Plant Regeneration from Septum Segment of a Water Plant Pak-bung (*Ipomoea aquatica*)

Kazuhiro Mori¹, Hiroyuki Igehara¹, Kazuya Yoshida², Atsuhiko Shinmyo² and Masanori Fujita¹

¹Department of Environmental Engineering, Graduate school of Engineering, Osaka University
/ 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
²Graduate School of Biological Sciences, Nara Institute of Science and Technology
/ 8916-5 Takayamacho, Ikoma, Nara 630-0101, Japan

ABSTRACT

Pak-bung (*Ipomoea aquatica*) is a water plant which is useful to the water purification due to the high nutrient-removal capacity. An efficient plant regeneration method from septum segment of pak-bung were established. The septum calli were induced on the modified MS solid (MMS) medium supplemented with 1 mg/l of 1-naphthalene-acetic acid (NAA) and benzyladenine (BA) respectively. Shoots were regenerated from septum calli with various combinations of BA (0 - 10 mg/l) and NAA (0 - 1 mg/l) and the highest regeneration frequency (77%) were observed on the medium containing 5 mg/l of BA. Roots were induced successfully from all of the regenerated shoots applied to the rooting test and plantlets were reproduced. There was no morphological difference between regenerated plants and plants grew up from seeds. Using the culture conditions established in this study, the regenerated plant of pak-bung can be produced in a short period (4 - 6weeks).

Key words: pak-bung (*Ipomoea aquatica*), plant regeneration, septum tissue

INTRODUCTION

Pak-bung (*Ipomoea aquatica*) is a water plant originated from Southeast Asia and very popular as a vegetable there with abundance of proteins and vitamins¹,². This plant grows quickly in warm condition and is tolerant to waste water, so is considered to be useful as a nutrient absorber in the advanced waste water treatment³. Waste water is purified in the rhizosphere of pak-bung by the nutrient uptake by the plants, filtration effect of the root hairs and oxidation effect of the microorganisms attached to them as known in many vascular or floating aquatic plants⁴ such as water hyacinth (*Eichhornia crassipes*)⁵-⁸, water lettuce (*Pistia stratiotes*)⁵,⁶,⁸ and duckweed (*Lemma spp.*)⁵,⁹. The suspended solids are filtrated by the roots and the dissolved organic carbons or ammonia are oxidized by the rhizosphere bacteria which are activated by dissolved oxygen transported from plant roots. Pak-bung shows high degrees of nitrogen and phosphorus removal capabilities (6.7 g-N/m²/month and 1.9 g-P/m²/month) and they compare favorably with those of water hyacinth (9.9 g-N/m²/month and 1.9 g-P/m²/month)⁵. Because of the weak tolerance to the low temperature, however, application of pak-bung to the wastewater treatment is limited to summer season
(from June to October) in Japan. Then, we considered that improvement of pak-bung manipulated by the genetical engineering techniques could overcome this weakness and improve the potential of the nutrient absorbency of pak-bung stronger. Plant DNA transformation techniques have been developed since 1980's mainly in the agricultural species\(^{10}\). There was, however, no study on the effective regeneration and genetic transformation system in such useful water plants to the wastewater treatment as pak-bung. We have already reported the callus induction from septum segment\(^{11}\), and in this paper we present an efficient plant regeneration from septum segment of pak-bung which will be useful in the manipulation of transgenic pak-bung.

**MATERIALS AND METHODS**

1. Plant materials

Pak-bung seeds were purchased from Takii co. (Kyoto, Japan). Seeds were surface-sterilized with 70% ethanol for 5 min, then in 0.5% NaOCl for 30 min, and rinsed 5 times with sterilized water. For germination, seeds were placed on Murashige and Skoog's (MS) medium\(^{12}\) supplemented with 3% sucrose and 0.25% gellan gum, pH 5.8 in vitro. The cultures were incubated at 25 °C under 16 h photo period (3000 lx). Two-weeks-old pak-bung seedlings was used as the source of tissue culture.

2. Callus induction and plants regeneration

Modified MS (MMS) medium consisted of two-times-diluted MS inorganic salts with m-inositol 100 mg/l, thiamin-HCl 0.1 mg/l, folic acid 0.5 mg/l, and sucrose 30 g/l supplemented with gellan gum 2.5 g/l was used for the basal medium of tissue culture. The septum segments and leaf disks of in vitro plants were cut off with sterilized knife in a petri dish and were cultured on the callus inducing medium which consisted of MMS salts, 1-naphthalene-acetic acid (NAA) 1.0 mg/l, benzyladenine (BA) 1.0 mg/l, and gellan gum 2.5 g/l. The calli were transferred to the MMS medium supplemented with various concentrations of NAA (0, 0.05, 0.2, 1.0 mg/l) and BA (0, 0.1, 0.5, 5.0, 10 mg/l) to examine the shoots regeneration. The cultures were incubated at 25 °C under 16 h photo period (3000 lx).

The regenerated shoots were cut off from the calli and transferred to the MMS medium in the test tube of 5 cm-height and covered with Milli-Wrap filter (Millipore Japan co., Tokyo, Japan) for rooting. The regenerated plants were transferred to bottles with tap water contained 0.2% Hyponex (Murakami Product co., Tokyo, Japan) and incubated at 25 °C under 16 h photo period (3000 lx).

3. Analytical methods

Nitrogen and phosphorus contents of pak-bung were analyzed according to the Analytical Methods for Agrotype Plants\(^{13}\).

**RESULTS AND DISCUSSION**

1. Callus formation from septum segment

Septum segments were incubated on the callus inducing medium containing NAA 1.0 mg/l and BA 1.0 mg/l. Small calli friable and light yellow in color were induced within 5 days. Auxin like NAA, 3-indolylacetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) is known to induce the callus formation in many plant species including some Ipomoea spp.\(^{14, 15, 16, 17}\). For high callus formation rate from septum tissue of pak-bung, the effectiveness of NAA as auxin, BA as cytokinin and the MMS medium as the basal medium was reported\(^{11}\). Especially, BA plays an important role in the callus formation of pak-bung. In this study we also used MMS medium containing 1 mg/l of NAA and BA.

2. Shoots regeneration from septum segment

The septum calli were transferred to the MMS medium with various combinations of
NAA and BA. Shoots were regenerated after more than 2 weeks incubation in various media testing here. The effect of the combinations of NAA and BA on shoot regeneration from septum calli were shown in Table 1. In general, the combinations of high concentrations of cytokinin and low concentrations of auxin induce the shoots regenerations as shown in tobacco\textsuperscript{10}. In case of pak-bung, BA was also effective and the highest shoots regeneration frequency (77\%) was observed in the MMS medium containing BA 5 mg/l without NAA. Shoot regeneration, however, was also observed in the medium containing neither BA nor NAA. But morphological differences were observed between in the regenerated shoots obtained in the medium containing high concentrations (5 or 10 mg/l) and low concentrations (0, 0.1 or 0.5 mg/l) of BA. In the MMS medium containing high concentrations of BA, more than two shoots with well developed leaves were mainly regenerated per one callus (Fig. 1a). On the other hand, one shoot with well developed stem were regenerated per one callus in the case of low concentrations of BA (Fig. 1b). The fact that shoots also regenerated on the MMS medium without BA and NAA suggests two possibilities. One is that the septum calli contained endogenous cytokinin or auxin. The other is that anlagen of the shoots had been already formed in the septum tissue cut off from the pak-bung plantlet and the shoots with well developed stems might originate from them.

In \textit{I. batatas}, Otani et al. also suggested that

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
BA (mg/l)* & NAA (mg/l)* & No. of calli & No. of calli with shoot (%) \\
\hline
0 & 0 & 14 & 7 (50.0) \\
0.05 & 0.05 & 23 & 6 (26.1) \\
0.2 & 0.2 & 18 & 7 (38.9) \\
1 & 0.1 & 13 & 6 (46.2) \\
0.5 & 0 & 24 & 11 (45.8) \\
0.05 & 0.05 & 22 & 11 (50.0) \\
0.2 & 0.2 & 33 & 15 (45.5) \\
1 & 1 & 32 & 6 (18.8) \\
0.5 & 0 & 25 & 7 (28.0) \\
0.05 & 0.05 & 14 & 7 (50.0) \\
0.2 & 0.2 & 14 & 7 (50.0) \\
1 & 1 & 33 & 4 (12.1) \\
5 & 0 & 13 & 10 (76.9) \\
0.05 & 0.05 & 23 & 13 (56.5) \\
0.2 & 0.2 & 30 & 12 (40.0) \\
1 & 1 & 15 & 2 (13.3) \\
10 & 0 & 33 & 15 (45.5) \\
0.05 & 0.05 & 29 & 13 (44.8) \\
0.2 & 0.2 & 32 & 15 (46.9) \\
1 & 1 & 13 & 2 (15.4) \\
\hline
\end{tabular}
\caption{Effect of the BA and NAA concentrations on shoots regeneration from septum callus of pak-bung.}
\end{table}

*The septum calli were incubated on the shoot inducing medium containing various combinations of BA and NAA.
the leaf calli of sweet potato contain endogenous cytokinin and exogenous supply of cytokinin inhibits the shoot regeneration \(^{16}\). But in pak-bung (\textit{I. aquatica}), the addition of \(5 \text{ mg/l} \) of BA increased the shoot regeneration frequency. So, we concluded that the MMS medium supplemented with \(5 \text{ mg/l} \) of BA is optimum as the shoot inducing medium for septum calli, not for the anlagen. The shoots regenerated in this medium were used in the following rooting experiments.

3. Callus formation and shoots regeneration from leaf disk In various \textit{Ipomoea} species including \textit{I. batatas} \(^{14}\) and \textit{I. trichocarpa} \(^{19}\), regeneration from leaf calli were reported. We also studied about the callus induction and shoots regeneration from leaf disks of pak-bung. Leaf disks were incubated on the callus inducing medium containing \(1.0 \text{ mg/l} \) of NAA and BA. Small calli were induced after 5 days incubation. The leaf calli were transferred to the MMS medium with various combinations of NAA and BA concentrations. But no shoots were regenerated (data not shown).

4. Roots induction from regenerated shoots The shoots regenerated from the septum calli on the MMS medium supplemented with \(5 \text{ mg/l} \) of BA were excised from the calli in aseptic condition and transferred to the MMS medium without BA and NAA. Roots were induced from all of the shoots after one week incubation (Fig. 2). There was no morphological difference between the regenerated plants and plants grew up from seeds after germination. The nitrogen and phosphorus contents of the regenerated plants were \(5\%\) and \(0.9\%\) of total dry weight respectively which are the same value as the plants grew up from seeds.

We could regenerated the plants from septum segments efficiently. The time required to produce the regenerated plants from septum segment under the culture condition of the present study is 4 to 6 weeks and it is shorter than that of another \textit{Ipomoea}
The regeneration frequency of pak-bung of this study is also very high (77%). As the regenerating manipulation did not affected the nutrient contents of pak-bung, the nutrient removal capabilities seemed to be not affected by regeneration. Some efficient plant transformation techniques like *Agrobacterium* method\(^{20-22}\) or particle bombardment method\(^{23,24}\) are developed and usually used to the production of transgenic plants. The former is originated to the natural transformation system of soil bacterium *Agrobacterium tumefaciens* harboring the Ti plasmid and mainly used to the dicotyledonous plants and the later is a physical gene transformation method and used to the monocotyledonous plants. But using these techniques, the step of the plant regeneration from the genetic transformed cell is finally needed. In case of production of transgenic pak-bung with various foreign genes which is useful to the wastewater treatment, effective regeneration system with high regeneration frequency and the short time needed for the regeneration is necessary. The effective regeneration system established in this study will be successfully used to the genetic transformation of pak-bung.

**CONCLUSION**

An regeneration method of water plant pak-bung which would be applicable to the breeding of transgenic plants useful to the water purification under various conditions were examined. The following conclusions were obtained.

1. Septum calli used to the shoot regeneration can be induced on the MMS medium supplemented with NAA 1.0 mg/l and BA 1.0 mg/l.
2. Shoots can be regenerated from the
septum calli on the MMS medium supplemented with BA 5.0 mg/l efficiently with high regeneration frequency of 77%.

(3) Roots can be induced from almost all regenerated shoots on the MMS medium without BA nor NAA and regenerated plants can be produced. It takes only 4 to 6 weeks to regenerate the plants.

(4) The regenerated plants show no morphological abnormality and the nutrient contents are also same as that of original plants grew up from seeds. These results indicate that this regeneration system can be used to the breeding of plant materials which are useful in the water purification.

ACKNOWLEDGMENTS

This work was supported by the Research for the Future Program (RFTF) (Project No. JSPS-RFTF9616001) of the Japan Society for the Promotion of Science (JSPS).

REFERENCE


16) Murata, T. and Y. Miyaji: Regeneration of


(Submitted 1998.1.20)

(Accepted 1998.9.16)