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Heat treatment of potato tubers: A study on the efficacy of thermotherapy in reducing potato leaf roll virus (PLRV) incidence

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Abstract
In the present study thermotherapy was used as a means to eliminate PLRV on the local landraces of potato of Assam, India. For this thermotherapy was applied to the tubers at temperatures of 37 °C and 40 °C for time intervals of 1 hour, 1 hour 30 minutes, 2 hours, 2 hours 30 minutes and 3 hours. The design was laid in Completely Randomized Design (CRD) with 11 treatments and 3 replications each. It was observed that no disease incidence was recorded at 40 °C for 2 hours 30 minutes where germination was 50 percent.

Keywords: Thermotherapy, potato, landrace, PLRV

Introduction

Solanum tuberosum L., commonly known as the potato, is an annual plant species belonging to the Solanaceae family. It is native to the Andean region of Peru and Bolivia, it renowned for its edible, starchy tubers. (Spooner *et al*., 2005) [9] Potato is one of approximately 150 species characterized by tuberous growth, a morphological adaptation wherein the underground stem swells to form a tuber. The plant's compound leaves exhibit a spiral arrangement, comprising a terminal leaflet and two to four pairs of leaflets, measuring 20-30 cm in length. The inflorescence features white, lavender, or purple flowers, typified by five fused petals and yellow stamens.

Among the various cultivated varieties of potato in Assam, there are also many local cultivars of potato. The tuber sizes of these potatoes are smaller as compared to the other commercially cultivated cultivars. The potato growers of Assam cultivate these local cultivars due to the preference of the customers and high market value. These local landraces of potatoes are highly acclimatized to the region of sub-Himalayan foothills. The two shapes of potatoes mostly found in this region are round shaped and nut shaped. (Chowdhury and Datta, 2020) [3] Above all the viruses that attack these indigenous cultivars are yet to be analyzed as there are no records regarding the diseases which results in yield loss.

Potato leaf roll virus (PLRV) is a phloem-limited, positive-sense RNA virus classified under the genus Polerovirus. This viral pathogen primarily mainly infects potatoes. This disease was initially described by Quanjer *et al*. in 1916. PLRV is a significant contributor to potato crop losses worldwide, particularly in resource-constrained regions. The virus is transmitted by aphid vectors, predominantly the green peach aphid (*Myzus persicae*). PLRV infection can result in substantial yield losses, as much as 50% in individual plants, and is characterized by symptoms such as leaf rolling, chlorosis, necrosis and leaf curling. (Taliansky *et al*., 2003) [10] To mitigate the spread of PLRV, seed certification programs enforce stringent standards, permitting only minimal virus levels in certified seed. However, even low virus percentages can pose a risk if aphid populations are abundant and appear early in the growing season. (Hull, 2002) [4] In years with favorable conditions for insect proliferation, the aphid population can expand rapidly, increasing the likelihood of virus transmission. In such scenarios, insecticide application for aphid control becomes the primary means of managing PLRV spread during production. Seed-borne infections typically result in stunted, severely impaired plants with reduced yields, both in tuber numbers and size.

Thermotherapy is one of the conventional techniques used to eradicate potato plants viral infection to reduce the concentration of virus. It was reported that the thermal sensitivity of viruses was lower than that of plant cells. This helps to apply thermotherapy as a means against the viruses as the damage caused to plants tissues by the thermal stress can be more easily reserved than viral damage (Spiegel *et al*., 1993) [8]. Thermotherapy was found to inhibit viral replication or caused virus RNA degradation, thus decreasing virus titer in the infected shoot tips (Wang *et al*., 2008) [11]. However, thermotherapy was found to be inefficient for the new synthesized viral particles as it was effective in eradication of viral particles already present in cells (Panattoni *et al*., 2013) [6]. However, the method of thermotherapy significantly depends upon the potato cultivar, type of virus, and the duration of heat treatment applied to eliminate potato virus (Waswa *et al*., 2017) [12].

Materials and Methods

Thermotherapy was given to the PLRV infected potato tubers. The two different temperatures regimes *viz*., 37 °C and 40 °C were taken with 5 different time intervals of 1 hour, 1 hour 30 minutes, 2 hours, 2 hours 30 minutes, 3 hours. (Table 1) Each lot had 10 tubers and two tubers grown in one pot in the net house placed in Completely Randomized Design (CRD). The untreated tubers were used as control. Regular observations were done and data was collected for germination percentage and disease incidence (%).

Table 1: Different temperatures and time interval for thermotherapy of potato tubers

Treatments	Conditions provided
T_1	1 hour at 37 °C
T_2	1 hour 30 minutes at 37 °C
T_3	2 hours at 37 °C
T_4	2 hours 30 minutes at 37 °C
T_5	3 hours at 37 °C
T_6	1 hour at 40 °C
T_7	1 hour 30 minutes at 40 °C
T_8	2 hours at 40 °C
T_9	2 hours 30 minutes at 40 °C
T_{10}	3 hours at 40 °C
T_{11}	Control

The thermotherapy treated plants were subjected to molecular analysis for the presence of PLRV. For the occurrence of the disease, the source of infection was natural vector transmission of PLRV. For this purpose RNA extraction, PCR analysis and gel electrophoresis were conducted on the potato plants.

Molecular analysis of PLRV-To identify the different potato viruses infecting the land races of potato they were subjected to molecular analysis by extraction of RNA, polymerase chain reaction (PCR) and gel electrophoresis.

a) **Plant RNA isolation:** Total genomic RNA from healthy and virus infected potato plants were extracted by following CTAB (Cetyl Trimethyl Ammonium Bromide) method of Lodhi *et al.* (1994) [5].
b) **Two step RT-PCR amplification:** RT-PCR combines reverse transcription of RNA into DNA *i.e.;* complementary DNA (cDNA) and amplification of specific DNA targets using PCR. The RNA template was converted to cDNA by using the Prime Script^{TM} 1st strand cDNA synthesis kit (TaKaRa-6110A) as per manufacturer's protocol. For the processing of PCR, EmeraldAmpGT (TaKaRa RR310A) Master Mix was used. The working volume of PCR master mix included Emerald Master Mix (4.0 µl), forward primer (0.5 µl), reverse primer (0.5 µl), sterile distilled water (4.5 µl) and template cDNA (0.5 µl) making a total volume of 10.0 µl. The primer pair used for PLRV was F: ATGAGTACGGTCGTGGTTAAAGG; R: GGCCTGCAGGCTATTTGGGGTTTTG as mentioned in Awasthi *et al*., 2014.
c) **PCR Cycle:** The PCR cycles were performed in a thermal cycler (Aligent SureCycler 8800, Aligent Technologies, USA) and the cycling protocol used for the detection of the viruses included 94 °C for 2 minutes denaturation. The PCR amplification was carried out for 30 cycles under the following conditions: denaturation at 94 °C for 30 seconds, annealing at 53 °C for 30 seconds, extension at 72 °C for 30 seconds and a final extension at 72 °C for 7 minutes and finally stored at 4 °C.
d) **Agarose gel preparation and analysis of PCR products:** The PCR products were resolved on 1.5 percent agarose gel in Tris EDTA (TAE) containing ethidium bromide (Sambrook and Russell, 2001) [7]. The gel was observed under an UV-transilluminator Gel-doc to observe the amplified bands.

Results and Discussion

A perusal of results for the thermotherapy treatment presented in Table 2 showed that the germination percent was observed to be 100 percent for the treatments T_1 (1 hour at 37 °C), T_2 (1 hour 30 minutes at 37 °C), T_3 (2 hours at 37 °C), T_6 (1 hour at 40 °C) and T_{11} (control). The treatments T_7 (1 hour 30 minutes at 40 °C) and T_4 (3 hours at 37 °C) had a germination percent of 90 percent and 80 percent, respectively. The disease incidence of PLRV as recorded for thermotherapeutic treatment for different treatments was observed to be lowest for T_9 (3 hours at 40 °C) with no incidence of PLRV, but in this case the germination percent was decreased by 50 percent only. The highest disease incidence was observed in the treatment T_{11} (control) with 90 percent incidence of PLRV. The percent disease reduction over control was observed highest in case of T_9 (3 hours at 40 °C) with 90 percent reduction in disease incidence followed by the treatments T_7 (1 hour 30 minutes at 40 °C) and T_8 (2 hours at 40 °C) with78.89 and 75.72 percent reduction in disease incidence over control. In case of the treatment T_{10} (3 hours at 40 °C) no germination was observed in the tubers.

Table 2: Effect of thermotherapy on germination and disease incidence of PLRV on potato plants

Treatments	Germination (%)	Disease incidence (%)	Percent disease reduction over control
T_1	100.00 (90.00)*	40.00 (39.23^{b**})*	50.00
T_2	100.00 (90.00)	30.00 (33.21^{bc})	60.00
T_3	100.00 (90.00)	30.00 (33.21^{bc})	60.00
T_4	80.00 (63.43)	25.00 (30.00^{bc})	65.00
T_5	50.00 (45.00)	20.00 (26.56^{bc})	70.00
T_6	100.00 (90.00)	20.00 (26.56^{bc})	70.00
T_7	90.00 (71.56)	11.11 (19.47^{bc})	78.89
T_8	70.00 (56.78)	14.28 (22.20^{bc})	75.72
T_9	50.00 (45.00)	0.00 (0.00^{c})	90.00
T_{10}	0.00 (0.00)	-	-
T_{11}	100.00 (90.00)	90.00 (71.56^{a})	-
CD (p=0.05)	-	0.36	-

*Figures in the parenthesis represent angular transformed values
**Values superscripted with same letters are significant at 5 percent
T_1-1 hour at 37 °C; T_2-1 hour 30 minutes at 37 °C; T_3-2 hours at 37 °C; T_4-2 hours 30 minutes at 37 °C; T_5-3 hours at 37 °C; T_6-1 hour at 40 °C; T_7-1 hour 30 minutes at 40 °C; T_8-2 hours at 40 °C; T_9-2 hours 30 minutes at 40 °C; T_{10}-3 hours at 40 °C; T_{11}-control

Considering both the temperatures used for thermotherapy it was observed that 37 °C for 2 hours 30 minutes and 40 °C for 2 hours could be selected as the best treatments for thermotherapy. The germinations were 70.00 percent and 90.00 percent while the disease incidence was observed as 25.00 percent and 11.11 percent.
Thermotherapy treatment could be a very effective and cost effective method for treating the potato tubers before plantation to get rid of the viruses present in the potato tubers. The presence of PLRV in the plants grown from thermotherapy treated tubers was also clearly observed in a band size of 629 bp in the gel documentation.

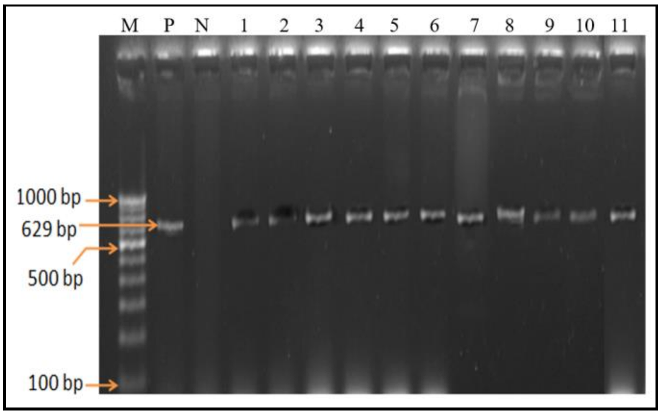

Fig 2: Gel Electrophoresis Representation of PLRV in Thermotherapy treated plants, M. 100 Bp ladder; P. Positive Control; N. Negative Control; 1. T_1 (1 Hour At 37 °C); 2. T_2 (1 Hour 30 Minutes At 37 °C); 3. T_3 (2 Hours At 37 °C); 4. T_4 (2 Hours 30 Minutes At 37 °C); 5. T_5 (3 Hours At 37 °C); 6. T_6 (1 Hour At 40 °C); 7. T_7 (1 Hour 30 Minutes At 40 °C); 8. T_8 (2 Hours At 40 °C); 9. T_9 (2 Hours 30 Minutes At 40 °C); 10. T_{10} (3 hours at 40 °C) and 11. T_{11} (Control)

Waswa *et al*. (2017) [12] where they studied the effect of thermotherapy duration against potato viruses. Thermotherapy was given at 37 °C to 40 °C for 16 hours of light and 30 °C to 34 °C for 8 hours of darkness for 2, 3 and 4 weeks. Similar experiment was also conducted by Abbas *et al*. (2016) [1] observed that PLRV could be removed from the tubers either partially or fully by giving a thermotherapy for 2 hours, 2 ½ hours and 3 hours. This result is in conformity with the results obtained in the present investigations.

Conclusion

Thermotherapy has shown a promising effect on improving potato tuber quality and also reducing disease incidence. Thermotherapy can help eliminate potato viruses and other pathogens, leading to higher yields and improved tuber quality. Overall, thermotherapy is a valuable tool in the management of potato tubers, offering a non-chemical approach to disease control and quality enhancement. Hence from this experiment the best thermotherapy temperature include 37 °C for 2 hours 30 minutes and 40 °C for 2 hour.

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