Note

Callus Induction and Shoot Generation from Flower Trichomes of *Nicotiana tabacum*

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Although the totipotency of plant tissues is an undoubted fact, up to now only a few organogenetic potentials have been revealed.[1] We succeeded in stigma culture of tobacco plants and found that only young stigma tissues could produce stigma-like tissues.[2] This seemed to contradict the idea that all plant cells have totipotency. Tobacco flower epidermis tissues have many trichomes[3] (Fig. 1). These trichomes are considered to be specifically differentiated, and exudate trichome-specific lipid compounds such as duvatrienediol (DVT) and labdanoid-diterpene.[4,5] We tried to culture trichome cells of tobacco so as to investigate their nature. Such investigation will help to study the biosynthesis and metabolism of diterpene compounds. Although we could not find any conditions that produce trichome cells directly from trichome cell culture, we succeeded in callus induction and shoot generation from flower trichomes of tobacco for the first fime.

Trichome cells of petals (*Nicotiana tabacum* cv. BY-4) were cut off aseptically by surgical blades under a microscope without contamination by epidermis cells. Trichome cells were cultured at 28°C under 12 hr photoperiods on Linsmaier–Skoog medium[6] with appropriate amounts of plant hormones. DVT contents of developing tissues were analyzed by gas chromatography after esterification with butylboronate.[7]

Callus induction from flower trichomes was observed when both NAA and BA were added to 0.1～1 ppm, while medium with only NAA did not induce callus formation. After 4 weeks of culture, greenish-white cell clusters were observed on the surface of the agar medium. They grew larger callus clusters thereafter. When these calli were subcultured under different hormonal conditions, morphological change occurred (Table I). The medium with

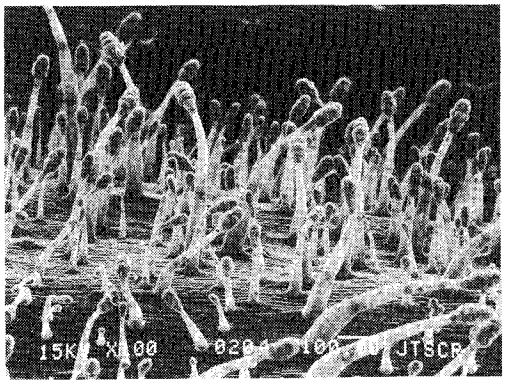

FIG. 1. Electron Microscope Photograph of Flower Trichomes of *Nicotiana tabacum* (× 100).

TABLE I. MORPHOLOGY, DRY WEIGHT, AND DUVATRIENDIOL (DVT) CONTENTS OF TRICHOME-DERIVED CELLS WHEN SUBCULTURED UNDER DIFFERENT HORMONAL CONDITIONS[a]

No.	Hormone[b] (ppm)					Morphology			Dry wt (mg)[c] /test tube	DVT (μg) /100 mg dry tissue
	IAA	NAA	2,4-D	BA	Zea	Callus	Shoot	Root		
1				1		+	+++		300	3.7
2		0.1		1		+	+++		330	5.8
3		1		0.1		++		+	397	6.2
4		1		1		++	+		386	3.0
5			1	1		++			168	6.3
6			0.01	1		+	+++		360	7.5
7	10			1		+	+++		345	6.3
8	1			5		+	++		181	5.5
9	1				5	+	++		172	5.9
BY-4 leaf										788

[a] Subcultured for 2 months. +～+++, growth index.
[b] IAA, indole acetic acid; NAA, naphthalene acetic acid; 2,4-D, 2,4-dichlorophenoxy acetic acid; BA, benzyl amino purine; Zea, zeatine.
[c] Mean value of 3 test tubes each (26 mmϕ × 88 mm).

1 ppm of NAA and 0.1 ppm of BA proliferated calli and roots, and the medium with 0.1 ppm of NAA and 1 ppm of BA proliferated calli and shoots. These results indicated that flower trichome cells easily dedifferentiated into calli and these calli easily differentiated into shoots and roots under appropriate hormonal conditions. DVT contents in these calli and differentiated tissues were less than 1/100 that of normal BY-4 leaf. This indicated chemically that cell culture derived from flower trichomes did not develop into trichome-natured tissues. However, the totipotency of tobacco trichome cells were shown in this study.

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