

The Determination of the Titers of Polyclonal Antibodies Obtained to Potato X Virus Using Elisa

Dilfuza Jovlieva¹, Abdirasul Vakhobov¹, Vokhid Fayziev²

¹National University of Uzbekistan, Tashkent, Uzbekistan

²Doctor of Biological Sciences, Associate Professor, Chirchik State Pedagogical University, Chirchik, Uzbekistan

Abstract In this article, the process of titrating polyclonal antibodies against potato virus X using elisa was studied. It is no secret that among the phytoviruses, the viruses infecting the potato plant are becoming more and more widespread and their epiphyticity is increasing. Timely detection of viruses requires the use of sensitive methods. IFA is such a method, it is based on the relationship between antibody (Ab) and antigen (Ag), where the titer of Ab is important. In this article, the titer of polyclonal Ab to potato X virus (PXV) isolated in Uzbekistan was determined using the DAS–ELISA method. For this, PXV antigen was isolated from the plant *Datura tatula* L. and virus–specific serum was prepared by means of rabbit immunization, and the prepared Ab titer was found to be 10–5 when determined by the sandwich variant of the enzyme immunoassay method. This allows the detection of the virus up to 10 µg.

Keywords Potato X virus, ELISA, Antigen, Antibody, Conjugate, Phytovirus, Determination of the serum

1. Introduction

Human health can be preserved and included among the serious puzzles facing humanity since ancient times. If we take into account that the number of people in the world exceeds 8 billion, and the number of people suffering from hunger is close to 9 million [24], the way to solve the food problem is to increase nutritious crops, damage to agricultural plants it is necessary to prevent the causing factors, search for the cause and search for ways to fight against them. The increase in the mutual export of agricultural products leads to the spread of plants, an increase in the level of disease of plants, and the emergence of new strains and isolates of viruses due to external factors. The potato plant, which is considered as one of the agricultural food plants, belongs to the group of plants that are highly infected by phytoviruses, and it is infected with 52 of more than 400 known viruses, including 36 common viruses [1]. Among the studied phytoviruses, 7 viruses (PLRV, Y, X, A, S, M, AMY) were found to be dangerous for potatoes, and potato X virus (PXV) was included among the 4 extremely dangerous viruses among them [1]. In addition, PXV, together with other viruses, causes an increase in the level of disease of potatoes. Transmission of PXV to plants has been determined mechanically, through insects and soil, and even through containers containing seedlings in greenhouse conditions [3,4]. It has been found that this virus causes scattered mosaic and simple mosaic

symptoms on plant leaves and coexists with other viruses in a latent (hidden) state [5,6].

Several methods have been developed for the detection of viruses, and among the methods used for the diagnosis of viruses, immunological methods are distinguished by the fact that they require little work and time, and can examine several samples at the same time [7,8,9,10,11].

The conducting such an analysis is based on the interaction of antigen (AG) and antibody (AT). One of these methods is immunoenzymatic analysis (IFA), which is also known as a process based on two components—an immune response and an enzymatic reaction.

If the immune reaction attempts to identify biological molecules, cell or microorganism elements by forming a bond, the enzymatic reaction allows to see the result of the immunological reaction. That is, the immune response is actually a part of a complex process that determines the desired antigen [6,12,19].

Immune reactions are specific reactions of binding between antigen and antibody with the formation of an immune complex. In enzyme immunoassay and any other immunochemical analysis, the primary process is the step of “recognition” of the analyzed compound by its specific antibody. Since the process of formation of immunochemical complexes occurs in a strictly quantitative ratio due to the concentration of components and reaction conditions, the initial concentration of the analyzed compound is determined by quantitative determination of the formed immune complexes [11,12].

A number of Uzbek scientists, including V.B. Fayziev,

A.H. Vakhobov, conducted a series of experiments to identify isolates of potato viruses found in Uzbekistan and study some of their characteristics using various methods. In particular, the effect of isolate PVXN-UZ 915 on the pigment content of *D. stramonium* plant was studied [16,17]. The necrotic isolate of PXV was found to be closely related to isolate GU384732.1 isolated in Australia based on PSR analysis and phylogenetic analysis [18]. Initially, it was determined that the specific serum for PXV and its titer was 1:16 by the double immunodiffusion method [19]. In addition, the spread of potato viruses X and L in reservoir plants and in the Samarkand region of Uzbekistan was studied using ELISA [20].

D. stramonium, *N. glutinosa*, plants such as *N. tabacum* burley were used [1,6,21]. Breeding of PXV *D. stramonium* var. *stramonium* (green shoot and white flowers) this was done in the plant because it showed symptoms of systemic disease very quickly in a clear mosaic state [2,19].

In some countries, hot sunny days limit the possibility of working with phytoviruses, because it is difficult to observe the symptoms of viruses in plants when the temperature is high. Therefore, when extracting PXV, *D. stramonium* var. *tatula* (red shoot and purple flowers) advantages of plant over other indicator plants were found, this plant was used to isolate the virus, and physiological changes in the plant under the influence of the virus were studied [22,23].

Due to the high sensitivity of immunological diagnostics for the detection of viruses and for the purpose of using them in the identification of viruses, it was aimed to determine the specific antibody titer obtained for PXV by the IFA method.

2. Material and Methods

Necessary reagents and equipment: to carry out the research, specific polyclonal serum obtained from PXV spread in the territory of Uzbekistan [22], antigen (*D. stramonium* var. *stramonium*, *D. stramonium* var. *tatula*) from the leaves of potatoes infected with the virus (Figure 1) amplified PXV (Figure 2)), conjugate (Antirabbit Ab+peroxidase), substrate (TMB), stop solution, Wash buffer (1% BSA) and polystyrene plates were used. In determining the results, IFA was determined from the analyzer and by visual inspection.



Figure 1. Potato leaves infected with PXV. a) sick b) healthy



Figure 2. The leaves are mechanically infected with PXV. c) *D. stramonium* L., d) *D. tatula* L

3. Performing a Diagnostic Test

First, Ab (Antibody) was diluted 1:100, 1:1000, 1:10,000, 1:100,000, 1:150,000, 1:200,000, 1:300,000 times. Specific Ab obtained for the virus was added to the polystyrene plates and left overnight at +4°C for 24 hours. After washing the excess Ab with 1% BSA, Ag was added and sorbed at 37°C for 1 h. After washing off excess Ag, Ab was placed on top of Ag and kept at 37°C for 1 h. After washing off excess Ab, it was added to Conjugate (Antirabbit Ab+peroxidase) and sorbed for 1 hour at 37°C. Excess Conjugate was also washed and TMB (tetramethylbenzidine) was added and kept in a thermostat for 1 hour at 37°C. After washing, the stop solution was added and after 20 minutes at 37°C in a thermostat, the result was visualized in an IFA reader at 405 nm.

4. Results and Discussion

It is important to determine the polyclonal serum specific for PXV. The reason is that determining the sensitivity of the necessary reagents in the course of the experiment determines the quality of the diagnostic. In order to determine the developed method, the experiment was carried out in the case where polyclonal serum was diluted in different amounts. Other reagents were diluted accordingly. The time and temperature for sorption were chosen at different levels. As a result of the conducted experiments, the antibody titer was determined based on the optimal variant of DAS-ELISA. The flow chart of the DAS-ELISA method used to determine the specific polyclonal Ab titer obtained for KXV is given below (Figure 3).

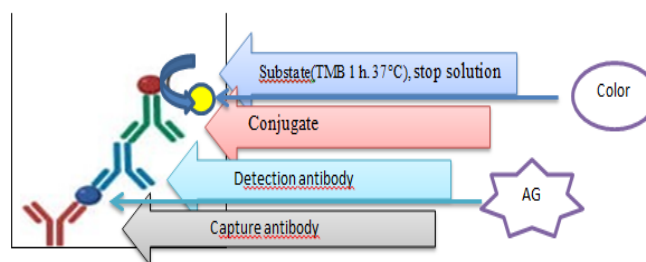


Figure 3. DAS-ELISA test scheme

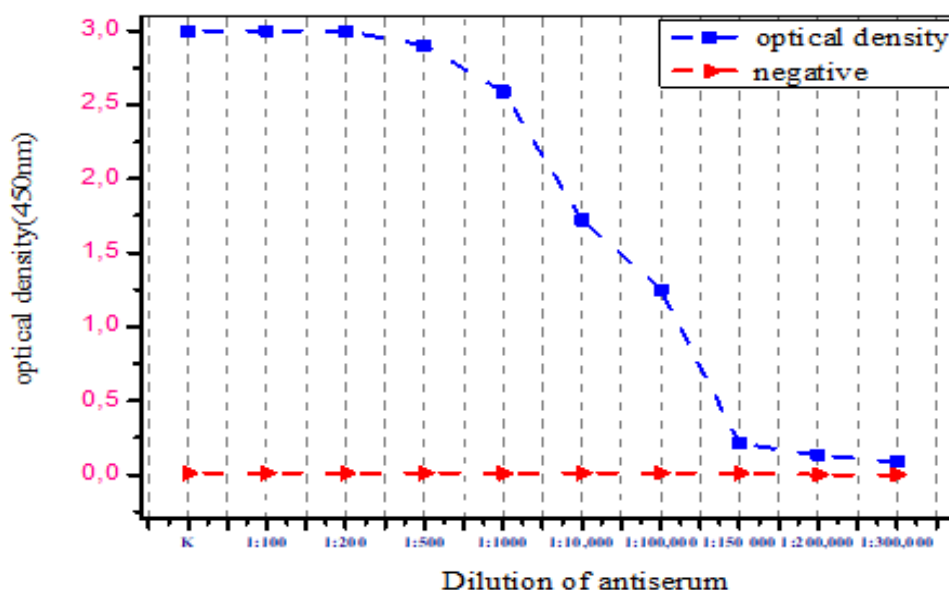


Figure 4. Graphic representation of polyclonal antibody titer obtained for PXV detected in DAS–ELISA

In the picture, the specific Ab titer for PXV was used using the sandwich variant of the enzyme immunoassay method (Figure 3). Pure KXV isolated from *D. tatula* L. plant and specific Antibody obtained for this virus were used as Ag. Conjugate used Antirabbit Ab+peroxidase and TMB as substrate. The test result was determined in the IFA reader at 405 nm [Table 1].

Table 1. IFA reader results of specific polyclonal antibody titers for PXV

Antibody dilution indicators	Optical density 450nm	Visual result
Control	OUT	++++
100	OUT	++++
1000	2,989	++++
10.000	1,722	++++
100.000	1,251	+++
150.000	0,211	–
200.000	0,134	–
300.000	0,087	–

Note: here 0.500–0.799 nm “+”; 0.800–1.199 nm “++”; 1.200–1.499 nm “+++”; 1.500 nm and above means “++++” and “–” means low titer. “OUT” is recorded when the optical density exceeds 3,000 when IFA is checked.

After the polystyrene was poured into the plates, a test was carried out and the results determined. The polyclonal agent was diluted as indicated in the Table 1. Ab titer at 100–fold dilution was found to be 2.989, at 100,000–fold dilution it was 1.151, and at 150,000–fold dilution and above, no Ab titer was detected. The result was marked by a “+” sign when counting the virus in the samples, depending on the light and dark blue colors formed during visual inspection. It is also known from the table that “++++” was detected when Ab was undiluted and when diluted 100, 1000 and 100,000 times, while it was colorless when diluted 150,000 and above. Marked with a “–” sign.

“OUT” was recorded when an optical density reading

above 3,000 was detected. This means that the concentration of Ab is high. In order to express the obtained result more accurately, the optical density indicator for determining the titer of Ab to the virus was depicted in a graphic form (Figure 4).

As a negative, the liquid separated from the clean plant was used. 1–represents the undiluted state of the antibody.

As depicted in the graph, it can be seen that the titer is high in the undiluted state of Ab, and as the dilution level increases, the titer decreases and approaches zero. A low titer was detected at a dilution of 1.5×10^{-5} . No optical density was recorded in this sample when a blank plant sample and buffer were used as a negative to check the titer.

5. Conclusions

As a result of the experiment, the polyclonal Ab titer obtained for KXV was found to be 100,000 (10–5). Therefore, the Ab obtained for KXV can be used for detection of viruses in a 10–5–fold dilution. Further continuation of the experiment allows to increase the antibody titer and use it in immunological methods. The authors express their gratitude to the staff of the Institute of Chemistry of Plant Substances for their assistance.

REFERENCES

- [1] Файзиев В.Б. Картошка X–вирусининг Ўзбекистонда тарқалган изолятини ажратиш, хусусиятларини ўрганиш ва унинг диагностикаси; док. дисс. Тошкент, 2019. 230 б.
- [2] Файзиев В.Б. Картошка вирусларининг замонавий иммунодиагностикаси (скрининг, мониторинг); Биол. фан. ном. дисс. Тошкент, 2011. 134 б.
- [3] Ахатов А.К., Джалилов Ф.С. и др. Защита растений от

- болезней в теплицах (справочник) / под ред. А.К. Ахатова. Москва: КМК, 2002. стр. 35.
- [4] Рогозина Е.В., Мироненко Н.В., Афанасенко О.С., Матсухито Ю. Широко распространенные и потенциально опасные для Российского агропроизводства возбудители вирусных болезней картофеля / Вестник защиты растений 4(90), 2016. стр. 24–33.
- [5] Вахабов А.Х. Характеристика наиболее распространенных фитовирусов в экологических условиях Узбекистана: Дисс. доктор. биол. наук. – Киев: Институт Микробиологии АН УР, 1989. стр. 254.
- [6] Вахабов А.Х. Умумий вирусологиядан амалий машгулотлар. И–жилд, –Тошкент: Университет, 2004. – 150 б.
- [7] Атабеков И.Г. Иммунодиагностика вирусов растений резервные миллиарды в сельском хозяйстве // Биотехнология. – Москва, 1984. стр. 238.
- [8] Вахабов А.Х. Ўсимлик вирусларини аниқлашда иммунология усулларини қўллаш. – Тошкент: ТошДД, 1991. – 36 б.
- [9] Жураева У.М. Выделение, очистка вирусов хлопчатника и их иммунодиагностика. Автореф. дис....канд. биол. наук. – Ташкент: АНРУз., 1996. стр. 29.
- [10] Одинец А.Г. Разработка и применение иммуноферментного анализа для диагностики вирусных болезней светочных культур. Дис....канд. биол. наук. – М.: 1986. стр. 125.
- [11] Умарова Г.М. Ғалла ўсимликлари вирусларини ажратиш, тозалаш ва уларни иммунодиагностикаси. Биол. фан. ном. дис... автореф. – Тошкент: ЎзРФА Микробиология институти, 2009. – 22 б.
- [12] С.В. Жаворонок, Д.В. Тапалский. Иммуноферментный анализ Учебное пособие для студентов 2–5 курсов).
- [13] Tsialtas J.T., E. Kostoglou, D. Lazari, I.G. Eleftherohorinos “Annual *Datura* accessions as source of alkaloids, oil and protein under Mediterranean conditions” *Industrial Crops & Products* 121 (2018) 187–194, <https://doi.org/10.1016/j.indcr.2018.05.015>.
- [14] Амбросов А.Л. Вирусные болезни картофеля и методы выращивания здоровых клубней. – Минск: Урожай, 1964. стр. 199.
- [15] Гнутова Р.В., Толкач В.Ф. Вирусы и их штаммы, поражающие овощные культуры (далневосточные изоляты) // *Агроэкологический журнал: Спец. вып. –Владивосток*, 2002. стр. 6–14.
- [16] Fayziev V., Jovlieva D., Juraeva U., Shavkiev J., Eshboev F. (2020). Effects of PVXN–UZ 915 necrotic isolate of Potato virus X on amount of pigments of *Datura stramonium* leaves // *Journal of Critical Reviews*, 7(9). – p. 400–403.
- [17] Fayziev V., Javlieva D., Eshboev F., Sattorov M. (2021). Comparative amino acid analysis of coat protein of necrotic isolate of virus x of potato plants // *Plant Cell Biotechnology and Molecular Biology* 22 (33&34). – p. 197–203.
- [18] V.Fayziev, D.Javlieva, Z.Kadirova, S.Chirkov, U.Jurayeva, A.Vakhabov. Study of some biological properties necrotic isolat of potato virus x and phylogenetic analysis // (2020). *International Journal of Psychosocial Rehabilitation*, Vol.24, Issue 09, ISSN: 1475–7192.
- [19] В.Б. Файзиев, Д.Т. Жавлиева, У.М. Жураева, З.Н. Кадилова, А.Х. Вахабов. Выделение, очистка и изучение иммунохимических характеристика ХВК // (4/2019), *Узбекский Биологический журнал*. стр. 19–24.
- [20] В.Б. Файзиев, Д.Т. Жавлиева, У.М. Жураева, А.Х. Вахабов, З.Н. Кадилова (2019). Изучение распространения и определения растений–резерваторов х и л вирусов картофеля методом иммуноферментного анализа // *Научное обозрение Биологические науки*, №2. стр. 79–86.
- [21] Аграновская А., Алиева Н.О.Х., Атабеков И.Г. и др. Практикум по общей вирусологии: Учеб. пособие / Под П68 ред. И.Г. Атабекова. – 2–е изд., перераб. И доп. – М.: Изд–во МГУ, 2002. стр. 184.
- [22] D.T. Jovliyeva, V.B. Fayziyev, A.H. Vakhobov. Isolation of Potato X–Virus from Collecting Plants // *Global Scientific Review*, Volume 10, December 2022. – p. 163–166.
- [23] D.T. Jovliyeva, V.B. Fayziyev, A.H. Vakhobov. Change of enzyme dynamics in *Datura tatula* plant under the influence of potato x virus. // *Science and innovation international scientific journal*. Volume 1, Issue 8. – p. 1031–1035.
- [24] <https://aniq.uz/uz/statistika/dunyo>.