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Shoot Regeneration in Root and Callus Cultures of *Hypericum perforatum*

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The plants in genus *Hypericum* (Guttiferae) have been commonly used as a hemostatic and astringent in Japan and China[1]. Recently some biological properties such as antiviral action[2] have been discovered in the phenolic constituents of the plants and they are regarded as an important crude anticancer medicine[3].

Several experiments on the proliferation of usual plants by shoot organogenesis in their root cultures have been reported[4-10]. In this study, we established the shoot regeneration from the root and callus of *H. perforatum* and the transplantation of the regenerated plantlets to soil.

Shoots (3 cm in length) cut from *H. perforatum* plants cultivated in the field were surface-sterilized with 3 % sodium hypochlorite with Tween 20 (1 drop/50 m*l*) and washed with sterilized water three times. The excised shoots (1 cm in length) were aseptically inoculated on hormone free Murashige-Skoog (MS) solid medium[11] (2 g/*l* gelrite, pH 5.7) and cultured at 25°C in the light (3,000 lux, 16 hr photoperiod). The roots of these plantlets grown on the medium were cut (3-4 cm in length) and transferred to the same solid medium (in petri dish, 9 cm in the diameter) in the light (3,000 lux, 16 hr photoperiod). After 2-3 weeks of culture, several shoots were differentiated directly from the roots (**Fig. 1-a~c**). With the growth of the shoots, the roots gradually elongated and after about 8 weeks of culture the regenerated plantlets grew to fill the dish with well developed roots. Shoot regeneration occurred each time the roots were subcultured in the same medium at 2 months interval.

The roots of *H. perforatum* were also inoculated into hormone free MS liquid medium (50 m*l*/100 m*l* flask) and cultured on a rotary shaker (100 rpm) at 25°C in the light (3,000 lux, continuous photoperiod). In this liquid culture, shoot regeneration occurred spontaneously and dircectly from the roots and the shoots proliferated sufficiently after 4 weeks culture (**Fig. 2-a~b**).

After acclimatization by dipping the root portion into water for 2 days in a 200 m*l* beaker, the plantlets regenerated from these root cultures were successfully transplanted to soil (in a greenhouse) (**Fig. 3**).

The calli of *H. perforatum* were derived from the leaf segments of the axenic plants cultured on MS solid medium containing 0.5 mg/*l* NAA and 0.1 mg/*l* BA at 25°C in the dark. When the calli

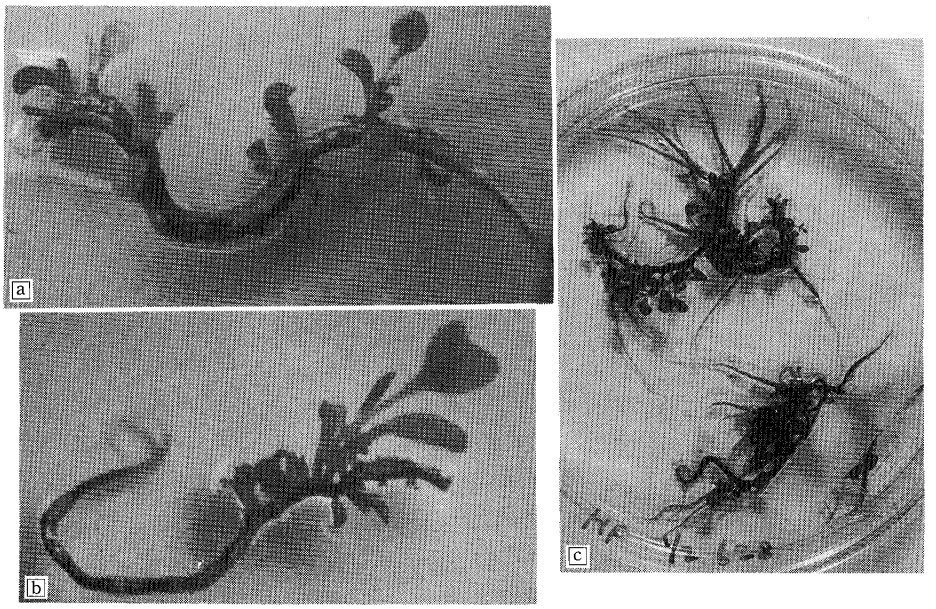

Fig. 1 a~c) Regenerated shoots from the roots of *Hypericum perforatum* cultured on hormone free MS solid medium.

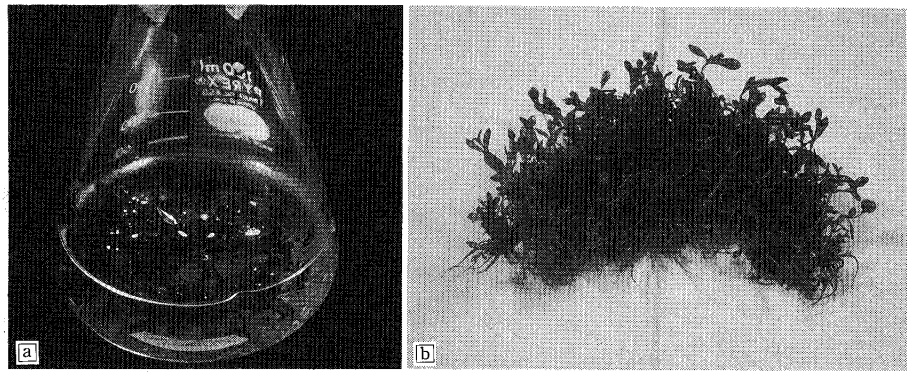

Fig. 2 a) Regenerated shoots from the roots of *Hypericum perforatum* cultured in hormone free MS liquid medium.
b) Plantlets regenerated from the roots cultured in liquid medium for 4 weeks.

Fig. 3 Two-month-old plantlets of *Hypericum perforatum* after transplant to a pot.

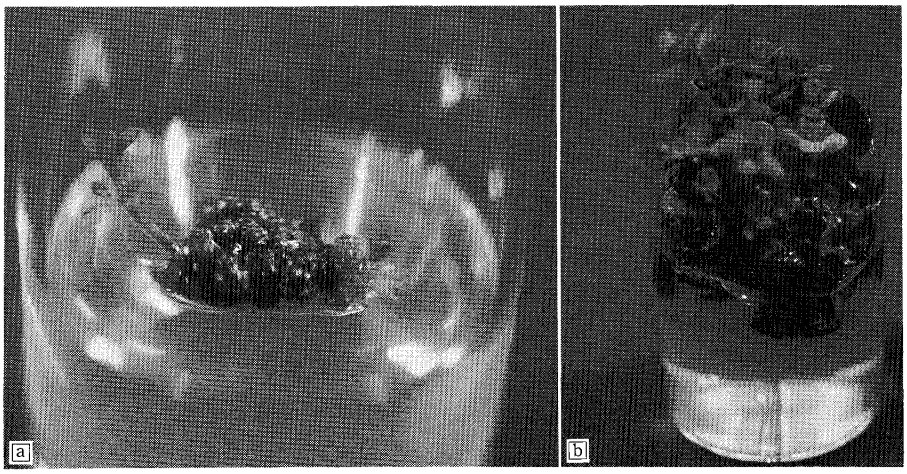

Fig. 4 a) Shoot regeneration from the callus of *Hypericum perforatum* cultured on MS solid medium supplemented with 0.5 mg/*l* NAA and 0.1 mg/*l* BA.
b) Multiple shoots regenerated from the callus.

grown on the same medium were placed under illumination (3,000 lux, 16 hr photoperiod), shoot proliferation from the calli were observed within 4 weeks of culture (**Fig. 4-a~b**).

Thus, in this experiment, we could easily obtain many shoots of *H. perforatum* in its root and callus cultures. It is noteworthy that the roots of this plant have the capability of shoot regeneration under normal culture conditions without any growth regulators. No morphological difference was observed between the plantlets of shoot cultures and the ones regenerated in the root and callus cultures. Concerning the study of tissue culture in *Hypericum* plants, only the production of phenolic compounds in the callus and multiple shoot of *H. erectum* was reported[12]. In the plant of this genus, this is the first report on the regeneration from the cultured root and callus. These cultures seem to be useful for the micropropagation of this medicinal herb.

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《和文要約》

セイヨウオトギリソウの根およびカルス培養におけるシュート形成

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セイヨウオトギリソウの根を照明下ホルモン無添加MS培地で培養すると，固型培地においても液体培地においても，根から直接多数のシュートが形成した．得られた再分化植物体は容易に馴化し栽培された．また，0.5 mg/l NAAと0.1 mg/l BA添加MS培地で培養したカルスからも，多数のシュート形成がみられた．根およびカルス培養はセイヨウオトギリソウの大量増殖に有効である．