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Development of a Reliable Technique to Eliminate *Sweet potato leaf curl virus* through Meristem Tip Culture Combined with Therapy of Infected *Ipomoea* Species

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Abstract - *In vitro* elimination of *Sweet potato leaf curl virus* (SPLCV) from infected sweet potato is difficult due to low number of virus-free plants obtained from meristem tip culture and long growth period required for the virus detection. In this study, efficient production of the SPLCV-free sweet potato by *in vitro* therapy coupled with a PCR assay for virus detection was investigated. Infected shoots cultured on Murashige and Skoog medium were treated at three different temperatures for 7 weeks followed by meristem tip culture on the medium with or without ribavirin at 50 mg/L. The regenerated plantlets were tested for virus infection by a PCR assay. The results showed that the both heat- and cold-treatments, and addition of the ribavirin did not have significant effect on efficiency of the virus elimination. The meristem size, however, greatly affected the survival rate. Meristems sized over 0.4 mm survived better than smaller ones (0.2-0.3 mm). The PCR assay was approved to be a rapid, sensitive and reliable for the SPLCV detection in regenerated plantlets. Therefore, combination of cultivating meristem tips sized 0.4-0.5 mm on the medium at 22℃ without ribavirin and detection of SPLCV in the regenerated plantlets by the PCR assay was an efficient system for the SPLCV elimination from infected sweet potato.

Key words - Meristem tip culture, *Sweet potato leaf curl virus*, *Ipomoea* spp. thermotherapy, ribavirin

Introduction

Sweet potato (*Ipomoea batatas* L.) is an economically important root crop in tropical and subtropical countries and ranks the seventh in food production in the world (FAOSTAT, 2006). It is propagated vegetatively and therefore, is prone to infection of many viruses which can significantly decrease the yield and quality of sweet potato. *Sweet potato leaf curl virus* (SPLCV) is a member of the genus *Begomovirus* in the family *Geminiviridae* and has geminate particles (~20 nm×28 nm) with a circular, single-stranded DNA molecule of approximately 2.8 kb (Fauquet et al., 2005). The virus does not induce visible symptoms on most cultivars of sweet potato, and is disseminated by propagation materials between growth seasons or areas. SPLCV has been reported in several countries (Lotrakul et al., 2002; Valverde et al., 2004; Briddon et al., 2006; Luan et al., 2007) and is commonly found in the genetic resources (Li et al., 2004). Despite the mild symptoms on the infected plants, SPLCV can cause significant yield reductions on certain cultivars (Clark et al., 2002). Therefore, elimination of the virus from infected plants is important not only to prevent yield decreases but also to prevent contamination in genetic resource collection centers, thereby facilitating safe movement of sweet potato genetic resources.

Meristem tip culture has been used frequently for virus-free plant production and germplasm conservation of many vegetatively propagated crops including cocoyam (*Colocasia esculenta*), potato (*Solanum tuberosum*), sweet potato, yacon (*Polymnia sanchifolia*) and other root crops (Alconero et al., 1975; Frison and Ng, 1981; Green and Lo, 1989; Frison, 1994; Truskinov and Rogozina, 1997; Malaurie et al., 1998; Fletcher et al., 1998; Mix-Wagner, 1999; Fletcher and Fletcher, 2001; Gao et al., 2000; Okamoto et al., 2001; Reyes et al., 2006; Yang et al., 2006; Zhang et al., 2006). This approach takes advantage of the tissue specificity of some viruses and uneven distribution of others within their hosts, resulting

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in the absence of the viruses in the meristem tips (Faccioli and Rubies-Autonell, 1982; Faccioli et al., 1988; Green et al., 1988; Ramirez-Malagon et al., 2006).

Meristem tip culture has been used routinely for the production of sweet potato plants free of Sweet potato chlorotic stunt virus, Sweet potato feathery mottle virus (SPFMV), Sweet potato latent virus (SPLV) and other viruses worldwide since the 1960's (Nielsen, 1960; Kuo et al., 1985; Gao et al., 2000; Sertkaya, 2002; Zhang et al., 2006 Wang and Valkonen, 2008 El Far and Ashoub, 2009). This technique has been employed in the U.S. quarantine programs for obtaining virus-free sweet potato plants from the infected accessions and worked well for elimination of SPFMV, SPLV and several RNA viruses, but not for SPLCV (our unpublished data). Green et al. (1992) reported that meristem tip culture, heat treatment of explants and addition of ribavirin to the culture medium, was effective in elimination of SPLCV from one infected sweet potato cultivar. However, the initial application of these methods to virus elimination from SPLCV-infected accessions of sweet potato in the U.S. quarantine programs resulted in a low virus elimination rate. Graft inoculation onto susceptible indicator, *I. setosa,* for indexing the regenerated plants in the greenhouse usually takes three months. This indexing method not only limited the number of plantlets that could be tested but also extended *in vitro* therapy time, resulting in accumulation of the infected accessions and extension of the quarantine holding time of those accessions. Therefore, a rapid, sensitive and reliable method for testing the virus in the regenerated plantlets was also vital for efficient production of the virus-free plant materials. Polymerase chain reaction (PCR) assay is sensitive and rapid, so it is more suitable for detecting DNA viruses in small amount of samples collected from the plantlets.

This study describes a strategy for efficient production of SPLCV-free sweet potato and other *Ipomoea* spp. by combining the PCR detection of virus with simple meristem tip culture and successful application of the system in eliminating SPLCV from a number of the infected accessions in the US quarantine program.

Materials and Methods

Plant materials

Seventeen SPLCV-infected sweet potato accessions imported from several sources to the US plant quarantine program were listed on Table 1. The infection of SPLCV in these accessions was determined by biological assay (Moyer et al., 1989) and PCR (Li et al., 2004). One accession, 42711, was obtained as an *in vitro* plantlet from the International Potato Center (CIP, Peru) and used for initial experiments. Several plants were established from each accession in a greenhouse. Shoots (3 cm in length) trimmed from each infected plant were cultured in 25x150 mm glass tubes containing 15 ml of culture medium after surface sterilization with a 10% (v/v) solution of commercial hypochloride and 0.05% (v/v) Tween 20 for 10-15 min and then sterile distilled water three times. Culture medium based on Murashige and Skoog (MS) basal

Table 1. List of infected sweet potato accessions and results of the *in vitro* therapy

Accession No.	Cultivar name	Source	Virus-free plantlets / Total plantlets (Elimination rate)
35661	IITA-TIS 83/1038	CIP	5/5 (100%)
37460	WT-59	CIP	4/16 (25%)
37461	WT-108	CIP	6/8 (75%)
37463	WT-129	CIP	10/18 (56%)
37464	WT-571	CIP	5/5 (100%)
37469	WT-325	CIP	3/5 (60%)
37472	WT-478	CIP	3/8 (38%)
37477	NO. 221	CIP	2/3 (67%)
37479	VICOSA	CIP	1/6 (17%)
42710	CN1421-68	CIP	3/6 (50%)
42711	CN1421-56	CIP	11/29 (38%)*
43785	Terlaje	Guam	1/3 (33%)
43787	GUY 2002-1	Guyana	2/7 (29%)
43788	GUY 2002-2	Guyana	1/3 (33%)
43791	GUY 2002-5	Guyana	1/2 (50%)
43792	GUY 2002-6	Guyana	5/6 (83%)
43793	GUY 2002-7	Guyana	1/9 (11%)

* Data from the control (untreated) group in the initial experiment.

salts (Murashige and Skoog, 1962) and vitamins, and 30 mg/L sucrose and 1.5 g/L gelrite (Sigma, St. Louis, MO) were supplemented. Shoot cultures were maintained under ambient temperature (approximately 22℃) with 16 hr of cool white fluorescent light (40 mol m-2 s-1) per day, the standard growth condition. The plantlets from the shoot cultures were tested again to be positive by the PCR assay before thermotherapy. The plantlets (3-5 cm in height) were then used to excise meristem tips after the thermotherapy. An uninfected plantlet was maintained in the standard growth condition and used as a negative control.

In vitro thermotherapy

Shoots (explants, 3-5 cm in length) of the infected plantlets were transferred onto the fresh MS basal medium in the glass tubes. For heat therapy, the shoots were incubated at 35℃ for the two days and then 32℃ in a growth chamber with a 12-hr light cycle for another two days followed by the condition cycles of 35℃ for 4 hr with light and 32℃ for 4 hr in dark for 46 days. For cold therapy, the shoots were incubated at 15℃ for two days and then at 12℃ for another two days in a growth chamber with a 12-hr light cycle followed by the condition cycles of 15℃ for 4 hr with light and 12℃ for 4 hr in dark for 38 days. A third set of the plantlets was maintained at the standard growth conditions described above.

Meristem tip culture and *in vitro* chemotherapy

Meristems sized 0.2-0.6 mm (average 0.38-0.47 mm) with one or two leaf primordial were excised from heat (109) cold treated (109) and untreated explants (128), separately. They were cultured on basal medium supplemented with 0.1 mg/L GA_3 and 0.04 mg/L kinetin at 22℃ with low light (20 mol m-2 s-1) for one week and then under the standard growth condition for another week. The meristems of each group were then divided into two equal portions. Approximately half of the meristems from each group were transferred onto the meristem culture medium containing 50 mg/L ribavirin (PhytoTechnology Laboratory, Shawnee Mission, KS) for testing the effects of ribavirin incorporation onto the medium on the virus elimination, and another half was transferred onto the same medium without ribavirin. The meristems of all six treatments were maintained under the standard growth condition for 4 weeks before transferring onto basal medium supplemented with 0.4 mg/L kinetin. The regenerated shoots or plantlets were transferred onto fresh medium every 4-5 weeks if they did not reach the testing size of 5 cm in height.

Plant establishment

Regenerated plantlets were tested by the PCR for SPLCV. PCR-negative plantlets (1-5 per accession) were transferred to 4-inch pots containing Promix HP potting mix (Griffin Greenhouse & Nursery Supply, Tewksbury, MA). To promote a high humidity, the pots were covered by magenta boxes and placed in a growth chamber at 27℃ with 14-16 hr light. After 2-3 days, the boxes were gradually opened to reduce humidity until the plants started to grow. After four weeks in the growth chamber, the plants were transferred to 8-inch pots and moved to the greenhouse.

Virus detection

Shoots from regenerated plantlets and leaves from greenhouse plants were tested by the PCR assay described by Li et al. (2004) for the detection of SPLCV. Virus-free plantlets and plants were used as the healthy control. When a plantlet was approximately 5-cm tall, the shoot tip (1 cm in length) was removed and transferred onto the fresh medium, the remaining shoot was used to extract total nucleic acids by the CTAB method (Li et al., 2008) for the PCR assay. Young leaves from the plants in the greenhouse were used for the extraction of the total nucleic acids. The plants established from PCR-negativeplantlets were also tested by graft inoculation onto *I. setosa* indicator plants. Two to three vine segments (2-4 nodes) were taken from each candidate plant and wedge-grafted onto young seedlings (4-7 weeks old) of the indicator plants. The indicator plants were observed for symptoms 2-5 weeks after grafting.

Data analysis

The numbers of surviving plantlets was counted after *in vitro* culture. Infection of the geminivirus in the plantlets was determined by PCR. Data obtained was analyzed using two-way ANOVA depending on each treatment and combination of treatments.

Results

Establishment and multiplication of meristem tip cultures. Meristems turned green 7-10 days after transfer to the basal medium under the standard growth conditions. All meristems regenerated slowly, especially those treated at lower temperature and those cultivated on the medium with ribavirin. Before enough shoots could be obtained for virus detection, a low concentration (0.4 mg/L) of kinetin was needed to promote shoot production from the meristems. Three to four months was required for shoots from untreated meristems to reach 5 cm in height, however, only one month was required for secondary shoots to reach this height, the size large enough for both subculture and virus detection. The regeneration of the meristems treated with low temperature and ribavirin was slower, and it took them 4-5 months to reach the 5-cm height. The shoots from the meristems of most accessions were vigorous in the basal medium under the regular growth condition, but few cultivars such as VICOSA, were slow and produced fewer plantlets with thinner stems and yellow leaves.

Effect of different factors on the meristem survival. No visual damage from the temperature stress was observed for the plantlets that underwent cold treatment, but the average meristem survival rate (37%) for the cold-treated group was lower than those of the heat-treated (51%) and untreated (56%) groups (Table 2A). Lower temperatures decreased the regeneration capability of the meristems. Addition of the antiviral chemical, ribavirin, to the medium did not have significant influence on the meristem survival rate (Table 3A). However, the chemical and/or cold treatments delayed the development of the plantlets, and the delay was up to three

Table 2. Effects of temperature and meristem size on survival, virus elimination and efficiency of *in vitro* therapy

A. Survival (%, number of survived plantlets/number of cultured meristem tips)						
Meristem size (mm) / Temperature[1]	≥0.2	≥0.3	≥0.4	≥0.5	≥0.6	Mean
Cold	0	28 cd	55 bc	52 bc	48 bc	37 bc
Control	29 cd	40 c	62 bc	92 a	55 bc	56 a
Heat	25 cd	50 bc	47 bc	71 b	61 bc	51 ab
Mean	17 c	39 bc	55 b	72 a	55 b	
B. Virus elimination (%, number of virus-free plantlets/number of survived plantlets)						
Meristem size (mm) / Temperature	≥0.2	≥0.3	≥0.4	≥0.5	≥0.6	Mean
Cold	0	75	46	44	26	38
Control	67	29	47	40	11	39
Heat	0	28	40	42	28	28
Mean	17	44	45	42	22	
C. Efficiency (%, number of virus-free plantlets/number of cultured meristem tips)						
Meristem size (mm) / Temperature	≥0.2	≥0.3	≥0.4	≥0.5	≥0.6	Mean
Cold	0	18	25	23	13	16
Control	16	12	29	35	8	20
Heat	0	14	18	29	17	16
Mean	5 b	15 cd	24 ab	29 a	12 cd	

[1]Temperature of the cold, control and heat treatments were 12-17℃, 22℃ and 32-35℃, respectively.
No significance on virus elimination by temperature, meristem size and their combination. Means of efficiency were significant by meristem size but not by temperatures. Same letters are same at level of p=0.05

Table 3. Effects of ribavirin and meristem size on survival, virus elimination and efficiency of *in vitro* therapy

A. Survival (%, number of survived plantlets/number of cultured meristem tips)						
Ribavirin \ Meristem size (mm)	≥0.2	≥0.3	≥0.4	≥0.5	≥0.6	Mean
Absent	18	44	47	78	60	46
Present	18	37	70	95	57	49
Mean	18 c	41 bc	59 b	87 a	59 b	
B. Virus elimination (%, number of virus-free plantlets/number of survived plantlets)						
Ribavirin \ Meristem size (mm)	≥0.2	≥0.3	≥0.4	≥0.5	≥0.6	Mean
Absent	52	31	52	54	27	38
Present	11	77	40	33	11	32
Mean	32	54	46	44	22	
C. Efficiency (%, number of virus-free plantlets/number of cultured meristem tips)						
Ribavirin \ Meristem size (mm)	≥0.2	≥0.3	≥0.4	≥0.5	≥0.6	Mean
Absent	7	13	23	36	15	18
Present	4	17	25	23	10	16
Mean	5 d	15 bc	24 ab	30 a	12 cd	

No significance on survival, virus-free and efficiency by ribavirin, meristem size and their combination at level of p=0.05.
Means of survival and efficiency by meristem size were significant at level of p=0.05. Same letters are not significant.

months in combination of the cold and chemical treatments (data not shown). The sizes of meristems also affected the meristem survival (Tables 2A and 3A). The meristems of 0.4 mm or larger were more viable than smaller ones (0.2 mm) although there was no direct correlation between the size and the survival rate of the meristems sized 0.4-0.6 mm. The average survival rate of the meristems of 0.2 mm in size was 17%, whereas the meristems of larger sizes had at least 39% survival rate. All the cold-treated meristems of 0.2 mm in size lost the regeneration capability. Compared with the heat-treated and control groups, the survival rates of the cold-treated meristems of 0.3 mm were also significantly lower, too.

Effect of different factors on the virus elimination. Statistical analysis of the data obtained showed that thermotherapy, ribavirin and meristem size had no effect on virus elimination (Table 2B and Table 3B). The average virus elimination rate (39%) for the control group was either similar to that of the cold-treated (38%) or higher than that of the heat-treated (28%) groups (Table 2B). Addition of the ribavirin at 50 mg/L also did not increase the virus elimination rate. Average virus elimination rates were 38% and 32%, respectively, in the medium without or with ribavirin (Table 3B). There was also no direct correlation between the virus elimination rates and the meristems sizes. Statistic analysis indicated that meristem size had no effect on the virus elimination rate although the average rates (42-45%) of virus elimination for meristems of 0.3-0.5 mm were higher than those of larger (22%, 0.6 mm) and smaller (32-34%, 0.2 mm) ones.

Efficiency of different factors on *in vitro* therapy. To measure the efficiency of *in vitro* therapy, the ratio of total virus-free plants obtained and total meristems cultured was calculated. The efficiency, therefore, was directly related to the survival and virus elimination rates. The average efficiencies were 16-20% for the cold-, heat- and untreated groups (Table 2C) and 16-18% for the groups without or with ribavirin (Table 3C). Unlike thermotherapy and chemotherapy, the size of the meristem tips had significant effect on efficiencies of obtaining virus-free plantlets. More virus-free plantlets were obtained from the meristem tips of 0.4-0.5 mm than from smaller (0.2-0.3 mm) and larger (0.6 mm) groups,

due to low survival rate from the smaller meristem tips and low virus elimination rate from the larger meristem tips. Since the temperature stress on the *in vitro* shoots and ribavirin to the medium for meristem tip culture did not have significant effect on the virus elimination rate, the efficiency of *in vitro* therapy for SPLCV elimination depended on the survival rate, which was greatly influenced by the meristem size. Therefore, cultivating meristem tips of 0.4-0.5 mm in size was more efficient than cultivating other sizes due to the high survival rates (Table 2C and 3C).

Virus detection. Shoots of the regenerated plantlets from meristems of all six treatments from the infected plants were tested by the PCR assay. Results showed that the 912-bp viral fragment was easily detected in infected plantlets and absent in virus-free plantlets (Fig. 1). Subcultures from tips of the virus-free plantlets were tested again by the PCR assay before transplanting into the pots, and the same results were obtained (data not shown). To check the reliability of the PCR assay for the SPLCV detection, virus-free plantlets (4-5) derived from each treatment were established in the greenhouse, and the plants were tested by both the PCR assay and the graft inoculation of the indicator plants. The results from the graft inoculation correlated completely with the results from the PCR assay (data not shown, Fig. 2).

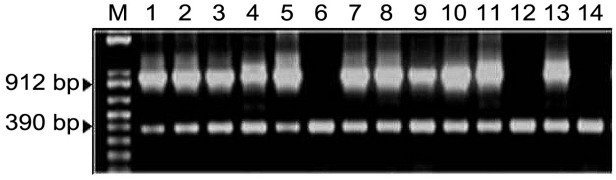

Fig. 1. PCR detection of the *Sweet potato leaf curl virus* (SPLCV) in some sweet potato plantlets regenerated from the cold-treated meristems of SPLCV-infected plants. Lane M, 1 kb plus DNA ladder; lanes 1-14, the regenerated plantlets. The upper band was the 912-bp PCR product amplified from the geminivirus, and the lower band was the 390-bp PCR product amplified from the malate dehydrogenase gene of the host plant, which was used as internal control in the PCR assay. The virus-specific band was absent in the lanes 6, 12 and 14, indicating those plantlets were virus-free.

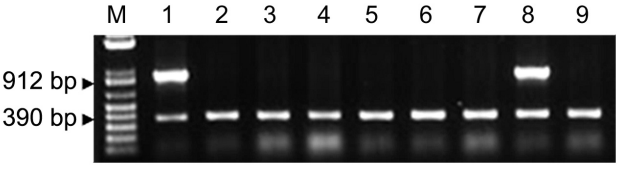

Fig. 2. PCR detection of the *Sweet potato leaf curl virus* (SPLCV) in indicator plants (*Ipomoea setosa*) graft inoculated with sweet potato plants regenerated from *in vitro* plantlets. Lane M, 1 kb plus DNA ladder; lane 1, from a SPLCV-positive plantlet; lanes 2-7, from SPLCV-free plantlets; lane 8, from positive sweet potato control; lane 9; from healthy sweet potato.

Evaluation of the system. This simple meristem tip culture coupled with the PCR assay was utilized to produce sweet potato plants free of SPLCV from 16 additional infected accessions accumulated in the US quarantine programs during this investigation (Table 1). Elimination rates of 11-100% (46% as average) were obtained from the plantlets regenerated from the meristem tips. At least one of the SPLCV-free plantlets was established in the greenhouse, and results of the graft inoculation and PCR assay confirmed that the plants from them were free of SPLCV.

Discussion

A simple meristem tip culture protocol was found in this study to be an efficient method for elimination of SPLCV from sweet potato when combined with a rapid, sensitive and reliable PCR assay for the virus detection in the plantlets. Elimination rates of 28-39% from different treatments in our study were much lower than the 75-80% reported by Green et al. (1992), and the results were consistent with our previous observation. A higher rate of the virus elimination was also obtained when greenhouse plants were used to dissect the meristem tips (C. Clark, Louisiana State University, personal communication). This difference may be the result of types of source plant and their growth conditions. The meristems in this study were dissected from the *in vitro* plantlets instead of the greenhouse plants (Green et al., 1992; Clark, personnel communication). Slow growth of initial shoots in *in vitro* culture might allow the spread of SPLCV to more meristems, resulting in low efficiency of virus elimination from the meristem tips. However, the advantage of using the *in vitro* plantlets as a starting material is that they are typically used in movement of sweet potato genetic resources and can be subjected to therapy directly if virus is detected in the recei-

ved materials, thus avoiding initial growth of plants in greenhouse and shortening the time for production of SPLCV-free plants.

Thermotherapy of the infected plant materials before meristem excision not only stimulates shoot elongation of plants but also impede replication and movement of the virus in its hosts, resulting in virus elimination from infected sweet potato (Green et al., 1992; Gao et al., 2000; Wang and Valkonen, 2008; El Far and Ashoub, 2009) and other root crops (Lizárraga et al., 1980; Adejare and Coutts, 1981; Faccioli and Rubies-Autonell, 1982; Truskinov and Rogozina, 1997; Fletcher et al., 1998; Fletcher and Fletcher, 2001). Green et al. (1992) showed that only the heat treatment of the shoot stems (1-cm stem pieces below the shoot tip) increased the SPLCV elimination rate. In consistent with the previous study, our results showed the heat treatment of the shoot tips (3-5 cm) at 32-35℃ before meristem excision did not improve either the meristem survival rate or virus elimination rate (Table 2). The cold treatment also did not improve the thermotherapy efficiency for the SPLCV elimination. Although the meristems sized 0.3 mm from the cold-treated shoot tips had a higher elimination rate, their average survival rate was low (28%) and their development was delayed. Therefore, its overall therapeutic efficiency was not great (18%).

Ribavirin, an anti-viral chemical, has been demonstrated to be effective on eliminating some RNA viruses from potato and Arracacha (Truskinov and Rogozina, 1997; Fletcher et al., 1998; Fletcher and Fletcher, 2001). Green et al. (1992) demonstrated that addition of the ribavirin at 50 mg/L to the medium for the shoot tip culture before meristem excision improved the SPLCV elimination rate to 86%. However, the effectiveness of the ribavirin at 50 mg/L in the meristem tip culture medium was limited for the SPLCV elimination from the infected plantlets (Table 3C). The low efficiency of the ribavirin on the virus elimination in this study may be due to substitution of the shoot stems (1-cm long) with the meristem tips. The ribavirin at 50 mg/L, therefore, is not recommended in the *in vitro* therapy for the SPLCV elimination due to its phytotoxic effect.

It is known that the meristem survival and virus elimination rate are size-dependent (Shang et al., 1996; Grout, 1999). The meristems of 0.2-0.5 mm in size are generally used in virus elimination in sweet potato (Kuo et al., 1985; Green et al., 1992; Gao et al., 2000 El Far and Ashoub, 2009). In the present study, none of the meristems of 0.2 mm from the cold treatment survived, and only a few meristems of 0.2 mm survived (Table 2A). Furthermore, the average virus elimination rate from the meristems of 0.2 mm (34%) was similar to those from larger sizes (22-45%), indicating that SPLCV could invade smaller meristems of the host plants. The meristems of ~0.5 mm had the highest average survival rate (72%), but the virus elimination rate for this group was similar to those for the meristems of 0.3-0.4 mm (Table 2). There was no correlation between the meristem size and virus elimination rate, indicating the SPLCV might be unevenly distributed in the meristems of infected plants.

The results of the present study show that the thermotherapy of *in vitro* plantlets, addition of at 50 mg/L ribavirin to the medium for meristem tip culture and meristem size had little effect on the SPLCV elimination. Since approximately 30% of survived meristems are naturally free of virus, obtaining more survived meristems is necessary for more efficient virus-free plant production. Therefore, use of meristems sized 0.4-0.5 mm will improve the efficiency of virus elimination from infected sweet potato.

A sensitive and reliable method of virus detection is also critical to index the plantlets. The detection of the virus by graft inoculation onto *I. setosa* plants requires growth of indicator plants in the greenhouse for 2-3 months. Use of a small amount (0.1 g or less) of shoot tissues directly from plantlets for the virus detection by the PCR assay took 1-2 days, and large numbers of plantlets could be tested at the same time. The reliability of the method was confirmed by the graft inoculation of 6 PCR-negative plants regenerated from in the accession 42711 in the greenhouse. The protocol was then used to produce at least one virus-free plant from 16 additional infected accessions from different sources (Table 1). These results demonstrated that the meristem tip culture when combining with rapid and sensitive virus detection by the PCR assay is an efficient system for production of SPLCV-free sweet potato plants. Such a system is not only simple, cost effective and reliable, but also reduces the time for the SPLCV indexing of regenerated plants after *in vitro* therapy. Thus it can shorten production of the SPLCV-free sweet

potato in quarantine and certification programs.

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