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DETERMINATION OF BIOLOGICALLY ACTIVE SUBSTANCES IN GERMINATING LEGUMES AND CEREAL PLANTS

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Abstract : *By the determination of the antioxidant system of plants and its high level lipid peroxidation led to an increase in the activity of antioxidant enzymes.The highest amount of water-soluble antioxidants was found among the legumes in soybean, with 800 µg/g on the first day of germination, and higher amount also was observed on the third day of the growing season - 620 mg/g. Vitamin C is a strong antioxidant and is also very important in redox reactions. The formation of vitamin C in barley from cereal plants in a 7-day-old seedling is 69.8 mg/%. In leguminous plants, for example, in soybean, the amount of vitamin C is greater than in a 7-day-old mung bean seedling and it is 48.4 mg /%.*

Key words: antioxidant, flavonoid, radicals, oxidation, plants

Introduction. As a result of metabolism in living organisms, oxidized products are formed, such as free radicals and peroxide compounds of organic and inorganic nature. The following groups of free radicals are known in an organism: peroxides; hydroxyls; various lipid peroxides and others. There are several specific antioxidant mechanisms in the cell, including superoxide distutase, catalase, peroxidase, and glutathione reductase. These antioxidant systems neutralize the amount of free radicals in an organism, that is, they bind reactive oxygen species: O_2^-, $H_2O_2^{\bullet}$, $HO^{\bullet}$(1). These radicals are also formed as a result of chronic stress and the accumulation of radioactive, various chemicals in an organism (2).

The structure and function of biological membranes is closely related to lipid peroxidation. Biological membrane lipids play an important role in cell metabolism (3). Lipid peroxidation has an importance in the regulation of lipid composition as well as permeability and lipid synthesis processes (4). The accumulation of lipid peroxide oxidation products leads to tissue damage. Lipid peroxidation is controlled by antioxidant defense systems.

It is well known that lipid peroxidation is an important indicator in determining the state of plant cells and their growth (5,6).

Therefore, the daily diet should be supplemented with natural antioxidants that protect the body from free radicals, increase its resistance to various adverse effects and slow down aging.

Natural antioxidants include ascorbic acid, citric acid, polyphenols, flavanoids, carotenoids, cysteine, phospholipids, tocopherols, vitamins A and K (7,16).

Flavanoids are not synthesized in the body of animals, they enter the body with nutrients. Flavanoids are biologically active compounds that have an antioxidant effect, protect the body from free radicals and thereby increase resistance to external adverse factors. Flavonoids are used to diminish and eliminate many diseases.

It is well known that fruits and vegetables are rich in antioxidants and are very useful for regular consumption. Antioxidants are most commonly found in the following foods: blueberries, plums, all kinds of beans, nuts, almonds, wild peanuts, pistachios, garlic, and onions. The radicals accumulated in an organism are released with the help of antioxidants (8). Various biologically active compounds with antioxidant properties have been found in cereals (9).

Vitamin C is a powerful antioxidant that plays an important role in slowing down oxidation and is involved in collagen synthesis, iron and folic acid metabolism, synthesis process of hormones and catecholamine, and a number of other important processes.

Herbs such as rosemary, bushes such as black currant, green currant, walnut are rich in ascorbic acid and their extracts, tinctures, and concentrates are widely used in medicine [10].

A number of sources indicate that fresh vegetables and fruits are the main source of ascorbic acid [11].

Various biologically active compounds with antioxidant properties have been identified in germinating cereals [12-13].

The purpose and tasks. The purpose of this work is to study the content of biologically active substances (antioxidants, flavonoids, vitamin C) during the germination of cereals and legumes.

Materials and the methods.Object: wheat, barley, oats, mung beans, soybeans. The study and observations were carried out on 1,3,5,7,9 day old seedlings of germinating seeds of such crops as wheat, barley, oats, mung beans, soybeans. The determination of lipid peroxidation is based on the reaction between malondialdehyde and thiobarbituric acid, since colored trimetine compounds are formed in a high temperature and acidic pH environment. The complex is measured in the range of 532 nm (1).

The method for determining the amount of water-soluble antioxidants is based on the oxidation of iron-III chloride by antioxidants. At the same time, iron-III-chloride is converted into iron-II-chloride, the amount of which is determined by the intensity of the color formed by the addition of o-phenanthroline (1).

To detect flavanoids in plant leaves, a solution of 1% Triton X-100 dissolved in 96% alcohol was used. The reaction is based on the formation of a stable color of flavanoids under the influence of a citric acid solution extracted from plant tissue. The obtained color complex of flavanoids is measured on a spectrophotometer 46 at 420 nm and the total amount of flavanoids in the germinating seedlings of cereals and legumes is determined (1).

The determination of vitamin C is based on the ability of ascorbic acid to reduce 2,6-dichloroindophenol under acidic conditions.

Under these conditions, the dark blue color (oxidized form) of the 2,6-dichloroindophenol indicator is restored in the presence of ascorbic acid [14].

On a spectrophotometer at a wavelength of 265 nm, depending on the amount of ascorbic acid, the activity of the enzyme ascorbate oxidase is determined [14].

At the same wavelength, ascorbic acid has the ability to recover as much as possible. Ascorbic acid is oxidized by ascorbate oxidase. The level of oxidation of ascorbic acid is directly proportional to the activity of the enzyme.

Results and their discussion.The results of our study showed that variations in lipid peroxide oxidation during the growing periods of cereals and legumes were different.

During the observation, it was found that on the 1st day of the wheat germination period, lipid peroxidation was 12.14 μmol/g, and on the 3rd day it was 22.83 μmol/g, and over the next days their number decreased. On the 5th day of the growing season, this indicationwas 10.64 μmol/g, and on days 7-9 – 9.75 μmol/g. The maximum lipid peroxidation during the growing season of oats appeared at 5 days and was 25.199 μmol/g.Lipid peroxidation during germination in oats was 11.484 μmol/g on the 1stday, 13.568 μmol/g on the 3rdday, 23.15 μmol/g on the 5th day, and 13.322 μmol/g on the 7thday. In barley, the highest concentration of lipid peroxidation was observed on the 5th day of the germination period and was equal to 26.787 μmol/g. In the following days of barley germination, a decrease in its content was observed.

Table 1
Lipid peroxidation during germination of cereals and legumes (μmol/g)

Germination days	**Cereals**			**Legumes**	
№	**wheat**	**oats**	**barley**	**soybean**	**mungbean**
Day 1	12,14	11,484	11,486	14,85	18,85
Day 3	22,83	13,568	22,833	12,55	13,95
Day 5	10,64	23,150	26,787	30,51	14,75
Day 7	9,75	25,199	21,781	34,15	27,09
Day 9	9,75	13,322	10,650	24,83	10,98

During the germination of leguminous plants of soybean and mung bean, the amount of lipid peroxidation was higher at 5,7,9 days of germination of soybean than mung bean and, respectively, was 30.51 μmol/g on day 5, 34.15 μmol/g on day 7, 24.83 μmol/g on day 9. The highest concentration of lipid peroxidation in mung bean was detected for 7 days and amounted to 27.09 μmol/g.

Data on lipid peroxidation in cereals is consistent with literature data (3).

The results show that the amount of water-soluble antioxidants in grain plants, that is, in wheat, varies, its highest content is observed on the first day of the growing period and is 600 μg/g, which decreases in the following days. On the third day of growing period, it was 340 μg/g, on the fifth day - 100 μg/g, on the seventh day - 80 μg/g.

The highest amount of water-soluble antioxidants was found on the third day of barley germination and amounted to 460 μg/g, on the fifth day - 400 μg/g, on the seventh day - 140 μg/g, and on the ninth day 80 μg/g.

The amount of water-soluble antioxidants in soybeans is higher than in mung beans. The highest amount of water-soluble antioxidants in soybeans was found on the first day of the growing period and amounted to 800 μg/g, on the third day of the germination period it was 540 μg/g, on the fifth day 400 μg/g. In the following days there was a decrease in its amount. The results obtained are consistent with the results of the scientific literature.

The results of the study also showed that water-soluble antioxidants were higher in legumes than in cereals, the amount was 800 µg/g on the first day of soybean cultivation, while in mung bean a high concentration of water-soluble antioxidants was observed on the third day of germination,it was 620 µg/g. In cereal crops, the highest concentration was found on the first day of wheat germination, with water-soluble antioxidants reaching 600 µg/g (Table 2).

Table 2
Water-soluble antioxidants in cereals and legumes (µg/g)

Germination days	**Cereals**		**Legumes**	
№	**wheat**	**barley**	**soybean**	**mungbean**
Day 1	600	160	800	440
Day 3	340	460	540	620
Day5	100	400	400	460
Day7	80	140	280	380
Day9	0	80	120	340

The amount of water-soluble antioxidants during the germination of soybean and mung bean was not the same. The highest concentration of antioxidants in soybean was 800 µg/g on the first day of vegetation, and in subsequent growing seasons, we observed a decrease in its content. We found that on the third day it retains 540 µg/g, on the fifth day 400 µg/g, on the seventh day 250 µg/g and on the ninth day 120 µg/g of the water-soluble antioxidant. The highest concentration of water-soluble antioxidants in mung bean was observed on the third day of the growing period at 620 µg/g, and it was found to decrease in subsequent days, reaching 340 µg/g on the ninth day of the growing period.

The results showed that the highest concentrations of water-soluble antioxidants in legumes were found in the first days of germination in soybean, which was 800 µg/g. A number of scientific studies have also shown that changes in antioxidant levels are closely related to the processes of cellular metabolism in cereals (15).

The results of the study also show that the amount of flavanoids in the germination of cereals and legumes varies widely and depends on their growing period. The highest concentration of total flavonoids during the growing season of cereals and legumes was found on the fifth day of vegetation and amounted to 37.4 mg/g in oats. In germinating grain crops –in wheat and barley, the amount of flavonoids was not high. On the fifth day of wheat germination, flavonoids were lower than in oats and amounted to 29.4 mg/g.

The results show that on the first day of germination in wheat, the amount of flavonoids was 14.3 mg/g, on the third day 15.8 mg/g, on the seventh day 28.95 mg/g and on the ninth day 11.1 mg/g . At the same time, we observed that the total amount of flavonoids in barley was lower than in wheat. On the fifth day of germination in barley, its maximum content was 19.9 mg / g. We found that on the first and third days of germination, the amount of flavonoids was 13.3 mg / g, on the ninth day - 10.1 mg / g. It was revealed that from leguminous plants in soybean and mung bean, the total amount of flavanoids is lower than in wheat. On the fifth day of germination, the total amount of flavanoids in soybean was 21.2 mg/g, and in mung bean 17.2 mg/g.

Besides, the results show that the amount of vitamin C, which exhibits antioxidant properties during the germination of cereals and legumes, depends on their vegetation.

It was determined that during the germination of cereal crops, the amount of vitamin C was higher in barley than in wheat and oats on days 1, 3 and 7 of the growing season.

Table 3
VitaminCcontent in cereal crops (mg /%)

Germination days	**wheat**	**oats**	**barley**
Day 1	15,54±0,3	27,28±0,88	18,48±0,88
Day 3	47,52±1,7	39,6±4,4	16,28±0,74
Day 5	25,08±0,42	36,08±5,8	1,98±0,44
Day 7	33,88±0,44	69,81±5,47	36,52±1,32

Day 9	24,2±1,3	18,48±0,88	22±3,52

The highest levels of vitamin C in cereal germination in barley were 27.28 mg/% on day 1, 39.6 mg/% on day 3, and 69.8 mg/% on day 7.

In legumes, the highest concentration of vitamin C during germination in soybeans was much higher than that of mung beans and on the seventh day was 48.4 mg /%.

Thus, the maximum amount of vitamin C in wheat is 47.52 mg /% on the 3rd day of the germination period, 69.81 mg /% on the 7th day in barley, 36.52 mg /% on the 7th day in oats and 49.2 mg /% on the 7th day in mung bean, 54.4 mg /% on 7 days in soybeans. The enzyme ascorbate oxidase is found in all plants. This enzyme catalyses the oxidation of L-ascorbic acid to the dehydroascorbic form. Ascorbate oxidase contains 0.26% copper. Enzyme activity was first detected on cabbage leaves.

It is known that dehydroascorbic acid is biologically active and found in plant tissues in combination with ascorbic acid and, under certain conditions, it turns into ascorbic acid under the action of an enzyme.

We have studied the activity of ascorbate oxidase during the growing season of cereals and legumes [9].

The results show that the activity of the enzyme ascorbate oxidase changes during the germination of cereals and legumes and depends on their vegetation. It was found that the activity of ascorbate oxidase during the growing period in cereal crops was higher on the 3rd day in wheat than in barley and oats, and amounted to 557.6 g/min. The highest activity of the enzyme in cereal crops was found in barley on the 7th day of vegetation and amounted to 730.2 g/min. In oats, the enzyme activity was 441.3 g/min.

It was also found that during the germination of legumes on days 1, 3, 5, 7 and 9, the activity of ascorbate oxidase was higher in soybeans than in mung bean, and the highest activity was manifested on the 7th day of the growing season, which was 583.95 g / min in and for mung bean was equal to 525 g/min.

Conclusion. Our results showed that during the periods of germination of cereal and legume plants, lipid peroxidation was different, in cereal plants during the germination period, the highest concentration of lipid peroxidation was in wheat 22.83 μmol/g on the 3rd day, in oats 25.19 μmol/g on the 7th day, in legumes –in soybeans 34.150 μmol/g on the 7th day, in mung bean on the 7th day 27.09 μmol/g. It is well known that lipid peroxidation determines the antioxidant system of the plant, and its high levels lead to an increase in the antioxidant enzyme systems - the enzymes superoxide dismutase and glutathione reuctase (6).

Changes in the amount of water-soluble antioxidants also depend on the growth processes of cereals and legumes.

In this way, the source of vitamin C, which is a powerful antioxidant, is germinating seedlings of cereals and legumes, the amount of which depends on their maturity. The highest levels of vitamin C were observed in wheat, barley, oats, mung beans on the 3rd day of growing period, and in soybeans on the 7th day of growing period.

It was also found that in germinating cereals and legumes, the amount of flavanoids during germination periods was different, and the highest concentration of flavanoids was observed on the 5th day of the germination period of oats, their concentration was 37.4 mg/g.

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