Regeneration of *Farfugium japonicum* Through Adventitious Shoot Formation from Leaf and Petiole Explants©

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Donor plants were produced from meristems on half-strength-MS medium supplemented with 0.5 mg·L^{-1} BA. From the in vitro plant, leaves and petioles were excised, and leaves were divided into two halves, distal and proximal ones. The petioles were successively divided into three segments. These were used for explants. Direct adventitious shoots were actively formed from these explants on the MS medium supplemented with 1 mg·L^{-1} thidiazuron (TDZ) and 0.1 mg·L^{-1} naphthaleneacetic acid (NAA). In the divided half of leaf, the position forming most actively adventitious shoots was the proximal end (cut section) of distal half. In petioles, the number of adventitious shoots increased toward the proximal direction. Such a polarity was considered to be due to a gradient in age of petiole tissue. The adventitious shoots rooted easily in the hormone-free medium. The regenerated plants flowered after about 1 year from the beginning of the explant culture.

INTRODUCTION

Farfugium japonicum, a perennial in the Asteraceae Family, is a plant native to Japan. The plant grows indigenously in the forest areas of the southeast coast of Japan and has yellow flowers in autumn. The long petioles of the plant have a nice taste when boiled with soy sauce. Therefore, the plant is not only utilized as an ornamental in the garden, but also cultivated in the field for food.

The plants are usually propagated by division or from seeds. Meristem aseptic culture is also used for clonal propagation in some nurseries. The micropropagation is expected to be effective for rapid propagation of elite clones. From this point of view, the authors reported on the propagation by in vitro division from the crown segment explants and through the formation of adventitious shoots from petiole explants (Yamamoto et al., 1999). Furthermore, micropropagation by direct formation of shoots from hypocotyl segments and two halves of cotyledons of donor plant were reported (Yamamoto et al., 2000). In these experiments, it was found that thidiazuron (TDZ) in the medium had promotive effect on the direct formation of adventitious shoots from the explants. In general, it is found that the explants from different organs or from different tissues within an organ vary in morphogenic capacity (George, 1993).

Therefore, we investigated the relationship between the potential formation of adventitious shoots and the position within two halves of leaves, as well as from successively divided petiole explants of *F. japonicum*.

MATERIALS AND METHODS

Farfugium japonicum growing on the campus of Minami-Kyushu University was used for experimental materials. The meristem tissue of the plants is formed on the crown in soil. After being excised from the crown tissue under a stereomicroscope, meristems were cultured on half-strength MS medium (Murashige and Skoog, 1962). The effect of BA on the development and growth was examined. As shown in Table 1, the survival of the meristem was the highest (81%) in the half-strength MS medium supplemented with 0.5 mg·L^{-1} BA. Accordingly, the medium was used for meristem culture to obtain donor plants in vitro.

Table 1. Effect of BA on the development and growth of plantlets from meristem cultures.

Benzyleadenine (mg·L^{-1})	Survival (%)	Number of leaves	Length of petiole
0	12	2.0	0.4
0.1	41	24.0	1.3
0.5	81	23.3	0.5

Each value was scored after 50 days of culture, medium 1/2 MS, length of petiole (cm)

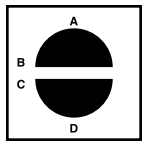
When the plant derived from the meristems attained approximately 4 cm in height and had several petioles with leaves, the leaves and petioles were excised from the plant. The leaves of about 1.5 cm in length were divided into two halves, distal and proximal ones. The position of distal and proximal ends and cut sections were symbolized as A, B, C, and D as shown in Table 2. These explants were cultured on MS medium supplemented with TDZ and NAA. The concentrations of hormones are shown in Table 2. The petioles of in vitro donor plants were successively divided into three segments (1 cm in length). The divided sections of the segments were symbolized as E, F, G, H, J, and I in proximal direction. The segment explants were cultured on MS medium supplemented with TDZ (1, 2, or 3 mg·L^{-1}) and 0.1 mg·L^{-1} NAA.

All the basal media used contained MS salts and 3% sucrose, and were solidified with 0.2% Gelrite. The pH was adjusted at 5.75 before autoclaving. All the cultures were kept at 25 °C and under 3,000 Lux with fluorescent lamps.

RESULTS AND DISCUSSIONS

Table 2 shows the number of adventitious shoots from the ends of two halves of leaves after 60 days of culture. The adventitious shoots were most actively formed from the proximal end of the distal half, which is, the cut section denoted B, and successively at the proximal end of proximal half which is close to the petiole. These results were observed commonly in both the two hormonal conditions shown in Table 2. Since no callus was found, these shoots were considered to be directly formed from the tissue. In the C and D areas formation of adventitious shoots was not observed.

Table 2. Number of adventitious shoots formed from the end of two halves of leaves. Numbers after TDZ and NAA are mg·L^{-1}.

End		TDZ (1) NAA (0.1)	TDZ (3) NAA (0.1)
A		0	0
B		9.6±2.0	9.0±4.1
C		4.4±1.7	1.3±0.3
D		6.4±1.3	3.8±1.3

Before dividing the leaves, the positions B and A contained identical tissue. After dividing the leaves, however, a difference was found in the potential of adventitious shoot formation. Such a tendency, that is, the higher activity of shoot formation of the cut section B than that of C has been recognized in our research, for example, with *Evolvulus glomeratus* (Yamamoto et al., 1996), *Eustoma grandiflorum* (Yamamoto and Watanabe, 1997), and *Primula sieboldii* (Yamamoto et al., 1999).

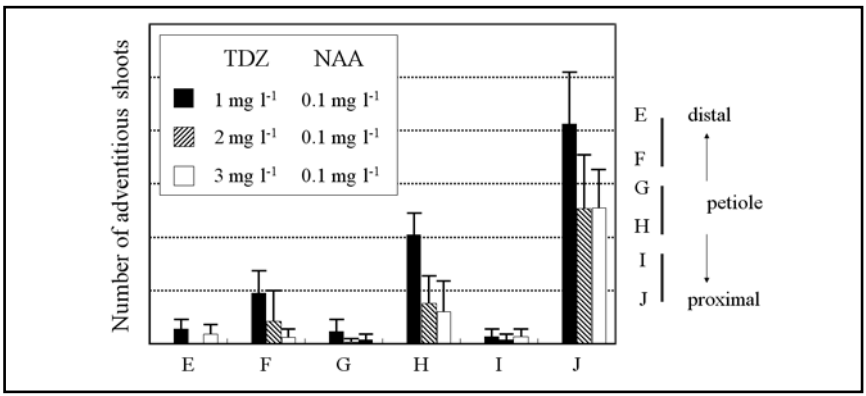


Figure 1. Polarity in the potential of adventitious shoot formation from petiole segments of *Farfugium japonicum*. Each value was scored 12 weeks after culture. n = 16.

Figure 1 shows the number of adventitious shoots formed from the cut sections of each segment of petiole. The combination of 1 mg·L^{-1} TDZ and 0.1 mg·L^{-1} NAA gave the best results for shoot formation. In each segment, adventitious shoots were more actively formed from the proximal section than from distal one. Furthermore, Fig. 2 shows the very clear tendency that the number of adventitious shoots formed increased toward the direction of proximal petiole. This was commonly observed in the three different concentrations of TDZ with 0.1 mg·L^{-1}.

NAA in the Media. No callus was observed in any of these cases. Such a polarity in the potential of direct adventitious shoot formation of petiole can be explained from the gradient in age of petiole tissue. In *F. japonicum*, the petiole tissue is younger in the more proximal position. In younger tissue, cell division is more active than in older tissue. Therefore, it is natural that proximal part of petiole of *F. japonicum* produces more shoot primordia than the distal part. We may ascribe the reason for polarity shown in Fig. 1 to a gradient in age of petiole tissue.

Adventitious shoots formed from the leaf and petiole explants were transferred to the MS medium without hormone where shoots rooted easily. The regenerated plants were transferred to vermiculite in plastic pots for acclimatization. Thereafter, the plants were grown in a greenhouse and flowered in November as shown in Fig. 2. We could obtain these flowering plants after about 1 year from the beginning of explant culture. The color and shape of flowers of the regenerated plants were same as those of indigenous plants.

Figure 2. Flowering of *Farfugium japonicum* regenerated through the methods of the present research.

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