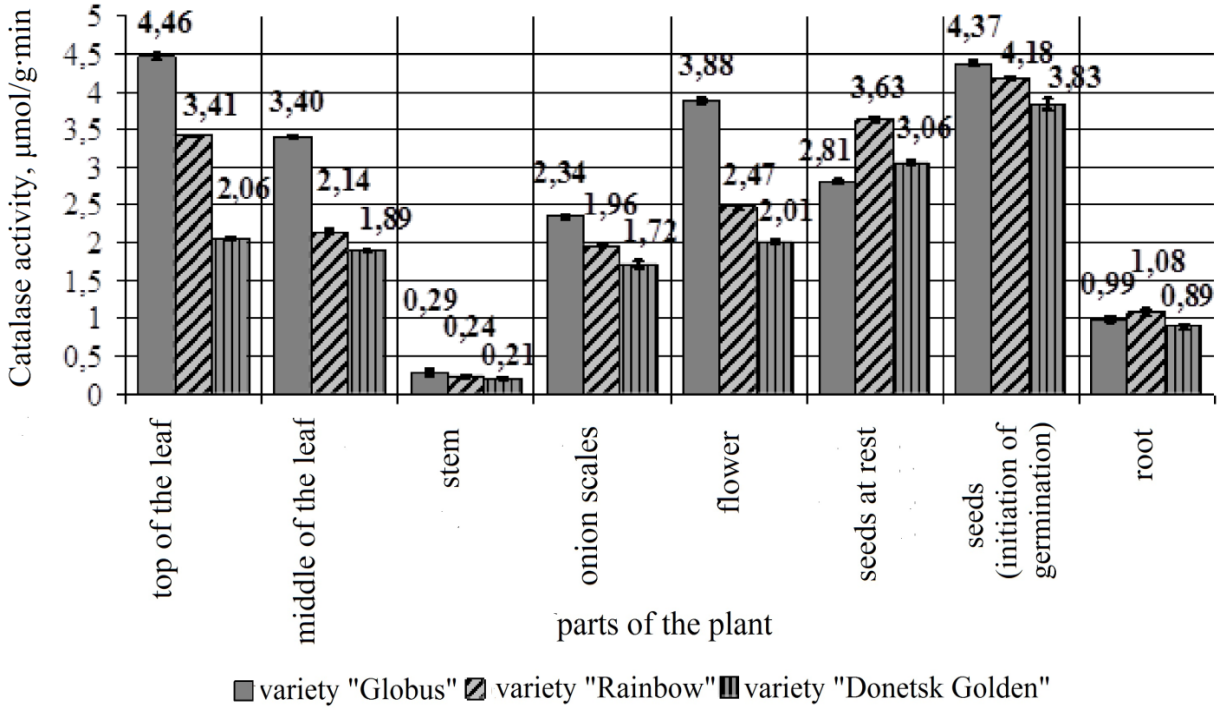

Fig. 5. Comparison of catalase activity in onion tissues

The significant similarity was also found in the series of organotropic decreases in catalase and GSH-peroxidase activity. According to the literature, a characteristic feature of plant catalase is its low specificity to the substrate and the increasing role of peroxidases in the utilization of H_2O_2. The compensatory role of GSH-peroxidase is probably explained by

the fact that the onion globe has the lowest catalase activity but the highest GSH-peroxidase activity, and the leaf apex tissues – on the contrary – are characterized by the highest catalase activity and one of the lowest GSH activity. It was found that changes in the activities of catalase and GSH-peroxidase of other organs show almost complete compliance.

As a result of the analysis of low molecular weight AO, it was found that the highest value of the concentration of AA is inherent in flower cells and all photosynthetic organs of onions of the three experimental varieties (Fig. 6). A possible explanation for this phenomenon is, experimentally confirmed by us, the highest level of generation $\bullet O_2$. In addition, AA is a major non-enzymatic antioxidant, a precursor to many plant metabolism compounds and a cofactor of enzymes. Thus, AA affects the synthesis of gibberellins, growth, stretching, cell morphogenesis. AA is a potential donor of hydrogen atoms and electrons used to reduce free radicals and H_2O_2, tocopherol, oxidized forms of many AOS enzymes such as ascorbate peroxidase, polyphenol oxidase, cytochrome oxidase, peroxidase. The analysis revealed the highest content of AA in those vegetative organs that are capable of photosynthesis (leaf, arrow), which naturally confirms the literature, which states that the concentration of AA in chloroplasts may exceed the concentration of chlorophyll, promoting its biosynthesis and recovery.

The lowest concentration of AA in seed cells, which is at rest, and the roots of all three experimental varieties of onions. A possible explanation for this distribution is that the synthesis of AA begins with UDF-glucose and is significantly enhanced with the appearance of the first photosynthetic leaves. An increase in the concentration of AA during the initiation of the germination process was recorded, which is probably associated with increased generation of ROS and the need for AOS by direct utilization of ROS and restoration of oxidized forms of antioxidants. A small percentage increase in the concentration of AA in the seeds during germination, compared with other AO, due to heterotrophic nutrition of the embryo using carbohydrate reserves before the first photosynthetic leaves and the inability to synthesize large amounts of AA from UDF-glucose. The participation of AA in the maintenance of the ascorbate-glutathione cycle is also known.

The highest concentration of GSH was found in the cells of the generative organs of onions of all experimental varieties, which may be explained by the participation of GSH in almost all stages of metabolism and its importance as a key AO of plant cells.

A characteristic feature of the transition of seeds to germination is a decrease in the level of GSH, which is inherent in the tissues of all experimental varieties of onions. This may be due to the use of GSH to restore oxidized forms of AO, which are actively used during germination to protect against elevated levels of AO in cells. For the same reason, the established concentration of GSH decreases during the transition from the tissues of the apex of the leaf to the photosynthetically active middle leaf of the onion varieties "Globus", "Rainbow" and "Donetsk Golden".

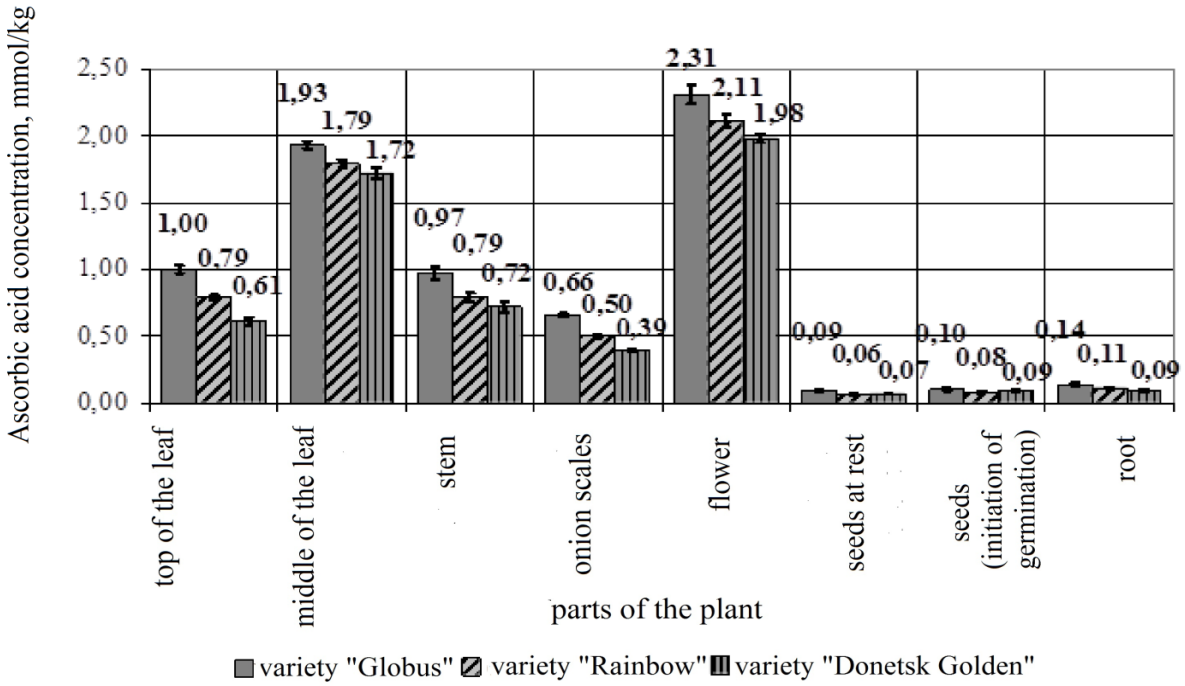

Fig. 6. Comparison of the concentration of ascorbic acid in the tissues of onions.

The lowest level of GSH is recorded in the roots of Globus and Veselka onions, which may be due to the constant use of GSH as a key participant in the detoxification of plant-absorbed xenobiotics and excretory metabolic products. The difference between the concentration of GSH levels in the tissues of the roots and arrows of the onion variety "Donetsk Golden" is insignificant, although the numerical value of the indicator is minimal, which does not contradict the general pattern and may be a variety-specific feature.

Experimentally established the highest level of generation $\bullet O_2^-$ in the cells of the onion flower of all three experimental varieties naturally causes the highest concentration of TBA_{ap}, both background and stimulated level. Similarly, a fairly high concentration of TBA_{ap}

in the seeds, which was initiated by germination and a significant increase in the level of TBA_{ap0} and $TBA_{ap1.5}$ during the transition of seeds from dormancy to germination (Fig. 7).

The sequence of changes in the indicator TBA_{ap0} and $TBA_{ap1,5}$ photosynthetic organs of onions "Globus" can be displayed as the following series: stem › middle of the leaf › top of the leaf, which may be due to the experimentally established most powerful enhancement of the activity cells of the middle of the leaf, which shifts the PAS towards the formation of prooxidants and the functional purpose of the stem with the proven minimum level of AOS (catalase, SOD, GSH) in comparison with other photosynthetic organs. It should be noted that for the fabrics of onion varieties "Rainbow" and "Donetsk Golden" the top of the leaf has a higher level of TBA_{ap0} compared to the middle of the leaf, and the tissues of the middle of the leaf were characterized by increased concentration of $TBA_{ap1.5}$ compared to the top.

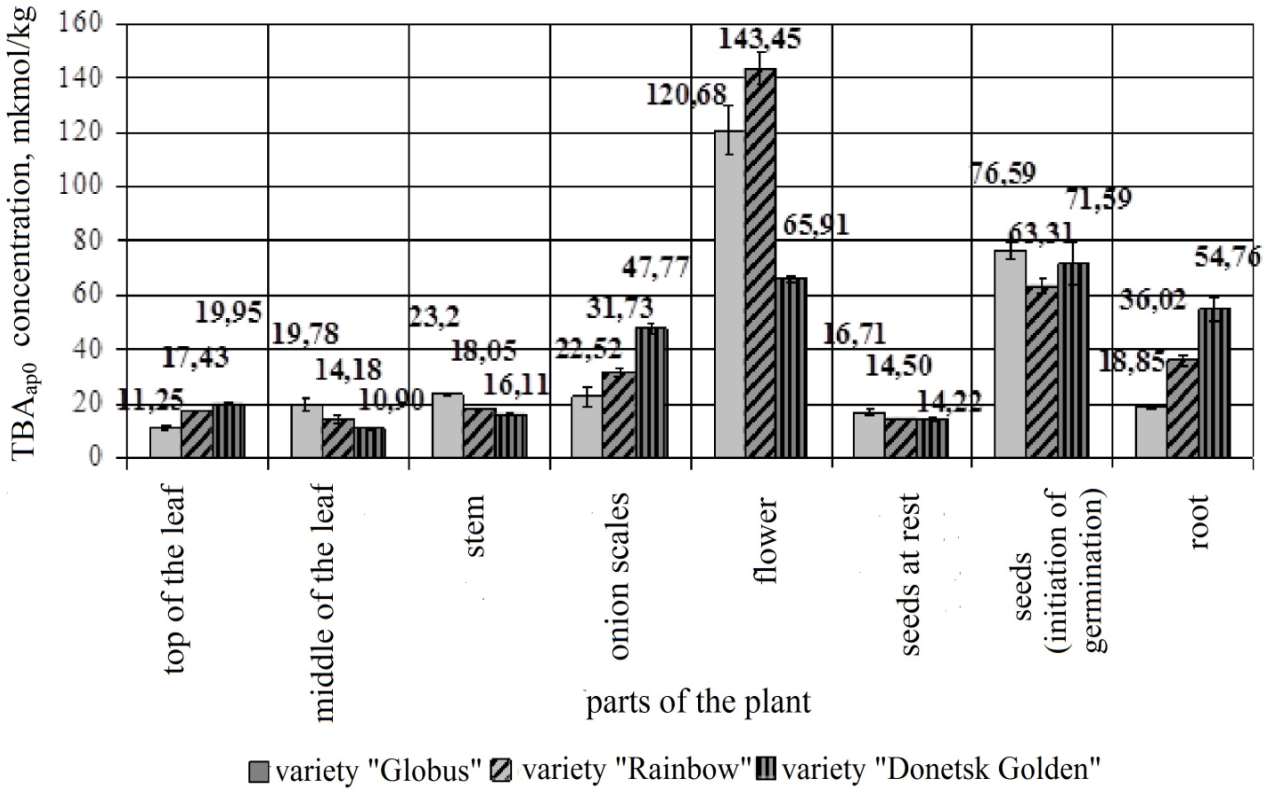

Fig. 7. Comparison of the TBA_{ap0} concentration of in the tissues of different onions

Since the highest level of $\bullet O_2^-$ generation is experimentally established in the tissues of the top of the onion leaf of all three experimental varieties, this discrepancy can be explained by the imperfection of the ROS utilization system, which is manifested in a decrease in the level of activity of the AOS system. Similarly, the increase in the concentration of TBA_{ap} in the tissues of the roots and scales of onion-turnip medium- and low-disease-

resistant variety can be explained. The decrease in the concentration of TBA_{ap} in the tissues of onion scales of the "Globus" variety can be attributed to the lack of a photosynthetically conditioned source of ROS generation. The lowest level of TBA_{ap0} among vegetative organs has onion root, which may be explained not only by the lowest level of generation $\bullet O_2^-$ but also by increased activity of GSH-peroxidase, SOD, and one of the highest levels of cytochrome oxidase compared to onion scales.

The marker of FRPO intensity is the activity of cytochrome oxidase, which transfers electrons from cytochromes to oxygen according to the scheme: $4H + 4e^- + O_2 \rightarrow 4H_2O$. Free radical peroxide degradation of membrane lipids reduces the activity of the enzyme, so the natural explanation for the low activity of cytochrome oxidase in the tissues of the apex of leaves, seeds, and flowers of onions of all experimental varieties is the highest level of generation of O_2 cells by these organs (Fig. 8).

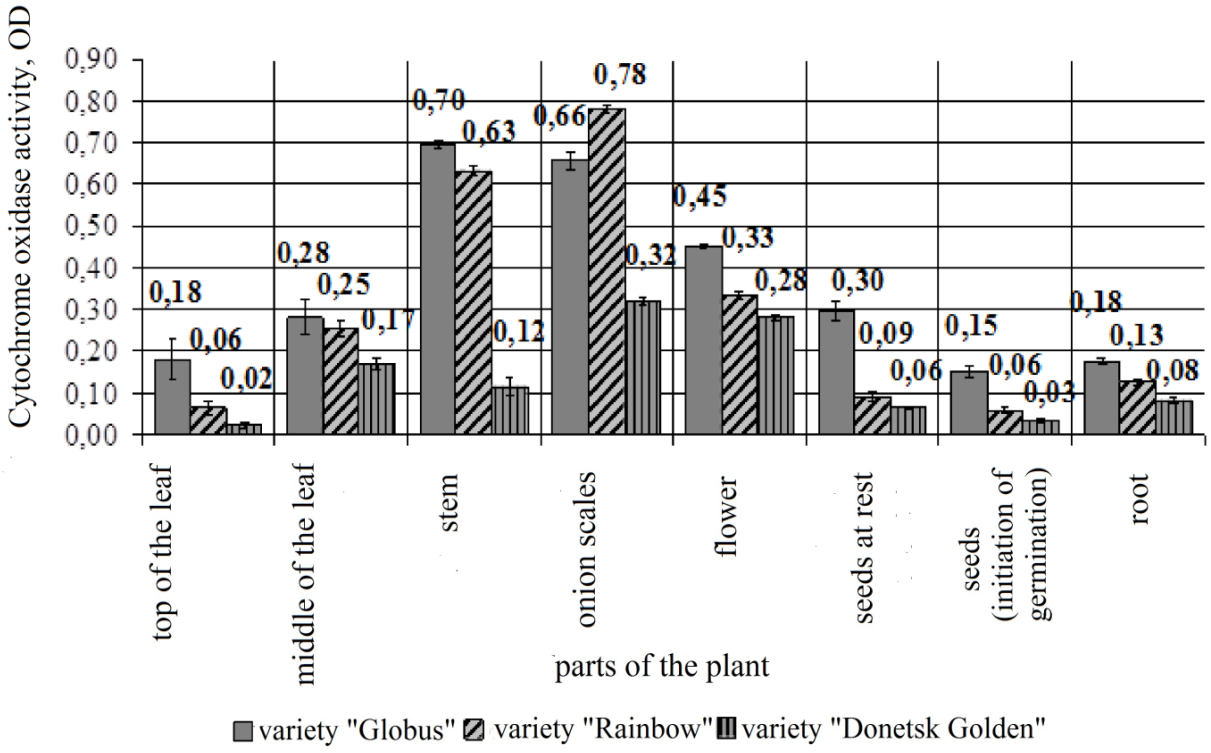

Fig. 8. Comparison of cytochrome oxidase activity in onion tissues.

The diagram shows that the highest level of enzyme activity among photosynthetic organs has a stem, among other vegetative organs – the root and scales of onions. As shown in Fig. 1 and 2, the tissues of these organs are characterized by a relatively low prooxidant activity, which is a possible explanation for this distribution of cytochrome oxidase activity.

As a result of statistical processing of experimentally obtained data, a characteristic feature of the state of PAS tissues of onion varieties "Globe" was a close relationship between concentrations of $\bullet O_2^-$ with GSH (r = + 0.65) and with catalase activity (r > + 0.77) in seed tissues, connection with AA (r > + 0.63) and SOD (r = + 0.75) in onion-turnip tissues, as well as with GSH-peroxidase in leaf apex tissues (r = + 0.93) and the middle of the leaf (r = + 0.67) onion. Variety "Rainbow" is characterized by a close dependence of the concentration of $\bullet O_2^-$ with AA (r > + 0.65) and catalase (r < -0.63) in the tissues of the apex of the leaf; catalase correlates with GSH-peroxidase (r = + 0.86) in the tissues of the middle of the leaf; in flower tissues, TBA_{ap} and SOD are closely related (r = + 0.67), $\bullet O_2^-$ and GSH-peroxidase (r = 0.86); in root tissues – $\bullet O_2^-$ directly correlates with AA, catalase, SOD and GSH (r > + 0.63), and in seed tissues – SOD with GSH and TBA_{ap} (r > + 0.63). For the tissues of the onion flower of the «Donetsk Golden» variety, a direct proportional relationship between the concentration of TBA_{ap} and the activity of catalase and cytochrome oxidase (r > + 0.63) was revealed; in seeds, the associated feedback is GSH-peroxidase and TBA_{ap} (r < -0.63); at the roots – directly associated catalase with TBA_{ap} (r = + 0.71); revealed an inverse relationship of TBA_{ap} with GSH and GSH-peroxidase (r < - 0.63) in the tissues of the scales and leaves of onions, as well as a direct connection with catalase (r > + 0.63) in the tissues of the stem, which is likely, are variety-specific traits.

Therefore, the results of correlation analysis indicate a possible connection of the synthesis of these components of PAS, their interdependent participation in the chains of biochemical reactions, including reactions to ensure the resistance of the variety to disease.

Comparative characteristics of quantitative indicators of the state of PAS of onions depending on the level of resistance of the variety to diseases. The results of the biochemical analysis and inter-varietal comparison of the values of indicators indicate that the tissues of the apex of the onion leaf variety "Globus" have the highest background level of generation $\bullet O_2^-$ compared to the variety "Rainbow" and "Donetsk Golden", but the level of TBA_{ap0} and $TBA_{ap1,5}$ is minimal and increases in the transition from stable to low-resistant variety. A possible explanation for this phenomenon is the experimentally confirmed significant predominance of catalase, SOD, and AA concentrations in onions of the highly disease-resistant Globus onion and the decrease in the value of these indicators in the transition to the low-resistance Donetsk Golden variety. The result of the established predominance is the lowest level of destructive

peroxide damage to tissue membranes of highly disease-resistant varieties, as evidenced by the almost predominance of cytochrome oxidase activity in the tissues of the leaf tips of the onion variety "Globus" in 2.81 ($p < 0.05$) and 8.18 ($p < 0.05$) times compared to "Rainbow" and "Donetsk Golden", respectively. Therefore, the obtained results confirm the connection between ROS and AO in ensuring the resistance of onion varieties to diseases.

Cross-varietal comparison of the values of the PAS of the tissues of the middle leaf of the onion also revealed the predominance of the background level of superoxide generation in the onion of the high-resistance variety. However, in the transition from the apex to the middle of the leaf there is a significant weakening of mitochondrial and enhancement of microsomal generation $\bullet O_2^-$, while in the tissues of medium- and low-resistant varieties there is a slight strengthening of both links of generation $\bullet O_2^-$, which may be varietal-specific.

It was found that mid-leaf tissues of all experimental onion varieties are characterized by a decrease in SOD and catalase activity, but a compensatory increase in GSH-peroxidase activity and AA concentration, which is associated with the increased photosynthetic activity of mid-leaf tissues and reflected in increasing TBA_{ap0} and $TBA_{ap1.5}$. The intensity of AOS increases in the transition from low-resistant to disease-resistant variety, resulting in a predominance of cytochrome oxidase activity in the transition from the variety "Donetsk Golden" to "Globe" and even an increase in the value compared to tissues of the apex, indicating a decrease free radical peroxide destruction of membrane biopolymers.

Analysis of cross-varietal comparison of the values of PAS tissue indicators of onion-turnip scales revealed an inverse dependence of the level of superoxide generation on the level of variety resistance to diseases with a significant increase in the share of microsomal products. The concentration of TBA_{ap0} and $TBA_{ap1.5}$ also increased during the transition from high-resistant to low-resistant varieties, but the analysis of cytochrome oxidase activity revealed a 6.05-fold predominance of the value of the Globus variety established for onions over Donetsk Golden ($p<0.05$). , and the advantage over the values of the tissues of the onion leaf in 3.02 times ($p <0,05$). It was found that the concentration of AA, the activity of SOD and catalase significantly increase according to the resistance of onion varieties to disease, and the activity of GSH-peroxidase and the concentration of GSH – decreases. The obtained

results allow us to conclude the strengthening of the role of the antioxidant part of PAS in ensuring the resistance of the tissues of onion scales to diseases.

Attention is drawn to the experimentally detected increase in the generation of $\bullet O_2^-$ in the tissues of the onion arrow with an increase in the resistance of the variety to disease while reducing the level of TBA_{ap0} and $TBA_{ap1,5}$. The inter-varietal difference in catalase, SOD, AA, and GSH activity is characterized by an increase in their numerical values during the transition to a highly disease-resistant variety, and cytochrome oxidase activity is generally highest among photosynthetic organs and decreases with decreasing variety resistance. A possible explanation for this distribution is the formation of other ROSs in addition to O_2 and the predominance of the AA link in ensuring tissue resistance to disease.

Intervarietal analysis of the value of the indicators of PAS of root tissues is similar to the tissues of onion scales, which is probably explained by the affiliation of these organs to non-photosynthetic vegetative. Thus, in the transition from high- to low-resistant onion varieties, a significant increase in superoxide generation, an increase in the concentration of TBA_{ap0} and $TBA_{ap1.5}$, a decrease in the activity of SOD and catalase, the concentration of AA. Instead, there is a slight increase in GSH peroxidase activity and GSH concentration. The degree of free radical peroxide destruction decreases with increasing resistance of the variety, as evidenced by the recorded increase in cytochrome oxidase activity during the transition to a highly resistant variety.

Cross-varietal comparison of the values of PAS of onion flower tissues shows that the level of background and stimulated generation $\bullet O_2^-$ decreases in accordance with the decrease in the stability of the variety. A similar series of decreases in the value of the indicator was found in relation to TBA_{ap0} and $TBA_{ap1,5}$. It is established that flower tissues have the highest value of all studied enzymatic and non-enzymatic antioxidants and are characterized by a significant increase in their activity during the transition from low-resistant variety "Donetsk Golden" to high-resistant variety "Globus". It was recorded that the tissues of the flower of the Globus variety also have the lowest level of FRPO of biopolymers of membranes, as evidenced by a significant increase in cytochrome oxidase activity during the transition from high- to low-disease-resistant varieties. Therefore, with increasing resistance of onion varieties to disease, there is an increase in both parts of the PAS in the tissues of its flower.

The inter-varietal analysis of the value of the indicators of the state of PAS of onion seeds, which is at rest, shows that the level of generation $\bullet O_2^-$ decreases with decreasing index of resistance of the variety. A similar pattern was found for the concentration of TBA_{ap0}. The concentration of low molecular weight antioxidants in onion seeds increases with increasing resistance of plant varieties to disease, which is also characteristic of most of the studied enzyme AO. In general, it was found that the level of FRPO biopolymers remains the lowest in the stable variety "Globus" and increases during the transition to "Donetsk Golden".

According to the experimentally obtained results, the initiation of the germination process stimulates an increase in the activity of enzymatic antioxidants, the values of which increase with increasing resistance of plant varieties to disease. Therefore, it can be concluded that for dormant onion seed tissues, the prooxidant PAS is predominant, which may play a role in controlling cell division (Janků et al., 2019; Tsebrzhinskiy, 1992), activating the germination process enhances the activity of both links of PAS, the values of which change according to changes in the resistance of onion varieties to disease.

The results of the analysis suggest that: first, the most significant indicators of PAS, which are associated with plant resistance to disease, in all parts of onions, are the concentration of $\bullet O_2^-$ and AA, catalase activity, SOD and cytochrome oxidase; secondly, when conducting biochemical studies that reflect the dependence of the resistance of the variety to diseases with the state of PAS of onions, it is advisable to use the tissues of the flower, leaf, and seed, activated before germination.

Conclusions

As a result of the conducted scientific research, the connection of the state of the prooxidant-antioxidant system with the level of resistance of the variety to diseases and the distribution of the components of the prooxidant-antioxidant system in different plant organs was established. This allowed us to draw the following conclusions:

1. The resistance of the variety to diseases depends on the following indicators of the prooxidant-antioxidant system of plants: level of generation $\bullet O_2^-$, the content of TBA-active products, ascorbic acid, glutathione, superoxide dismutase activity, glutathione peroxidase, cytochrome oxidase.

2. In the tissues of photosynthetic vegetative organs of onions, there is a strengthening of both parts of the prooxidant-antioxidant system; in tissues that are not capable of photoproduction, there is an advantage of the antioxidant link in accordance with the increased resistance of the variety to disease.

3. The greatest activity of both parts of the prooxidant-antioxidant system is characteristic of onion flower cells, which is associated with their receptor, protective, and regulatory value. Onion seed tissues, which are at rest, have the advantage of a prooxidant link and increase the concentration of low molecular weight antioxidants. Initiation of germination processes enhances the activity of both parts of the prooxidant-antioxidant system, as evidenced by an increase in the basic concentration $\bullet O_2^-$ by 26.98% ($p < 0.05$), an increase in the stimulated concentration with NADP●H – by 6.55% ($p < 0.05$), stimulated by yeast – by 175.39% ($p<0.001$), stimulated by NaF – by 362.34% ($p < 0.001$); ascorbic acid – by 4.5% ($p < 0.05$), catalase activity – by 30.31% ($p <0.05$), superoxide dismutase – by 76.82% ($p < 0.001$), cytochrome oxidase – by 58, 69% ($p <0.001$).

4. A characteristic feature of the state of PAS of onion varieties "Globe" was a close dependence between the concentrations of $\bullet O_2^-$ with GSH ($r = + 0.65$) and with the activity of catalase ($r > + 0.77$) in seed tissues, the connection with AA ($r > + 0.63$) and SOD ($r = + 0.75$) in onion-turnip tissues, as well as with GSH-peroxidase in leaf apex tissues ($r = + 0.93$) and leaf middle ($r = + 0.67$) onions. Variety "Rainbow" is characterized by a close dependence of the concentration of $\bullet O_2^-$ with AA ($r > + 0.65$) and catalase ($r < – 0.63$) in the tissues of the apex of the leaf; catalase correlates with GSH-peroxidase ($r = + 0.86$) in the tissues of the middle of the leaf; in flower tissues, TBA_{ap} and SOD are closely related ($r = + 0.67$), $\bullet O_2^-$ and GSH-peroxidase ($r = 0.86$); in root tissues – $\bullet O_2^-$ directly correlates with AA, catalase, SOD and GSH ($r > + 0.63$), and in seed tissues – SOD with GSH and TBA_{ap} ($r > + 0.63$). For the tissues of the onion flower variety "Donetsk Golden" revealed a directly proportional dependence of the concentration of TBA_{ap} with the activity of catalase and cytochrome oxidase ($r > + 0.63$); in seeds, the associated feedback is GSH-peroxidase and TBA_{ap} ($r < – 0.63$); at the roots – directly associated catalase with TBA_{ap} ($r = + 0.71$); revealed an inverse relationship of TBA_{ap} with GSH and GSH-peroxidase ($r < – 0.63$) in the tissues of the scales and leaves of onions, as well as a direct connection with catalase ($r > + 0.63$) in the tissues of the arrow, which is likely, are variety-specific traits.

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