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Establishment of Mass Propagation System of Virus-Free Sweetpotato Plants and Conservation

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ABSTRACT Sweetpotato fields in Korea are highly infected with virus and virus like diseases that greatly diminish both yield and quality as indicated by field observations and laboratory tests. In order to solve this problem, there is an urgent need to produce and mass propagate virus-free planting materials for distribution to the farmers. These experiments were conducted, firstly, to determine the most appropriate culture media, nutrient solution, and cutting intervals to maintain growth and vigor of tissue cultured plantlets as mother plants for propagation in insect-proof greenhouse. And as a labor saving method, the production efficiency of plug trays for rapid propagation of stem cuttings as a source of planting materials was likewise evaluated. Results showed that plants grown in medium B supplied with 0.5 and 1.0 strength of MS nutrients had high growth rate, and 20-day cutting interval was the best. 72-plug tray was better than 128-plug. Secondly, it was to develop a technique for the production of first-generation seed roots using hydroponics cultivation system. The yield of virus-free plants propagated in the non-insect proof and open-field cultivation was 2,402 kg/10a, 6% higher than those in the insect-proof cultivation, and the rate of virus re-infection was 18% higher compared to 3.3% with insect-proof cultivation. Lastly, it was to investigate the growth performance of virus free plants in farmers' field. Differences were existed in the yield depending on the variety used, but virus free plants showed an increase of 6~24% over virus infected plants.

Keywords : sweetpotato, virus-free, mass propagation, preservation

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Sweetpotatoes, which served as supplementary food item during the times of food shortage, are recently performing a new role as a healthy food item. At the moment, sweetpotatoes are being cultivated in approximately 17,000 ha throughout the country. The main producing districts include Yeoju, Nonsan, Iksan, Haenam and Tongyeong. The field is displaying the trend of slight increase in area cultivated each year.

Important factors of sweetpotatoes sold for table use are taste and quality of outer appearance, such as shape or skin color, etc. (Jeong *et al.*, 2002). Quality of outer appearance is closely regarded to soil texture of the field and also influenced by virus. As a vegetative propagation plant, sweetpotatoes, once infected by virus, pass on the infection into the successive generations. This disables manifestation of the unique characteristics of each variety and lowers both production quantity and quality of outer appearance. Virus, also referred to as toxin or filterable virus, is very small and can only be observed through electronic microscope (Villordon *et al.*, 2003; Hahn *et al.*, 1981). Sweetpotato virus disease is often found in cultivation field (Moyer and Salaza, 1990). However, the symptoms are masked when temperature increases. Therefore, farmers often fail to recognize and overlook the severity of damages (Bryan *et al.*, 2003). Certain types of virus do not cause significant impact on production quantity or quality of sweetpotatoes. However, other types exert very serious damages. Complicated infection by two or more types of viruses often produces severe damages (McCreight, 2000;

Mihovilovich *et al.*, 2000; Abad. *et al.*, 1992). It is being known that over 10 types of viruses are causing damages in sweetpotatoes throughout the world. Main symptoms to appear on storage roots are diverse, such as russet crack, inter-cork phenomenon, quantity reduction, deterioration of shape and surface discoloration, etc. Therefore, in order to prevent the damages of virus and to produce high-quality sweetpotatoes, Japan and China are widely practicing the cultivation method to use virus-free plants. In the near future, Korea will also need to distribute such cultivation technology (Chun and Kozal, 2001). In this study, experiment was administered to verify the method of mass-propagating virus-free plants obtained through tissue culture in net screen house by preventing re-infection and the effect of virus-free plant cultivation by comparing quality of sweetpotatoes produced from virus-free plants and general plants.

MATERIALS AND METHODS

Establishment of virus-free plants mass propagation technique

Disease-free, tissue-cultured sweetpotato plantlets of Yulmi, Shinhwangmi, and Sinjami were used as samples for this experiment. After the plantlets undergo acclimatization, they were transplanted and grown in horticultural box filled with the culture media. The media used were a mixture of rockwool and perlite (R+P), commercial soils A (EC : 0.8~1.2 ds/m, NH_4^+-N : 120~150 mg/L, NH^{3-}-N : 210~270 mg/L, P_2O_5 : 270~330 mg/L, K_2O : 60~90 mg/L, CEC : 9~11 $cmol^+/L$) and commercial soils B (EC : 0.7~0.9 ds/m, NH_4^+-N : 60~90 mg/L, NH_3^--N : 1800~230 mg/L, P_2O_5 : 230~280 mg/L, K_2O : 60~100 mg/L, CEC : 8~10 $cmol^+/L$). Commercial soil A has low level of fertilizer, small particle size, and hold water very long. Commercial soil B has high level of fertilizer, larger particle size than A, and hold water at a shorter time. Concentration of nutrient solution was set in three levels of 0.5 MS, 1.0 MS and 1.5 MS nutrients. Collection of cuttings was done with an interval of 10, 15, and 20 days.

In order to determine the propagation efficiency of plug trays, 72-plug and 128-plug trays were used. Main stem length, branching stem length, total vine weight and total root weight were examined using 1 and 2-node cuttings.

Maintenance of virus-free plants

Since sweetpotatoes are highly susceptible to virus and virus like diseases, it is important to ensure that virus-free plants stayed disease-free during propagation. To determine the rate of re-infection during propagation, virus-free sweetpotatoes were planted in insect-proof greenhouse using net screen as protection and in plastic house. The leaves from the upper, middle, and lower parts were sampled and observed under electron microscopy.

Production of virus-free seed roots using hydroponics

To produce virus-free seed roots using hydroponicsin insect-proof greenhouse, virus-free cuttings of Yulmi, Shinhwangmi, and Sinjami were planted on the 22nd of June in plastic bed of 25×73×18 cm. The three types of media used were sand, mixture of perlite and rockwool, and loess. MS nutrient was used as the nutrient solution and it was supplied 1 to 3 times per day with 20 liters per application. After harvesting on the 11th of November, quantity and status of virus infection were investigated.

Virus-free plants field trials

Virus-free cuttings were planted and grown in the field of selected farmers on the 15th of June. On the 20th of August, at which plant growth was thriving, foliar symptoms of infection caused by virus were investigated on the field. After harvesting on the 16th of October, yield and quality such as starch, brix, skin color, and appearance quality were investigated. All field cultivation was followed by standard cultural practices of Rural Development administration.

RESULTS AND DISCUSSION

Establishment of virus-free plants mass propagation technique

After sampling and culturing apical meristems, status of virus infection was evaluated on each plantlet. Materials

Table 1. Effect on the multiplication by the concentration of nutrient solution, medium and cutting interval on Yulmi varieties in sweetpotato.

Conc. of Nutrient solution		1/2 MS			1.0 MS			1.5 MS		
Medium	Cutting interval	TVW† (g)	SW (g/shoot)	RM	TVW (g)	SW (g/shoot)	RM	TVW (g)	SW (g/shoot)	RM
R+P	10	857	1.8	39.3	867	1.7	28.6	910	2.1	27.9
	15	751	1.8	27.9	1,592	2.6	43.2	1,599	2.6	41.4
	20	1,487	2.4	40.0	1,432	2.7	34.4	1,276	2.4	42.6
A	10	1,113	1.9	38.7	1,339	2.3	39.1	826	1.8	23.8
	15	1,470	2.7	35.9	1,324	2.5	37.6	1,451	2.3	43.8
	20	1,585	2.9	36.9	1,645	2.7	43.6	1,604	2.7	41.7
B	10	1,145	2.1	38.1	771	2.2	19.7	782	1.9	36.9
	15	1,544	2.5	43.9	1,475	2.5	41.4	1,663	2.6	45.5
	20	2,668	2.8	64.4	2,189	2.8	52.2	1,906	2.6	51.3

†TVW : Total vine weight, SW : Shoot weight, RM : Rate of multiplication, R+P : Rockwool + perlite, A : Commercial soil A, B : Commercial soil B

that showed negative for virus symptoms were used as samples. They were potted and grown in incubation room. When the plantlets had obtained 4 to 5 nodes, they were prepared for multiplication in insect-proof greenhouse.

In order to determine the proper condition for mass propagation of virus-free plants, total vine weight, shoot weight, and rate of multiplication were evaluated per cutting interval, concentration of nutrient solution, and type of medium used. Results showed that multiplication rate varied among the cultivars. Yulmi had the lowest multiplication rate while Sinjami had the highest among the three cultivars used. In Yulmi, total vine weight was high in commercial soil B. The highest multiplication rate of 64 times was obtained in experimental plot supplied with 0.5 MS nutrients with a cutting interval of 20 days. On the other hand, the rate of multiplication was very low in rockwool+perlite in cutting interval of 10 days (Table 1). In case of Shinhwangmi, plants grown in rockwool+perlite displayed higher rate of multiplication with cutting intervals of 15 and 20 days compared to 10-day interval. In commercial soil A, multiplication rate of 70 times was observed in experimental plot supplied with 1.5 MS nutrients and cutting interval of 15 days. In commercial soil B, the highest multiplication rate of 75~79 times was observed in experimental plot to use 15, 20 days of interval and 1.0 and 0.5 MS nutrients (Table 2). Sinjami was the cultivar that showed the highest multiplication rate. In commercial soil A and B to use 0.5 MS nutrients, it respectively displayed high multiplication rates of 136 and 120 times when employing cutting intervals of 20 days. In case of employing cutting interval of 15, 20 days by using commercial soil B, high multiplication rate was displayed regardless of the concentration level of nutrient solution. On the other hand, shoot weight, compared to other varieties, was found to be relatively lower (Table 3). Thus, plants that were grown in commercial soil B and supplied with 0.5~1.0 MS nutrients had high growth, and 20-day cutting interval was the best.

As a labor-saving method, the propagation efficiency of plug trays for rapid propagation, stabilization and production of virus-free cuttings throughout the year was also determined. Virus-free plantlets were grown in incubation room until they developed 20 nodes, which was approximately 20 days. Stem cuttings with 1 and 2 nodes each were taken from these plants and then planted in 72-plug and 128-plug trays. The cuttings were grown in growth chamber of 27/25℃ in daytime and nighttime with a photoperiod of 16/8 hours. In order to develop a technique

Table 2. Effect on the multiplication by the concentration of nutrient solution, medium and cutting interval on Shinhwangmi of varieties in sweetpotato.

Conc. of Nutrient solution		1/2 MS			1.0 MS			1.5 MS		
Medium	Cutting interval	TVW† (g)	SW (g/shoot)	RM	TVW (g)	SW (g/shoot)	RM	TVW (g)	SW (g/shoot)	RM
R+P	10	1,069	1.9	31.5	1,380	2.0	51.1	1,275	2.0	43.1
	15	1,554	2.1	43.4	2,237	2.4	60.9	1,975	2.3	59.1
	20	2,330	2.6	59.2	2,484	2.8	61.6	2,080	2.5	56.4
A	10	1,573	2.2	49.0	1,610	1.7	59.6	1,830	2.1	58.1
	15	1,792	2.4	51.5	1,970	2.5	55.4	2,099	2.0	70.0
	20	2,638	2.8	64.4	2,507	2.6	65.9	2,133	2.6	56.8
B	10	2,138	2.2	66.0	1,505	2.2	42.3	1,847	2.2	57.3
	15	2,171	2.4	79.2	2,318	2.3	69.9	2,445	2.4	71.0
	20	3,262	3.0	75.3	2,730	2.4	76.8	2,563	2.6	68.4

†TVW : Total vine weight, SW : Shoot weight, RM : Rate of multiplication, R+P : Rockwool + perlite, A : Commercial soil A, B : Commercial soil B

Table 3. Effect on the multiplication by the concentration of nutrient solution, medium and cutting interval Sinjami varieties in Yulmi.

Conc. of Nutrient solution		1/2 MS			1.0 MS			1.5 MS		
Medium	Cutting interval	TVW† (g)	SW (g/shoot)	RM	TVW (g)	SW (g/shoot)	RM	TVW (g)	SW (g/shoot)	RM
R+P	10	1,057	1.3	59.5	1,694	1.6	78.2	1,643	1.5	76.4
	15	1,537	1.6	71.4	1,594	1.5	76.5	1,998	1.8	80.0
	20	1,728	1.7	79.7	1,954	1.8	77.6	1,959	1.8	80.5
A	10	1,991	1.7	92.3	1,779	1.6	81.1	1,790	1.6	80.4
	15	1,824	1.8	76.5	2,083	1.7	89.9	1,957	1.6	88.4
	20	3,535	1.9	136.3	1,915	1.9	78.7	2,428	1.8	98.7
B	10	1,993	1.5	95.3	2,108	1.5	88.5	1,951	1.6	84.7
	15	2,494	1.8	103.3	2,604	1.9	99.2	2,249	1.8	93.3
	20	3,193	1.9	119.7	2,541	1.8	97.9	2,489	1.8	104.9

†TVW : Total vine weight, SW : Shoot weight, RM : Rate of multiplication, R+P : Rockwool + perlite, A : Commercial soil A, B : Commercial soil B

for the production efficiency of plug trays in net screen house, the growth rate was investigated by 1, 2 cutting nodes with leaves from the plants grown in 72-plug and 128-plug trays. The result showed that the cuttings with 1 and 2 nodes planted in 72-plug tray had longer branch length and heavier total vine weight compared to 128-plug tray (Table 4). Also, in order to investigate the seedling quality in accordance with the type of medium used, tray

Table 4. Production efficiency of plug trays on multiplication of shoot in Yulmi Variety.

Tray type	No. of node	Main Stem length (cm)	Branching stem length (cm)	TVW† (g/shoot)	TRW (g/shoot)
128-plug (18.5 ml)	1	4.09a	-	1.71b	0.48a
	2	4.38a	2.04a	2.54b	0.48a
72-plug (32.0 ml)	1	5.58a	-	1.84b	0.37a
	2	5.58a	4.66b	4.00a	0.68a

†TVW : Total vine weight, TRW : Total root weight, SW : Shoot weight

Table 5. Shoot soundness according to nutrient solution, medium and number of tray hole in in Yulmi Variety.

Variety	Nutrient solution	Medium	No. of tray hole	Vine length (cm)	No. of node (No./shoot)	SR† (%)	TVW (g/shoot)	VI (%)
Sinjami	Water	Commercial soil	72	18.3	9.5	72.0	33.0	0
			128	14.6	9.0	72.0	53.0	0
		Vermiculite	72	12.1	8.0	84.5	46.0	0
			128	10.5	6.5	77.0	27.0	0
	Nutrient solution	Commercial soil	72	18.1	10.0	93.0	52.7	0
			128	17.0	8.0	81.0	56.4	0
		Vermiculite	72	15.7	8.0	87.5	29.7	0
			128	13.5	7.0	79.0	33.0	0
Shin-hwangmi	Water	Commercial soil	72	17.8	9.5	86.0	36.5	0
			128	13.0	7.0	85.5	37.0	0
		Vermiculite	72	16.2	7.0	89.0	24.1	0
			128	14.0	6.0	69.0	18.5	0
	Nutrient solution	Commercial soil	72	16.9	8.5	94.5	47.1	0
			128	16.7	6.5	73.0	42.5	0
		Vermiculite	72	15.0	7.5	92.0	39.4	0
			128	13.2	6.0	79.0	38.9	0

†SR : survival rate, TVW : Total vine weight, VI : Virus infection

size and concentration of nutrient solution, 2-node seedling was planted on the 12th of June. Then, number of node, diameter, vine weight, survival rate and total vine weight were observed on the 16th of July. In terms of the medium used, commercial soil displayed superiority to vermiculite. In terms of the size of tray, 72-plug tray was found to be better than 128-plug tray (Table 5).

Maintenance of virus-free plants

It takes approximately 5~6 months to sample apical meristem from stems of sweetpotatoes, to culture, and to finish the stage of multiplying cultured seedlings equipped with the complete leaves and stems in greenhouse. Since sweetpotatoes are highly susceptible to virus and virus like diseases, it is important to ensure that virus-free plants stayed disease-free during propagation. Appropriate method of virus prevention is yet to be developed and chemical control is also not possible. Therefore, it is important to block off the sources of contamination in advance (Karyeija *et al.*, 2000; CIP, 1990). Viruses are spread by aphids or greenhouse whitefly (Trialeurodes vaporariorum) to sweetpotatoes and infected through planting material. Therefore,

the most important management method of all is to keep the plants in net screen and to apply several types of chemicals on a regular basis.

Net screen was installed in order to lower the rate of re-infection in the stages of virus-free plant multiplication and supply. Then, compared against those without installation of net screen house, the degree of virus infection was observed (Table 6). For approximately 20 days, during which original virus-free plants grew up to 10~15 joints, the rate of virus infection was found to be 0% in net experimental plot. However, it was indicated as 5% in non-net experimental plot. While the rate was 0% in net experimental plot during the course of cutting 2 joints each of original virus-free plants and of primary multiplication of them for 30 days in 72-plug tray, it was observed to be high at 27.6% in non-net experimental plot. Also, the result of administering secondary multiplication through isolated cultivation on seedlings sampled from the primary multiplication indicated the rate to be 0% in net experimental plot. However, it was found to be high at 31.4% in non-net experimental plot. Such results are estimated to have a close relevance to influx of insect vector, such as aphids. In particular, greenhouse has conditions suitable for propagation of insect vectors in terms of the temperature, humidity, etc. Therefore, it is crucial to administer thorough preventive measures in advance.

In addition, as a result of producing virus-free seedlings in net isolated cultivation and non-net isolated cultivation, the average yield in the case of non-net isolated cultivation was 2,402 kg/10a, which was 6% higher compared with 2,268 kg/10a in net isolated cultivation. Also, the rate of virus infection was found to be 3.3% in net isolated cultivation and 18% in non-net isolated cultivation (Table 7). These results indicated that viral re-infection can be effectively prevented even in farmhouses without glass house by installing net screen house when administering isolated cultivation of virus-free plants.

Virus-free seed roots production using hydroponics

In order to multiply and maintain virus-free plants, there are ways to continuously propagate the plants by node in net screen house or to produce virus-free seedlings by hydroponics and to sow them in seedling bed the following year.

For development of virus-free seedling multiplication technology by using hydroponics system, virus-free plants of Yulmi, Sinjami and Shinhwangmi were cultivated in rockwool + perlite, loess and sand as the media for investigation on their production yield (Table 8). Production yield was higher by 39~59% in Yulmi, 68% in Sinjami and 21~40% in Shinhwangmi in sand medium than in rockwool + perlite or loess. Virus infection rate was 0%.

Table 6. Prevention of virus infection on multiplication and growth stage in Yulmi Variety.

Growth stage	Virus free shoot (Green house)		First multiplication (Green house)		Second multiplication (Upland field)	
	20 days		30 days		30 days	
	Control	Net	Control	Net	Control	Net
Virus infection (%)	5	0	27.6	0	31.4	0

Table 7. Prevention of virus infection on soil multiplication of Yulmi, Shinhwangmi, and Sinjami in sweetpotato.

	Upland field cultivation(control)				Upland field cultivation(Net house)			
	Yulmi	Shinyulmi	Shin-hwangmi	Mean	Yulmi	Shinyulmi	Shin-hwangmi	Mean
Marketable storage root (ton/ha)	21.4	23.3	27.4	24.0	20.5	22.4	25.1	22.7
Infection rate (%)	18	15	21	18	4	3	3	3.3

Table 8. Virus - free seed root production using nutriculture system

Variety	Medium	Length of main vine (cm)	No. of vines	Vine weight (kg/plastic bed)	Marketable storage root (kg/plastic bed)	Infection rate of virus
Yulmi	Rockwool + perlite	203	5.4	6.97	1.46	0
	Loess	213	4.0	12.00	1.83	0
	Sand	228	4.9	10.73	3.42	0
Sinjami	Rockwool + perlite	125	7.9	7.30	2.80	0
	Loess	155	9.8	9.07	1.39	0
	Sand	124	6.1	6.87	3.18	0
Shin-hwangmi	Rockwool + perlite	235	5.2	9.50	2.35	0
	Loess	228	4.9	15.77	1.92	0
	Sand	315	4.5	12.40	3.18	0

Table 9. Growth, storage root yield and appearance quality of virus-free cutting of sweetpotato.

Variety	Cutting	Vine weight (ton/ha)	Marketable tuberous root (ton/ha)	Index	Infection rate of virus (%)	Starch (%)	Brix° %	Skin Color (Hunter-a)	Appearance quality (1~9)
Yulmi	virus-free	3,556	3,002	116	3.5	26	5.4	20.7	7
	Control	3,556	2,599	100	100	23.9	4.2	17.6	3
Sinjami	virus-free	4,028	2,886	106	4.5	21.2	3.4	17.9	7
	Control	4,417	2,729	100	100	21.1	4.8	25.3	3
Shin-hwangmi	virus-free	3,333	3,810	124	4	18.8	6.3	29.7	7
	Control	3,389	3,079	100	100	18	3.1	21	3

Plants field trials

Sweetpotatoes undergo vegetative propagation, therefore, when infected by virus, the infection continues onto the successive generations. The infection rate may also increase by insect vector. Therefore, even plant varieties of superior properties may not produce their intrinsic qualities in the end (Abad *et al.*, 1992).

After cutting virus-free plants multiplied in greenhouse into the field of selected farmers, their production quantity, degree of virus re-infection and quality-related characteristics were investigated. While there was no difference in quantity per plant variety, virus-free plants yielded an increase by 6~24% compared to general plants. Also, the rate of re-infection was found to be 3.5~6.0% (Table 9). Results of analysis on external quality of sweetpotatoes indicated improvement in coloration as the most significant quality improvement of virus-free plants. Using color meter (CM-508d, Minolta, Japan), surface color of sweetpotatoes were indicated as a value among L value (whiteness), a value (redness) and b value (yellowness). Although there were differences among plant varieties, redness of virus-free plant was found to be 29.7 in Shinhwangmi, which was higher than 21.0 of general plants. Also, the overall assessment on external qualities produced the result of 7 for virus-free plants, which was higher than 3 for general plants (Table 9).

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