Approaches to Multiplication and Supply of Virus-Free Japanese Yam (*Dioscorea japonica*) Seed Tubers©

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We are students majoring in biological engineering at Iyo Agricultural High School. Since 2008 numerous attempts have been made by us to provide virus-free Japanese yam (*Dioscorea japonica* Thunb.) seed tubers with the help of the local Japanese yam production guild in Hirota village. In the past the guild had received the seed tubers from the Ehime Prefectural Agriculture, Forestry and Fisheries Examination Institution but they are not now supplied.

In Stage 1 of the original micropropagation method for the production of virus-free plantlets, the shoot apexes were cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA) phytohormones (Table 1). In the current study, however, we used hormone-free medium, because we should not overlook that there is a possibility of epigenetic changes when phytohormones are used. The explants used for the study are not the meristem apexes but larger segments including the meristem. We also need to take measures to prevent a virus from entering these explants.

Table 1. Murashige and Skoog (MS) medium growth regulator and sucrose treatments.

Material	MS strength	NAA	BA	Sucrose
MS	1/1	0.02 mg/L	0.2 mg/L	30 g/L
MS	1/1	0	0	30 g/L
Amaotome	4.6	9.7		

The objective of the current study was investigation into the possibility of producing a stable supply of virus-free (Editor's note: refers to free from Japanese yam mosaic virus) Japanese yam seed tubers from microbulbils by using explants that are not infected with Japanese yam mosaic virus (JYMV) as determined by using the reverse transcription-polymerase chain reaction primers (RT-PCR, Table 2).

From July through August, 160 stem segments with the axillary buds were taken from the vines of one Japanese yam plant. The length of the each segment was about 3 cm. The segments were soaked in the alkaline detergent (MYPET, Kao Co.,

Table 2. Specific primers used in the present study to detect Japanese yam mosaic virus (JYMV).

Target virus	Primer	Sequence	Ampricon size (bp)	Reference or accession
JYMV	125F	5`-TTGGATGATAATTCAATGCAA-3`	241	AB430808
	12345R	5`-GTGGCATATACGCTTTTTC-3`		AB430808

Japan) for 10 sec and washed with water; for surface sterilization, segments were soaked in 10% chlorine bleach (HYTER, Kao Co., Japan) for 10 min and rinsed with sterilized water. The ends of the surface sterilized segments were trimmed (about 5 mm) prior to placing on hormone-free MS medium. To develop micro-bulbils from the shoots, the culture conditions were maintained at a constant temperature of 23 °C under a 14-h photoperiod by white fluorescent lamps.

Some axillary buds became micro-bulbils; the others have elongated shoots with micro-bulbils. Finally, elongated shoots were induced from all segments, 90 elongated shoots out of 160 could have 1 or 2 micro-bulbils each that weighed 0.15 g on average. These micro-bulbils were kept in cold storage, and sprouted after planting.

In conclusion, the reproduction of Japanese yam micro-bulbils is possible by using stems with axillary buds. In addition, micro-bulbils developed on them can be preserved at 4 °C in a refrigerator and they need no acclimatization.

Hirota Japanese yam production guild has a greenhouse (about 1 acre) for seed tubers production. Every spring, the superior seed tubers were cut into pieces of about 10 g each and planted five pieces each into 120 large pots; in autumn, they produce about 60-kg seed tubers in all. The guild supplies these tubers to the farmers.

For our produced micro-bulbils to apply to this seed-tubers production system future research will be needed to examine how large the seed tubers will become from micro-bulbils. In addition, we have been trying to supply virus-free Japanese yams stably by the use of reverse transcription loop-mediated isothermal amplification (RT-LAMP) methods.

LITERATURE CITED

Iida, T. 2001. Sin-tokusan-sirizu jinenjyo. noubunkyo. 7-6-1 Akasaka, Minato-ku, Tokyo, Japan.

Kajihara, H., K. Muramoto, S. Fuji, S. Tanaka, and **S. Ito.** 2008. Shimultaneous detection of Japanese yam mosaic virus and yam mild mosaic virus from yam leaves using a tube capture reverse transcription-polymerase chain reaction assay. J. Gen. Plant Pathol. 75:72–75.

Murashige, T., and **F. Skoog.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473–479.