

***In vivo* thermotherapy: attempt to eliminate virus in potato tuber**

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Abstract. Potato is one of an important vegetable crop in Indonesia, including Bali. Main potato production areas in Bali are at Bedugul region, 1.200 m above sea level. Potato production in Bali continued to decrease due to diseases infection, such as early blight, late blight, black leg and virus diseases. Potato farmers in Bali usually set aside their harvest as seed potatoes, resulting in virus diseases being carried out on the next planting seasons and eventually would decrease potato production both in quantity and quality. Four types of virus were confirmed: PVY, PVX, PVS and PRLV. A number of studies have reported thermotherapy technique has been employed to eliminate potato virus *in vitro*. However, this technique is not readily available for farmers, since there is no established tissue culture laboratory to support. Therefore, there is an urgent need to develop a more practical method. The objective of this study was to eliminate virus on seed potatoes using thermotherapy on tuber. Seed potatoes with 1 cm sprout which were virus positive were placed on sterile charred rice paddy husk, and then put into a humidified incubator. Tubers were exposed to 37°C for four days followed by 34°C for three days alternately for two weeks and three weeks duration. Four tubers received heat exposure regime for each virus type. After thermotherapy, potato tubers were transferred to pots containing charred rice paddy husk and maintain for three weeks until new leaves emerge for virus analyses. Results show that seed tubers experienced delayed growth after thermotherapy. Control plants sprout one week after thermotherapy, while treated plants were not yet sprouting. Experiment is currently underway. It is expected that heat treatment on tuber will give a practical method for farmers to eliminate virus of seed potatoes.

Keywords: DAS-ELISA, heat treatment, *Solanum tuberosum*

1. Introduction

Potato (*Solanum tuberosum* L.) belongs to Solanaceae family. Potato is the fourth most important crop around the world including Indonesia. Potato is susceptible to various pathogens including fungi, bacteria, nematodes, and viruses. Virus is the most contributors for yield reduction in potato crop throughout the world. Among the viral diseases potato leaf-roll virus (PLRV), potato virus Y (PVY), potato mop top virus (PMTV), potato virus X (PVX), potato virus S (PVS) and potato virus A (PVA) is the major viral pathogens found throughout the world. As compare to the other pathogens, viruses



damage plants and cause much more economic losses by a reduction in yield and quality of plant products. The severity of individual virus disease may vary with the locality, virus species, stage of infection and crop variety [1].

In Bali, potatoes grow at Bedugul highland region (1.200 m above sea level). Potato production in Bali continues to decrease, due to high production cost and diseases infections, resulting in declining number of farmers wanting to grow potatoes. Farmers could not afford to buy certified seed potatoes which can reach Rp. 50.000 per kg for third generation seed potatoes (G3). Farmers, therefore, would buy uncertified seed potatoes or set aside their harvest as seed potatoes. Using later generation (G3 and more) as seed potatoes has caused potato plants become very susceptible to pest and diseases, particularly virus [2].

Observation on some of potato fields in Pancasari Village, Bedugul region, revealed that approximately 70% of potato plants show virus symptoms on leaves and plant structure/habitus. Detection using DAS-ELISA technique shows four type of virus was confirmed, i.e., PVY, PVX, PVS and PLRV (data not shown). Tuber produces on these plants are mostly small, and many of them have a secondary infection from bacteria and fungi; causing tubers become easily rotten during storage. It is, therefore, an urgent need to find out practical methods to eliminate virus on potato tuber to be used for seed potato.

There are some successful methods have been employed to eliminate virus on potato, i.e. meristem culture, thermotherapy and cyrotherapy [3]. Encapsulation-dehydration followed by plunging in vitro shoot tips into liquid nitrogen (cryotherapy) was possible, but recovery rate was very low [4]. On in vitro thermotherapy technique, potato plantlets were incubated on 34°C/37°C night/day temperature for six weeks and then tested using ELISA for virus confirmation. If the virus was detected, repeat incubation until plantlets are virus free [5]. This repeat incubation slows down effort on providing virus free seed potatoes.

A more simple and practical method for virus elimination for local farmers need to be developed. An alternative method is to expose potato tubers with heat treatment. Evaluation of the effectiveness of thermotherapy to inactivate Potato leaf-roll virus (PLRV) from the potato tubers has been done [6]. The potato tubers were then treated with hot water at average 37°C for various intervals of time. It was concluded that thermotherapy at 37°C for 2 hours, 2½ hours and 3 hours in hot water treatment were in full or partially effective in elimination of PLRV from potato tubers. Exposing potato tubers to 35°C for 56 days or 36°C for 39 days resulting in significant effect on potato leaf roll virus elimination from small lots of seed of particular value in a breeding program; however, 50% of treated seed were dead [7].

The aim of this study was to find out response and survival rate of seed potato tubers after thermotherapy for different duration. This current work was conducted to explore the possibility of eliminating virus on potato tubers cultivar Granola using heat treatment regime. Using heat therapy close to death point of tuber might reduce the need for more complex technique to eliminate the virus.

2. Methods

The research was conducted at Biology Laboratory, Udayana University, Jimbaran campus, from October to August 2017. The equipment used in this experiment is a humidified chamber containing a thermostat, two light bulbs, and a thermometer.

Seed potato tubers cultivar 'Granola' was collected from farmer's field at Pancasari Village, Bedugul region. Collected seed were from plants with virus infection, and the virus type has been confirmed using DAS-ELISA method, i.e. PVY, PVX, PVS and PLRV. Seed potatoes were stored for ten weeks until sprouting. Seed potato tubers with 1 cm sprouts were placed in a humidified chamber and exposed alternately to 34°C temperature for three days, and 37°C for four days, for two and three weeks. Seed tubers were placed on charred rice paddy husk on the base to keep them moist. There are four tubers treated for each virus type. After thermotherapy treatment, tubers were transferred to 15 cm diameter pots containing charred rice paddy husk as the media and watered daily. Seed potato tubers with no virus and do not receive heat treatment were planted on the same media as the control.

3. Results and Discussion

Results show that there was no growth observed during thermotherapy on treatment 1 (37°C/34°C for 3 weeks) and 2 (37°C/34°C for 2 weeks). After one week exposure to heat, green sprouts started to change into brown color but tubers remain firm. On the second week, tubers on treatment 1 and 2 started to degrade due to heat. At the end of third week, all sprouts on treatment 1 were browning (Table 1). Only 50% of sprouts with leaf-roll and malformed symptom were survived on treatment 2 (Table 2).

Observation at one week after transferred to pots shows that only control tubers produced sprouts, started at five days after transferred. Heat treated tubers on treatment 1 was not survived; tubers were shrunk or dried out (Figure 1). Tubers receiving treatment 2 showed green sprouts but there was no further growth after 4 weeks in pots. Heat treatment delayed development of sprout and leaf emergence. This result is in agreement with [7] in which hot water treatment delayed shoot growth.

Exposing potato tubers rather than meristem tips has the advantage of less damaging and also allows use of larger meristem as the explant, and would be easier to develop into culture [9]. The mechanism of virus elimination using heat is still unclear, but induced changes in cell environment may inhibit virus multiplication. Heat-dependent alteration of cellular metabolism may cause the formation of o-quinones, activation of ribonucleases, and inhibition of replicases, and a reduction in ribosomes [3]. In this report, serological test DAS-ELISA has not been done.



Figure 1. Post-thermotherapy tubers at two weeks after transplanting. a) Control tubers, b) Treated tubers. The arrow shows the browning sprouts.

Table 1. Seed tubers performance after exposure to heat treatment 37°C for 4 days followed by 34°C for 3 days alternately, for 3 weeks period.

No	Treatment 1	Potato tubers symptom, survival rate (%)		
		Mosaic	Leaf roll	Malformation
1	Green sprouts, firm tuber	-	-	-
2	Brown sprouts, firm tuber	-	25	50
3	Brown sprouts, squashy tuber	-	25	25
4	Brown sprouts, shrunk tubers	50	50	25
5	Brown sprouts, tuber rotten	50	-	-

Table 2. Seed tubers performance after exposure to heat treatment 37°C for 4 days followed by 34°C for 3 days alternately, for 2 weeks period.

No	Treatment 1	Potato tubers symptom, survival rate (%)		
		Mosaic	Leaf roll	Malformation
1	Green sprouts, firm tuber	-	50	50
2	Brown sprouts, firm tuber	50	25	50
3	Brown sprouts, squashy tuber	50	25	-
4	Brown sprouts, shrunk tubers	-	-	-
5	Brown sprouts, tuber rotten	-	-	-

4. Conclusion

Thermotherapy by exposing tubers to 37°C/4 days followed by 34°C/3 days for two weeks has the highest survival rate (50%). Exposing tubers for three weeks resulting in most tubers dead after therapy and survival tubers eventually died after two weeks transferred.

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