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ANTIOXIDANT STATUS OF CALLUS CULTURE AND NATIVE PLANTS OF POTATO UNDER VIRAL INFECTION CONDITIONS

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Abstract

Potato (*Solanum tuberosum L.*) is a vital tuberous crop consumed around the globe following rice, wheat, and maize. The biotic factors, such as fungi, viruses, bacteria, and viroid infections, significantly impact the potato plant's metabolism, altering its physiological, biochemical, and intermolecular reactions to these stress factors. To investigate the activity levels of antioxidant enzymes (AOEs) in the oxidative stress of potato plants and assess their resistance levels to viral infections, callus culture is often employed as a primary model. This study examines the activity profiles of AOEs, specifically peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD), in two entities – potato callus tissue and native plants – under potato virus X (PVX) virus infection conditions.

The research results demonstrate distinct AOE profiles in callus tissues and leaves of infected potato plants. The POD activity in callus tissue is over 50% higher on the 3 dpi compared to plant leaves.

The dynamics of increased AOE activity follow a pattern, peaking on the 3 dpi and declining by the 7 dpi. CAT activity remains consistent until the 7 dpi across all five studied varieties. SOD activity, as the primary enzyme in hydrogen peroxide deactivation, increases by 16-42% post-infection. The AOE activity exhibits a consistent pattern during PVX virus infection in callus and leaf tissues, with the preinfection activity level being over 100 units/gram dry weight lower in callus tissue than in leaf tissue.

Moreover, the nature of increased activity differs between the two entities.

Thus, drawing definitive conclusions regarding potato variety resistance based solely on AOE activity in callus tissue is challenging. Therefore, a mandatory examination of AOE levels in native plants in non-sterile conditions is essential.

Key words: potato; S*olanum tuberosum*; antioxidant enzymes; peroxidase; catalase; superoxidase.

Introduction

Potato (*Solanum tuberosum L.*), an autotetraploid member of the Solanaceae family, is a starchy tuber crop that ranks fourth in the world in terms of production [1]. Potatoes are subject to a wide range of biotic stresses, among which bacterial, fungal, and viral diseases cause significant economic damage. Viruses are among the most harmful pathogens affecting potato crops, with more than 40 viruses reported Crops are affected. The most important viruses are potato virus Y (PVY), potato leaf roll virus (PLRV), potato virus A (PVA), PVX, potato virus S (PVS) and potato virus M (PVM) [2].

PVX can cause losses of 10-40% (average 25%) in single infections and is particularly damaging in combination with potato Y or A viruses. This is due to its synergism with both *Potyviruses,* leading to tuberose yield losses of up to 80% [3].

One of the earliest responses of plant cells to infection with a pathogen, including a viral one, is the formation of reactive oxygen species (ROS), which is called an "oxidative explosion" [4].

To protect against potentially cytotoxic forms of activated oxygen, the plant cell has a powerful defense system that includes both enzymatic and non-enzymatic antioxidant systems [5]. The main elements of the enzymatic system are enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), which protect the plant cell by directly deactivating radicals, converting them to less reactive forms [6,7].

Inhibition of the activity of one of the enzymes of the antioxidant system can lead to an excessive accumulation of reactive oxygen species and cell destruction [8]. High constitutive levels or high induced levels of antioxidants in a plant cell may confer resistance to stressors. It should be noted that in resistant plant forms, the activity of enzymes involved in protection from oxidative stress increases to a greater extent compared with unstable ones [9].

At the same time, it is known that callus culture is considered a model object in the study of the level of resistance to various types of stress. However, the nature of the production of enzyme antioxidants in potato culture has not been studied [10]. At the same time, the resistance of Kazakh varieties to viral infections also remains an issue that has not been fully studied. The study of the level of resistance of varieties of Kazakh selection in the conditions of viral infection will make it possible to select the most resistant varieties in a short time and study their level of immune response to the invasion of the pathogen.

In this regard, the purpose of this study is to conduct a comparative analysis of the activity of the components of the enzyme antioxidant system (peroxidase, catalase, superoxidase) of potatoes in vivo and in vitro on the example of five promising varieties of Kazakhstani selection under conditions of infection with the PVX virus (family *Alphaflexiviridae*, genus *Potexvirus*).

Materials and methods

The objects of the study were potato plants of five promising varieties of Kazakhstani selection ("Ulan", "Tokhtar", "Babaev", "Alliance" and "Narli") at the age of 4 weeks, grown in a phytochamber under 15000 lux, temperature 240°C during the day and 18°C at night, humidity 70% and 16-hour light period.

Callus was prepared from leaves using Murashige-Skooga medium with the addition of 2,4-D (1 mg/mL) and kinetin (0.05 mg/mL). For plant regeneration, callus was transplanted to a medium with 6-BAP (1 mg/ml) and IAA (0.5 mg/ml). Callus induction and plant regeneration were carried out in a phytochamber in compliance with the above regimes.

Infection of plants with PVX virus was carried out by inoculation through leaf blades by means of microlesions with carborandum and application of a freshly prepared mixture of virus particles and phosphate buffer to the damaged surfaces. The inoculation mixture contained 10 mM sodium–phosphate buffer (pH 6.9–7.0 (pH meter, Consort C931, Belgium) and carborandum (d–0.037 mm).

The callus was infected by cocultulating loose callus tissue with a viral inoculum. Plants and calli were tested for infection by PCR [11]. The general scheme of the experiment is shown in Figure 1.

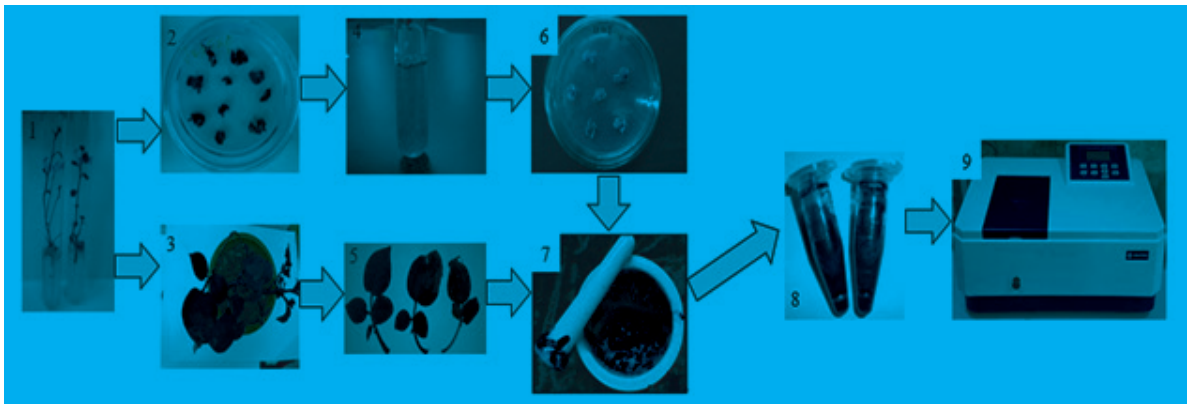

Figure 1 – Scheme of preparation of infected calli and plants for spectrophotometry
(1 – test tube potato plants, 2 – induction of callus from leaf blades, 3 – planting of test tube plants in the ground, 4 – cultivation of the obtained loose callus with a viral inoculum, 5 – selection of leaves on the 3rd, 5th, 7th day, 6 – production of infected callus, 7 – homogenization in a porcelain mortar, 8 – obtaining an extract from leaf and callus tissue, 9 – spectrophotometric analysis)

PCR identification of viral infection.

Total RNA was extracted from leaves infected or uninfected with PVX virus using TRIZOL with modifications [12]. A single-stage RT-PCR reaction was performed using the RT-PCR Kit (Thermo Fisher Scientific, USA). The mixture was incubated at 45° C. for 30 min and denatured at 94°C for 5 min. This was followed by 30 cycles: 94°C for 30 s, 50.7°C for 30 s, and 72°C for 30 s, with a final extension of 72°C for 5 min. RT-PCR products (5 µL) were analyzed by electrophoresis in 1.5% agarose gel. The sequence of the capsid protein gene was used for amplification [13].

Primers: F CACTGCAGGCGCAACTCC; R GTCGTTGGATTGTGCCCT [11]. Product size 565 bp cDNA was amplified by PCR in a VeritiPro (Applied Biosystems) in a volume of 20 µL containing 2 µL of 10x PCR buffer, 1.5 µL of 25 mmol/L MgCl2, 2 µL of 2 mmol/L dNTP, 0.2 µL of Taq DNA polymerase (Thermo Fisher Scientific, США), and 1 µL of cDNA. Whereas in classical RT-PCR, 1 µL of each primer was used in each of several reactions. The PCR mode consisted of 94°C - 1.5 min (initial denaturation), 38 cycles of 94°C - 45 sec (denaturation), 53°C - 45 sec (annealing primers) and 72°C - 1 min (elongation) followed by final elongation at 72°C for 5 min. 10 µL of products were analyzed by electrophoresis in 2% agarose gel. Leaf and callus samples were collected on 3, 5, and 7 dpi and immediately prepared for the determination of peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) enzyme activities.

Peroxidase activity. The degradation rate of H2O2 at 590 nm for 120 sec was used to calculate POD activity. The extract was prepared with the addition of 20 µL of 100 mM FMSF (phenylmethylsulfonyl fluoride). The experimental and control cuvettes contained 0.01% benzidine hydrochloric acid solution. The reaction was triggered by the addition of 0.3% hydrogen peroxide [14].

Catalase (CAT) activity. The degradation rate of H2O2 at 240 nm was used to calculate CAT activity [15]. The enzyme extract (50 µL) was mixed with a buffer solution of potassium phosphate (1 mL, 25 mM, pH 7) containing H 2O2 (10 mM). CAT activity was expressed in µmol/g fresh weight.

Superoxide dismutase (SOD) activity. The ability of superoxide dismutase (SOD) to inhibit the photochemical reduction of nitrosine tetrazolium (NBT) has been determined [15]. The mixture consisted of KH2PO4 (50 mM, pH 7.8), NBT (75 mM), L-methionine (10 mM), EDTA (0.1 mM), and riboflavin (20 mM) with enzyme extract (100 µL). After 15 minutes of exposure to light, the tubes were incubated at 25°C under two 15W fluorescent lamps. Finally, absorption at 560 nm was calculated. SOD activity was expressed in µmol/g FW.

Statistical data processing. All experiments were carried out in threefold repetition. Statistical processing was carried out taking into account the calculation of the mean value, standard deviation and p-value of the Student's test. The data analysis was carried out in Microsoft Excel 2010.

Results

Infected plants and callus samples, according to the protocol, were examined for Potato Virus X (PVX) after 3 dpi. On the same day, the first sample was collected for the analysis of antioxidant enzyme activity (AOE). Based on the results of the polymerase chain reaction (PCR) analysis, all samples of native plants and callus (after co-cultivation with viral particles) subjected to infection were confirmed to be infected. The PCR results are presented in Figure 2.

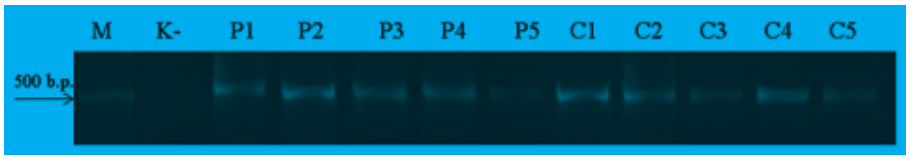

Figure 2 – PCR Analysis Results for Potato Virus X Identification in Potato Callus and Plants. M – marker; NC – negative control; P1 – Ulan variety plant, P2 – Tokhtar variety plant, P3 – Babayev variety plant, P4 – Aliyans variety plant, P5 – Narli variety plant; C1 – Ulan variety callus, C2 – Tokhtar variety callus, C3 – Babayev variety callus, C4 – Aliyans variety callus, C5 – Narli variety callus.

Symptoms of infection on plants were observed at the infection site as early as 3 dpi, and virus spread was noted on 7 dpi. Typically, signs of infection on plants manifest within 5-7 dpi. In the studies by Aguilar E. et al., it was demonstrated that infected tobacco plants exhibited symptoms of PVX infection within 6 days, as revealed by Western blotting [16]. Even resistant potato varieties carrying the Rx gene become infected with Potato Virus X as early as 5 dpi [17].

Furthermore, according to the research design, changes in peroxidase (POD) enzyme activity were assessed for the five examined potato varieties in leaf explants and callus tissues on the 3, 5, and 7 dpi (Figure 3).

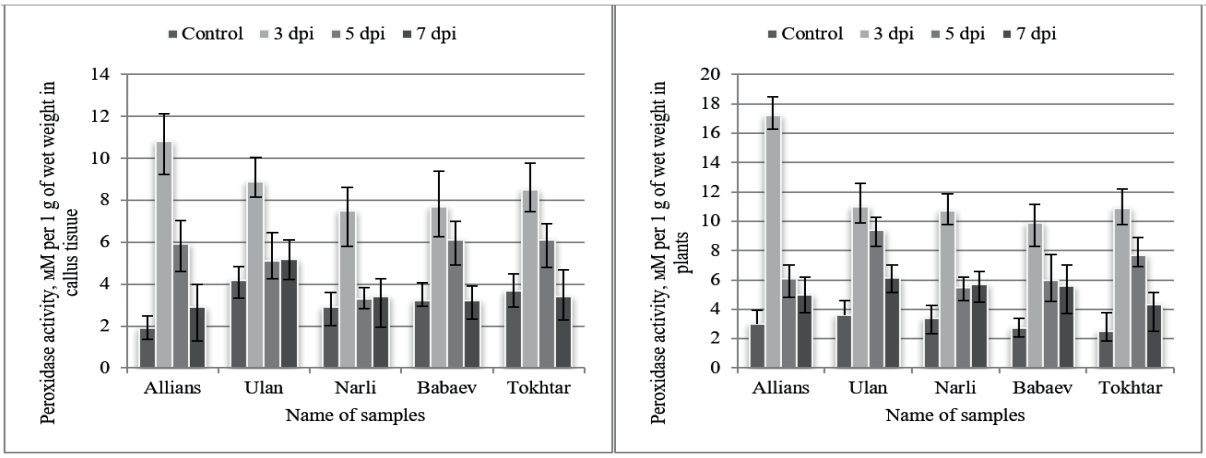

Figure 3 – Variation in Peroxidase Activity in Calli and Plants of Five Potato Varieties of Kazakhstani Breeding

As revealed by the results, the initial antioxidant status in callus cells was 58% lower ($p<0.001$) compared to plants. Subsequently, upon infection, a significant increase in peroxidase activity by 5.6-5.9 times ($p<0.001$) was observed in both samples on the 3 dpi. The Alliance variety exhibited the highest responsiveness to viral introduction, showing the maximum level of activity enhancement in both plants and callus within the antioxidant system (AOS). Following closely in activity was the Tochtar variety, which demonstrated a 4.4-fold increase ($p<0.001$) in peroxidase activity compared to the baseline.

In plants, Ulan, Narli, and Babaev varieties showed a threefold increase in AOS levels on the 3 dpi, compared to the initial point. Simultaneously, the callus reaction to infection in these varieties was 2-2.5 times higher ($p<0.001$) than before infection. Peroxidase activity indicators decreased by almost 50% ($p<0.001$) on the 5 dpi for all varieties, suggesting a reduction in the first level of defense against the pathogen and virus spread in all cells.

As presented in the results by Khaled El

Dougdoug et al., an increase in SOD, CAT, and POD levels is primarily observed in tolerant and resistant varieties compared to susceptible and healthy varieties [18]. According to Cristina Aguilar-Sánchez et al., it can be inferred that the increase in AOS occurs as early as 4 hours after infection for CAT and POD [19]. Many scientists associate the activation of AOS with the functioning of POD. Although plant PODs may exhibit substrate-specific activity *in vitro*, this does not necessarily imply the same effect *in vivo* [20].

Peroxidase activity data indicated that the Alliance, Ulan, and Tochtar varieties have higher activity levels after 3 dpi. This suggests that these varieties have a higher level of primary activation of the immune system during viral infection. The dynamics of changes in the AOS level in plant and callus objects are nearly identical, although it is essential to consider that the initial AOS level is significantly lower in the callus, as it is maintained in sterile conditions with low levels of stress factors.

Another significant enzyme in deactivating peroxidation is catalase. As known, catalase activity is influenced by the concentration of hydrogen peroxide – its main substrate [21]. Catalase has very high reaction rates but low affinity for hydrogen peroxide, requiring large amounts of substrate [22]. The results of studying catalase activity in plants and callus culture under viral infection conditions are presented in Figure 4 below.

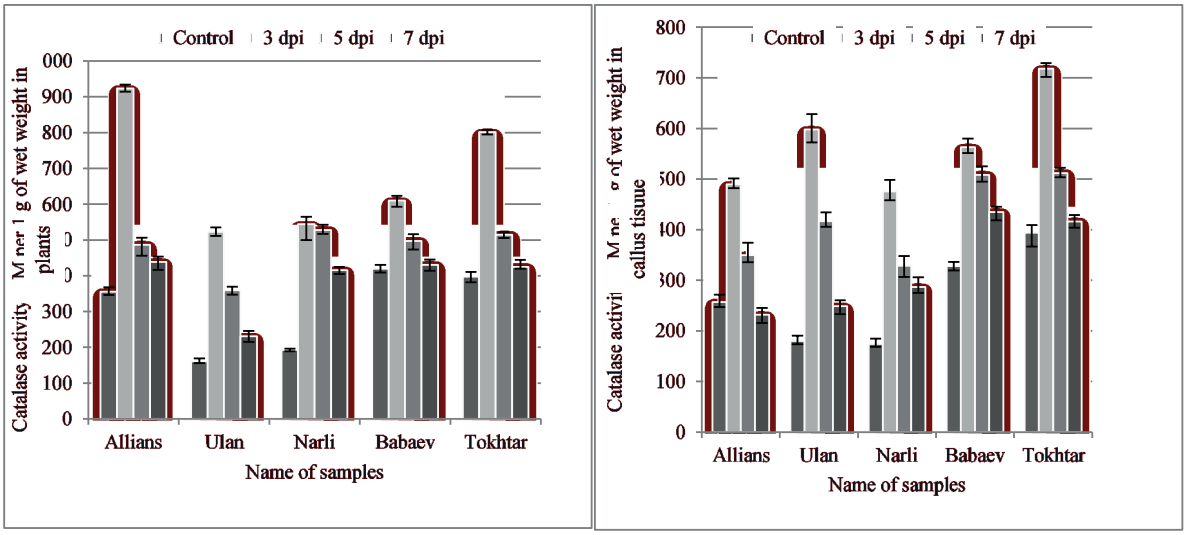

Figure 4 – Changes in catalase activity in callus tissues and plants of five varieties of Kazakhstan i-selected potatoes

Catalase activity in the callus tissue showed significantly higher levels compared to plant cells. It is important to note that the activation of activity occurs on the 3 dpi, and significantly high levels persist until the 7 dpi. This is particularly evident in the Ulan, Narli, and Babayev varieties, where the activity on the 7 dpi exceeded by 27% ($p<0.01$), 39.3% ($p<0.001$), and 24.6% ($p<0.01$), respectively. This may indicate a low level of catalase degradation and a high level of stress factors. Meanwhile, the Alliance variety showed a value 11% lower than the initial measurement on the 7 dpi ($p\geq0.05$), and the Tokhtar variety exhibited an activity 5% higher than the initial measurement on the 7 dpi ($p\geq0.05$). For the Babayev variety, both in callus tissue and native plants, catalase activity on the 3 dpi was the lowest, being 41% ($p<0.001$) and 31% ($p<0.01$) higher than the initial measurement, respectively.

The low affinity for hydrogen peroxide indicates that there is almost always a linear relationship between catalase (CAT) activity and peroxide concentration, even at supraphysiological concentrations. Therefore, accurately determining the Michaelis constant for CAT is very challenging. It is believed to be in the range of 40-600 mmol/ [23]. Thus, CAT operates significantly below its maximum capacity and maintains its activity for an extended period. Additionally, CAT remains stable during changes in the cell's redox status, making it more resistant to stress compared to other components of the antioxidant system. Under physiological conditions, catalase enzyme activity can be up to 10,000 times higher than peroxidase activity [24]

As demonstrated in recent studies, viral proteins of viral particles are directly aimed at deactivating catalase. For example, the 2b protein

of the Cucumber Mosaic Virus (CMV) directly interacts with catalases (CAT) and inhibits their activity, as shown in the research by Ting Yang et al. (2022). [25]. The same group of scientists in 2020 demonstrated that the helper component protease (HcPro) encoded by the Chilean Pepper Vein Mottle Virus (ChiVMV) can directly interact with catalase 1 (CAT1) and catalase 3 (CAT3) in the cytoplasm of tobacco plants *(Nicotiana tabacum*) to facilitate the spread of viral infection. [26].

Thus, the catalase activity levels in the five potato varieties indicated a high level of infection. In this context, the Alliance, Ulan, and Narli varieties exhibited a higher level of the primary response to infection in plants. The difference in catalase degradation in the two objects may be associated with differences in physiological processes.

One of the indicators of the resistance and susceptibility of various plant species to stress factors is the increase in superoxide dismutase (SOD) activity in cells. During the superoxide dismutation reaction, hydrogen peroxide is formed, which is less active compared to the superoxide radical and is neutralized by other enzymes. The main pool of hydrogen peroxide is neutralized by catalase (CAT), while peroxidases (POD) can neutralize peroxide inaccessible to CAT due to their high affinity for hydrogen peroxide. Moreover, this neutralization occurs thanks to the presence of peroxidases in numerous cellular organelles.

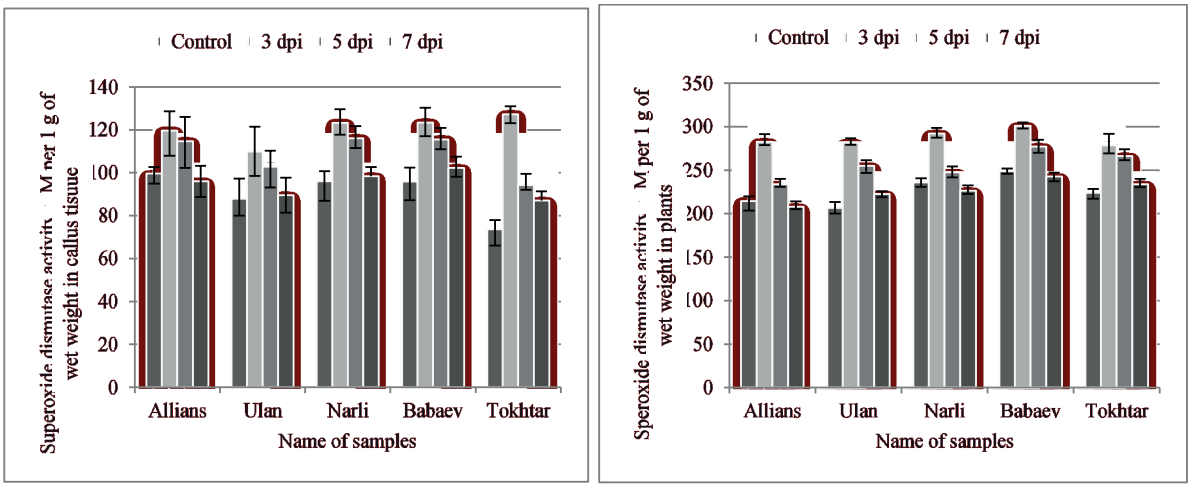

Figure 5 – Changes in superoxide dismutase activity in callus tissues and plants of five varieties of Kazakhstan i-selected potatoes

As seen in the presented figure, changes in the activity of the SOD enzyme are observed as early as the 3rd dpi. Significant differences in enzyme activity in the callus are noted for the Allians and Tokhtar varieties, being 16% ($p< 0.01$) and 42% ($p<0.001$) higher than in the uninfected callus. In the Ulan, Narli, and Babaev varieties, almost the same level of activity is observed, which is 20% ($p< 0.01$), 21% ($p<0.01$), and 22% ($p<0.01$) higher than the initial indicator. In native plants, this indicator was highest in the Alians and Ulan varieties, being 24% ($p<0.01$) and 26% ($p<0.01$) higher. For the Narli and Tokhtar varieties, this indicator was at the level of 19.3-19.6% ($p<0.01$). At the same time, the minimum difference in this indicator was noted for the Babaev variety. The dynamics of activity in the callus and in the native plant for this enzyme is ambiguous. For the Allians variety, the activity indicator in the callus culture is the lowest, while in the plant, it is one of the highest. The opposite situation is observed for the Tokhtar variety, in the callus tissue of which the maximum activity of SOD is noted, whereas in the plant, it is one of the lowest. According to Serenko et al. (2011) resistance to stress factors in transgenic plants of *Solanum lycopersicum L.* was observed in plants with increased SOD activity. It is shown that the cell quickly responds to stress by increasing SOD activity. The results of Viola Kunos et al. (2022) showed that, despite differences in the reactions of the studied 5 barley varieties when infected with three isolates of *Pyrenophora teres* f. *teres*, significantly increased SOD activity was observed in all studied varieties at early stages of infection. At the same time, the lowest SOD activity was observed in the most resistant

variety [27]. According to Quancheng Zhang et al. (2022) after powdery mildew infection in cucumber leaves, the chlorophyll and free proline content decreased, while the activities of POD and SOD, as well as the content of soluble protein and malondialdehyde, increased [28].

Thus, the activity of the SOD enzyme remains ambiguous in both callus tissue and plant leaves during viral infection. It is not possible to unequivocally identify a resistant variety based solely on the level of activity of this enzyme. Comparing the three enzymes presented in the study, it can be concluded that the Alians, Ulan, and Tokhtar varieties showed the highest enzyme activity in both objects. At the same time, the level of enzyme activity in the Babaev variety was minimal in all examined variants. It is worth noting that the ambiguity of the increase and decrease in enzyme activity levels in callus and leaf tissue in potatoes may not fully reflect the dynamics of antioxidant enzyme activity under infection conditions. Additionally, it is important to consider the differences in the growth conditions between callus and plants.

Discussion

In living organisms, two functionally distinct sources of ROS exist. They are generated during normal metabolism and play a role in signal transduction, so a living system can never completely eliminate all ROS [29]. However, ROS can also be formed as a result of various environmental stresses, and this excess amount of ROS is scavenged by protective antioxidants or the antioxidant defense system. The primary forms of ROS include superoxide anion (O2−•), hydrogen peroxide (H2O2), and hypochlorous acid (HOCl), as the reactivity of these compounds is analogous to ions with unpaired electrons [17]. In cells, ROS are primarily produced in mitochondria, chloroplasts, apoplasts, and peroxisomes, as well as in cell walls and plasma membranes. If prooxidants (i.e., free radicals) accumulate, for example, due to stress, a three-level antioxidant defense system is activated. O2−• is formed as a result of the reaction between molecular oxygen and electrons generated in electron transport chains. This free radical is abundantly produced in response to stress and is eliminated by direct enzymatic pathways as the first protective component of the antioxidant system [5]. Superoxide dismutase (SOD) absorbs O2−• by catalyzing its dismutation into H2O2. A high concentration of H2O2 inactivates SOD, so its dissociation to water is facilitated by catalase (CAT), which has high activity but low substrate affinity. Removal of H2O2 can be carried out by glutathione peroxidase (GPX), which is one of the inducible key enzymes in membrane protection when membranes undergo oxidative stress, using reduced glutathione (GSH) as a substrate [29].

In this study, changes in the activity of antioxidant enzymes in callus tissue and native potato plants in virus-free and infected samples were investigated over time of infection and antioxidant activity. Catalase (CAT) and peroxidase (POD) activities were the lowest on the 7 dpi, while superoxide dismutase (SOD) activity was the highest.

Earlier studies have shown that varieties with different susceptibility exhibit different enzymatic activities, which can serve as a marker for ranking potatoes in breeding. The most significant differences in physiological response were observed in POD activity in microclones derived from virus-resistant potato varieties, while a decrease in activity was noted in microclones from susceptible varieties. However, microclones from susceptible varieties showed higher CAT activity than other genotypes. The highest SOD activity was observed in microclones from resistant potato varieties compared to other genotypes [30].

Substantial differences in antioxidant enzyme activity may reflect the importance for the adaptive response to stress factors in potato callus [31].

When studying the influence of antioxidants on the regenerative activity of callus regeneration obtained from hypocotyls of *Mesembryanthemum crystallinum* seedlings, differences in the activity of CuZn-superoxide dismutase (SOD) were shown to be comparable in all calluses, but the activity of FeSOD and MnSOD varied depending on the activity of photosystem II and the regenerative potential of tissues. CAT activity was associated with H2O2 concentration and depended on cultivation conditions and the morphogenic potential of calluses [32].

Antioxidant enzymes form a unified network due to ROS and various phenolic substrates, which in one enzymatic reaction can be products of interaction and substrates for the next reaction.

The functioning of the antioxidant defense system is based on a complex multi-stage mechanism of action. Interactions between antioxidant components can be diverse: additive,

synergistic, and antagonistic, and antioxidants themselves are diverse in structure, nature, and functions, operating conjugately or in different directions.

In general, the functioning of the antioxidant defense system in cells is determined by local and cell-wide cyclic and cascading interactions of antioxidants. Simultaneous action of different components of the antioxidant defense system gives rise to unique antioxidant electron transfer chains. Thus, the switching of electron flow may be the reason for the correlation between CAT and SOD activity, which may be localized in different parts of the cell. The simultaneous operation of individual parts of the antioxidant defense system is the basis for forming organism resistance to the action of ROS. Antioxidants must be continuously synthesized and delivered to the site of their consumption, providing antioxidant protection determined by specific 'antioxidant structures.'

The level of intracellular antioxidants is genetically determined. The need for the study of antioxidant systems consisting of many components is emphasized. However, such studies are not widely spread.

Examples of such cascading interactions are found in the literature. For instance, in drought-stressed woody plants of various families, a positive correlation was observed between SOD activity and the activities of CAT and POD. CAT, in turn, positively correlated with POD [33]. Additionally, many works describe an increase in CAT and POD activities as a result of a high demand for neutralizing the formed hydrogen peroxide through the superoxide dismutase reaction [7].

Conclusion

Through conducted research, a discernible association emerged between viral infection and the antioxidant status of potatoes within in vitro and in vivo culture, specifically focusing on callus culture and local varieties Alliance, Ulan, Narli, Babaev, and Tokhtar, exhibited varying activity levels contingent upon the timing of contamination. The dynamics of enzyme accumulation in callus tissue displayed distinctive attributes owing to the sterile growth conditions in contrast to the indigenous infected potato plants. The activity levels observed in the callus were notably an order of magnitude higher than those in tuberous plants, a phenomenon potentially elucidated by the absence of additional environmental stressors in the controlled *in vitro* environment. It is imperative to acknowledge disparities in growth conditions, necessitating further exploration through additional in *vivo* studies, particularly focusing on the assessment of antioxidant activity. The comprehensive understanding of the differences between callus and plant reaction on viral infection will contribute significantly to the advancement of knowledge pertaining to the intricacies of potato plant responses to stress factors.

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PVX ВИРУСТЫҚ ИНФЕКЦИЯСЫ ЖАҒДАЙЫНДА КАРТОП ПЕН ӨСІМДІКТЕРДІҢ КАЛЛУС ДАҚЫЛЫНЫҢ АНТИОКСИДАНТТЫҚ МӘРТЕБЕСІ

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Түйін

Картоп (*Solanum tuberosum L.*) - күріш, бидай және жүгеріден кейін бүкіл әлемде тұтынылатын маңызды алқа тұқымдас дақыл. Саңырауқұлақтар, вирустар, бактериялар және вироидты инфекция сияқты биотикалық факторлар картоп өсімдігінің метаболизміне айтарлықтай әсер етеді және олардың аталған стресс факторларына физиологиялық, биохимиялық және молекулалық реакцияларын өзгертеді. Өсімдіктердің антиоксидантты ферменттерінің (АОФ) тотығу стрессінің белсенділігі мен вирустық инфекцияларға төзімділік деңгейлерін зерттеу және анықтау үшін көбінесе бастапқы үлгі нысаны ретінде каллус мәдениетін пайдаланады. Бұл зерттеуде АОФ белсенділігінің профильдері, атап айтқанда пероксидаза (POD), каталаза (CAT) және супероксид дисмутаза (SOD) - екі нысанда – картоп каллус тіні мен жергілікті өсімдіктерде PVX вирусын жұқтыру жағдайында зерттелді. Зерттеу нәтижелері АОФ профильдері каллус тіндерінде және вирус жұқтырған картоп өсімдіктерінің жапырақтарында әртүрлі екенін көрсетті. Атап айтқанда POD каллус тініндегі белсенділік деңгейі инфекцияның үшінші күнінде өсімдік жапырақтарына қарағанда 50% - дан жоғары. АОФ белсенділігінің жоғарылау динамикасы инфекциядан кейінгі 3-ші күні деңгей жоғарылау және 7-ші күні төмендеу заңдылығын көрсетті. SAT белсенділік деңгейі зерттелген барлық 5 сортта инфекциядан кейін 7-ші күнге дейін сақталды.Сутегі асқын тотығын залалсыздандырудағы негізгі ферменті ретінде СОД белсенділігі инфекцияға дейін 16-42% жоғары болды. АОФ белсенділігі каллус пен жапырақ тінінің мысалында PVX вирусын жұқтырған кезде өзіндік үлгіге ие: инфекцияға дейінгі белсенділік деңгейі жапырақ тінінен қарағанда құрғақ масса 100 бірлік/гр. аз. Сонымен қатар, екі объектідегі белсенділіктің арту сипаты әртүрлі. Осылайша, картоп сорттарының төзімділік деңгейі бойынша тек каллус тініндегі АОФ белсенділік деңгейіне негізделген нақты тұжырымдар жасау мүмкін емес. Сондықтан стерильді емес жағдайларда жергілікті өсімдіктердегі АОФ деңгейін міндетті түрде зерттеу қажет.

Кілт сөздер: картоп; *Solanum tuberosum;* антиоксидантты ферменттер; пероксидаза; каталаза; суперпероксидаза.

АНТИОКСИДАНТНЫЙ СТАТУС КАЛЛУСНОЙ КУЛЬТУРЫ КАРТОФЕЛЯ И НАТИВНЫХ РАСТЕНИЙ В УСЛОВИЯХ ВИРУСНОЙ ИНФЕКЦИИ PVX

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Аннотация

Картофель (*Solanum tuberosum L.*) является важной пасленовой культурой, потребляемой во всем мире после риса, пшеницы и кукурузы. Биотические факторы, такие как грибы, вирусы, бактерии и вироидная инфекция, существенно влияют на метаболизм растения картофеля и изменяют его физиологические, биохимические и молекулярные реакции на эти стрессовые факторы. Для изучения уровня активности антиоксидантных ферментов (АОФ) окислительного стресса растений и выявления уровни устойчивости при вирусных инфекциях часто используют в качестве первичного модельного объекта каллусную культуру. В данном исследовании изучены профили активностей АОФ, в частности, пероксидазы (POD), каталазы (CAT) и супероксиддисмутазы (SOD) на двух объектах – каллусной ткани картофеля и нативных растениях в условиях инфицирования вирусом PVX. Результаты исследований показали, что профили АОФ различаются в каллусных тканях и в листьях инфицированных растений картофеля. Так уровень активности в каллусной ткани POD более чем на 50% выше на третий день инфицирования, чем в листьях растения. Динамика повышения активности АОФ имеет закономерность, повышения уровня на 3-ий день после инфицирования и понижения к 7-му дню. Уровень активности CAT сохранялся до 7-го дня после инфицирования у всех 5-ти изученных сортов. Активность СОД, как основного фермента при дезактивации перекиси водорода, была выше на 16-42%, чем до инфицирования. Активность АОФ имеет свою закономерность при инфицировании вирусом PVX на примере каллусной и листовой ткани, так уровень активности до инфицирования на более, чем 100 ед/гр.сухой массы меньше, чем в листовой ткани. При этом характер увеличения активности в двух объектах различна. Таким образом, однозначных выводов по уровню устойчивости сортов картофеля исходя только из уровня активности АОФ в каллусной ткани сделать невозможно. Поэтому необходимо обязательное изучение уровня АОФ в нативных растениях в нестерильных условиях.

Ключевые слова: картофель; *Solanum tuberosum;* антиоксидантные ферменты; пероксидаза; каталаза; супероксидоксидаза.