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Multiple Shoot Regeneration from Root Cultures of Prairie Gentian (*Eustoma grandiflorum*)

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ユーストマの根組織培養系におけるシュートの再生

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Root organ cultures of prairie gentian (*Eustoma gradiflorum*) were established from seedling in vitro. One cm long terminal segments of roots were cultured in the MS medium supplemented with 0.1 mg/l 1-naphthaleneacetic acid and 20 g/l sucrose for 40 days. The cultured roots were transferred into solid MS medium free of growth regulator. Many adventitious shoots regenerated from the cultured roots. The shoots rooted easily into rock wool and grew up young plants in a greenhouse.

ユーストマ (*Eustoma grandifloum*) の根組織から不定芽を誘導する培養系を検討した．無菌は種した実生の根を根端より1 cm切り取り，0.1 mg/1のNAAを含むムラシゲスクーグの液体培地で40日間施回培養すると旺盛に増殖した．この根をホルモンフリーの固形培地に移植すると，多数の不定芽が根組織より直接再生した．再生した植物は，容易に発根し完全な植物体に生長した．

Introduction

Prairie gentian (*Eustoma grandiflorum* (Graeiseb.) Schnners) has been one of the most grown flower crops in the last decade. Seedlings of the plant show wide range of variation in some important charactors such as flower color, flower shape, stem lengh and days to flower. In vitro propagation of prairie gentian from shoot tip[5], leaf[3] and stem[8] have been reported. But their proliferation rates were not high enough to be useful in commercial production. Recently Furukawa et al.[4] reported the shoot regeneration from the roots of intact seedlings of prairie gentian in vitro. Establishment of root organ cultures would be useful for micropropagation of selected prairie gentian. In this paper we report root multiplication and adventitious shoot induction from the cultured roots of prairie gentian and discuss the applicability of the root culture for micropropagation.

Materials and Methods

Seeds of prairie gentian cv. 'Fukushihai' were surface sterilized with sodium hypocloride solution (1% active chlorine) for 15 min. followed by washing two times with sterilized water. The seeds were sown aseptically in the medium containing 3% Hyponex (complete soluble fertilizer, N-P-K: 6.5-6-19, Hyponex

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Japan Co. Ltd.), 20 g/l sucrose and 8 g/l agar. The cultures were incubated under a constant light of 1500 lux at 25°C.

After 40-50 days from sowing, one cm long terminal root segments were excised from the seedlings and put into liguid Murashige and Skoog (MS) medium[6] supplemented with 20 g/l sucrose and 1-naphthaleneacetic acid (NAA) or 3-indolebutiric acid (IBA) in a range of 0 to 1 mg/l. Ten root segments were cultured in a 100 ml Erlenmeyer flask containing 50 ml liquid medium on a rotary shaker operated at 100 rpm at 25°C in the dark for 40 days. The experiment had 3 replicates.

The roots cultured in the liquid MS medium containing 0.1 mg/l NAA for 40 days were recultured on solid medium as follows. The cultured roots were divided into two segments, and transferred into 50 mm x 80mmϕ plastic culture vessel containing 50 ml MS medium supplemented with 20 g/l sucrose, 8 g/l agar and 6-benzylaminopurine (BA) and or NAA at a level of 0 or 0.1 mg/l. These were recultured under the same condition of germination for 60 days. Each treatment consisted of 4 replicates of 6 segments.

Results

Effects of auxins on growth of root segments

In the auxin free medium, terminal meristem of the original explant only grew but the initiation of lateral roots was poor. Both NAA and IBA in the medium depressed growth of the original meristem but stimulated initiation of lateral roots except 0.01 mg/l IBA. The fresh weight of roots and number of lateral roots was increased but extension of lateral roots was depressed as the auxin concentration of the medium increased. NAA was more effective than IBA for initiation of lateral roots. The explants grew into callus-like multiple roots in NAA at 1.0 mg/l (Table 1).

Table 1 Effects of auxin concentration on the growth of the root segments

Auxin	Conc. (mg/l)	Fresh weight per vesel (mg)	Length of root (mm)	No. lateral roots
NAA	0.01	163	11.4b	16.3b
	0.1	413	11.1b	32.1a
	1.0	1080	callus	multiple roots*
IBA	0.01	50	18.2a	3.4c
	0.1	303	11.1b	19.5b
	1.0	1095	11.3b	multiple roots*
Control		30	16.6a	0.6d

* No. of lateral roots was uncountable.
Means with the same letter are not significantly different at $P = 0.05$.

Effects of BA and NAA on shoot regeneration form the cultured roots

The cultured roots turned green a few days after recultured. The terminal meristems of the lateral roots grew actively again in the medium (Fig. 1-1). Adventitious shoot regeneration from the cultured roots was observed within 60 days of culture regardless of growth regulators content in the medium. The addition of NAA to the medium depressed shoot production but BA stimulated it (Table 2).

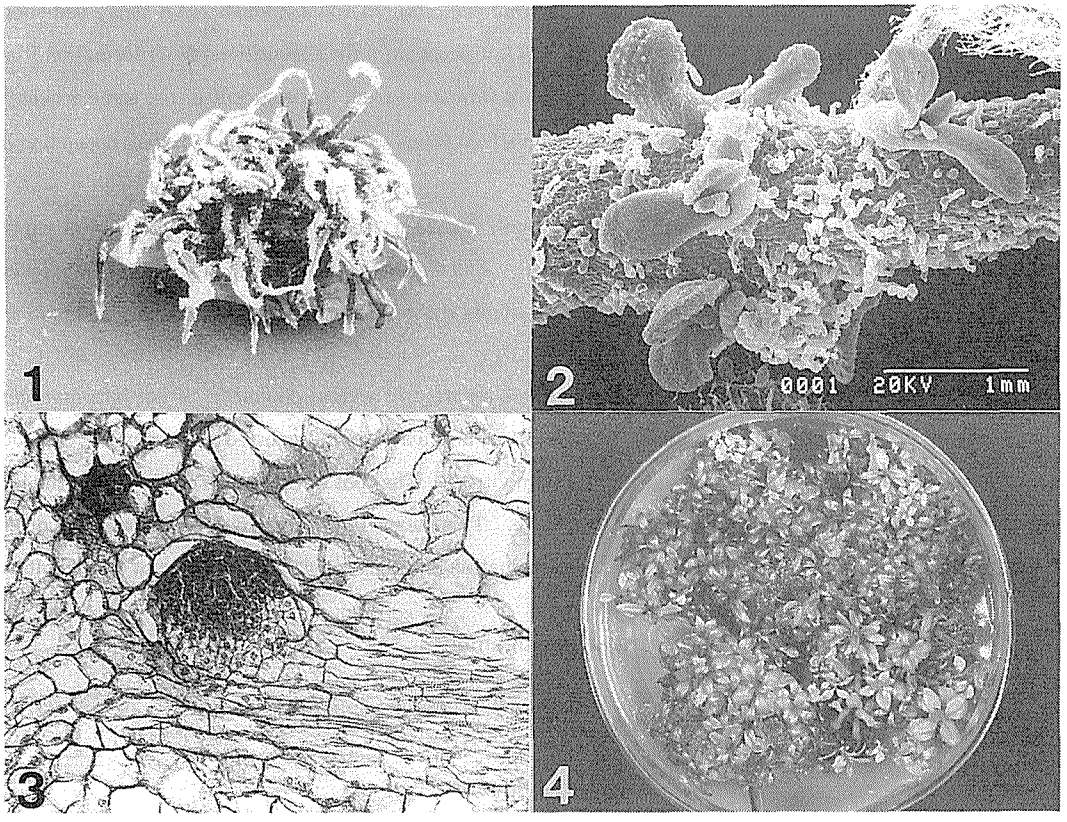

Fig. 1 Adventitious shoot formation from the cultured roots of prairie gentian.
1. The regrowth of the cultured roots on the hormone free medium.
2. Micrograph of adventitious shoot on the cultured root.
3. Micrograph of primodium of adventitious shoot.
4. Multiple shoots from the cultured roots after 150 days of reculture.

Table 2 Effects of BA and NAA on the shoot regeneration from the cultured roots

BA (mg/l)	NAA (mg/l)	Freq. shoot regeneration (%)	No adventitious shoot per explant
0	0	95.8a	14.2b
0.1	0	100.0a	19.7a
0	0.1	91.5a	7.9c
0.1	0.1	95.8a	17.4b

Means with the same letter are not significantly different at $P = 0.05$.

Adventitious shoot appeared on the green and thick roots (more than 1mm in diameter), away from the terminal end of the roots (Fig. 1-2). The shoot arose endogenously. Its initiation was observed at the pericycle bordering the protoxylem, where the primodium developed into a dome structure (Fig. 1-3).

Discussion

The results showed that auxin in the medium was required for the proliferation of roots of prairie gentian and that 0.1 mg/lNAA was the optimal concentration without callus formation. On the contrary, growth

regulators were not essential for shoot regeneration from the cultured roots, though NAA depressed shoot production and BA stimulated it. It was shown that NAA was essential for adventitious shoot formation from stem tissue of prairie gentian[6] but it depressed the shoot formation from leaf segment of the same species[3]. These facts show that the effect of growth regulators on adventitious shoot formation is different depending on the organ or tissue even though in a same species.

The regenerated shoots grew actively on the growth regulator free medium. Many additional shoots appeared in succession up to 150 days of culture (Fig. 1-4). The terminal part of the shoots with 4-6 leaves were cut off and transferred to rock wool for rooting. The shoots rooted easily and grew into young plants in the green house.

Adventitious shoot induction from cultured roots was reported in several plants,[1,2,7,9,10] but information is lacking on the root organ culture for micropropagation. Furukawa et al. reported successful adventitious shoot formation from leaf segments of prairie gentian in vitro.[3] The root of the shoots derived from the leaf segment culture are also available for root organ culture. This means that effective micropropagation of the selected prairie gentian could be established by the combination of leaf segment culture and root organ culture.

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