

The Production of *Pelargonium graveolens* Oil by Shoot and Plant Tissue Culture¹

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Abstract

The production of essential oil from *Pelargonium graveolens* by plant tissue culture was investigated. Since the essential oil could not be obtained by callus culture, shoot culture was investigated. Sterile plants were induced from intact plants by using solid medium with appropriate hormones. Young shoots which also were obtained from sterile plants, were well cultivated in liquid medium by a method of agitating with air. The odor of the essential oil obtained from cultured shoots was found to be similar to that of the natural essential oil. Furthermore, the GC pattern of the volatile compounds in the essential oil from cultured shoots was similar to that of natural essential oil. The content of citronellol, a major constituent of natural *Pelargonium* leaf oil, was slightly less in the cultured shoots than in the intact plant.

Key Word Index

Pelargonium graveolens, tissue culture, shoot culture, essential oil production, citronellol.

Introduction

As essential oils are normally obtained by distillation from natural plants, their quality and quantity are both influenced by the weather. We have tried to produce essential oils by employing plant tissue culture techniques, which if successful could stabilize the quality and quantity of oil produced. In this paper, we report our results on the production of *P. graveolens* oil which is found throughout the entire plant.

Experimental

The *P. graveolens* used, which was the same clone of geranium grown for commercial oil production, was obtained from cuttings raised in our own greenhouse. The method of tissue and shoot culture is summarized as follows:

- A. *Sterilization of plant.* An intact plant was cut into pieces and sterilized by the standard method with ethanol and NaClO.
- B. *Medium.* LS medium was used unless otherwise mentioned (1). Plant hormones in medium were needed to obtain young shoots from the cut leaf. Indoleacetic acid, indolebutyric acid and naphthylacetic acid as an auxin, and 6-benzyl adenine, and kinetin as a cytokinin were investigated.

In addition the LS medium was modified by the inclusion of C sources: sucrose, glucose, fructose; N sources: NH₄OH, HNO₃, KNO₃; minerals: KH₂PO₄, CaCl₂, MgSO₄, FeSO₄, Na₂EDTA, MnSO₄, H₃BO₃, ZnSO₄, CuSO₄, Na₂MoO₄, KI, CoCl₂; vitamins: myoinositol, thiamine-HCl, pyridoxine, nicotinic acid and an amino acid: glycine.

- C. *Method of cultivation.* Solid medium with agar was used to obtain sterile plants and young shoots. A liquid medium then was employed to cultivate young shoots. A static method, as well as methods of shaking by rotation or with a shaker, agitation with a stirrer, by air, and by both stirrer and air were investigated to optimize cultivation of young shoots, using 100 mL flasks with 50 mL medium. Light intensity from 0 to 20,000 lux were tested to examine its effects on growth and oil production. A 5 L jar fermentor (Model KMJ-5, Mitsuwarika) with 3 L modified LS medium containing 10⁻⁶ indoleacetic acid and 10⁻⁵ M 6-benzyl adenine was used for fermentor culture. In this study, stirrers and vanes were removed from the jar fermentor, and a fused glass filter was used as a sparger in order to agitate with air.
- D. *Extraction of essential oil.* Cultivates were homogenized with Polytron (Kinematica Co.) in CH₂Cl₂. The layer of CH₂Cl₂ was separated by centrifugation. The essential oil was obtained from the layer of CH₂Cl₂ by evaporation.

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Table I. Hormonal balance of differentiation

Indoleacetic acid (M)	6-Benzyl Adenine					
	0	10 ⁻⁸	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴
0	C	S	S	S	S	
10 ⁻⁸				S	S	
10 ⁻⁷				S	S	S
10 ⁻⁶				S	S	S
10 ⁻⁵	C	S		S	S	S
10 ⁻⁴	C			C	C	C

C: Callus; S: Shoot

Table II. An example of a good medium

Major component	C source	Sucrose 3%
	N source	KNO ₃ 0.3%
Minor component	Minerals	KH ₂ PO ₄ , CaCl ₂ , MgSO ₄ , FeSO ₄ , Na ₂ EDTA, MnSO ₄ , H ₃ BO ₃ , ZnSO ₄ , CuSO ₄ , Na ₂ MoO ₂ , KI, CoCl ₂
	Vitamins	myoinositol, thiamine-HCl, pyridoxine, nicotinic acid, glycine
PII		5.8

E. *GC and GC/MS Analysis.* The essential oil was subjected to GC analysis using a Hitachi Model GC16 gas chromatograph with a flame ionization detector and FFAP fused silica capillary column. The oil was also subjected to GC/MS analysis (JEOL Model JMS-D 300 mass spectrometer with JEOL Model JGC-20K GC and JEOL Model JMA-2000 data system).

Results and Discussion

In this study the production of essential oil of *P. graveolens*, which contains its essential oil throughout the whole plant, was investigated by using plant tissue culture technique.

The germ-free plant was obtained from the intact plant by the standard method. Callus and shoots were induced from sterile plants on a solid medium, and their odors were examined. The odor of the shoot was found to be similar to that of the intact plant, while the odor of the callus was very weak.

Suitable combinations of the hormones such as auxin and cytokinin were investigated in order to induce young shoots from the cut leaf efficiently. The results are shown in Table I. The combination of 10⁻⁶ indoleacetic acid and 10⁻⁵ M 6-benzyl adenine was the best combination, and it was used for further study.

Cultivation of shoots in liquid media was studied in order to produce essential oil efficiently. The optimum composition of medium to cultivate young shoots was investigated. A modified LS medium with altered N source, minerals, vitamins and amino acid gave a good result (see Table II). The modified LS medium was differed from the original LS medium as follows: As an N source, only 0.3% KNO₃ was used. As minerals, CaCl₂ was used in a concentration of one third the original concentration, and MgSO₄ was three times as much. As vitamins and an amino acid, 5 x 10⁻⁶% pyridoxine, 5 x 10⁻⁶% nicotinic acid and 2 x 10⁻⁵% glycine were added. Methods of

Table III. Comparison of cultivation methods using a liquid medium

Cultivation method	Result
Static	poor
Shaking by rotation	fair
Shaking with a shaker	poor
Agitating with a stirrer	poor
Agitating with air	good
Agitating with a stirrer and air	poor

Table IV. Conditions and data

Cut leaf	Young shoot	Shoot
Temperature	26°C	26°C
Period	7-10 days	14 days
Growth rate (Final/Initial)	3-5 times	20-30 times

liquid culture to cultivate young shoots were investigated and the method of agitating with air was found to give the best result (see Table III).

Light had a significant influence on the production of essential oil but little on growth. The light intensity of 1,000 to 6,000 lux gave the best result in the production of the essential oil. From the results mentioned above, the 5 L jar fermentor culture method was established (see Figure 1). The best conditions and data of the cultivation of young shoots from cut leaf and that of shoots from young shoots are shown in Table IV.

The results of GC analysis are shown in Figure 2. The main components of the essential oil from the cultivates were identified with GC/MS. Citronellyl formate, citronellol and geraniol, which were found in the intact plant, also were identified in

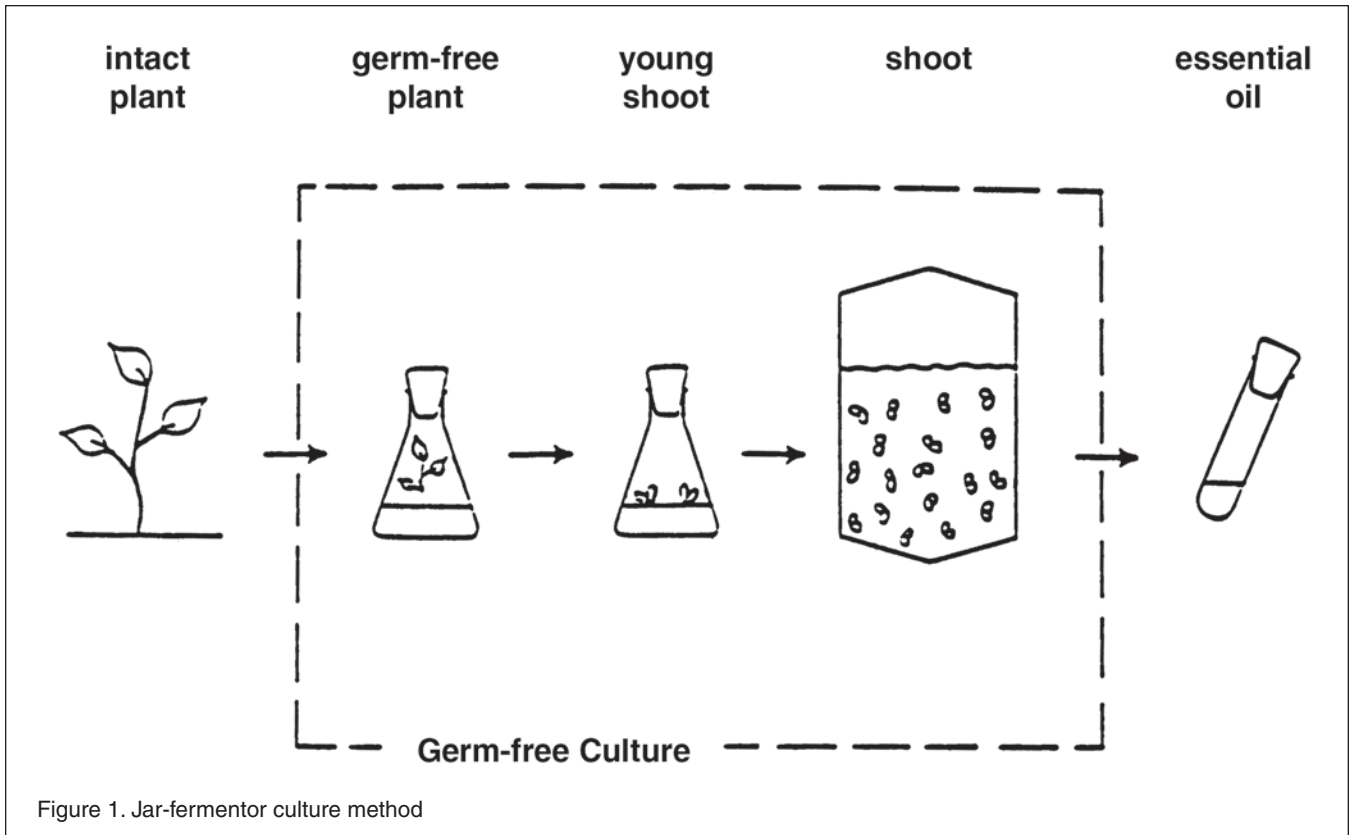


Figure 1. Jar-fermentor culture method

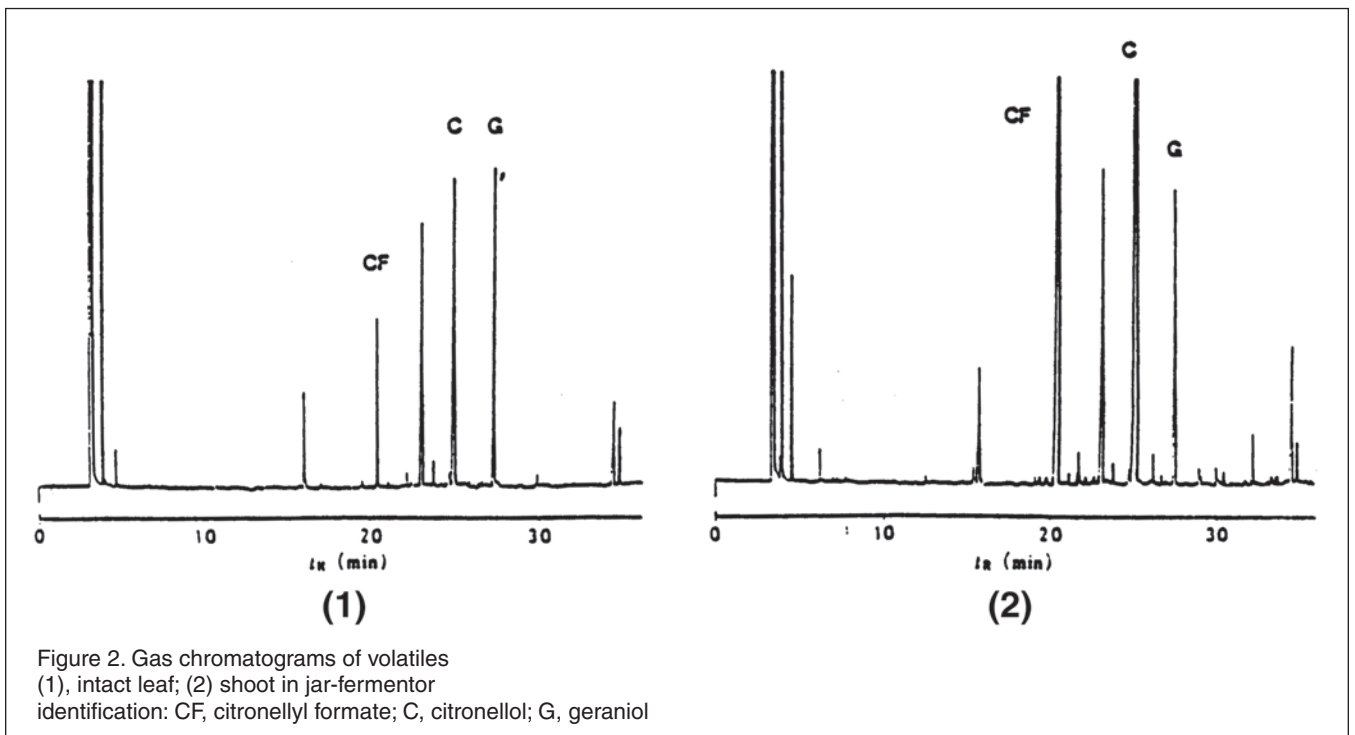


Figure 2. Gas chromatograms of volatiles (1), intact leaf; (2) shoot in jar-fermentor identification: CF, citronellyl formate; C, citronellol; G, geraniol

the essential oil from the cultivates. The content of citronellol in the cultivate after 24 days cultivation period was 104 ppm, about half its concentration in the intact plant.

References

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