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DETERMINATION OF ACTIVITY OF CATALAZA ENZYME DURING THE GROWTH PERIOD OF GRAIN AND LEGAL PLANTS

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Abstract

As a result of metabolism in a living organism, "products" are formed "free radicals" and peroxide compounds of organic and inorganic substances. It is known that free radicals damage the cell. Enzymes of the body's antioxidant defense system superoxide dismutase, catalase, glutathione reductase, glutathione peroxidases, toxic products of peroxidation of lipids convert free radicals into harmless substances.

In this study, the activity of one such enzyme, catalase, was studied. The activity of the enzyme catalase in the germination periods of cereals and legumes varies, the highest activity of the enzyme catalase in the germination period of cereals was found on the 5th day of the germination period: wheat - 5.644 mg H_2O_2 / g, barley - 4.08 mg H_2O_2 / g, oats - 5,134 mg H_2O_2 / g. In legumes, the highest activity was found on the 3rd day of the germination period: in the shade - 11.832 mg H_2O_2 / g, and in the moss - 8.126 mg H_2O_2 / g.

Keywords: harvested oats, wheat, barley, soybeans, moss; antioxidant enzymes; catalase, enzyme activity, extract.

The structure and function of biological membranes depend on the peroxidation of lipids. Biological membrane lipids play an important role in cell metabolism. [6]. Accumulation of lipid peroxidation products leads to tissue damage. Peroxidation of lipids is controlled by antioxidant defense systems.

Metabolism in living organisms produces oxidized products - "free radicals" and peroxides of organic and inorganic substances. Adverse conditions can accelerate this process. It is known that free radicals damage the cell [7], there are several specific mechanisms against oxidation in the cell, including superoxide dismutase, catalase, peroxidase, glutathione reductases.

These antioxidant systems neutralize the amount of free radicals in the body, that is, bind the active forms of oxygen: O_2^-, H_2O_2, HO^0 and increase their resistance to various adverse external influences [9].

Various biologically active compounds with antioxidant properties have been identified in extracted cereals. [1,2,3,4]. The importance of natural antioxidants is that they can be used to correct a number of diseases [5,10].

Catalase (Greek catalysis disorder) is an enzyme belonging to the class of oxidoreductases that catalyzes the decomposition of hydrogen peroxide into oxygen by water (H_2O):

$$2H_2O_2 = O_2 + 2H_2O$$

Catalase is present in all animal and plant tissues as well as in aerobic microorganisms.

Catalase enzymes have been studied in many plants and animals, and their amounts vary depending on time, conditions, and the type of organism.

The main purpose of our research is to determine the activity of the enzyme catalase, which has antioxidant effects during the germination of seeds of crops such as wheat, barley, oats, soybeans, moss.

Object of research: harvested wheat, barley, oats, soybeans, moss.

The essence of the method. The method of determining the activity of the enzyme catalase in the object under study is based on the determination of the decomposed amount of hydrogen peroxide by titration with a solution of potassium permanganate. The reaction proceeds as follows:

$$5H_2O_2 + 2KMnO_4 + 4H_2SO_4 = 5O_2 + 2KHSO_4 + 8H_2O + 2MnSO_4$$

Reagents:

1. Aqueous extract of cereals and legumes.

Take 2 grams of the sample of the crushed plants, place them in a porcelain dish, crush them with a pestle and transfer them to a 50 ml volumetric flask. The forfor container and the pesticide are rinsed with 20 ml of water into a flask. Add 2-3 drops of toluene to the flask, mix well, fill the flask to the mark with water and leave at room temperature for 2 hours. The liquid is then centrifuged for 15 minutes at 4,000 rpm. The catalase activity is determined in the centrifuge.

2. 1% solution of freshly prepared hydrogen peroxide.

3. 10% sulfuric acid solution.

4. 0.1M $KMnO_4$ solution.

5. Distilled water.

Required equipment: 50 ml heat-resistant conical flasks.

Procedure: Two flasks are centrifuged for 20 ml - experiment and control. The control flask is boiled for 5 minutes to denature the enzyme.

Experiment, add 20 ml of distilled water to the boiled control flasks, add 3 ml of 1% hydrogen peroxide and leave at room temperature for 30 min. After 30 minutes, add 5 ml of 10% sulfuric acid to the flasks for incubation and titrate the remaining hydrogen peroxide with hydrogen peroxide.

The activity of the enzyme catalase is determined by the amount of milligrams of hydrogen peroxide decomposed by the enzyme in 1 gram of test material for 30 minutes. The activity of the enzyme is determined using the following formula:

$$\mathbf{A}= \frac{1{,}7\cdot(a-b)V_1}{V_2\cdot m}$$

Where **A** is the catalase activity, mg H_2O_2 / g; a is the amount of $KMnO_4$ used to titrate the control probe in ml; **b** is the amount of $KMnO_4$ used to titrate the experimental sample, in ml (equivalent to 1.7 mg of H_2O_2 in 1 ml of $KMnO_4$ solution); **V1** - the volume of the plant obtained for analysis in ml; **V2** is the volume of solution obtained for titration; **m** - mass of the sample taken for the study, grams.

Results: The results show that the activity of the enzyme catalase, which is part of the antioxidant enzyme system, depends on the period of germination of cereals and legumes, and its activity varies.

The results showed that the high activity of catalase was 5,644 mg of H_2O_2 / g in oats and 5,134 mg in oats in 5 days. (Table 1, Fig. 1), catalase activity decreased in the following days of the germination period. On day 9 of the germination period, 3.06 mg of H_2O_2 / g was found in wheat and 4.726 mg in oats.

Table 1

Catalase activity during the germination of cereals and legumes (mg H_2O_2/g)

Growth days	Legumes		Cereals		
	Soy	Mung bean	Wheat	Barley	Oats
1-day	2,924 ± 0,01	2,72 ± 0,01	1,564 ± 4,75	1,02 ± 0,01	4,624 ± 0,1
3-day	11,832 ± 1,5	8,126 ± 0,95	4,964 ± 0,01	2,006 ± 0,15	4,42 ± 1,25
5-day	7,922 ± 1,05	7,14 ± 0,55	5,644 ± 0,01	4,08 ± 2,65	5,134 ± 1,25
7-day	7,446 ± 0,25	3,196 ± 0,3	4,046 ± 1,15	2,108 ± 0,05	4,896 ± 1,1
9-day	7,31 ± 0,45	3,128 ± 0,4	3,06 ± 2,1	2,856 ± 1,6	4,726 ± 0,55

During the germination period of barley, the activity of the enzyme catalase was found to be lower than that of harvested wheat and oats (Figure 1). The highest catalase activity was on day 5 of the germination period, at 4.08 mg H_2O_2 / g. 2,108 on day 7, 2,856 mg H_2O_2 / g on day 9.

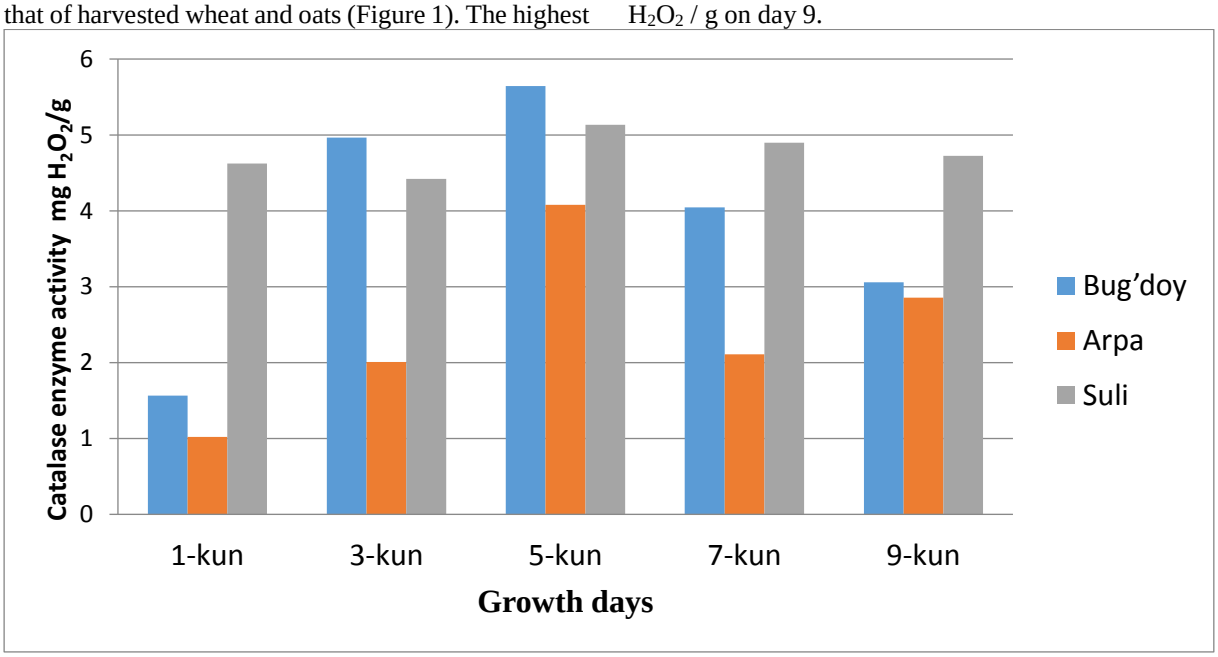

Figure 1. The activity of the enzyme catalase during the germination of cereals

It was found that the activity of the enzyme catalase varies during the germination period of legumes. Shade germination showed the highest catalase activity on day 3 of the germination period, at 11,832 mg H2O2 / g. On days 5,7,9, the enzyme activity decreased. On day 9, its activity was 7.31 mg H2O2 / g. (Figure 2).

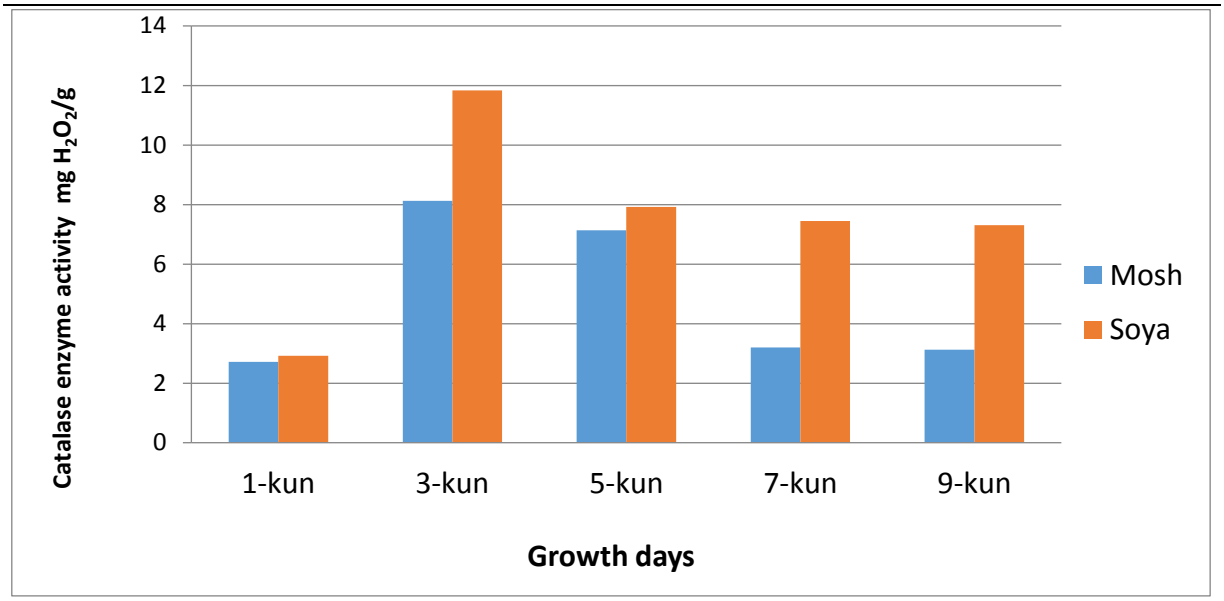

Figure 2. The activity of the enzyme catalase during the germination of legumes

We also found that moss had the highest activity on day 3 of germination, at 8,126 mg H_2O_2 / g, and decreased on day 7.9 of germination, on day 9 of germination, at 3,128 mg H_2O_2 / g (Figure 2).

A number of scientific studies have shown that changes in the activity of antioxidant enzymes depend on metabolic processes in the cell during the germination of cereals [9].

Conclusion. The results show that the activity of the enzyme catalase in the germination periods of cereals and legumes varied, and the highest activity was found in the 5 days of germination of cereals. At the same time, the high activity of catalase was 5,644 mg in 5 days of wheat germ from cereals, 5,134 mg H_2O_2 / g in oats, and 4.08 mg H_2O_2 / g in barley.

In legumes, the highest activity was observed on day 3 of the germination period. In the shade -11,832 mg H_2O_2 / g, in the moss - 8,126 mg H_2O_2 / g.

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