Journal of Phytopathology and Pest Management

eISSN: 2356-6507

Volume (1), Issue (2)

2014

Heat and freezing pre-thermal treatments as a means of freeing potatoes from mosaic virus and its effects on potato plants quality characters

N. A. Younes[1*], M. M. El-Sheikh Aly[2], M. H. Aboul-Nasr[3]

[1]Department of Horticulture, Faculty of Agriculture, Al-Azhar University (Assiut Branch), 71524 Assiut, Egypt
[2]Department of Botany, Faculty of Agriculture, Al-Azhar University (Assiut Branch), 71524 Assiut, Egypt
[3]Department of Horticulture, Faculty of Agriculture, Assiut University, 71526 Assiut, Egypt

Abstract

The aim of the study was to control of potato mosaic virus by using hot-air, hot-water and freezing as a physical pre-thermal treatments. Infected tubers of Lady Rosetta and Mondial potato (*Solanum tuberosum* L.) cultivars were treated by hot-air and hot-water treatments at 55± 2˚C and freezing treatment at -18±2˚C for 1, 2, 3 and 4 hours. Hot-air and freezing treatments showed complete elimination of mosaic virus from treated tubers. Hot-water treatment caused tuber damage at different exposure times. In this respect, hot-air treatment at 55± 2˚C and freezing treatment at -18±2˚C for two hours gave the best results of eliminating the virus from tubers and did not affect on economic characters of potato cultivars.

Key words: Potato, *Solanum tuberosum*, viral diseases, thermal treatment, freezing treatments

Introduction

Potato (*Solanum tuberosum*) is an annual, herbaceous plant belonging to the family Solanaceae. Each 100 g of potato tuber contains 79.8 g water, 76 calories, 2.1 g protein, 0.1 g lipids, 17.1 g carbohydrates, 0.5 g fiber and 0.9 g ash as well as it contains a little quantity of nutrient elements and some vitamins. It contains 0.1 mg thiamin, 0.4 mg Riboflavin, 1.5 mg Niyasin and 20 mg Ascorbic acid (Hassan, 2003). Potato is the world's leading vegetable crop. It is grown in about 140 countries (Haase, 2008). In Egypt, potato crop has an important position among all vegetable crops, where about 20% of the total area devoted for vegetable production is cultivated with potato. (Abd-Elgawad and Youssef, 2008). Because Potato is an important crop for the Egyptian consumer its acreage was 381379 feddans (feddans = 2400 m^2) in 2013 which yield a total of 4265342.74 tons (Agriculture Directorates of Governorates. Area, yield and Production of fall Vegetable Crops, 2013. Publisher: Economic Affairs Sector). Potato tubers can transfer many diseases and pests and these cause degeneration of the seed tuber and plants. Potato production is being seriously hampered due to certain viruses (Rolot & Seutin, 1999), like potato virus Y (PVY) which is the most dangerous virus.

* **Corresponding author:** nabel_aly77@yahoo.com

This virus was detected in commercial fields in single or mixed infection (Biswas et al., 2005; Nascimento et al., 2003). Attempts to sanitize virus-infected plants or portions of diseased plants to obtain new plants has not led to miraculous results, even if some recent technologies seem to offer new opportunities for facing difficulties that occur during sanitation procedures (Panattoni et al., 2013). Thermotherapy treatment consists of keeping plants, or more frequently a part of them, at temperatures between 35°C and 54°C, within the physiological tolerance limits of each plant, for an appropriate period. In practice, the selected temperature represents the best compromise between virus degradation and plant survival, taking into account that the threshold of thermal sensitivity of some viruses is lower than that of plant cells and that the damage caused to plant tissues by the thermal stress can more easily be reversed than viral damage (Spiegel et al., 1993). Kassanis, (1949) provided an interpretation of the results obtained by the treatment, basing it on identification of the infected cell as the environment where virus particles are in a dynamic equilibrium between newly formed particles and degraded ones. Therefore, thermal treatment produces a shift in this balance towards greater viral degradation, when repeated over time, can lead to elimination (Cooper & Walker, 1978; Kassanis, 1957). The principal alterations in viral particles as a result of thermal treatment above 35°C.are related to the rupture of hydrogen and disulfide bonds of the capsid protein, followed by nucleic acid phosphodiester covalent bonds, and consequently, even deterioration of viral infectivity which can include selective inhibition of viral replicate, changes in pH and cellular ionic strength, increase of lytic enzymes, competition between viral RNA and messenger for ribosome bonds. In the field of thermotherapy, cryotherapy represents a whole new approach (Nukari et al., 2009; Wang & Valkonen, 2009; Wang et al., 2009). The freezing of shoot tips (*i.e*. in liquid nitrogen, and subsequent thawing and regeneration to shoots) was found to result in virus-free plants with high efficiency. Moreover cryotherapy takes only a few days: a minor addition to the whole procedure of virus elimination which requires several months. Meristem culture of shoot tips was also used to enhance thermotherapy virus elimination as the elimination ratio of viruses is higher when the size of isolated tissue (*i.e.* shoot tip) is smaller (Mori & Hosokawa, 1977).

The aim of the present study is to control of potato mosaic virus by using hot-air, hot-water and freezing as a physical pre-thermal treatments to free plants and tubers of the virus and to study the effect of such thermal treatments on the quality of potato plants.

Materials and methods

Virus detection and identification: Field surveying were done every ten days depending on visual observation of virus symptoms. Symptoms on plants were used to characterize potato mosaic disease. Also, many factors such as host plant cultivar/variety, time of infection, vector transmission and the environment

were used to simple identification of virus (Matthews, 1980). Two cultivars of potato dormant tubers which yielded from virus infected plants were used to study the effect of pre thermal treatments on diseased potato tuber to minimize the viral diseases. The two cultivars were namely, Lady Rosetta cv., as a susceptible, and Mondial cv., as a tolerant to viral diseases.

Thermotherapy treatments of tubers: The experiment was conducted in the Vegetable research Laboratory, Department of Horticulture and in a clay soil of the Experimental Farm, Faculty of Agriculture, Al-Azhar University, Assiut Branch, Assiut, Egypt. Three thermal treatments and four exposure times of each one (1, 2, 3 and 4 hr.) were applied to all cultivars as follows:

A- Hot-air treatment of tubers (HA): Four bags, that have 20 tubers each, were placed in a growth chamber (1m X 1m X 1m). The air temperature in the chamber was maintained at 55 ± 2 ˚C with an Xpelair 3 kw fan heater, and relative humidity was kept at or above 75%.

B- Hot-water treatment of tubers (HW): The dormant tubers were treated for various times at 55± 2 ˚ C in a circulating water bath. The tubers were placed in a wire-screen cage that was submerged in the hot water, then; tubers were dried at room temperature for 24 hours.

C- Freezing treatment of tubers (Fr): The dormant tubers were kept in paper bags in freezer at a temperature of 18 ± 2 ˚ C. After freezing treatment tubers of each cv., remained at room temperature in the paper bags until planted.

D- Control treatment (Cont): Untreated tubers remained at room temperature in paper bags until planted.

All the thermal treatments and control tubers were placed in a closed container and were treated for 36-48 hr with a Rindite solution (a mixture of ethylene chlorohydrin, ethylene dichloride and carbon tetrachloride (7:3: 1, v/ v) at the rate of 0.5 ml/ kg of tubers to induce sprouting. Tubers were stored at room temperature until sprouting tubers were placed in sterilized sandy soil in 15-cm-diameter plastic pots and kept under greenhouse conditions to induce rooting. After rooting, tubers were planted in sterilized soil in 15 cm diameter plastic pots. Plants were sprayed periodically with pesticides to control insects and mites. The experiment was laid out in the Randomized Complete Block Design (RCBD) with three replications, each one consist of 45 mini tubers. Normal cultural practices were applied as recommended on potato commercial production.

Virus incidence reduced: The effect of pre thermal treatment on Lady Rosetta and Mondial mini tubers cultivars were estimated. The presence of the virus percentages on sprouts and plants after 45 days of cultivation was estimated by using the following formula according to Mohamed, (2010):

$$\%\,Inhibition = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

To be sure that the plants are free from viruses, growing plants were tested biologically using specific indicator plants for virus. Some leaves from each plant were grinding in 0.01 M pH 7.0 phosphate buffer and inoculating the following hosts: *Datura metale* and *Solanum tuberosum* (Robert Kahn and Monroe 1970).

Measurements of growth parameters: Some vegetative characters were calculated such as; tuber sprouting percentage after treatment *in vitro*, the field stands after cultivation, the number of stems per plant, stem height (cm) and the numbers of tubers/plant after 45 days of cultivation.

Statistical Analysis: All obtained data were statistically analyzed separately for each cultivar according to Gomez and Gomez (1984). The Least Significant Differences test (LSD) at the 5 % probability level was used to find out the significance among the mean values.

Results

The surviving tubers after thermal and freezing treatments were tested for the presence of the virus on sprouts and plants after 45 days of cultivation. It was observed that all the tubers which were exposed to either hot air at 55 ± 2 ˚C or freezing at - 18± 2 ˚C at 1, 2, 3 and 4 hours were found free from the virus diseases. While, untreated tubers were 100% infected with viruses. On the other hand, tubers that were treated with hot water at 55 ± 2˚C for all time periods were totally damaged (Table, 1).

Table 1: The effect of pre-thermal and freezing treatments on the percentage of virus free tubers (after sprouting and 45 days from cultivation).

Exposure time (hour)	Percentage of Virus Elimination After Sprouting							
	Lady Rosetta				Mondial			
	HA	HW	Fr	Cont.	HA	HW	Fr	Cont.
1	0.0	----	0.0	100	0.0	----	0.0	100
2	0.0	----	0.0	100	0.0	----	0.0	100
3	0.0	----	0.0	100	0.0	----	0.0	100
4	0.0	----	0.0	100	0.0	----	0.0	100
Percentage of Virus Elimination After 45 Days of Cultivation								
1	0.0	----	0.0	100	0.0	----	0.0	100
2	0.0	----	0.0	100	0.0	----	0.0	100
3	0.0	----	0.0	100	0.0	----	0.0	100
4	0.0	----	0.0	100	0.0	----	0.0	100

HA= hot-air treatment, HW= hot-water treatment and Fr= freezing treatment.

The effect of pre-thermal and freezing treatments on the percentage of potato tubers sprouting and the field stands after cultivation is shown in Table, 2. Different pre-thermal treatments and exposure times was significantly effected on trails. The negative effect of the hot air and freezing treatments on sprouting percentage increased as the time increased. Further, Mondial cv. exhibited positive effect on sprouting percentage with the time increasing of the freezing treatment. All thermal treatments had no affected on the field stand after cultivation.

Table 2: Effect of heat and freezing treatments on sprouting percentage of two potato cultivars tubers and the field stand after cultivation.

Exposure time (hour)		Percentage of Tuber Sprouting After Treatment									
		Lady Rosetta					Mondial				
		HA	HW	Fr	Cont.	Mean	HA	HW	Fr	Cont.	Mean
1		86.00	----	81.33	98	66.33	94.67	----	60.33	100	63.75
2		88.67	----	72.00	100	65.17	98.00	----	77.67	98	68.42
3		63.33	----	65.33	99	56.92	88.33	----	87.33	97	68.17
4		68.00	----	66.67	98	58.17	87.33	----	98.00	99	71.08
Mean		76.50	----	71.33	98.75	---	92.08	----	80.83	98.5	---
LSD =0.05	A			1.67					1.68		
	B			1.67					1.68		
	AxB			3.34					3.36		
		Percentage of the Field Stand After Cultivation									
1		100	----	100	100	---	100	----	100	100	75
2		100	----	100	100	---	100	----	100	100	75
3		100	----	100	100	---	100	----	100	100	75
4		100	----	100	100	---	100	----	100	100	75
Mean		100	----	100	100	---	100	----	100	100	---
LSD =0.05	A			1.67					1.49		
	B			NS					NS		
	AxB			NS					NS		

HA= hot-air treatment, HW= hot-water treatment, Fr= freezing treatment, A= heat and freezing treatments and B = exposure time.

The number of stems per plant, stem height and the number of tubers per plant is presented in Tables, 3 and 4. Different exposure time applications of the pre-thermal treatments had no significant effect on the stems number/plant and the stem height as compared to the control treatment. While, the different pre-thermal treatments was significantly effected on trails.

On the other hand, thermal treatments and exposure times of application had a significant effect on the number of tubers per plant. Applications of hot-air at 55 ± 2℃ or freezing at 18± $^{2^\circ}$C for two hours gave the best effect on the number of tubers per plant in the two cultivars under his study as compared with the untreated tubers.

Table 3: Effect of heat and freezing treatments on the number of stems/ plant and the stem height.

Exposure time (hour)		Numbers of Stems / Plant									
		Lady Rosetta					Mondial				
		HA	HW	Fr	Cont.	Mean	HA	HW	Fr	Cont.	Mean
1		4.00	----	81.33	4.63	3.52	3.78	----	3.33	4.22	2.83
2		3.78	----	72.00	5.39	3.52	2.67	----	4.00	4.50	3.04
3		2.85	----	65.33	4.42	2.85	3.33	----	3.22	3.88	2.61
4		2.73	----	66.67	4.11	2.73	3.67	----	2.67	3.61	2.49
Mean		3.42	----	71.33	4.64	---	3.61	----	3.31	4.06	---
LSD =0.05	A			0.64					0.53		
	B			0.64					NS		
	AxB			NS					NS		
		Stem length (cm)									
1		14.67	----	16.33	18.33	12.33	12.33	----	17.33	20.00	12.42
2		13.33	----	16.33	16.67	11.58	12.33	----	18.33	17.33	12.00
3		16.00	----	18.00	18.33	12.33	13.00	----	19.00	17.00	12.25
4		16.00	----	18.00	17.67	12.92	19.67	----	17.33	16.00	13.25
Mean		14.25	----	17.17	17.75	---	14.33	----	18.00	17.58	---
LSD =0.05	A			1.40					2.38		
	B			NS					NS		
	AxB			NS					NS		

HA= hot-air treatment, HW= hot-water treatment, Fr= freezing treatment, A= heat and freezing treatments and B = exposure time.

Table 4: Effect of heat and freezing treatments on the number of tubers/ plant of two potato cultivars.

Exposure time (hour)		Numbers of Tubers / Plant									
		Lady Rosetta					Mondial				
		HA	HW	Fr	Cont.	Mean	HA	HW	Fr	Cont.	Mean
1		5.36	----	5.26	6.11	4.18	6.48	----	7.85	10.11	6.10
2		6.29	----	5.18	5.33	4.20	9.55	----	6.29	9.67	6.38
3		3.33	----	3.18	5.33	2.96	4.37	----	3.12	9.17	4.16
4		2.49	----	0.89	5.89	2.32	4.56	----	2.66	9.77	4.25
Mean		4.37	----	3.63	5.67	---	6.23	----	4.98	9.68	---
LSD =0.05	A			0.33					0.34		
	B			0.33					0.34		
	AxB			0.65					0.68		

HA= hot-air treatment, HW= hot-water treatment, Fr= freezing treatment, A= heat and freezing treatments and B = exposure time.

Discussion

This study has demonstrated clearly the effectiveness of using the pre-hot-air (55± 2 ˚C) and pre-freezing (-18 ± 2˚C) treatments on the total elimination of viruses from small batches of tubers of Lady Rosetta and Mondial potato cultivars. Since its introduction in 1949 (Kassanis, 1949), heat therapy has been extensively used to rid tubers of PLRV (Nagaich & Upreti, 1964). However, heat treatment has not been successful in freeing potato tubers from other viruses, including potato acuba mosaic virus, potato virus A (Rozendaal, 1952), potato virus S, potato virus X and tobacco rattle virus (Rozendaal & Brust, 1955) and the viroid-induced potato spindle tuber disease (Fernow et al., 1962). In a bulletin published in 1977 by the International Potato Center, Lima, Peru, on the major diseases and nematodes of potato, PLRV was the only virus listed as being eliminated from potato tubers by heat treatment (International Potato Center, 1977). Heat therapy and meristem-tip culture have been used extensively, singly and in combination, to free potatoes of several viruses, (Quak, 1972; Hollings, 1965). Using the traditional heat-thereby in a combination with meristem tip culture can be very efficiently eliminated SPFMV from sweet potato infected plants (Mervat ElFar & Ashoub, 2009). Eliminated PVY from in vitro potato plants by using temperatures between 35 and 38˚C (Luciana et al., 2003).

Thermotherapy procedures may be of interest and can be used by research institutions and plant quarantine stations where there are fears of introducing virus-induced diseases in imported potato germplasm source material, and to those that distribute potato germplasm for research and commercial purposes (Kaiser, 1980). Our results and those of others (Roland, 1952; Kassanis, 1950) disagree with those of (Upreti & Nagaich, 1968; Nagaich & Upreti, 1964). Several factors could have contributed to these discrepancies; e.g., different virus.

It recommended trying these pre-thermal treatments at the scale of commercial production. Further effort could be needed to reach a satisfactory level under such large scale.

References

Abd-Elgawad MMM, Youssef M, 2008. Programs of research development in Egypt. First International Workshop on Ecology and Management of Plant-parasitic Nematode Communities in South-Mediterranean Ecosystems 17-19 March, 2008-Sousse, Tunisia.

Biswas MK, De BK, Nath PS, 2005. Rate of spread of PVX, PVY and PLRV diseases to potato varieties. Annals of Plant Protection Sciences **13**: 165-178.

Cooper VC, Walker DGA, 1978. Thermal inactivation of cherry leaf roll virus in tissue culture of Nicotiana rustica raised from seeds and meristem tips. Annals of Applied Biology **88**: 273-326.

Fernow KH, Peterson LC, Plaisted R L, 1962. Thermotherapy of potato leafroll. American Potato Journal **39**: 445-451.

Gomez KA, Gomez AA, 1984. Statistical procedures for agricultural research. Second edition, John Wiley & Sons, Inc., New York, USA. pp. 680.

Haase NU, 2008. The canon of potato science: 50. The nutritional value of potatoes. Potato Research **50**: 415-417.

Hassan AA, 2003. Potato. Dar-Al-Arabia publication, Cairo, Egypt.

Hollings M, 1965. Disease control through virus-free stock. Annual Review of Phytopathology **3**: 367-396.

International Potato Center, 1977. The Potato; Major Diseases and Nematodes. Centro Internacional de la Papa, Lima, Peru.

Jan H, Khan SB, 1995. Incidence and distribution of potato viruses in the upper Kaghan velly of Pakistan. Pakistan Journal of Phytopathology **7**: 13-16.

Kaiser WJ, 1980. Use of thermotherapy to free potato tubers of alfalfa mosaic, potato leaf roll, and tomato black ring viruses. Phytopathology **70**:1119-1122.

Kassanis B, 1949. Potato tuber freed from Leaf roll virus by heat. Nature **164**: 881.

Kassanis B, 1957. Some effects of varying temperature on the quality and quantity of Tobacco mosaic virus in infected plants. Virology **4**: 187-199.

Kassanis B, 1950. Heat inactivation of leaf-roll virus in potato tubers. Annals of Applied Biology **37**: 339-341.

Luciana CN, Gilvan Pio-Ribeiro, Lilia W, Genira PA, 2003. Stock Indexing and Potato Virus Y Elimination From Potato Plants Cultivated *in Vitro*. Scientia Agricola 60: 525-530

Matthews, R.E.F. 1980. Host plant responses to virus infection. Comprehensive virology, virus-host interaction, viral invasion, persistence, and diagnosis, edited by H. Fraenkel-Conrat and R.R. Wagner. Plenum Press, New York, USA. pp. 297–359.

Mervat ElFar M M, Ashoub A, 2009. Utility of Thermotherapy and Meristem Tip for Freeing Sweet Potato from Viral Infection. Australian Journal of Basic and Applied Sciences **3**: 153-159.

Mohamed EF, 2010. Antiviral Properties of Garlic Cloves Juice Compared with Onion Bulbs Juice Against Potato Virus Y (PVY). Journal of American Science 6(8). (Add pages)

Mori K, Hosokawa D, 1977. Localization of viruses in apical meristem and production of virus-free plants by means of meristem and tissue culture. Acta Horticulturae **78**: 389-396.

Nagaich BB, Upreti G C, 1964. Heat inactivation of potato leaf roll virus. Indian Potato Journal **6**: 96-102.

Nascimento LCG, Pio-Riberio L, Willadino GP, Andrade, 2003. Stock indexing and Potato Virus Y elimination from potato plants cultivated in vitro. Scientia Agricola 60: 525-530.

Nukari A, Uosukainen M, Rokka VM, 2009. Cryopreservation techniques and their application in vegetatively propagated crop plants in Finland. Agricultural and Food Science **18**: 117-128.

Panattoni A, Luvisi A, Triolo E, 2013. Review. Elimination of viruses in plants: twenty years of progress. Spanish Journal of Agricultural Research **11**: 173-188.

Quak F, 1972. Therapy. Viruses of Potatoes and Seed-Potato Production. J. A. de Bokx, ed. Centre for Agricultural Publishing and Documentation (PUDOC), Wageningen, The Netherlands. pp. 158-166.

Robert P, kahn , Monroe RL, 1970. *Datura metel* L. as a virus-Indicator plant. U.S. Plant Introduction Station, Glenn Dale, Maryland 20769.

Roland G, 1952. Quelques reserches sur renroulement de la pomme de terre. (Solanum virus 14, Appel & Quanjer). Parasitica **8**:150-158.

Rolot JL, Seutin H, 1999. Soilless production of potato minitubers using a hydroponic technique. Potato Research **42**: 457-469.

Rozendaal A, 1952. Demonstration of experiments with potato viruses. First Conf. Potato Virus Diseases, Lisse-Wageningen, The Netherlands, 1951. pp. 63-65.

Rozendaal A, Brust AH, 1955. The significance of potato virus S in seed potato culture. Second Conf. Potato Virus Diseases, Lisse-Wageningen, The Netherlands, 1954. pp. 120-123.

Spiegel S, Frison EA, Converse RH, 1993. Recent development in therapy and virus detection procedures for international movements of clonal plant germ plasm. Plant Disease 77: 176-1180.

Upreti GC, Nagaich BB, 1968. Inactivation of potato leaf roll virus in tubers by hot water treatment. American Potato Journal **45**: 373-377.

Wang Q, Panis B, Engelmann F, Lambardi M, Valkonen JPT, 2009. Cryotherapy of shoot tips: a technique for pathogen elimination to produce healthy planting materials and prepare healthy plant genetic resources for cryopreservation. Annals of Applied Biology **154**: 351-363.

Wang Q, Valkonen JPT, 2009. Improved recovery of cryotherapy- treated shoot tips following thermotherapy of *in vitro*-grown stock shoots of raspberry (*Rubus idaeus* L.). Cryoletters **30**: 171-182.