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Effect of Different Thermotherapy Conditions on Eradication of Potato Viruse M and Microclonal Propagation of Potato Varieties

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ABSTRACT:

Potato seed production remains a problem in Georgia. Large farmers prefer imported elite seed, which is quite expensive, while small farmers use low-quality seed produced on their plots, which may be infected with viruses. To assure potato production and supply of high-healthy planting material effective ways of eliminating viral infection must be developed.

This study was conducted to evaluate the effect of thermotherapy with the combination of apical meristem culture on the four potato varieties "Picasso", "Raya", "Santé" and 'Arizona" infected with Potato virus M (PVM) in Georgia.

Tree types of temperature: 33^0C, 36^0C, 39^0C with combination of 2, 3 and 4 h exposure were used for thermotherapy on the four potato varieties "Picasso", "Raya", "Santé" and "Arizona" infected with Potato virus M. After 27 days Enzyme-linked immune sorbent assay (ELISA) were used to evaluate the virus infection rate.

The most effective result (100% virus eradication) was obtained by temperature- 36^0C with 2h exposure during 27 days for potato varieties "Picasso" and "Raya". Results show that on treatment 36^0C/4h during 27 days was effective for potato varieties "Sante" and "Arizona". Sprouts generated on 39^0C with 4h exposure started to degrade due to heat. Based on the results virus free potato accessions were added collection of Biotechnology Center.

1. Introduction

Currently, potato is the fourth-most important food crop in the world with a production of 376 million tons it is the staple food of 1.3 billion people. The highest production is by China (22%), followed by India, Russia, Ukraine, and USA [1], [2], [3].

The cultivation of healthy potato seed is based on the in vitro apical meristem method, which is recognized as a successful approach in the world. The quality of potato seed material is one of the main factors for its yield [4], Multiple reproduction in the open field causes various types of potato diseases in which viral infections are one of the leading reasons for the degeneration of sustainable potato seed production [5]-[8] and economic losses due to their negative impacts on yield and tuber quality [9], [10].

40 species of viruses were known which can infect potatoes worldwide [11]. The yield reduction may go up to 75% depending on viral varieties. Potato virus X (PVX) potato leaf roll virus (PLRV) and potato virus Y (PVY) were characterized by relatively high distribution in Georgia, potato virus A (PVA), potato virus M (PVM), potato virus S (PVS), are found in limited geographical areas [12].

Among potato viruses, a significantly negative effect on crop production can be caused by PLRV, PVA, PVM, PVS, PVX, and PVY virus infection [13].

The virus's local economic impact is mostly determined by its incidence, virus strain virulence, and potato cultivar tolerance. Potato virus M is geographically widespread, even mild strains are globally very important. Even mild symptoms can cause a 10-18% reduction in tuber production [14]. Infection of intolerant

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cultivars with more severe isolates, which is mostly widespread in some Eastern European countries, might result in 40-75% tuber production losses [15].

Effective management of viral infections includes the preservation of potato germplasm and the international interchange of genetic resources [16]. Complete eradication is considered the most effective measure to control infected plants. To assure potato production and supply of high-healthy planting material effective ways of eliminating viral infection must be developed [17], [18].

Meristem culture methods both alone and in combination with thermotherapy have been used to eliminate virus's infection in potato plants Chemotherapy and electrotherapy both also are popular virus eradication techniques. [19]- [22].

Despite many efforts including the usage of the application of shoot tip cryotherapy as a novel method the complete eradication of viral particles especially in mixed infections, remains a problem In the case of mixed infection potato leaf roll virus (PLRV) and potato virus Y (PVY) cryogenic treatments were completely successful with high efficacy whereas completely failed during the presence of PVM- and PVS-mixed infection [19], [20], [23], [24].

Potato virus eradication shown more effective by combining two or more in vitro-based techniques than just a single application were virus eradication than just a single technique [25]-[28].

It seems that the efficiency of virus reduction depends on the virus varieties, infection level (single or mixed, virus titer), and virus-host combination [22], [26], [28]. The success of virus eradication can also be influenced by the thermotherapy treatment's duration and temperature, the types and quantities of antiviral treatments used during chemotherapy, and the size of the excised shoot tip. Therefore, it is essential to establish virus eradication techniques, especially when plants have a mix of infections [27]-[29].

Potato seed production remains a problem in Georgia. Large farmers prefer imported elite seed, which is quite expensive, while small farmers use low-quality seed produced on their plots, which may be infected with viruses. In recent years (2020-2023), reduced yields have had a direct impact on the domestic market, increasing the cost of food potatoes for consumers. In this situation, 60% of the local population can no longer afford potatoes, while potatoes are the second most consumed food in Georgia after cereals [30].

This study was conducted to evaluate the effect of thermotherapy with the combination of apical meristem culture on the four potato varieties "Picasso", "Raya", "Santé" and 'Arizona" infected with Potato virus M (PVM) in Georgia. Enzyme-linked immune sorbent assay (ELISA) was used to evaluate the virus infection rate, based on the results virus free potato accessions were added to the collection at Biotechnology Center.

2. Materials and Methods

The Thermotherapy method based on Skiada et al. and Das-Elisa methods as reported by Clark and Adams were used in this study [31].

Thermotherapy

Samples were collected from Tsalka region of Georgia.

Tubers were harvested in September and kept at 4–8 °C until the following spring. Tuber transmission of PVM was examined using the progeny tubers from the inoculated plants, which were harvested in 2021-2022 for PVM. Upper leaves were sampled and examined by TAS-ELISA as described above.

Tree types of temperature: 33ºC, 36ºC, 39ºC with the combination of 2, 3, and 4h exposure were used for thermotherapy on the four potato varieties "Picasso", "Raya", "Santé" and "Arizona" infected with the potato virus M.

Potato seeds harvested from open fields were tested on the viral infection using the Elisa method.

Potato virus M infected tubers (with PVM virus) were placed at different temperatures with the combination of different exposures in the dark condition in the Thermostat.

- 33 ºC exposure for 2h; 3h; and 4h every 24 hours.
- 36 ºC exposure for 2h; 3h; and 4h every 24 hours.
- 39 ºC exposure for 2h; 3h; and 4h every 24 hours.

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After 27 days of incubation, the sprouts generated from potato tubers were tested for potato virus M infection using an Enzyme-linked immune sorbent assay (ELISA) to evaluate the effect of the thermotherapy method.

Das –Elisa Method

The DAS-ELISA test was conducted by using extracts from potato tubers of the collected samples according to the manufacturer's instructions for commercial kits (BIOREBA AG Switzerland). 200 µL of coating buffer (pH 9.6) was used for a dilution of polyclonal antibodies of the respective virus and were coated to each well of microtiter plates (200 µL/well) for 24 h at 4°C.

After three washes with PBS-Tween, the plates were covered with crushed samples [(w/v) 1:5] in extraction buffer (pH 8.2) containing 2% polyvinylpyrrolidone (PVP MW 24,000), 0.02% NaN3 and 0.05% Tween 20 for 24 h at 4°C following four washes with PBS-Tween.

Conjugate antibody (200 µL/well) (alkaline phosphate conjugated polyclonal virus-specific antibody) was coated in each well for 5 h at 30°C. Next after final washing, the plates were incubated with fresh pNPP (para-nitrophenyl phosphate) substrate buffer [adding p-nitrophenyl phosphate tablets (1 mg mL–1) in substrate buffer] for 3 min at 25°C preferably in the dark. Absorbance was determined at 405/450 nm on ELX800 Microplate Reader (Bio-Tek Instruments, Winooski, VT) and the sample was considered positive if its optical density was 3 times higher than the negative control.

Apical Meristem Method

Apical shoot tips (1.5 mm length) generated from sprouted potato tubers were excised and sterilized with 0.1% mercury chloride during 1-3 minutes followed 3 times wash in double distillate water under biosafety cabinet condition and placed in clear tissue culture vessels with 10 ml were cultured in Murashige and Skoog (MS) medium supplemented 30g/l sucrose (3% medium), pH was adjusted to 6.1 and placed under 16h photoperiod, temperature-23-25 ºC. After 25-30 days, the explants (with 4-5 nods) were ready for inoculation.

After 2 days of culture under standard conditions, the culture vessels containing shoot segments were moved into a growth chamber with 40% relative humidity at a photosynthetic photon flux density of 70 µmol s−1 m −2 provided by cool-white fluorescent tubes. The shoots were grown in an alternating temperature regime of 28°C for 8 h in darkness and 40°C for 16 h with light for 2 weeks. Apical shoot tips (1 mm length) were excised from heat-treated shoots and cultured on RM in the dark at 24°C for 1 week before transfer to standard conditions.

3. Results and Discussion

We determined the effects of temperature to assess PVM viral infection. The plant infection rates with PVM depend on potato varieties and thermotherapy conditions. In general, the rate of virus eradication rises with increasing temperature and exposure time. Thermotherapy frequently depends primarily on the virus type, plant species, and virus-host combination. [32], [33]. The choice of a thermotherapy regiment should permit the treated plant to live while also inactivating the virus, producing plants free of the virus.

The DAS-ELISA method was used for evaluating the virus infection status of four potato cultivars "Picasso", "Raya", "Santé" and "Arizona".

Potato varieties differently reacted to the various thermotherapy conditions: the most effective result (100% virus eradication) was obtained by temperature-36ºC with 2h exposure during 27 days for potato varieties "Picasso" and "Raya". 84% of potato varieties "Picasso" and 88% of " Raya" free from M virus sprouts were obtained at the temperature of 33ºC with 2h exposure, among 3 h exposure on 36^0C was most effective (44%) for "Picasso" followed 33ºC/3h (24%), other thermotherapy conditions were not effective in eradication potato virus M on potato variety "Picasso", sprouts generated on 39 ºC with 4h exposure appeared dark spots after 5 days and complete necrosis developed after 15 days on the sprouts of the potato varieties "Picasso" and "Raya", tubers started to degrade due to heat (Table 1).

Plants are stressed by high temperatures, and this stress increases according to the rise in temperatures. [34], [35]. High temperatures and their long-term durations were found to decrease the survival of treated shoots and shoot tips excised from treated shoots, as well as the regeneration potential and micrografting success of shoot tips excised from treated shoots. However, higher temperatures and longer durations of thermotherapy improved virus eradication. [36]-[38].

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Table 1. Effect of thermotherapy method on PVM virus eradication of the potato varieties "Picasso" and" Raya"

Potato varieties	Temperature/Exposure								
	Duration 27days								
	33^0C/2h	36^0C/2h	39^0C /2h	33^0C/3h	36^0C/3h	39^0C /3h	33^0C/4h	36^0C/4h	39^0C /4h
Sante	8	11	0	16	18	0	21	45	0
Arizona	5	9	0	11	14	0	17	30	0
Results	**36^0C/4h**								
Eradication	**30-45%**								

Results show that treatment 36ºC/4h during 27 days was effective for potato varieties "Sante" and "Arizona".

A 55% decrease by complete necrosis of yield of sprouts was observed in the potato variety "Sante" and from the generated buds 100% completely virus-free potato plants were obtained. In the "Arizona" variety, only 30 % of sprouts were generated, from which 100% eradication of PVM virus was observed in the obtained plants (Table 2).

Table 2. Effect of thermotherapy method on PVM virus eradication of potato varieties: "Sante" and "Arizona"

Potato varieties	Temperature/Exposure								
	Duration 27 days								
	33^0C/2h	36^0C/2h	39^0C /2h	33^0C/3h	36^0C/3h	39^0C /3h	33^0C/4h	36^0C/4h	39^0C /4h
Picasso	84	100	0	24	44	0	0	0	0
Raya	88	100	0	30	48	0	0	0	0
Results	**36^0C/2h**								
Eradication	**100%**								

Enhancing the exposure time the 33ºC only increased viral reduction from 8% to 15%, for potato varieties "Arizona" and "Santé" while only 50% of sprouts survived on treatment 39 ºC tubers were shrunk or dried out (Table 2) other tubers on 39 ºC heat treatment delayed development of sprout (Fig.1).

Our results correlate with Abbas et al., 2016 that hot water treatment delayed shoot growth [39]. It is less hazardous to expose potato tubers rather than meristem tips it also enables the use of more meristems as explants, which are easier to develop into culture [40]. Although the mechanism of virus elimination via heat is unknown, induced changes in the cell environment might block virus growth.

The high temperature assists in the formation and dissemination of necrotic areas on all four cultivars of potato.

The in vitro potato collection of four potato varieties "Picasso", "Raya", "Santé" and "Arizona" were established using the apical meristem method and added to the potato in vitro collection of the Biotechnology Center.

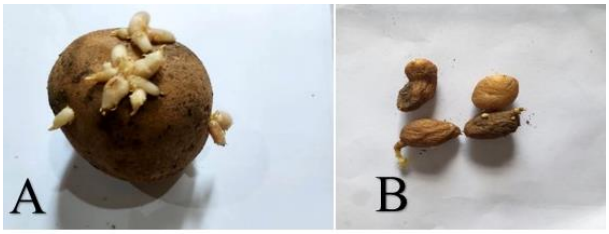

Fig. 1. Potato variety "Sante", A- Generated sprouts; B- Completed necrosis of sprouts.

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4. Conclusion

An earlier study revealed that higher temperatures could reduce plant resistance to PLRV infection [41]. Webb reported that the maximum temperature at which plants could sustain resistance was 27ºC. This is in line with our results in which 26 ºC was effective for eradication of virus M in all potato varieties, low temperature (33 ºC) despite increasing exposure was not effective in eliminating the virus in any of the tested potato cultivars, while high temperature caused necrosis of tubers and delay in the development of sprouts from potato tubers.

Potato viruses are affecting the productivity of potato yield, so finding a reliable and efficient method for producing certified potato seed tubers is still a problem in Georgia. One way to promote this process is to establish effective ways to control and limitation of spreading virus infection. The work implemented by the Biotechnology Center supports improving the potato virus elimination method for further development of potato virus-free seed production system in Georgia.

Thus, within this work, the best thermotherapy conditions for four potato cultivars: “Picasso”, “Raya”, “Santé” and “Arizona” were selected.

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