Indian Journal of Agricultural Sciences **86** (4): 541–4, April 2016/Article
https://doi.org/10.56093/ijas.v86i4.57570

An efficient protocol of *potato virus A* eradication by thermotherapy coupled with *in vitro* culture of potato (*Solanum tuberosum*)

YANLIN LI[1], CHANGZHENG HE[2], XINXI HU[3], MINGYUE LIU[4], QING ZHOU[5] and XINGYAO XIONG[6]

College of Horticulture and Landscape, Hunan Agricultural University, Changsha, Hunan 410 128, China

Received: 3 October 2015; Accepted: 13 November 2015

ABSTRACT

With the aim of developing an effective protocol for virus elimination from potato (*Solanum tuberosum* L.) plantlets, thermotherapy coupled with isolating the first nodal cuttings by *in vitro* culture was successful to potato virus A (PVA) elimination. The survival ratio of potato plantlets was affective by thermotherapy temperatures and durations. The optimal thermotherapy temperature was 36±1 °C with highest survival ratio and effective elimination. The results of RT-PCR indicated that the regenerated plantlets obtained from the first cycle (four weeks) of thermotherapy in daytime at 36±1 °C with light intensity 40 µmole/m/s for 12 hr, and 20±1 °C in darkness for 12 hr had PVA infected. While isolated the first nodal cuttings and followed by thermotherapy at the first cycle conditions for another two weeks, the PVA could be eliminated. Thermotherapy was given by culturing the nodal cutting from the infected of PVA for six weeks in total on MS medium, and the PVA-free plantlets were obtained. In concluded that the protocol of thermotherapy coupled with isolating the first nodal cuttings by *in vitro* culture in the study can be effectively used for virus free plantlets in potato, and probably also for other vegetable propagated plant species.

Key words: *In vitro,* Potato, *PVA,* Thermotherapy, Virus eradications

Virus diseases constitute a major constraint to production of high qualitative and quantitative yields in all major potato (*Solanum tuberosum* L.) cultivated areas in the world, because of they are transmitted from generation to generation (Faccioli and Colombarini 1996, Garg *et al.* 1999, Wang *et al.* 2006). It is known that viruses are uneven distribution in plants (Wang *et al.* 2009), and the concentration of phytoplasmas or virus is found to be different with increasing distance from the meristem (Hogenhout *et al.* 2008, Wang *et al.* 2009). Therefore, virus-free potato plants can be obtained by fast virus eradication methods, such as meristem culture (Cooper and Walkey 1978, Faccioli and Colombarini 1996, Wambugu *et al.* 1985), thermotherapy (Cieslinska 2002, Paprstein *et al.* 2008), thermotherapy coupled with meristem culture (Arkorful *et al.* 2015, Gopal 2011, Koubouris *et al.* 2007, Manganaris *et al.* 2003, Sharma *et al.* 2008, Tan *et al.* 2010), cryotherapy (Wang *et al.* 2006), cryotherapy coupled with meristem culture (Wang *et al.* 2009) and other approaches (Klein and Livingston 1982, Verma *et al.* 2005, Yang *et al.* 2014). Infected plants were introduced to tissue culture, and then heat treatment of plant *in vivo*/*in vitro* can reduce virus titers and improve the efficiency of virus eradiation (Kassanis 1957, Tan *et al.* 2010), and this method is wildly used in crops (Faccioli and Colombarini 1996, Paprstein *et al.* 2008, Sharma *et al.* 2008, Tan *et al.* 2010, Wang *et al.* 2006). Potato virus A (*PVA*), a member of the family Potyviridae, occurring worldwide, and can cause yield losses of about 10%~40% (Barker 1997, Jones 1990, MacLachlan *et al.* 1954) . The aim of the present study was to develop a new virus eradication protocol of *PVA*-free plant of *potato* Zhongzhaihuangpi for commercialization and long-term preservation.

MATERIALS AND METHODS

Potato Zhongzhaihuangpi, widely grown in central and southern Hunan province in China, was used for initial materials. The field-growing potato plants, growing in Hunan Provincial Engineering Research Center for Potatoes, Changsha, Hunan, China, naturally infected with *PVA* (as confirmed by ELISA and RT-PCR), were used as explants for *in vitro* culture. The shoots from one mother plant were washed by flowing water for 30 min, and surface disinfested with 70% alcohol, and then immerged into 0.1% solution of mercuric chloride for 10 min, followed by washed with sterile water for three times. After sterilization, the segments were cultured in glass bottle, each with 30 ml of Murashige and Skoog (MS) medium gelled with 0.6% (W/V) agarose, supplemented with sucrose 30 g/l. The pH of the medium was adjusted to 5.8 with 1M sodium hydroxide before

[1] e mail: lyl843@163.com, [2] e mail: hecz@hotmail.com, [3] e mail: huxingxi163@163.com, [4] e mail: liumingyue58@126.com, [5] e mail: 544538760@qq.com, [6] e mail: xiongxingyao@caas.cn.

autoclaving. The culture was maintained at 25±1 °C with light intensity 40 µmole/m/s for 16 hr.

ELISA was used to analyze the reaction of PVA specific monoclonal antibodies according to the manufacture's guidelines with potato leaves. The absorbance at 405 nm (A405) was recorded using a Multiskan MK3 microplate reader. It was performed on the following: (1) leaves from the field-growing plants; (2) leaves from plants *in vitro* culture before the application of thermotherapy; (3) acclimatized potato plants which were negative by RT-PCR detection.

Indirect RT-PCR was also employed to detect *PVA*. It was performed on the following: (1) leaves from the field-growing plants; (2) leaves from plants *in vitro* culture before the application of thermotherapy; (3) leaves from all thermotherapy treatment; (4) acclimatized potato plants. The RT-PCR detection procedure of *PVA* was described by Singh *et al.* 2000, Singh 1998 and Hu *et al.* 2009.

The thermotherapy was applied with *in vitro* culture on the MS medium gelled with 0.6% (W/V) agarose, and supplemented with 30 g/l sucrose. The cultures were incubated in a heat chamber with different temperatures (44 °C, 42 °C, 40 °C, and 36 °C, respectively) in daytime with light intensity 40 µmole/m/s for 12 hr, and 20 °C at darkness for 12 hr. The thermotherapy with optimal survival and plant growth treatment was chosen to the following treatments.

Following the thermotherapy with mentioned above, potato plantlets which cultured for one week were thermotherapy at elevated temperatures as follows: 1) Primary thermotherapy with 36 °C/20 °C. *In vitro* plantlets were incubated in a growth chamber at 36 °C with light intensity 40 µmole/m/s for 12 hr and at 20 °C in darkness for 12 hr, and which maintained for four weeks. Then the first nodal cuttings were dissected from treated shoots and sub-cultured with fresh MS medium gelled with 0.6% (W/V) agarose, and supplemented with 30 g/l sucrose. 2) The two successions thermotherapy at 36°C/20°C or 25°C/20°C, respectively. First nodal cuttings, isolated from plantlets treated at 36°C/20°C for four weeks, were continuous thermotherapy with 36°C/20°C or 25°C/20°C for another two weeks, respectively. And the light intensity and photoperiod were the same with the primary conditions.

All the treatments were performed in triplicate, and each treatment consisted of 50 plantlets. The data obtained was statistically analyzed using SPSS 13.0 for windows standard version for compare means – one way ANOVA, and Post Hoc Test-Duncan at level of significance ±=0.05 was performance.

RESULTS AND DISCUSSION

Virus indexing

Parent plants, including of field and *in vitro* cultures were found to be *PVA* positive by ELISA. And the RT-PCR results of the parent plants showed amplification of 270 bp fragment of partial coat protein gene indicating the presence of *PVA*. ELISA is routinely used for the detection of other plants viruses (Sharma *et al.* 2008, Tan *et al.* 2010). However, ELISA test lacks the sensitivity of detection for low concentrations in plant tissues, so RT-PCR is widely used for plant virus detection for its higher sensibility (Manganaris *et al.* 2003, Tan *et al.* 2010, Yang *et al.* 2014). In the present study, the result of RT-PCR was much sensitive compared with ELISA.

Survival ratio under different temperatures and durations

The effect of different heat treatments on *in vitro* culture plantlets which infected PVA are presented in Table 1. An increase survival ratio was observed with a decrease in thermotherapy temperatures, while an opposite variation tendency on durations at particular temperature. The thermotherapy temperature is a vita factors between optimal plant survival and virus elimination (Sharma *et al.* 2008). Dissimilar thermotherapy temperature showed a significant difference for survival ratio of meristem tissue culture and virus elimination in potato plantlets, and increase temperature during thermotherapy can decrease the survival rate of potato plantlets (Ali *et al.* 2014). In our study, 36°C/20°C treatment was an optimal temperature groups for thermotherapy on potato plantlets that of infected PVA.

Plants have different endurances on duration under different temperatures, which are effective on their survival ratio and virus eradiation. The survival ratio of pear plants decreases to 34.2 and 14.2% when thermotherapy under 37 °C lasted for 30 and 35 days, respectively, (Tan *et al.* 2010).

Table 1 Effectiveness of different temperature treatments on survival ratio of PVA infective plantlets

Duration (day)	Survival ratio(%)			
	44°C/20°C	42°C/20°C	40°C/20°C	36°C/20°C
0	100.00± 0.00a	100.00± 0.00a	100.00± 0.00a	100.00± 0.00a
1	55.6± 1.25b	69.88± 2.38b	100.00± 0.00a	100.00± 0.00a
2	48.89± 3.83c	60.92± 1.99c	94.20± 4.12b	100.00± 0.00a
3	33.33± 2.71d	44.87± 4.07d	81.76± 2.76c	100.00± 0.00a
4	24.44± 2.95e	29.73± 3.01e	75.17± 2.89d	92.86± 2.24b
5	17.78± 1.23f	20.00± 2.34f	68.33± 3.71e	92.86± 1.24b
15			30.14± 4.52f	80.00± 3.46c
20			10.47± 6.47g	80.00± 2.85c
30			3.01± 1.24h	80.00± 4.13c

Data shown are mean ± SD of three experiments, each treatment consisted of 50 replicates. Mean followed by the same letter are not significantly different from each other (General factorial – Duncan's HSD at P > 0.05).

Considering the temperature on potato plantlets in our study, the survival ratio was decreased with durations. The survival ratio of plants under 44°C/20°C and 42°C/20°C was sharply reduced with thermotherapy for one to five days, since only 17.8% and 20% plants survived, and 0% when lasted for 15 days, respectively. The percentage of plants survived was 3.0% under 40°C/20°C for 30 days. While treated at 36°C/20°C showed higher survival ratio on *in vitro* potato plantlets; it was observed a little dropping down from one to fifteen days, and then a constant ratio of about 80% when lasted for 30 days. With a higher survival ratio, thermotherapy with 36°C/20°C combination was used to do the following treatments.

Thermotherapy applied to in vitro plantlets

In this study, the primary thermotherapy at 36 °C/20 °C lasted for four weeks could not get a perfect effective elimination. The excised big cuttings for *in vitro* culture coupled with thermotherapy have low effective virus elimination, which is the main reason for researchers try new protocols for virus elimination, such as chemotherapy (Wambugu *et al.* 1985), meristem tissue culture (Faccioli and Colombarini 1996), thermotherapy coupled with shoot tip grafting (Sharma *et al.* 2008), thermotherapy coupled with meristem culture (Ali *et al.* 2014, Gopal 2011, Sharifi Nezamabad *et al.* 2015), stem-disc dome coupled with thermotherapy (Ghaemizadeh *et al.* 2014). However, different results were obtained when the first nodal cuttings were excised and sub-cultured into a fresh medium, at 25°C/20°C or at 36°C/20°C for another two weeks. The RT-PCR results showed that two of three plants detected amplification of 270 bp fragment of partial coat protein gene, indicating the presence of PVA, by sub-culturing and incubating at 25°C/20°C. While the sub-cultured plantlets that were at thermotherapy at 36°C/20°C for another two weeks didn't amplified any of fragments. Continuously, the results of RT-PCR indicated that the sub-culture regenerated plantlets under primary culture's condition after six weeks of thermotherapy at 36°C/20°C and the second sub-culture regenerated plants were all PVA free plants. In the course of *in vitro* culture and thermotherapy treatments, no morphological abnormalities were observed. Two weeks later, the regenerated clones of the *PVA*-free plantlets were 3 to 7 cm height, and they rooted well.

In conclusion, *in vitro* culture potato plants subjected to thermotherapy (36±1°C) for four weeks, and then isolating the first nodal cuttings to sub-culture and followed by thermotherapy (36±1°C) for another two weeks can be effectively used for virus PVA in potato.

ACKNOWLEDGEMENT

The research was supported by Modern Agriculture Industry Technology System of China.

REFERENCES

Ali M, Nasiruddin K, Haque M, Faisal S. 2014.Virus elimination in potato through meristem culture followed by thermotherapy. *SAARC Journal of Agriculture* **11**: 71–80.

Arkorful E, Appiah A and Dzahini-Obiatey H. 2015.Screening for sweet potato (*Ipomoea batatas* L.) leaf curl virus (SPLCV) and its elimination using thermotherapy-meristem tip culture technique. *Journal of Agricultural Sciences* **10**: 1–9.

Barker H. 1997. Extreme resistance to potato virus V in clones of *Solanum tuberosum* that are also resistant to potato viruses Y and A: evidence for a locus conferring broad-spectrum potyvirus resistance. *TAG Theoretical and Applied Genetics* **95**: 1 258–62.

Cieslinska M. 2002. Elimination of apple chlorotic leaf virus (ACLSV) from pear by in vitro thermotherapy and chemotherapy. *Acta Hort (ISHS)* **596**: 481–4.

Cooper V C and Walkey D G A. 1978. Thermal inactivation of cherry leaf roll virus in tissue cultures of *Nicotiana rustica* raised from seeds and meristem-tips. *Annals of Applied Biology* **88**: 273–8.

Faccioli G and Colombarini A. 1996. Correlation of potato virus S and virus M contents of potato meristem tips with the percentage of virus-free plantlets produced in vitro. *Potato Research* **39**: 129–40.

Garg I, Singh M, Khurana S P and Gopal J. 1999. Virus analysis of indigenous potato germplasm collection. *Journal of Indian Potato Association* **26**: 111–7

Ghaemizadeh F, Dashti F, Khodakaramian G and Sarikhani H. 2014. Combination of stem-disc dome culture and thermotherapy to eliminate Allexiviruses and Onion yellow dwarf virus from garlic (*Allium sativum* cv. Hamedan). *Archives of Phytopathology and Plant Protection* **47**: 499–507.

Gopal J. 2011. An efficient protocol of chemo-cum-thermotherapy for elimination of potato *(Solanum tuberosum* L.) viruses by meristem-tip culture. *Indian Journal of Agricultural Sciences* **81**.

Hogenhout SA, Oshima K, Ammar E-D, Kakizawa S, Kingdom H N and Namba S. 2008. Phytoplasmas: bacteria that manipulate plants and insects. *Molecular Plant Pathology* **9**: 403–23.

Hu X, He C, Xiao Y, Xiong X and Nie X. 2009. Molecular characterization and detection of recombinant isolates of potato virus Y from China. *Arch Virol* **154**: 1 303–12.

Jones RAC. 1990. Strain group specific and virus specific hypersensitive reactions to infection with potyviruses in potato cultivars. *Annals of Applied Biology* **117**: 93–105.

Kassanis B. 1957. Effects of changing temperature on plant virus diseases. *Adv Virus Res* **4**: 221–41.

Klein R E and Livingston CH. 1982. Eradication of potato virus X from potato by ribavirin treatment of cultured potato shoot tips. *American Potato Journal* **59**: 359–65.

Koubouris G, Maliogka V, Efthimiou K, Katis N and Vasilakakis M. 2007. Elimination of plum pox virus through in vitro thermotherapy and shoot tip culture compared to conventional heat treatment in apricot cultivar. *Bebecou Journal of General Plant Pathology* **73**: 370–3.

MacLachlan D, Larson R, Walker J. 1954. Potato virus A. *American Journal of Potato Research* **31**: 67–72.

Manganaris G A, Economou A S, Boubourakas N and Katis N I . 2003. Elimination of PPV and PNRSV through thermotherapy and meristem-tip culture in nectarine. *Plant Cell Reports* **22**: 195–200.

Paprstein F, Sedlak J, Polak J, Svobodova L, Hassan M and Bryxiova M. 2008. Results of in vitro thermotherapy of apple cultivars. *Plant Cell, Tissue and Organ Culture* **94**: 347–52.

Sharifi Nezamabad P, Koohi Habibi M, Dizadji A and Kalantari S.

2015. Elimination of Bean yellow mosaic virus through thermotherapy combined with meristem-tip culture in gladiolus corms. *Journal of Crop Protection* **4**: 533–43.

Sharma S, Singh B, Rani G, Zaidi A, Hallan V, Nagpal A, Virk G. 2008. In vitro production of Indian citrus ringspot virus (ICRSV) free Kinnow plants employing thermotherapy coupled with shoot tip grafting. *Plant Cell, Tissue and Organ Culture* **92**: 85–92.

Singh R P, Nie X Tai GCC. 2000. A novel hypersensitive resistance response against potato virus A in cultivar 'Shepody'. *TAG Theoretical and Applied Genetics* **100**: 401–8.

Singh RPS, M. 1998. Specific detection of potato virus A in dormant tubers by reverse transcription polymerase chain reaction. *Plant Disease* **82**: 230–4.

Tan R, Wang L, Hong N and Wang G. 2010. Enhanced efficiency of virus eradication following thermotherapy of shoot-tip cultures of pear. *Plant Cell, Tissue and Organ Culture* **101**: 229–35.

Verma N, Ram R and Zaidi A A. 2005. In vitro production of Prunus necrotic ringspot virus-free begonias through chemo- and thermotherapy. *Scientia Horticulturae* **103**: 239–47.

Wambugu F, Secor G, Gudmestad N. 1985. Eradication of potato virus Y and S from potato by chemotherapy of cultured axillary bud tips. *American Potato Journal* **62**: 667–72.

Wang Q, Liu Y, Xie Y, You M. 2006. Cryotherapy of Potato Shoot Tips for Efficient Elimination of Potato Leafroll Virus (PLRV) and Potato Virus Y (PVY) . *Potato Research* **49**: 119–29.

Wang QC, Panis B, Engelmann F, Lambardi M, Valkonen JPT. 2009. Cryotherapy of shoot tips: a technique for pathogen eradication to produce healthy planting materials and prepare healthy plant genetic resources for cryopreservation. *Annals of Applied Biology* **154**: 351–63.

Yang L, Nie B, Liu J, Song B. 2014. A reexamination of the effectiveness of ribavirin on eradication of viruses in potato plantlets in vitro using ELISA and quantitative RT-PCR. *American Journal of Potato Research* **91**: 304–11.