DEPÓSITO LEGAL ZU2020000153
ISSN 0041-8811
E-ISSN 2665-0428

Revista de la Universidad del Zulia

Fundada en 1947
por el Dr. Jesús Enrique Lossada

Ciencias Exactas, Naturales y de la Salud

Año 13 N° 37
Mayo - Agosto 2022
Tercera Época
Maracaibo-Venezuela

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ANIVERSARIO

REVISTA DE LA UNIVERSIDAD DEL ZULIA. 3ª época. Año 13 N° 37, 2022
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DOI: http://dx.doi.org/10.46925//rdluz.37.23

Features of changes in prooxidant-antioxidant balance of tissues during activation of seed germination

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ABSTRACT

Aim of the research: to identify changes in the value of indicators of the state of the prooxidant-antioxidant system (PAS) in seed tissues at rest and the initiation of its germination processes. The **subject** of the research is the role of individual components of the PAS in ensuring the activation of seeds before germination. **Methodology**. Quantitative determination of indicators of the state of PAS was performed on tissue samples of seeds of the following plants: *Glycine max L., Helianthus annuus L., Fagopyrum esculentum L., Linum usitatissimum L., Sinapis alba L., Chenopodium quinoa L., Panicum miliaceum L., Oryza sativa L., Avena sativa L., Zea mays L., Hordeum vulgare L., Triticum durum Desf.* The concentration of superoxide anion radical ($\bullet O_2^-$), TBA-active products, cytochrome oxidase activity, superoxide dismutase activity, catalase, the concentration of ascorbic acid, glutathione was determined. The **results** of the research show that for the tissues of seeds of experimental Magnoliopsida plants at rest, both links of PAS are more powerful than in Liliopsida, the level of free radical peroxidation (FRPO) is lower, which is achieved by both enzymatic and low molecular weight antioxidants (AO). Germination activation enhances both links of PAS in all experimental groups of plants, however, in Magnoliopsida, we observe the stronger generation of $\bullet O_2^-$, and the predominance of protection by enzymatic AO, and in Liliopsida - low molecular weight.

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

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Recibido: 09/02/2022 Aceptado: 06/04/2022

DOI: http://dx.doi.org/10.46925//rdluz.37.23

Características de los cambios en el equilibrio prooxidante-antioxidante de los tejidos durante la activación de la germinación de semillas

RESUMEN

Objetivo de la investigación: identificar cambios en el valor de indicadores del estado del sistema prooxidante-antioxidante (SPA) en tejidos de semillas en reposo y el inicio de sus procesos de germinación. El tema de la investigación es el papel de los componentes individuales del SPA para garantizar la activación de las semillas antes de la germinación. Metodología. La determinación cuantitativa de indicadores del estado de SPA se realizó en muestras de tejido de semillas de las siguientes plantas: Glycine max L., Helianthus annuus L., Fagopyrum esculentum L., Linum usitatissimum L., Sinapis alba L., Chenopodium quinoa L. , Panicum miliaceum L., Oryza sativa L., Avena sativa L., Zea mays L., Hordeum vulgare L., Triticum durum Desf. Se determinó la concentración de radical anión superóxido (●O2-), productos activos de TBA, actividad de citocromo oxidasa, actividad de superóxido dismutasa, catalasa, concentración de ácido ascórbico, glutatión. Los resultados de la investigación muestran que para los tejidos de semillas de plantas experimentales de Magnoliopsida en reposo, ambos enlaces de SPA son más potentes que en Liliopsida, el nivel de peroxidación de radicales libres (PRL) es menor, lo que se logra tanto por enzimática como por bajo antioxidantes de peso molecular (AO). La activación de la germinación potencia ambos enlaces de SPA en todos los grupos experimentales de plantas. Sin embargo, en Magnoliopsida observamos la mayor generación de ●O2-, y el predominio de la protección por AO enzimático, y en Liliopsida -de bajo peso molecular.

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

Introduction

The transition of a plant from a latent state to a pregenerative one is the initial stage of its ontogenesis. The starting and the most responsible process is the germination process. Induction, maintenance, and exit of seeds from dormancy are controlled by complex physiological and biochemical mechanisms, which are influenced by a wide range of endogenous and exogenous factors. The study of changes in the components of the prooxidant-antioxidant system (PAS) that initiate the process of seed germination opens the possibility of regulating and correcting this stage of plant ontogenesis, increasing germination, and friendliness of crops is particularly relevant and economically justified in

DOI: http://dx.doi.org/10.46925//rdluz.37.23

terms of crop intensification. The use of human processes of seed germination initiation in everyday life deserves special attention. Thus, among supporters of a healthy lifestyle and a balanced diet, attention to cereals and so-called "live cereals" has increased significantly, in the preparation of which pre-soaking and minimal heat treatment is recommended to preserve the maximum amount of biologically active substances in food. Given the growing number of supporters of this issue among physicians, nutritionists, and scientists (Baiano & del Nobile, 2015; Xu et al., 2017; Pacheco et al., 2018), the study of biochemical changes that accompany the processes of seed germination is of great practical importance.

Aim of the research: to investigate the change in prooxidant-antioxidant homeostasis in seed tissues at rest and the initiation of its germination processes.

To achieve this aim, the following **tasks** were identified:

1. To investigate the state of PAS in the tissues of seeds of experimental plants at rest.
2. To investigate the changes in the values of PAS in the tissues of seeds of experimental plants in the initiation of the germination process.
3. To compare how the initiation of sampling affects the prooxidant (PO) and antioxidant (AO) links in the tissues of experimental Magnoliopsida and Liliopsida plants.
4. To determine species-specific features of PAS change of tissues of seeds of experimental plants of different systematic categories.

1. Literature review

The seeds of wild and cultivated plants are characterized by a state of forced dormancy. In this case, the ability of seeds to be at rest provides plants with the opportunity to experience unfavorable for their existence periods of the year. However, with increasing hydration in the seeds, the main metabolic processes are activated, and respiration increases to the maximum level, which characterizes their growth and development. The period of seed germination is divided into three stages: 1) activation of metabolism (stage of physical swelling of seeds); 2) preparation for the beginning of growth by stretching (gluing of seeds due to transition to stretching of cells of axial organs of a germ); 3) the actual growth of the organs of the seedling (Nonogaki, 2017; Wolny et al., 2018). In wet seeds, there is an active consumption of oxygen, which can cause oxidative tissue damage (Oracz & Karpinski, 2016). Reactive oxygen species (ROS) play a role in the development of oxidative stress: $\bullet O_2^-$, H_2O_2, HO^-, $HOCl$, etc. (Apel & Hirt, 2004; Halliwell, 2006)

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Reactive oxygen species (ROS) are currently considered "dual agents" (Dat et al., 2000; Mittler, 2017). They either directly initiate intense oxidative stress, accompanied by damage or death of cells and the body, or act as signaling molecules that induce physiological and biochemical reactions that increase the body's resistance (Vranova et al., 2002; Jaspers & Kangasjarvi, 2010; Kolupaev & Karpets, 2010; Kreslavski et al., 2012).

Recent studies have shown that ROS are involved in the regulation of the most important physiological processes, including growth and development, response to biotic and abiotic stresses, and programmed cell death (Kreslavski et al., 2012; Suzuki et al., 2012; Silvina et al., 2016; Gautam et al., 2017). The greatest progress in understanding the role of ROS in growth and development has been achieved in the study of the early embryogenesis of Fucus and the formation of Arabidopsis root hairs (Silvina et al., 2016). Using similar approaches, it was previously shown that growth processes in plant somatic cells also depend on the level of extracellular ROS (Gautam et al., 2017). Two mechanisms have been proposed to explain this phenomenon. According to the first of them, some ROS can act as signaling factors; according to the second, ROS are involved in the chemical modification of the polymer matrix of the cell wall and thereby affect its mechanical properties (Mittler et al., 2004).

ROS generation in plants occurs in cell walls, plasma membrane, chloroplasts, mitochondria, peroxisomes, and, possibly, in other compartments (Foyer & Noctor, 2009). The greatest contribution to the formation of ROS during seed germination is made by mitochondria, which is associated with the activation of cellular respiration, as well as the cell wall of actively dividing cells (Kolupaev et al., 2019). Complexes I and III are considered to be the main sites of electron leakage in plant mitochondria (Rhoads et al., 2006; Cvetkovska & Vanlerberghe, 2013). In plants, the relationship between electron transport, oxidative phosphorylation, and ROS generation is complicated by the presence of an alternative oxidase that catalyzes the oxidation of ubiquinone and the reduction of molecular oxygen to water. In this case, the likelihood of the formation of $\bullet O_2^-$ due to the leakage of an electron from complex III is prevented. Along with this, the transport of electrons bypassing complex III, cytochrome c, and complex IV reduces mitochondrial re-reduction, their membrane potential, and, as a consequence, the likelihood of ROS formation (Moller, 2001; Kolupaev & Karpets, 2014).

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During the germination process, the plant cell is exposed to exogenous environmental factors. ROS accompany the formation of the metabolic reaction of plants to the first influence of abiotic factors, take part in the formation of the adaptation reaction, as well as the response to stress changes (Gill & Tuteja, 2010; Hasanuzzaman et al., 2019; Kolupaev & Karpets, 2019; Pacheco et al., 2018). Thus, using hypothetical sensors (Los et al., 2010; Hirayama & Shinozaki, 2010), the cell surface perceives signals of hyperthermia, osmotic action, or salinity. This leads to the activation of transmembrane NADPH-oxidase, apoplastic forms of peroxidase and increased generation of $\bullet O_2^-$. The latter, with the help of apoplastic forms of superoxide dismutase (SOD), can be converted into H_2O_2, which freely penetrates the cytoplasm through the plasma membrane. In addition, under certain conditions $\bullet O_2^-$ can be converted to hydroperoxyl and pass through membranes (Sagi & Fluhr, 2006). Simultaneously, under the action of stressors, an increase in the stochastic formation of ROS in chloroplasts and mitochondria and activation of photorespiration can occur (Foyer & Noctor, 2009). An increase in the concentration of H_2O_2 in cells leads to the modification of intracellular protein redox sensors. Ultimately, it is likely that the ROS signal leads to a change in the state of transcript factors that control the genes of antioxidant enzymes and enzymes involved in proline synthesis, and other protective reactions (Suzuki et al., 2012).

ROS signals are also closely integrated with the signals of phytohormones, in particular, ethylene, abscisic, salicylic and jasmonic acids, brassinosteroids (Galiba et al., 2013; Bartoli et al., 2013), regulating cell metabolism during germination and rest (Kolupaev & Karpets, 2014; Oracz & Karpinski, 2016).

However, the accumulation of ROS in cells leads to disruption of the course of transcription and replication processes, changing the composition of membrane lipids. $\bullet O_2^-$ modify proteins, disrupt the structure of DNA, destroy hormones and other functionally active substances. Therefore, the study of the manifestation of compensatory mechanisms in seeds on the action of ROS is a general biological task [5. The control over the content of ROS in seeds is carried out by antioxidants (AO) (Apel & Hirt, 2004; Kumar et al., 2011).

The key low molecular weight AO is ascorbic acid (AA) and glutathione (GSH), and the enzymatic ones are SOD, catalase, and peroxidase (Polesskaja, 2007; Kolupaev et al., 2019). Recently, the relevance of publications on a significant increase in the amount of antioxidants in the tissues of germinated seeds, but comprehensive information on the

DOI: http://dx.doi.org/10.46925//rdluz.37.23

consistency of the components of prooxidant and antioxidant systems in the initiation of germination is absent or fragmentary, which necessitates comprehensive research and systematization.

2. Research methodology

Quantitative determination of PAS was performed on seed tissue samples of the following plants: *Glycine max L., Helianthus annuus L., Fagopyrum esculentum L., Linum usitatissimum L., Sinapis alba L., Chenopodium quinoa L., Panicum miliaceum L., Oryza sativa L., Avena sativa L., Zea mays L., Hordeum vulgare L., Triticum durum Desf.* Seed analysis was performed on objects that were in a state of physiological rest. In parallel, the seeds were examined during the initiation of germination, which was carried out by the previous 12-hour soaking in water. Each experimental group included 10 samples, so the experiment analyzed 1680 samples.

NBT test (spectrophotometric variant) was used to determine the concentration of *superoxidanion radical*: 0.1 g of tissue was homogenized in 0.9 cm^3 of phosphate buffer (pH = 7.4). The obtained homogenate and buffer solution (0,05 cm^3 each) were mixed in equal proportions, shaken (time – 2 min), 0,05 cm^3 NBT was added. After 30 minutes incubation of the resulting solution (at 24^0C) was added 2 cm^3 of a solution of dimethyl sulfoxide-chloroform (2:1). The extraction was performed for 1 min, centrifuged for 5 min (at 1500 rpm). The supernatant of the cetrifugate solution was photometered against the control at 540 nm (cuvette 1 cm^3, thickness 0,5 cm).

Distilled water was used instead of the homogenate to prepare the control sample. Superoxide production was calculated according to the calibration graph, for the construction of which the extinction of the samples was determined from 0,01, 0,02, 0,05, 0,07, 0,1, 0,2 cm^3 NBT (w = 0.2%), 0,1 cm^3 1 M KOH and 0,1 cm^3 AK solution (18 mg/10 cm^3).

To determine the increase in the level of *TBA-active products* (Δ TBAap), their baseline level (TBA_{ap0}) and stimulated ($TBA_{ap1,5}$) level were determined. The final value of the indicator (in µmol/kg) was calculated by the formula:

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

To determine TBA_{ap0} 0,5 g of the test object was homogenized in 4,5 cm^3 of tris-buffer solution (pH = 7,4). 2 cm^3 of homogenate were taken, mixed with trichloroacetic acid (w = 30%) and centrifuged (conditions: 30 min, at 3000 rpm).

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The 2 cm^3 centrifuge supernatant was mixed with 3 cm^3 of thiobarbituric acid solution (w = 0,338%, ex tempore preparation). The resulting trimethine complex was photometered at 540 nm against a control that did not contain homogenate (reagent control composition: 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). Determination of $TBA_{ap1,5}$ was performed by a similar method, with pre-incubation of the sample in prooxidant iron-ascorbate buffer for 90 min. The concentration of TBAap was calculated by 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.

Superoxide dismutase (SOD) activity was determined in the following sequence: 0,5 g of test tissue was homogenized in 0,5 cm^3 of water. To precipitate pigments after 10 minutes was added 2 cm^3 of a mixture of ethanol chloroform (5:3), incubated at -4^{0}C. After 24 hours, stirred and centrifuged for 15 min at 3000 rpm. 0,1 cm^3 of the centrifuge was mixed in a cuvette (1 cm) with a prepared solution containing 4,4 cm^3 of carbonate buffer (pH = 10,2) and 0,5 cm^3 of a solution of adrenaline (C = 0,01 mol/dm^3) in lemon acid (C = 0,01 mol/dm^3). Extinction was determined every minute until it stopped increasing. 0,1 cm^3 of distilled water was introduced into the control sample instead of the centrifugate. The calculation of SOD activity was carried out according to the formula:

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

where 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%.

Catalase activity was determined by the following method: to a flask with 7 cm^3 distilled water was added 1 cm^3 of aqueous tissue homogenate of the test object (0,1 g in 20 cm^3 of H_2O) and 2 cm^3 of H_2O_2 (w = 1%), shaken every 10 minutes. After 30 minutes 3 cm^3 of H_2SO_4 solution (w = 10%) was added and titrated with 0,1 M $KMnO_4$ solution to a pale pink color that did not disappear for 30 seconds.

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The calculation of catalase number (A) (in µmol of substrate per unit time per unit mass of protein) was carried out by the formula:

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

where $V_{control}$ and $V_{experimental}$ is the volume of $KMnO_4$ solution spent on titration of the control and test samples, respectively, cm^3 (boiled homogenate was used in the control sample); 1,7 – amount of H_2O_2 (mg), which corresponds to 1 cm^3 of $KMnO_4$ solution; t – incubation time of the sample (30 s); $M(H_2O_2)$ – molar mass of H_2O_2 (34 g/mol).

When determining the *concentration of GSH*, the homogenate was prepared on trichloroacetic acid (0,1 g of tissue with 2,4 cm^3 of trichloroacetic acid) and left for 10 minutes. Subsequently, the samples were centrifuged for 15 min at 3000 rpm. 0,2 cm^3 of centrifugate was mixed with 0,05 cm^3 of NaOH solution (w = 20%) and 5 cm^3 of tris-buffer (pH = 8,05) and 0,1 cm^3 of Elman's reagent (99 mg DTNBK in 25 cm^3 ethanol). After 20 minutes keeping the samples in the dark, performed their photometry at 412 nm in a cuvette per 1 cm against control of reagents that did not contain homogenate. GSH concentration was determined according to a standard calibration schedule.

When determining the *concentration of AA* used acid homogenate (1 g of tissue in 9 cm^3 of HCl solution (w = 2%)), which after 10 minutes filtered. 3 cm^3 of the filtrate was titrated with a solution of 2,6-dichlorophenolindophenol (0,001 mol/dm^3) to a stable pink color. Boiled filtrate in the presence of 3 drops of 3% H_2O_2 was used in the control sample. The concentration of AA (in mmol/kg) was calculated by the formula:

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

where Q is the amount of AA corresponding to 1 cm^3 of a solution of 2,6-dichlorophenolindophenol (0,088 mg); V_0 – total amount of extract, cm^3; V_1 - volume of extract taken for titration, cm^3; a – weight of the sample, g; A_{contr}, A_{exp} – the volume of a solution of 2,6-dichlorophenolindophenol spent on titration of the control and experimental sample, cm^3.

To determine the *activity of cytochrome oxidase*, a tissue homogenate of the test object was prepared in phosphate buffer (ratio 0,5 g in 4,5 cm^3) at pH 7,6. To 1 cm^3 of homogenate was added 1 cm^3 of the reaction mixture containing 0,25 cm^3 of α-naphthol in 50 cm^3 of ethanol (w = 22%), 0,35 cm^3 of aqueous solution of N,N-dimethyl-para-phenylenediamine hydrochloride (w = 0,1%), 0,25 cm^3 of phosphate buffer, 0,15 cm^3 of cytochrome *c* solution (w = 0,02%). After 5 minutes diethyl ether and ethanol were added in a volume ratio of 9:1 (V =

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10 cm^3), kept at 4^{0}C for 30 minutes, shaking periodically. The extract was photometered at 540 nm against the control, which instead of the homogenate contained 1 cm^3 of diluted buffer solution. The calculations were performed 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;

10 – breeding; 5 – incubation time.

All the results of the determination of biochemical indicators of the state of the prooxidant-antioxidant system were processed statistically according to generally accepted methods.

3. Results and discussion

The results of determining the indicators of the PO link of PAS and FRPO tissues of dormant seeds are shown in table 1, AO link of PAS - in table 2. The effect of activation of germination processes on PO link of PAS and FRPO is shown in table 3, the effect on AO link - in table 4.

Table 1. The results of determining the prooxidant activity and the level of FRPO in seed tissues at rest

Experimental plants	Indicators of prooxidant activity		Cytochrome oxidase activity, OD
	NBT test (base level), nmol $\bullet O_2^-/(g*s)$	Δ TBA_{ap}, %	
Magnoliopsida			
Glycine max L.	0,072 ±0,011	66,15 ± 5,01	0,314 ±0,019
Helianthus annuus L.	1,134 ± 0,042	42,23 ± 1,06	0,204±0,003
Fagopyrum esculentum L.	0,287 ± 0,019	99,22 ± 4,11	0,183±0,005
Linum usitatissimum L.	1,006 ± 0,011	29,88 ± 1,44	0,262±0,009
Sinapis alba L.	0,778 ± 0,021	35,18 ± 1,22	0,240±0,004
Chenopodium quinoa L.	0,122 ± 0,014	85,14 ± 3,67	0,436 ±0,011
Liliopsida			
Panicum miliaceum L.	1,086±0,011	136,49 ± 6,22	0,118±0,006
Oryza sativa L.	0,437±0,010	21,63 ± 1,10	0,398 ±0,006
Avena sativa L.	0,036±0,004	11,27 ± 2,01	0,418 ±0,009
Zea mays L.	1,273±0,015	111,83 ± 5,19	0,159±0,008
Hordeum vulgare L.	0,091±0,009	128,45 ± 18,35	0,276±0,005
Triticum durum Desf.	0,090 ±0,009	27,86 ± 4,11	0,346 ±0,001

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Table 2. The results of determining the antioxidant activity in the tissues of seeds at rest

Experimental plants	Enzymatic antioxidants		Low molecular weight antioxidants	
	Catalase activity, $\frac{\mu mol}{kg*min}$	SOD activity, OD	Concentration of AA, $\frac{mmol}{kg}$	Concentration of GSH, $\frac{mmol}{kg}$
Magnoliopsida				
Glycine max L.	0,48 ± 0,02	0,53 ± 0,02	0,293 ± 0,03	59,32 ± 0,95
Helianthus annuus L.	0,19 ± 0,01	0,28 ± 0,01	0,096 ± 0,01	39,11 ± 0,72
Fagopyrum esculentum L.	0,31 ± 0,02	0,28 ± 0,02	0,141 ± 0,02	43,22 ± 0,96
Linum usitatissimum L.	0,11 ± 0,01	0,30 ± 0,01	0,135 ± 0,02	46,79 ± 0,48
Sinapis alba L.	0,25 ± 0,01	0,36 ± 0,03	0,110 ± 0,01	41,01 ± 0,63
Chenopodium quinoa L.	0,36 ± 0,01	0,44 ± 0,02	0,120 ± 0,02	51,67 ± 0,11
Liliopsida				
Avena sativa L.	0,39 ± 0,03	0,46 ± 0,02	0,111 ± 0,03	54,19 ± 0,34
Oryza sativa L.	0,31 ± 0,01	0,42 ± 0,01	0,092 ± 0,01	45,18 ± 0,78
Hordeum vulgare L.	0,23 ± 0,02	0,29 ± 0,01	0,076±0,01	48,05 ± 0,10
Triticum durum Desf.	0,09 ± 0,02	0,22 ± 0,01	0,057 ± 0,01	40,79 ± 0,25
Zea mays L.	0,09 ± 0,01	0,19 ± 0,01	0,085 ± 0,02	37,16 ± 0,99
Panicum miliaceum L.	0,07 ± 0,01	0,16 ± 0,01	0,037 ± 0,01	43,14 ± 0,67

Among the researched Magnoliopsida, the lowest level of $\bullet O_2^-$ generation has the seeds of *Glycine max L.*, the highest is characteristic of the tissues of *Helianthus annuus L.*, *Linum usitatissimum L.*, and *Sinapis alba L.* It is noteworthy that the reserve substances in the last three experimental plant species are lipids, while in *Glycine max L.* – proteins. Moreover, the level of $\bullet O_2^-$ generation is higher the lower the content of polyunsaturated fatty acids (PUFA) among lipid inclusions. The lowest values of ΔTBAap are also characteristic of plants that specialize in lipid storage, within this group we also observe a decrease in ΔTBAap with increasing PUFA content. The largest difference in ΔTBAap is characteristic of the tissues of *Fagopyrum esculentum L.* and *Chenopodium quinoa L.*, whose reserve substance is carbohydrates. However, according to the literature, among these two plant species, the seeds of *Chenopodium quinoa L.*, in addition to carbohydrates, have a significantly higher percentage of protein in the reserve than *Fagopyrum esculentum L.*, and according to the results - a lower level of generation $\bullet O_2^-$ and ΔTBA_{ap}. All of the above suggests that the accumulation of tissue tissues of experimental plants of protein inclusions, or PUFA helps to reduce the generation of $\bullet O_2^-$

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and ΔTBAap. The degree of FRPO can be judged by the activity of cytochrome oxidase. Thus, in the tissues of *Glycine max L.* and *Chenopodium quinoa L.* it is the highest, and in the tissues of *Helianthus annuus L.*, *Linum usitatissimum L.*, and *Sinapis alba L.* we again observe a decrease in enzyme activity following a decrease in PUFA content in lipid inclusions.

The activity of enzymatic antioxidants in the tissues of experimental Magnoliopsida is 1,35 times higher than that of Liliopsida (the predominance of the average activity of catalase is 1.44 times, SOD – 1,26 times). The increase in the activity of enzymes within the experimental group is also manifested against the background of increasing protein content in the reserve components of seeds. The highest activity of both SOD and catalase was found in the tissues of *Glycine max L.* and *Chenopodium quinoa L.*, which confirms the fact that SOD supplies a substrate for catalase. A parallel decrease in the activity of these two enzymes is observed in a number: *Sinapis alba L.*, *Helianthus annuus L.*, and *Linum usitatissimum L.* (dependence on PUFA content was not detected). In the tissues of *Fagopyrum esculentum L.*, catalase activity remains high, while the activity of SOD decreases, which may be explained by the participation of catalase in the inactivation of PO, the source of which is not SOD.

Table 3. The results of determining the prooxidant activity and the level of FRPO in seed tissues, with the activation of germination processes

Experimental plants	Indicators of prooxidant activity		Cytochrome oxidase activity, OD
	NBT test (base level), nmol $\bullet O_2^-/(g*s)$	Δ TBA$_{ap}$, %	
Magnoliopsida			
Glycine max L.	0,111 ±0,06	30,41 ± 1,26	0,465 ±0,011
Helianthus annuus L.	2,444 ± 0,003	106,84 ± 1,88	0,220±0,006
Fagopyrum esculentum L.	0,364 ± 0,006	58,15 ± 2,01	0,230±0,009
Linum usitatissimum L.	1,809 ± 0,022	61,29 ± 1,55	0,299±0,008
Sinapis alba L.	1,464 ± 0,018	80,80 ± 2,16	0,263±0,004
Chenopodium quinoa L.	0,197 ± 0,011	52,94 ± 1,43	0,572 ±0,020
Liliopsida			
Panicum miliaceum L.	1,506±0,021	154,07 ± 8,02	0,126±0,004
Oryza sativa L.	0,826±0,022	33,38 ± 2,65	0,644 ±0,016
Avena sativa L.	0,062±0,003	17,36 ± 1,28	0,610 ±0,023
Zea mays L.	1,809±0,026	129,14 ± 3,89	0,177±0,005
Hordeum vulgare L.	0,124±0,005	144,29 ± 4,07	0,337±0,009
Triticum durum Desf.	0,112 ±0,004	53,97 ± 1,88	0,364 ±0,008

DOI: http://dx.doi.org/10.46925//rdluz.37.23

Table 4. The results of determining the antioxidant activity in seed tissues, with the activation of germination processes

Experimental plants	Enzymatic antioxidants		Low molecular weight antioxidants	
	Catalase activity, $\frac{\mu mol}{kg*min}$	SOD activity, OD	Concentration of AA, $\frac{mmol}{kg}$	Concentration of GSH, $\frac{mmol}{kg}$
Magnoliopsida				
Glycine max L.	1,21 ± 0,07	0,88 ± 0,05	2,063 ± 0,03	94,43 ± 3,44
Helianthus annuus L.	0,27 ± 0,01	0,34 ± 0,01	0,137 ± 0,02	41,07 ± 0,18
Fagopyrum esculentum L.	0,64 ± 0,02	0,42 ± 0,02	0,845 ± 0,02	51,44 ± 2,18
Linum usitatissimum L.	0,15 ± 0,01	0,38 ± 0,03	0,469 ± 0,01	50,96 ± 0,83
Sinapis alba L. **горчица**	0,39 ± 0,01	0,41 ± 0,01	0,318 ± 0,01	46,16 ± 0,99
Chenopodium quinoa L.	0,79 ± 0,04	0,70 ± 0,03	0,614 ± 0,02	70,42 ± 1,76
Liliopsida				
Avena sativa L.	0,69 ± 0,04	0,86 ± 0,03	1,703 ± 0,09	62,88 ± 0,22
Oryza sativa L.	0,64 ± 0,02	0,80 ± 0,02	0,481 ± 0,02	50,49 ± 0,93
Hordeum vulgare L.	0,33 ± 0,03	0,40 ± 0,02	0,749 ± 0,08	54,91 ± 0,19
Triticum durum Desf.	0,12 ± 0,02	0,29 ± 0,02	0,459 ± 0,04	45,34 ± 0,21
Zea mays L.	0,12 ± 0,01	0,23 ± 0,01	0,690 ± 0,03	42,17 ± 1,04
Panicum miliaceum L.	0,09 ± 0,01	0,18 ± 0,01	0,101 ± 0,01	46,51 ± 0,34

Initiation of germination causes an increase in the level of generation ●O_2^- in all experimental plants. Thus, in the tissues of *Glycine max L.* this figure increased by 54,17%, in the tissues of *Helianthus annuus L.* – by 115,52%, *Fagopyrum esculentum L.* – 24,46%, *Linum usitatissimum L.* – 79,82%, *Sinapis alba L.* – 88,17%, *Chenopodium quinoa L.* – 61,47%. Moreover, among Magnoliopsida, the largest increase in the level of TBA-active products was found in the tissues of *Helianthus annuus L.*, *Linum usitatissimum L.*, and *Sinapis alba L.*, where we again observe a connection with the content of PUFA. However, in the tissues of these three plant species, cytochrome oxidase activity does not decrease and even increases by a small percentage (7,82% for *Helianthus annuus L.*, 14,18% for *Linum usitatissimum L.* and 9,56% for *Sinapis alba L.*) at a slight increase in the activity of enzymatic AO and the content of low molecular weight. A possible explanation for such results is that with the strengthening of the link, the level of non-structural FRPO increases, namely reserve lipids, which are used to meet the energy needs of the body, which is especially important in the transition from rest to active growth. The largest increase ●O_2^- with the smallest increase in TBAap level and the

DOI: http://dx.doi.org/10.46925//rdluz.37.23

highest increase in cytochrome oxidase activity (48,15%) was found in the tissues of *Glycine max L.* A similar pattern is observed for *Chenopodium quinoa L.* but with slightly lower values (increase in cytochrome oxidase 31,24%). In the tissues of *Fagopyrum esculentum L.*, cytochrome oxidase activity increases by 26,03% and occupies an intermediate position between the results described above.

Analyzing the effect of germination activation on the AO link in the tissues of the researched Magnoliopsida, we also observe the largest increase in enzyme AO in the tissues of *Glycine max L.* (66,04% for SOD and 152,08% for catalase). A significant increase in the value of AO enzymes is observed in the tissues of *Fagopyrum esculentum L.* (51,28% for SOD and 107,01% for catalase) and *Chenopodium quinoa L.* (58,22% for SOD and 119,16% for catalase).

The increase in the activity of antioxidant enzymes in Magnoliopsida's tissues, that specializing in lipid inclusions is insignificant, depending on the amount of PUFA was not detected. Thus, for *Helianthus annuus L.*, the increase in catalase activity was 40,22%, SOD – 28,01%, for *Linum usitatissimum L.* – 33,79% and 28,28%, respectively, for *Sinapis alba L.* – 54,13% and 14,93%

Analyzing the results of determination of low molecular weight AO, it can be stated that the highest base level of AA has *Glycine max L.*, which is characteristic of plants of the Fabaceae, and more than 2 times higher than ascorbate content in all other experimental plants. *Helianthus annuus L.* seed tissues have the lowest AA content, which also has the smallest increase in AA content when germination is activated (1,43 times). Activation of germination processes increases the amount of ascorbate in the tissues of *Fagopyrum esculentum L.* by 5,99 times, *Chenopodium quinoa L.* – by 5,12, *Linum usitatissimum L.* – by 3,48 times, *Sinapis alba L.* – by 2,89 times. *Glycine max L.* has the largest increase in the concentration of AA, which is 7,04 times.

Seed tissues of *Glycine max L.* have the highest content of GSH, both at rest and the largest increase in germination (59,18%), which is naturally due to the presence of a large number of amino acids L-cysteine, L-glutamic acid, and glycine required for its synthesis. *Helianthus annuus L.* seed tissue, which specializes in lipid inclusions, has the lowest level of GSH and has the lowest protein content among all experimental plants. Activation of germination processes increases the amount of GSH in the tissues of *Chenopodium quinoa L.* by

DOI: http://dx.doi.org/10.46925//rdluz.37.23

36,28%, *Fagopyrum esculentum L.* – by 19,01%, *Sinapis alba L.* - by 12,55%, *Linum usitatissimum L.* – 8,92%. *Helianthus annuus L.* has the smallest increase in GSH concentration, which is 5,02%. The average GSH growth rate for experimental Magnoliopsida is 26.11%.

Summarizing the results obtained on experimental Magnoliopsida, we can say that in the tissues of seeds at rest, the average level of generation $\bullet O_2^-$ is 0,567 nmol $\bullet O_2^-$/(g*s), ΔTBA_{ap} – 59,63%, SOD – 0,37 OD, catalase – 0,28 µmol/(kg*min), AA – 0,149 mmol/kg, GSH – 46.85 mmol/kg, cytochrome oxidase – 0,273 OD. When activating the germination processes, the average level of generation $\bullet O_2^-$ was 1,065 nmol $\bullet O_2^-$/(g*s), ΔTBAap - 65.07%, SOD – 0,52 OD, catalase – 0,58 µmol/(kg*min), AA – 0,741 mmol/kg, GSH – 59,08 mmol/kg, cytochrome oxidase – 0,342 OD. Thus, the activation of germination caused an increase in the value of the average PAS as follows: for the generation of $\bullet O_2^-$ growth is 87,83%, for ΔTBAap – 9,12%, for SOD – 40,54%, catalase – 107,14% (2.07 times), AA – 397,32% (4,97 times), GSH – 26,11%, cytochrome oxidase – 25,28%.

Analyzing the state of prooxidant activity of seed tissues at rest, we can say that among the experimental Liliopsida the highest base level of $\bullet O_2^-$ generation have *Zea mays L.* and *Panicum miliaceum L.* The same plants have the highest ΔTBAap and rather low values of cytochrome oxidase activity, indicating a high degree of FRPO macromolecules in their cells. This assumption is natural, because, according to the results, both in the tissues of *Zea mays L.* and *Panicum miliaceum L.* found the lowest level of activity of enzymatic antioxidants and close to the lowest content of AA and GSH. A similar prooxidant pattern is observed in the tissues of *Hordeum vulgare L.*, however, increased generation of low molecular weight AO in combination with increased SOD and catalase activity suggests a lower level of FRPO and is confirmed by significantly higher cytochrome oxidase activity compared to the previous two experiments. *Avena sativa L.* tissues have the most powerful link in AO protection, cytochrome oxidase activity is also the highest, TBAap value is the lowest, but the base level of $\bullet O_2^-$ is also low. The peculiarity found in the tissues of *Oryza sativa L.*: so at a fairly high level of generation $\bullet O_2^-$ we observe one of the lowest levels of ΔTBAap and the level of cytochrome oxidase activity, which does not differ significantly from the value set for *Avena sativa L.* Thus, the AO link of PAS in the tissues of *Oryza sativa L.* is more powerful than for *Avena sativa L.*, which is achieved both by SOD and catalase and several other AOs.

DOI: http://dx.doi.org/10.46925//rdluz.37.23

Analyzing the results of the research of PAS of Liliopsida, it can be argued that the initiation of germination causes an increase in the level of $\bullet O_2^-$ generation in the tissues of *Panicum miliaceum L.* by 38,67%, in the tissues of *Oryza sativa L.* – by 88,97%, *Zea mays L.* – 42,14%, *Avena sativa L.* – 72,14%, *Hordeum vulgare L.* – 36,36%, *Triticum durum Desf.* – by 24,39%. The largest increase in the level of ΔTBA_{ap} is characteristic of *Triticum durum Desf.* (26,11%), the least - for *Avena sativa L.* (6,09%). Germination initiation increases the activity of cytochrome oxidase in the tissues of *Avena sativa L.* by 46,02%, *Panicum miliaceum L.* by 6,99%, in the tissues of *Oryza sativa L.* – by 61,80%, *Zea mays L.* – 11,42%, *Hordeum vulgare L.* – 22,16%, *Triticum durum Desf.* – at 5,18%. Catalase activity is enhanced in experimental plants by the following values: *Avena sativa L.* by 75,79%, *Panicum miliaceum L.* by 21,04%, in tissues of *Oryza sativa L.* – by 105,13%, *Zea mays L.* – 29,99%, *Hordeum vulgare L.* – 42,62%, *Triticum durum Desf.* – at 33,91%. By increasing the growth of SOD experimental plants can be placed in the following order: *Panicum miliaceum L.* (15,11%), *Zea mays L.* (23,20%), *Triticum durum Desf.* (30,40%), *Hordeum vulgare L.* (38,46%), *Avena sativa L.* (86,03%), *Oryza sativa L.* (91,07%). Analyzing the content of low molecular weight antioxidants, it should be noted that the concentration of ascorbate in the seed tissues of experimental Liliopsida plants is on average 1,96 times lower than in Magnoliopsida. Oats *Avena sativa L.*, *Oryza sativa L.*, and *Zea mays L.* have the highest background level of AA, *Panicum miliaceum L.* has the lowest.

Activation of germination processes increases the amount of ascorbate in the tissues of *Avena sativa L.* by 15,34 times, *Hordeum vulgare L.* by 9,86, *Zea mays L.* – by 8,15 times, *Triticum durum Desf.* – 8,06 times, *Oryza sativa L.* – 5,23 times. The smallest increase in the concentration of AA has *Panicum miliaceum L.*, which is 2,74 times. The content of GSH has a similar tendency, so the average concentration of GSH in the tissues of experimental Magnoliopsida is 1,49 times higher than in Liliopsida. The maximum value of the indicator for dormant seeds recorded for *Avena sativa L.*, the minimum – for *Zea mays L.* Activation of germination processes increases the amount of GSH in the tissues of *Avena sativa L.* by 16,04%, *Hordeum vulgare L.* – by 14,29%, *Zea mays L.* – by 13,48%, *Triticum durum Desf.* – by 11,23%, *Oryza sativa L.* – 11,76%. The smallest increase in the concentration of GSH has *Panicum miliaceum L.*, which is 7,81%. As a result of the analysis of changes in the amount of low molecular weight AO, it can be assumed that when activating the processes of germination of AA seeds has a more protective value

DOI: http://dx.doi.org/10.46925//rdluz.37.23

compared to GSH. The increase in the concentration of AA is more species-specific, while for GSH is more uniform.

Summarizing the results obtained on experimental Liliopsida, we can say that in the tissues of seeds at rest, the average level of generation $\bullet O_2^-$ is 0,502 nmol $\bullet O_2^-$/(g*s), ΔTBA_{ap} – 72,79%, SOD – 0,29 OD, catalase – 0,20 µmol/(kg*min), AA – 0,076 mmol/kg, GSH – 44,75 mmol/kg, cytochrome oxidase – 0,241 OD. When activating the germination processes, the average level of $\bullet O_2^-$ generation is 0,740 nmol $\bullet O_2^-$/(g*s), ΔTBAap – 88,70%, SOD – 0,46 OD, catalase – 0,33 µmol/(kg*min), AA – 0,697 mmol/kg, GSH – 50,38 mmol/kg, cytochrome oxidase – 0,376 OD.

Thus, the activation of germination caused an increase in the value of the experimental average PAS as follows: for the generation of $\bullet O_2^-$ growth is 41,47%, for ΔTBAap – 21,64%, for SOD – 58,62%, catalase – 68,37%, AA – 817.11% (9,17 times), GSH – 12,58%, cytochrome oxidase – 56,02%.

Comparison of the average values of the obtained results makes it possible to identify the following patterns:

– At rest in experimental Magnoliopsida compared to Liliopsida, the level of generation $\bullet O_2^-$ is higher by 12,96%, the level of ΔTBAap is lower by 13,29%, cytochrome oxidase activity is higher by 13,29%. The activity of SOD is higher by 27,59%, catalase - by 40%, the concentration of AA - by 96,06%, GSH - by 4.69%.

– When germination is activated, we have the following predominance of indicators: the level of $\bullet O_2^-$ generation is higher by 43,92%, the level of ΔTBAap is lower by 33,63%, the activity of cytochrome oxidase is lower by 9.94%. The activity of SOD is higher by 13,04%, catalase – by 75,76%, the concentration of AA – by 6,31%, GSH – by 17,27%.

– Thus, for the tissues of seeds of experimental Magnoliopsida, which are at rest, both links of PAS are more powerful than in Liliopsida, the level of FRPO is lower, which is achieved due to both enzymatic and low molecular weight antioxidants. Germination activation enhances both levels of PAS in all experimental groups of plants, but in Magnoliopsida, we observe the stronger generation of $\bullet O_2^-$, and the predominance of protection by enzymatic AO, and in Liliopsida - low molecular weight

– If we characterize the effect of germination activation on individual components of the AO protection system, we can see that germination activation enhances

DOI: http://dx.doi.org/10.46925//rdluz.37.23

SOD activity in Liliopsida more intensely than in Magnoliopsida, which in turn show increased catalase activity. Among low molecular weight AO, we also observe a cross pattern: a significant increase in the generation of AA Liliopsida and the concentration of GSH Magnoliopsida.

When comparing changes in the values of the indicators of the PAS of different species of plants, the following assumptions can be made:

– The level of AO protection during germination activation depends on the quantitative and qualitative composition of reserve inclusions in the composition of seed tissues. For example, an increase in the content of protein inclusions promotes the increased synthesis of enzyme AO and GSH, and an increase in the content of PUFA among lipid inclusions reduces the intensity of FRPO.

– According to the growth of AO content during germination activation, experimental plants can be placed in the following order: Glycine max L., Chenopodium quinoa L., Avena sativa L., Fagopyrum esculentum L., Oryza sativa L., Hordeum vulgare L., Linum usitatissimum L ., Sinapis alba L., Panicum miliaceum L., Zea mays L., Helianthus annuus L. This is a biochemical basis for practical recommendations on the feasibility and benefits of pre-soaking cereals in their culinary processing.

Conclusions

1. In the tissues of seeds of experimental Magnoliopsida at rest, the average level of generation $\bullet O_2^-$ is 0,567 nmol $\bullet O_2^-$/(g*s), ΔTBA_{ap} – 59,63%, SOD – 0,37 OD, catalase – 0,28 μmol/(kg*min), AA – 0,149 mmol/kg, GSH – 46.85 mmol/kg, cytochrome oxidase – 0,273 OD. For Liliopsida: the level of generation $\bullet O_2^-$ is 0,502 nmol $\bullet O_2^-$/(g*s), ΔTBAap – 72.92%, SOD – 0,29 OD, catalase – 0,20 μmol/(kg*min), AA – 0,076 mmol/kg, GSH – 44.75 mmol/kg, cytochrome oxidase – 0,241 OD.
2. Activation of germination caused an increase in the value of the average PAS in the tissues of experimental Magnoliopsida as follows: for generation $\bullet O_2^-$ growth is 87,83%, for ΔTBAap – 9,12%, for SOD – 40,54%, catalase – 107,14% (2.07 times), AA – 397,32% (4,97 times), GSH – 26,11%, cytochrome oxidase – 25,28%. For Liliopsida: for generation $\bullet O_2^-$ growth is 41,47%, for ΔTBAap – 21,64%, for SOD – 58,62%, catalase – 68,37%, AA – 817,11% (9,17 times), GSH – 12,58%, cytochrome oxidase – 56,02%.

3. For seed tissues of experimental Magnoliopsida, which are at rest, both links of PAS are more powerful than in Liliopsida, the level of FRPO is lower, which is achieved due to both enzymatic and low molecular weight AO. Germination activation enhances both links of PAS in all experimental groups of plants, however, in Magnoliopsida, we observe the stronger generation of $\bullet O_2^-$, and the predominance of protection by enzymatic AO, and in Liliopsida – low molecular weight. Activation of germination enhances the activity of SOD in Liliopsida more intensely, compared with Magnoliopsida, which in turn revealed enhanced catalase activity. Among low molecular weight AO, we also observe a cross pattern: a significant increase in the generation of AA in Liliopsida and the concentration of GSH in Magnoliopsida.
4. As the growth rate of generation $\bullet O_2^-$ increases during germination activation, the experimental plants form the following series: *Triticum durum Desf., Fagopyrum esculentum L., Hordeum vulgare L., Panicum miliaceum L., Zea mays L., Glycine max L., Chenopodium quinoa L., Avena sativa L., Linum usitatissimum L., Sinapis alba L., Oryza sativa L., Helianthus annuus L.* According to the growth of AO content during germination activation, experimental plants can be placed in the following order: *Helianthus annuus L., Zea mays L., Panicum miliaceum L., Sinapis alba L., Linum usitatissimum L., Oryza sativa L., Hordeum vulgare L., Fagopyrum esculentum L., Avena sativa L., Chenopodium quinoa L., Glycine max L.* This is a biochemical basis for practical recommendations on the feasibility and benefits of pre-soaking cereals in their culinary processing. The results of the study also indicate the need for amendments to the methods of biochemical analysis of seeds to differentiate dry homogenization and its pre-soaking during sample preparation.

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