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Elimination of SPFMV from Virus-infected Sweet Potato Plants through Apical Meristem Culture

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ABSTRACT

Sweet potato infected with a viral disease (SPFMV) showed irregular chlorotic patterns, so called feathering associated with faint or distinct ring spots that have purple-pigmented borders. SPFMV was eliminated from sweet potato plants using meristem tip culture. MS medium supplemented with BAP (2 mg/L) and NAA (0.05 mg/L) was used for shoot proliferation and 1/2 MS medium for rooting of the plants. Highest percentage of regenerated plants (60%) was obtained from the optimum size (0.3-0.5 mm) meristem tips. Of these, 60% plants were found negative for SPFMV by RT-PCR. Virus detection by RT-PCR was found to be a reliable method. Meristem-tip culture to produce SPFMV-free quality sweet potato and virus detection by RT-PCR is an efficient, time saving and reliable method for production of SPFMV-free tissue culture raised plants.

Key Words **:** Sweet potato, meristem tip culture, shoot proliferation, SPFMV, RT-PCR.

INTRODUCTION

Sweetpotato [Ipomoea batatas L. (Lam)] is not only a widely grown food crop, but also an important forage and industrial material. It is widely cultivated in the developing countries from tropical to temperate regions, such as in Asia, Africa and Latin America. According to statistics from the Food and Agriculture Oragnization of the United Nations (FAO), in 1997 the cultivated area of sweetpotato in the world was about 9.0 million hactors and its production ranked the 7th in worldwide crop production.

Many surveys have shown that the sweet potato feathery mottle virus (SPFMV) is the most prevalent and widespread pathogen in the world where sweet potatoes are cultivated (Cali and Moyer 1981; Moyer and Salazar 1989; Colinet and Kummert 1993). SPFMV generally induces irregular chlorotic patterns, so called feathering associated with faint or distinct ring spots that have purple-pigmented borders (Moyer and Salazar 1989).

Detection of virus in plants is usually based on the use of antisera and/or monoclonal antibodies in an enzyme-linked immunosorbent assay (ELISA), but this

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method suffers from a lack of sensitivity (Koenig and Paul 1982; Yoon *et al.* 1991). Nucleic acid amplification techniques can increase the sensitivity of the detection of plant viruses, compared with serological methods. The use of RT-PCR as a sensitive method for the detection of RNA viruses has been reported (Kinard and Scott, 1996; Lim *et al.*, 1993; Griesbach 1995; Hu *et al.* 1995; Li *et al.* 1998; Singh and Singh 1998; Jeong *et al.* 2001, 2003).

In vitro meristem-tip culture is an efficient method for obtaining virus-free plants either alone or combined with heat treatment from a wide range of plants (Wang and Hu, 1980 Kartha, 1986).Meristem-tip culture combined with heat treatment has been used to eradicate viruses (Shang *et al.*, 1994, 1996; Yang *et al.*, 1998) from sweet potato in China, but no attempt has been made to eradicate SPFMV from sweet potato in Korea.

In the present communication, we report the detection of SPFMV in sweet potato by RT-PCR and in vitro production of SPFMV-free sweet potato by meristem tip culture. This will help to minimize virus infection and hence produce quality SPFMV-free sweet potato plants in Korea.

MATERIALS AND METHODS

Collection and maintenance of viral culture

Sweet potato cv. Beniotome, Haenam (local var.), and Kokei 14 showing characteristic symptoms on leaves were collected and the cuttings from them were grown in plastic pots containing sterilized soil mixture (1 soil: 2 compost: 1 sand). From these plants, young leaves and meristem tips were chosen for further detection and to produce virus-free plants.

Virus detection by RT-PCR

Young leaves (100 mg) from virus infected sweet potato (both under field condition and in vitro grown) were taken and homogenized in a 1.5 mL micro centrifuge tube with sterilized drill tip containing 200 μl of extraction buffer (3 M guanidium thiocyanate, 10 mM 2-ethane sulfonic acid pH 6.5 and 30 mM EDTA)(Lee *et al.* 1996). The total RNA extraction was followed method as reported earlier by Jeong et al. (2003). RT-PCR was performed in thermo-cycler (Perkin Elmer Model 480, USA). Primer pair based on the conserved region of SPFMV coat protein gene was used that resulted in an amplification product of 411 bp as reported earlier by Jeong *et al.* (2003). For reverse transcription, 20 μl reaction mixture was prepared containing 4 μl sample RNA, 20 pmol primer, 2.5 mol dNTP, 30 units reverse transcriptase (Bioneer, Korea), 5 units RNasin, and 1 unit Taq DNA polymerase (Bioneer, Korea). The mixture was incubated at 42℃ for 45 min followed by incubation at 70℃ for 5 min (for enzyme inactivation) and then placed on ice immediately. For the amplification of cDNA, The reaction was preheated to 96℃ for 2 min and then 40 cycles each consisting of denaturation at 94℃ for 30 sec, annealing at 63℃ for 30 sec and elongation at 72℃ for 1 min were given followed by a final elongation step at 72℃ for 10 min. The PCR product was run on 1.7% agarose gel at 50 V. The gel was stained in ethedium bromide (1 mg/mL) and visualized in UV transilluminator.

In vitro production of sweet potato

Shoot tips (2 cm long) with two or three leaves were chosen from infected plants of sweet potato cv. Beniotome, Haeanm, Kokei 14. Shoot tips were thoroughly washed in running water for 30 min and then in sterilized double distilled water containing few drops of Tween-20 (Sigma). These were further surface sterilized with 70% ethanol and 2% sodium hypochlorite for 10 sec and 10 min, respectively, in laminar flow followed by washing (3-4 times) with sterilized double distilled water. Then the shoot tips

were trimmed with sterilized knife to 0.3-0.5, 0.5-1.0 and 1.0-1.5 mm long and were implanted vertically into MS medium (Murashige and Skoog, 1962) supplemented with BAP (1 mg/L) and NAA (0.05 mg/L). The pH of the medium was adjusted to 5.8 and autoclaved at 121℃ for 15 min. The culture was maintained at 25±2℃ under PPFD 40 μmol · $m^{-2}s^{-1}$ with 70-80% relative humidity.

After 7-8 weeks, developed shoots were subcultured on half-strength MS medium containing 6 g/L agar for rooting. As the roots developed and established in the medium, the rooted plants were taken out from the medium, transferred in plastic pots containing sterilized soil mixture, and were transferred to the hardening chamber (80% relative humidity, 16-h light and 8-h dark period and 25±2℃ temperature). After 30-40 days, the plants were transferred to the sterilized pots containing sterilized soil in an insect proof net house. The plants were routinely checked for virus presence.

RESULTS AND DISCUSSION

Virus detection

In RT-PCR, the expected amplification of 411 bp was obtained in virus infected plants (naturally and in vitro grown) while no amplification was obtained in SPFMV free plants (Fig. 1).

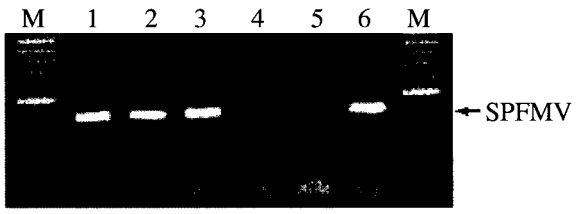

Fig. 1. Gel photograph of one step RT-PCR amplification of in vitro grown sweet potato plants. Lane 1-3shows an amplification of 411 bp in virus infected sweet potato. Lane 4 and 5 show no amplification in virus-free samples. Lane 6 shows a band in positive control, Lane M shows 100 bp DNA ladder.

Meristem culture and shoot multiplication

About 55.0-65.5% meristem tips cultured in MS medium supplemented with BA (1 mg/L) and NAA (0.05 mg/L) sprouted within 2 weeks of culture (Fig. 2A) and shoot differentiation occurred in 5-6 weeks. The shoots were further multiplied in the same medium. About 3-5 long shoots regenerated from a single meristem. Callus was also formed near the base of the meristem and then small shoots appeared from the callus (Fig. 2).

Plant growth regulators, for example, indole acetic acid (IAA) naphthalene acetic acid (NAA), 6-benzyl aminopurine (BA), gibberellic acid (GA_3), are also essential factors for shoot tip culture, and their concentration and ratio in MS medium directly affect growth and differentiation (Yan *et al.*, 1997). To

Table 1. Effect of plant growth regulator on shoot formation from the meristem culture of sweet potato on MS medium after 7 weeks culture

Plant growth regulator (mg/L)		Cultivar					
		Beniotome		Haenam		Kokei 14	
NAA	BA	Shoot/No of explant	Growth response	Shoot/No of explant	Growth response	Shoot/No of explant	Growth response
0.05	1.0	12/20	++++	12/20	++++	12/20	++++
0.05	2.0	10/20	+++	10/20	+++	11/20	+++
0.1	1.0	6/20	++	6/10	++	4/20	++
0.1	2.0	10/20	+	6/10	+	10/20	+

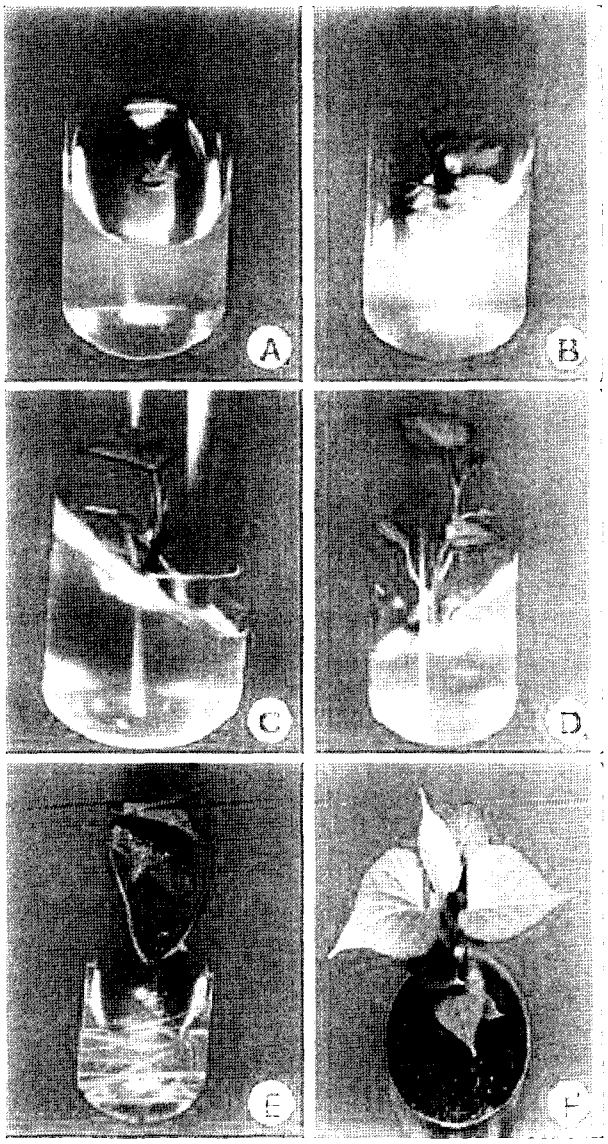

Fig. 2. Production of SPFMV-free sweet potato using meristem tips. (A) initiation of proliferation of shoots along with callus after 2 weeks of culture, (B) proliferation of several shoots after 3 weeks of culture, (C-D) proliferation of virus tested normal shootsafter 5, 6 weeks of culture, (E) in vitro plant with roots after 8 weeks of culture, (F) plantlets in soil after 3 month of culture.

improve the regeneration rate of sweet potato during shoot tip culture, Cheng *et al.* (1990) developed a suitable MS medium supplemented with 1.0 mg/L BA, 0.01 mg/L NAA and 1.0 mg/L GA_3. BA is an essential plant growth regulator for sweet potato shoot tip culture, but a high concentration easily induced the formation of callus, so a low concentration (0.5-1.0 mg/L) should be used (Tang *et al.*, 1994).

The use of meristem and shoot-tip culture for pathogen-free plants is a common practice for the production of virus-free stocks of vegetatively propagated plants. Meristem tips have been used to propagate sweet potatoin MS medium supplemented with various growth regulators, BA and NAA (Eun and Kim, 1999; Ichi 1991) and BA (Litz and Conover 1978). In our experiment, the best response was obtained in MS medium containing BAP and NAA for shoot proliferation and in half-strength MS medium for rooting. Several small shoots also arose from the callus that showed good growth later.

Effect of meristem size on its establishment and virus elimination

Production of virus-free sweet potato plantlets was influenced by the size of shoot tip. A small size meristem (0.3-0.5 mm) produced callus and shoots and 60% SPFMV-free plants were obtained (as indexed by RT-PCR) from this explant. As the size of meristem increases, the percentage of obtaining the virus-free plants decreased and with 1.0-1.5 mm long meristem,

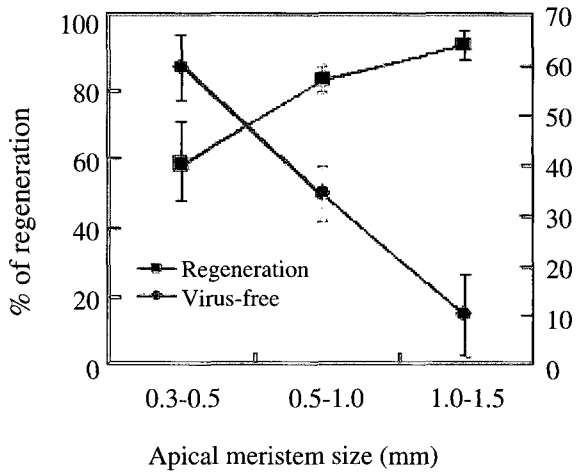

Fig. 3. Effect of apical meristem size on regeneration and ratio of virus-free.

only 10% of virus-free plants were obtained (Fig. 3). Generally, the size of excised shoot tip is negatively correlated with the percentage of virus-free plantlets obtained, and is positively correlated with the percentage of plantlet regeneration (Shang et al., 1996).

Virus elimination depends on various factors such as meristem size, the virus concerned, physiological condition of mother plants and meristem position. The larger the size of meristem, the greater is the number of regenerated plants, while the number of virus-free plantlets obtained, is inversely proportional to the size of the cultured tips (Faccioli and Marani, 1998). In our experiments, meristem tips size of 0.3-0.5 mm was found to be the optimum for eliminating SPFMV from sweet potato.

Rooting, hardening and acclimatization

Proliferated shoots after 8 weeks were transferred to 1/2 MS medium containing 6 g/L agar for rooting and 100% rooting was observed (Fig. 2E). The rooted plants were taken out from the medium and were transferred to the hardening chamber.

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