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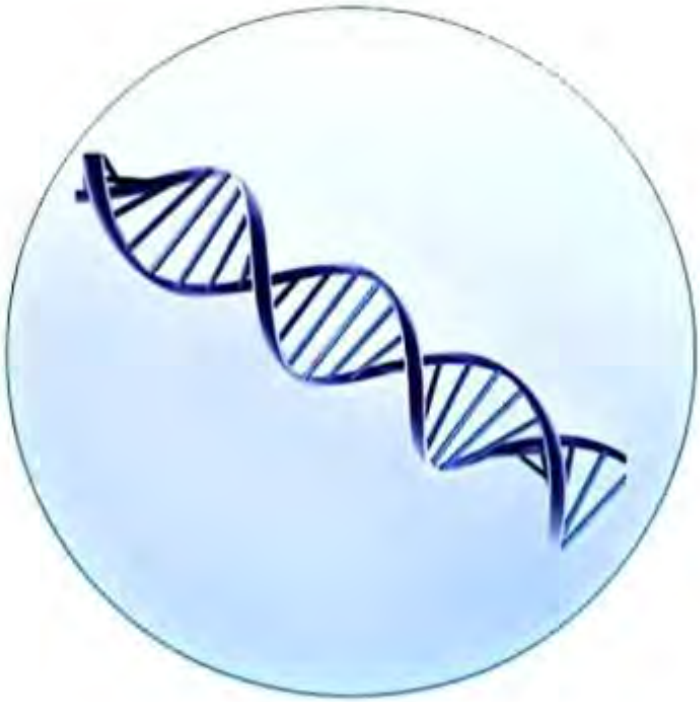

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STUDY OF DYNAMICS OF PEROXIDASE ENZYME IN HEALTHY AND VIRUS-INFECTED PLANT *PHASEOLUS VULGARIS* L.

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Annotation. *Leguminous grain products and pulses have an incomparable place among the foods that people eat throughout their lives. Vitamins in them are very important for the human body. One of the representatives of such leguminous cereal products is beans. Beans are the 10th most consumed food in the world. Today, a number of phytopathogenic viruses infecting agricultural plants have been identified, which negatively affect the quantity and quality of cultivated products and cause great economic damage. The bean plant has been infected by a number of viruses in recent years, causing a decrease in yield as well as a deterioration in the quality of the product. The main reason for this is that the bean plant is infected with various microorganisms, including phytopathogenic viruses. One of these phytopathogenic viruses is bean mosaic virus (BMV), which damages leguminous crops and affects plant growth, development, and yield, reducing yield up to 80%. In this article, the dynamics of the peroxidase enzyme in the leaves of BMV infected and healthy bean plant (Phaseolus vulgaris L) was studied.*

Key words: *Peroxidase, phaseolus mosaic virus, Phaseolus vulgaris, enzyme, activity, morphology, phytopathogen microorganism.*

ИЗУЧЕНИЕ ДИНАМИКИ ФЕРМЕНТА ПЕРОКСИДАЗЫ У ЗДОРОВЫХ И ЗАРАЖЕННЫХ ВИРУСОМ РАСТЕНИЙ *PHASEOLUS VULGARIS* L.

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Аннотация. *Зернобобовые продукты и бобовые занимают несравненное место среди продуктов, которые люди едят на протяжении всей своей жизни. Витамины в них очень важны для организма человека. Одним из представителей таких зернобобовых продуктов является фасоль. Фасоль занимает 10-е место среди наиболее потребляемых продуктов питания в мире. На сегодняшний день выявлен ряд фитопатогенных вирусов, поражающих сельскохозяйственные растения, которые отрицательно влияют на количество и качество выращиваемой продукции и наносят большой экономический ущерб. За последние годы фасоль была заражена рядом вирусов, что привело к снижению урожайности, а также ухудшению качества продукта. Основная причина этого в том, что растение фасоли заражено различными микроорганизмами, в том числе вирусами фитопатогенного происхождения. Одним из таких фитопатогенных вирусов является вирус мозаики фасоли (BMB), который повреждает зернобобовые культуры и влияет на рост, развитие и урожайность растений, снижая урожайность до 80%. В данной статье изучена динамика фермента пероксидазы в листьях инфицированных BMB и здоровых растений фасоли (Phaseolus vulgaris* L.*).*

Ключевые слова: *Phaseolus vulgaris, пероксидаза, вирус мозаики фазеолуса, фермент, активность, морфология, микроорганизм-фитопатоген.*

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the important legumes and is known as a seed of hope in poverty-stricken countries due to its high nutritional value such as protein, trace elements, vitamins and antioxidants. Common beans are a suitable food substitute for meat in developing and underdeveloped countries [17]. As population growth is an important factor in the increase in food demand, farmers need to obtain a satisfactory yield of beans to guarantee food security in some areas [1]. However, high yield losses are often observed as a result of leaf damage caused by a number of viral diseases, particularly BMV. BMV is the most important and fatal foliar disease of beans. BMV usually occurs on the lower surface of bean leaves and leaf veins [4, 7]. Although BMV can appear on both sides of the leaf, the first symptoms of infection are usually seen on the leaf surface along the veins. As the disease progresses, chloritic spots first appear on the tip of the bean leaf, then the leaf curls and narrows the intervening space until it spreads along the border and eventually the entire leaf surface [4, 7].

Plants develop according to environmental conditions and constantly interact with various biological substances. These interactions cause many positive and negative effects on plant metabolism. Many viruses associate with different plant species and change their metabolism. In addition, virus-plant interactions alter the expression of many plant enzymes [7]. Enzymes act as biological catalysts in all living organisms. Enzymes are very important compounds in the life of plants and control a number of biochemical processes. One of the most important enzymes involved in biochemical reactions in plant cells is the peroxidase enzyme [15]. Peroxidases are a class of enzymes widely distributed in plants and can be easily isolated from most plant cells and organs. Peroxidase enzyme controls oxidation-reduction processes in biochemical processes in plant cells. this enzyme belongs to the class of oxidoreductases and has a unique coenzyme and specificity [3]. Peroxidase is a two-component enzyme that contains compounds of active groups, these groups react chemically with the substrate, or otherwise interact with kalloid proteins with the help of these active groups to increase catalytic activity. The peroxidase enzyme is a globular protein with a diameter of 50A and 43% α-helical parts in the protein part. Peroxidase enzyme is an enzyme that acts on hydrogen peroxide as an

acceptor, adopted in 1979 international convention on enzyme nomenclature [13]. The primary structure of peroxidase was studied by Velinder et al. They found that 203 to 300 amino acid residues can enter the primary protein structure [5]. The main properties of peroxidase are catalyzing the oxidation of chemical compounds with the formation of intermediate complexes with different spectral characteristics due to peroxide oxygen.

The enzyme is not only a peroxidase, but also an oxidase, in which it catalyzes the oxidation of a number of compounds at the expense of unactivated molecular oxygen. The oxidase function was first identified by Teorelyu in some plants, he identified an enzyme that oxidizes dihydroxyfumaric acid (ODE) by absorbing oxygen and that the enzyme is peroxidase [18]. The oxidase function of the enzyme is manifested in the interaction with various chemical compounds. A necessary condition for the oxidase reaction is the presence of manganese cofactor ions and various phenolic compounds [7]. Hydro-naphthoquinones, indolyl acetic acid, reduced coenzymes NAD*H2 and NADP*H2 serve as substrates for the manifestation of the oxidase function of peroxidase. also, research was conducted to study the conditions of oxidation of NAD and NAD*H by peroxidases [13]. Determining the isozyme spectrum of this enzyme, that is, peroxidase, not only in plants, but also in other living organisms, and its role in cellular processes in the life of a living organism, will allow solving a number of problems of today's agriculture and medicine. in addition, considering the use of this enzyme in today's biotechnological practical processes, it is important to solve the problems of purification [8].

Literature analysis and methodology

In recent years, one of the enzymes widely used all over the world is the peroxidase enzyme. Peroxidase is one of the most common enzyme proteins and is of increasing interest in its study. The presence of this enzyme in the tissues of plants and animals, as well as in the composition of fungi and bacteria, gives grounds for considering it as an important combination of higher and lower organisms. according to the information of a number of authors, this enzyme is a stress enzyme, especially in plants growing in various extreme conditions (arid and saline soils), in plants infected with various phytopathogens, the amount of this enzyme is higher than healthy o several in relation to plantshas been shown in many literatures [12]. When pathological conditions occur in plants, it causes the amount of peroxidase enzyme to change, that is, to increase. It enhances the catalytic functions of the enzyme. Currently, high-sensitivity biosensors have been produced based

on recombinant peroxidases, which are used in complex multi-component mixtures, as well as for the detection of various compounds in the analysis of environmental pollution. In recent years, peroxidase preparations isolated from new sources appeared on the market [12]. The most effective enzyme today is peroxidase with high stability under extreme conditions, which is a recombinant variant of fungal peroxidase [13].

A number of basic biochemical processes in every living organism, including respiration, nutrition, and energy exchange, are carried out with the participation of enzymes. An example of these enzymes is the peroxidase enzyme found in plants [4]. Peroxidase enzyme is of special importance among plant enzymes. It is an enzyme that responds to the immune system in plants [5]. In infected plants, the peroxidase enzyme changes dramatically.

BMV was first identified in Russia in 1894 and has been known in the United States since 1917, when the disease became a serious problem and continues to pose a severe threat to the plant [17]. BMV manifests itself as an irregular mosaic of pale yellow and green or dark green streaks along the veins of green leaves, hardening of leaves, twisting, curling. This viral disease will eventually kill the bean. this virus is widely spread all over the world and causes great damage to agriculture, causing a decrease in productivity up to 80% [6]. Therefore, it is one of the important issues to study the bioecological characteristics of this virus in our country, such as reservoir plants and spreader. We know that viruses affect the plant's immune system, causing a drastic reduction in plant growth and development.

Unfortunately, 20-40% of crops are lost every year due to microbial attacks, pest infestation, nutrient deficiency and poor soil quality [8]. Among phytopathogens, BMV infections cause approximately 70–80% of all microbial diseases in agricultural systems [9]. BMV spores have been found to survive in the field for several years [10]. After infecting plant parts, the virus affects the flow of water and nutrients in the plant, resulting in yellowing, wilting, and plant death [3]. Initially, the use of resistant varieties and chemical fungicides can reduce the virus disease to some extent. however, due to variability in pathogenicity, the development of new pathogenic species is an ongoing problem [15].

Discussion and results

To date, the peroxidase enzyme has been isolated from a number of plants such as potato, radish, wheat, mash, beans, and it has been determined that the peroxidases isolated from all of them differ from each other in terms of their activity and isoenzyme spectrum. In recent years, the presence of

peroxidases weakly bound to the cell wall on the surface of plant cells and the ability of these peroxidases to be easily separated from the cell wall and able to circulate through the apoplast of the whole plant, where it encounters a pathogen, is "immuneinformation about the development of "answer" appeared [1;18]. The enzymes located in this type are the first to encounter the "attack" of the pathogen and resist the entry of the virus into the internal environment of the plant cell.

Until now in our country *Ph.vulgaris* scientific research on phytoviruses that cause disease in the vulgaris plant has not been conducted. Therefore, the scientific research conducted in this direction is considered urgent. For the research, phenological observations were made on bean plants grown in the territory of Tashkent region. taking into account the participation of the peroxidase enzyme in the protective function of plants, it was aimed to study the peroxidase freely bound to the plant cell membrane and soluble. For this, diseased local bean varieties "Kora Koz" and "Ravat" were selected. In which the bean plant infected with BMV Ph. The peroxidase enzyme bound to the plant cell and soluble in the vulgaris leaf was determined. For this purpose, it was aimed to determine the dynamics of the peroxidase enzyme in the leaves located in the parts (upper, middle, lower) of bean varieties infected with the virus. The substrate for peroxidase enzyme detection is prepared as follows.

For this, a leaf of a naturally infected bean plant is taken, an equal amount of 10 mg of the leaf tissue is taken using an electronic scale and placed in a porcelain mortar. Then 10 ml of a 0.04 M solution of acetate (CN3-COOH) buffer (pH=4.7) was added to it in a ratio of 1:1, it was thoroughly crushed in a mortar, and the resulting mass was filtered using a four-layer gauze into test tubes was poured. centrifuge each homogenate at 4000 rpm for 15 min. it is centrifuged and cleaned of cell components. After centrifugation, the supernatant was poured into specially numbered test tubes, and the precipitate was discarded. The resulting supernatant liquid was removed and the enzyme was completely separated from the leaf cells, kept in a refrigerator (+4°C) until the buffer (benzidine) was mixed, and the amount of enzymes was determined by spectrophotometry (Agilent Cary 60 UV-Vis, Ger.)). control and different levels of virus in different light absorption ranges Peroxidase activity was studied in plants infected with peroxidase from two forms of peroxidase weakly bound to the cell wall and the activity of peroxidase enzyme in the soluble ratio (weakly bound to the cell wall 625, soluble 640 nm).

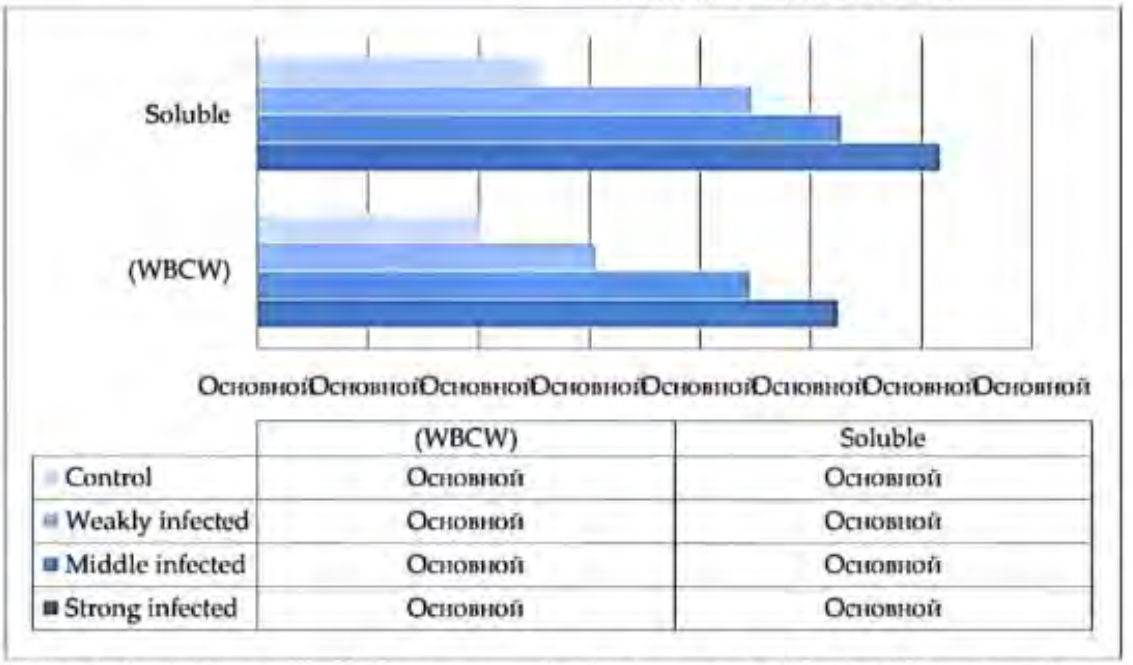
For this, enzyme activity was determined using spectrophotometry (Agilent Cary 60 UV-Vis, Ger.) by

preparing samples from infected and healthy plants. The results were calculated based on A.H. Boyarkin's quick method of determining the activity of peroxidase biochemistrical – formula:

$$A = (D_2 - D_1)VV_2 \times = \frac{60}{(t_2 - t_1)V_1 H}$$

in the formula; D_1 - headlight absorption index; D_2 is the final light absorption index; t_1 and t_2 – start and end time; V_1 is the volume obtained for the reaction; V_2 – cuvette volume; 60 – coefficient of rotated minutes; H - plant tissue weight [2].

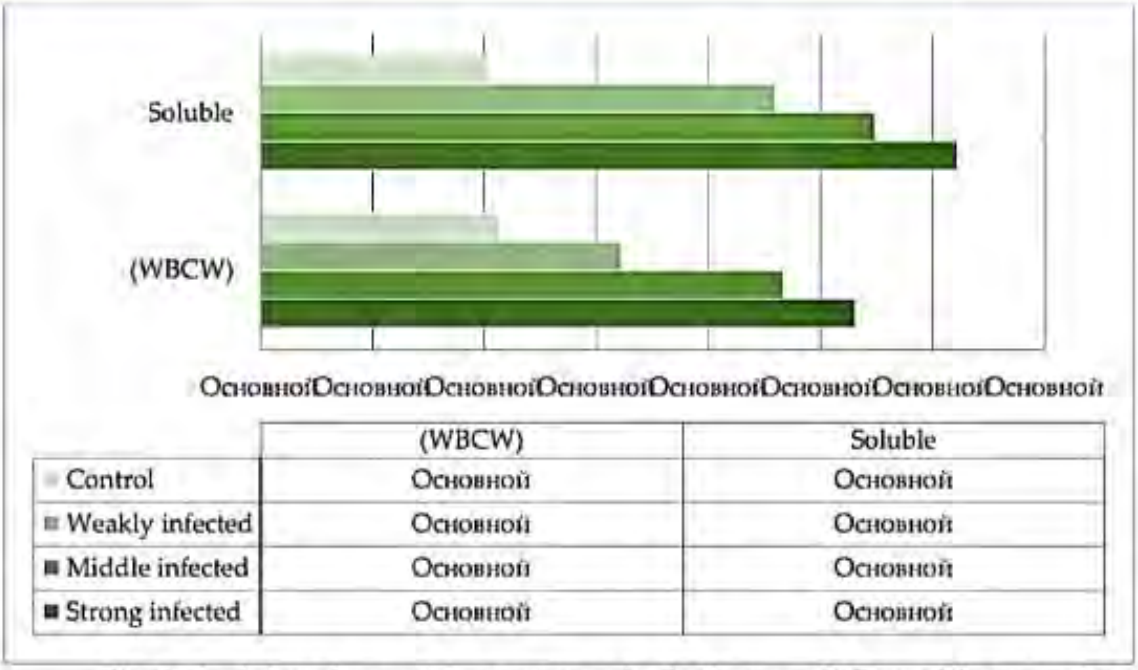
As can be seen in the diagram, it was found that the soluble form of the enzyme is higher than the weakly bound form of the enzyme in the bean plant of "Qorako'z" and "Ravot" varieties (Fig. 1, 2). The obtained results showed that two types of peroxidase found in the plant: weakly bound to the cell wall and soluble form of peroxidase were studied by comparing the enzyme activity in the plant in 2 types of beans, "Qorako'z" and "Ravot".



	(WBCW)	Soluble
Control	Основной	Основной
Weakly infected	Основной	Основной
Middle infected	Основной	Основной
Strong infected	Основной	Основной

Fig. 1. Activity of peroxidase enzyme in the leaves of "Qorako'z" bean variety

Soluble form, weakly bound to the cell wall (WBSW), the amount of peroxidase enzyme increased depending on the degree of leaf damage. Especially in weakly bound to the cell wall (WBSW) and in the soluble form, the enzyme indicators in the control (healthy) leaves are on the same line with a partial difference, the amount of the enzyme is increased compared to the leaf samples of weak, moderate and severe diseaseis clearly expressed in Figures 1 and 2.



	(WBCW)	Soluble
Control	Основной	Основной
Weakly infected	Основной	Основной
Middle infected	Основной	Основной
Strong infected	Основной	Основной

Fig. 2. Activity of peroxidase enzyme in the leaves of "Ravot" bean variety

Therefore, the reproduction of the virus in plant leaf tissues is the period of the enzyme's pathological conditions in plants. In such a trend, the peroxidase enzyme increases, which increases the immune and catalytic functions of the enzyme.

Table 1

Peroxidase activity in the leaves of bean plant "Qorakoz" and "Ravat".

Number of experiment	QORAKOZ		RAVAT	
	Weakly bound to the cell wall (WBCW) M±SE	Soluble M±SE	Weakly bound to the cell wall (WBCW) M±SE	Soluble M±SE
	Activity o enzym mmol/ml			
Control	2,02	2,55	2,13	2,04
Weakly infected	3,06	4,47	3,22	4,59
Middle infected	4,45	5,28	4,67	5,48
Strong infected	5,25	6,17	5,31	6,22

*- *control is normal plant*

The conducted experiment showed that as the level of infection with the virus increased, it was found that the amount of enzyme in the plant leaf increased. that is, the value of the enzyme activity of a healthy leaf sample was 2.02 ml, while in a heavily damaged leaf, this indicator showed 5.25 ml, that

is, it was found that it increased up to 1.5 times. therefore, based on the obtained results, the increase of the peroxidase enzyme at this level is related to the occurrence of pathological processes in plants.

Conclusion

A number of basic biochemical processes in every living organism, including respiration, nutrition, energy metabolism, are carried out with the participation of enzymes. An example of these enzymes is the peroxidase enzyme found in plants. peroxidase enzyme is of particular importance among plant enzymes [4]. It is an enzyme that responds to the immune system in plants. When pathological conditions occur in plants, it causes the amount of peroxidase enzyme to change, that is, to increase. it enhances the catalytic functions of the enzyme.

Currently, high-sensitivity biosensors have been produced based on recombinant peroxidases, which are used in complex multi-component mixtures, as well as for the detection of various compounds in the analysis of environmental pollution. in recent years, peroxidase preparations isolated from new sources appeared on the market [12].

In short, the peroxidase enzyme has a very high biological value in the plant organism. Peroxidase enzyme activity is different in plant leaves (upper, middle, lower). especially, it was observed in the experiment that the amount of enzyme activity in the diseased plant differs from that of the healthy plant, and that the peroxidase enzyme is higher in the diseased plant. From the obtained results, it was determined that there are differences between the types of enzymes in these plants. It was observed experimentally that enzyme activity increased in bean plants infected with the virus. In general, in this study, when the activity of the peroxidase enzyme in the leaf of a virus-infected and healthy plant was studied, the value of the enzyme activity was 2.02 ml, while in a severely infected leaf, this indicator was 5.25 ml. showed that it increased up to 1.5 timeswas determined.

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