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Shoot Regeneration from the Roots of Prairie Gentian (*Eustoma grandiflorum* (GRISEB.) SCHINNERS)

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Shoot regeneration occurred *in vitro* from the roots of intact regenerated plants of *Eustoma grandiflorum* and from the roots of intact seedlings, and also from the root segments of regenerated plants. These roots were cultured on a medium (3 g/*l* Hyponex® (Hyponex Japan, 6.5-6-19), 8 g/*l* agar, 20 g/*l* sucrose) without plant growth regulators. Shoot regeneration occurred spontaneously and directly from the roots. These shoots were transferred to a modified MS medium without plant growth regulators. Root formation occurred in all of these shoots. Rooted plantlets were successfully transferred to the soil.

Prairie gentian (*Eustoma grandiflorum* (GRISEB.) SCHINNERS) is commonly propagated by seedling. However, in some cultivars, such as those with marginal variegation, these seedlings show a wide range of variation because of their heterozygous character. Recently, the methods for micropropagation of prairie gentian have been developed and many plants were regenerated from stem, leaf, meristem explants.[1,2] In this report, shoot regeneration from the roots of intact plants and the root segments on the medium without plant growth regulators is described.

Materials and Methods

Two marginal variegation lines, E. G.-R-1 and E. G.-R-2 and two cultivars "Saga no Murasaki" and "Saga no Yuki" were used as materials. And three kinds of roots indicated below were used for this experiment.

(1) Root of intact regenerated plant (E. G.-R-2); Intact regenerated plants which were obtained as previously described[3] were used 30 days after rooting.

(2) Root segment of regenerated plant (E. G.-R-2); Root segments (5 mm length) excised from the regenerated plants were used 30 days after rooting.

(3) Root of intact seedling; Seeds of 2 cultivars ("Saga no Murasaki" and "Saga no Yuki") and 2 marginal variegation lines (E. G.-R-1 and E. G.-R-2) were sterilized with 70% ethanol for 5 sec, 1% sodium hypochlorite for 5 min, and rinsed three times with sterilized distilled water. These seeds were sown on the Murashige and Skoog medium[4] supplemented with 0.05 mg/*l* biotin, 0.5 mg/*l* folic acid, 20 g/*l* sucrose and 8 g/*l* agar (modified MS medium). The 60-days-old seedlings were used.

The roots thus obtained were placed on the H medium containing 3 g/*l* Hyponex® (Hyponex Japan, 6.5-6-19), 20 g/*l* sucrose and 8 g/*l* agar. All media were adjusted to pH 5.7 before autoclaving for 15 min at 121℃. These roots were transferred every two months to the fresh H medium.

Regenerated shoots were transferred to the modified MS medium. The cultures were maintained at 25℃ with 16 hr illumination under the fluorescent light (3,000 lux).

Results and Discussion

Results for three kinds of roots are summarized in **Tables 1-3**. On the three kinds of roots, callus formation did not occur and shoots appeared to be breaking through the epidermis of the root (**Figs. 1, 2**). Most shoot regeneration occurred in the roots which were placed on the surface of the culture medium. Shoot regeneration from the thick roots (ϕ : 1 mm$\leqq$) occurred at a higher frequency than that from the thin roots (ϕ : 1 mm$>$). These shoots were regenerated spontaneously, continuously and directly from the roots. The roots of an intact regenerated plants produced an average of 85.0 shoots after 110 days of culture (**Fig. 3**).

These shoots were transferred to the modified MS medium without plant growth regulators. After 14 days of transfer, root was formed in all of the shoots. Rooted plantlets were successfully transferred to the soil.

Semeniuk and Griesbach first observed shoot regeneration in prairie gentian from callus on the modified MS medium supplemented with naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP). On the other hand, shoot regeneration from callus developed on the roots of plantlets was reported on the cherry rootstock and of the apple rootstock on the modified MS medium supplemented with plant growth regulators.[5] In the present experiment, however, shoot regeneration in prairie gentian occurred directly

Table 1. Shoot regeneration from the roots of intact regenerated plants of *Eustoma grandiflorum* (after 60 days of culture).

Number of cultured plants	Number of plants with shoot regeneration	Mean number of shoots/ cultured plant
20	14	5.0

Table 2. Shoot regenaration from the roots of intact seedlings of *Eustoma grandiflorum* (after 90 days of culture).

Genotype (cultivar)	Number of cultured plants (A)	Number of plants with shoot regeneration (B)	Freq. ((B/A)×100)
EG-R-1	55	52	95.5
EG-R-2	57	52	91.2
"Saga no Murasaki"	70	52	74.3
"Saga no Yuki"	78	51	65.4

Table 3. Shoot regenaration from the root segments of *Eustoma grandiflorum* (after 60 days of culture)

Number of cultured segments (A)	Number of segments with shoot regeneration (B)	Freq. ((B/A)×100)
20	7	35

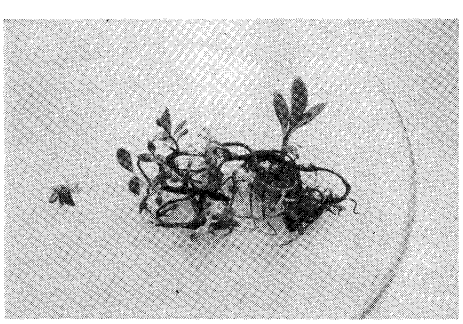

Fig. 1. Regenerated shoots from the root of intact regenerated plant after 60 days of culture.

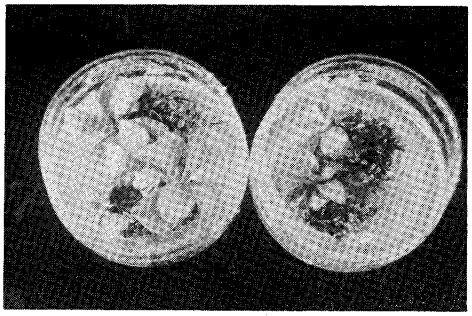

Fig. 2. Regenerated shoots from the root of intact seedling after 60 days of culture.

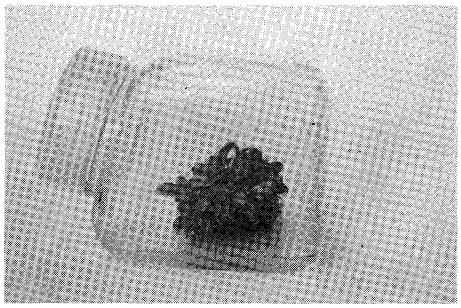

Fig. 3. Regenerated shoots from the root of intact regenerated plant after 90 days of culture.

from the roots on the medium without plant growth regulators. The same phenomenon was observed on the root segments of red raspberry.[6]

In this study, many shoots were easily regenerated from the roots. Therefore, it seems that root organ culture may be useful for the micropropagation of prairie gentian.

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References

1) Semeniuk, P., R. J. Griesbach, 1987. Plant Cell Tissue Organ Cult., **8**: 249-253.
2) Griesbach, R. J., P. Semeniuk, 1987. J. Hered., **78**: 114-116.
3) Furukawa, H., K. Kishida, S. Fukai, 1988. Plant Tissue Cult. Lett., **5**: 96-97.
4) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-497.
5) Jones, O. P., J. A. Gayner., R. Watkins, 1984. J. Hort. Sci., **59**: 463-467.
6) Borgman, C. A., K. W. Mudge, 1986. Plant Cell Tissue Organ Cult.. **6**: 127-137.

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トルコギキョウの根からのシュート形成

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トルコギキョウの再分化植物や実生あるいは再分化植物の細断した根を植物生長調節物質を含まない培地（ハイポネックス® (6.5-6-19) 3 g/*l*，ショ糖 20 g/*l*，寒天 8 g/*l*）で培養したところ，カルスを形成することなく，これら3種類の培養体の根から多数のシュートが形成した．シュートは根の表皮を破って次々と出現した．これらのシュートを植物生長調節物質を含まない MS 修正培地に移植したところ，すべてのシュートが根を形成した．こうして得た植物は容易に馴化し栽培することができた．これらの結果は根培養はトルコギキョウの大量増殖に有効であることを示している．