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Rotenoid Production by Root-like Organ Induced from *Derris elliptica*

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Root-like organs of *Derris elliptica* BENTHAM observed in the cultured tissue and formed like imperfectly differentiated rootlets were induced for the production of rotenoids. As already found a large amount (160 μg/gdw) of rotenoids was accumulated in the root-like organ. In this paper, a viny derris previously reported[1] was investigated to clarify mechanisms for the induction and culture conditions of root-like organ, and a woody derris was also investigated to compare differences in rotenoid content between original plant tissues and induced root-like organs. Root-like organs were induced and cultured by the same method described previously,[1] and the composition of media (modified from MS medium) except for plant hormones was also the same unless otherwise stated. Methods for extraction and colorimetric determination of rotenoids have been also described.[1] The induction and succeeding culture of tissues formed were performed over 6 weeks. Usually about 30 slant cultures were applied in each experiment.

Root-like organs were induced at 26.5, 30 and 37℃ on a medium containing naphtaleneacetic acid (NAA) and kinetin. They grew accompanied with callus tissues at 26.5 and 30℃, but did not grow at 37℃. No clear difference was observed between rotenoid content in the root-like organs induced at 26.5 and those induced at 30℃. Although the alteration of sugar concentration (1.0–5.0% glucose) in the medium with 1.0 μg/ml NAA and 0.2 μg/ml kinetin did not cause a clear effect, high concentration of sucrose or glucose with 0.4 μg/ml NAA and 0.2 μg/ml kinetin stimulated both root-like organ induction and rotenoid accumulation. Higher glucose concentration (10%) inhibited the growth of root-like organ. Coconut milk (15% in basal medium) was effective for rotenoid accumulation, and the rotenoid content was 580 μg/gdw root-like organ while only 100 μg/gdw of rotenoids were accumulated without coconut milk (plant hormones: 1.0 μg/ml NAA and 0.2 μg/ml kinetin). When 1.0 μg/ml of kinetin was added together with 1.0 μg/ml NAA into the medium, rotenoid accumulation was poor (44 μg/gdw). Addition of organic nutrients such as 0.3% yeast extract, 0.05% casamino acid, and 0.05% N-Z amine instead of 15% coconut milk caused inhibitory effects on the induction and growth of root-like organ (plant hormones: 1.0 μg/ml NAA, 0.2 μg/ml kinetin).

The influence of the addition of 10–100 μg/ml of IAA was tested with a medium containing 0.5 μg/ml 2,4-D and 1.0 μg/ml kinetin. Addition of 20–50 μg/ml IAA induced root-like organ and rotenoid accumulation, but the growth was very slow compared with that in the medium added with suitable concentration of NAA. IAA added to the medium as a sole auxin brought about a formation of adventitious fine roots though their rotenoid content did not exceed the highest value observed in root-like organs induced most frequently with various concentrations of NAA together with 0.2 μg/ml kinetin. No other synthetic cytokinin (e.g. 0.2–1.0 μg/ml BA) was effective for the induction of root-like organs and rotenoid accumulation though callus was induced abundantly. The optimum condition for the induction of root-like organ and rotenoid accumulation was as follows; addition of 1.0 μg/ml of NAA and 0.2 μg/ml of kinetin, 3.0–5.0% sucrose or glucose and 15% coconut milk to the basal

Table 1. Rotenoid content of original plant roots and induced root-like organs of *Derris elliptica*.

	Root (8 mmϕ)	Root (0.5 mmϕ)	Root-like organ (μg/gdw)
Viny derris	8,100	120	580
Woody derris	450	160	470

medium and incubation temperature at 26.5–30℃. The highest rotenoid content was 580 μg/gdw after 6 week incubation of viny derris under these conditions.

Table 1 shows the rotenoid content in roots and root-like organs obtained from viny and woody derris (the optimum culture condition of viny derris was employed). The rotenoid content in the root-like organs induced from viny derris was higher than that in fine roots (diameter 0.5 mm) of original plant, but lower than that in thick roots (diameter 8 mm) where a large portion of rotenoids is known to be accumulated. On the other hand, the root-like organs induced from woody derris contained a little higher amount of rotenoids than thick roots of the original plant.

In the whole culture, callus tissues and root-like organs usually exist in a mixed state. Actually, callus containing much less amount of rotenoids grow more rapidly than root-like organs, therefore the reduction of rotenoid content was observed during subculture of the whole culture. However, we can subculture only root-like organs accumulating much rotenoids under the defined conditions (data not indicated here). This fact indicates the possibility of obtaining secondary metabolites from plant tissue culture by controlling differentiation of plant organs through regulation of plant hormones.

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Reference

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≪和文要約≫

デリス根様器官によるロテノイドの生産

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ロテノイド生産を目的として，マメ科植物デリスの茎葉部からの，根様器官の誘導および培養のための条件を検討した．ロテノイド含量は根様器官の誘導時の培地条件により大きく異なった．ロテノイドは根様器官中に誘導後6週間で最高 580 μg/gdw 蓄積された．