Rapid Clonal-propagation of *Matricaria chamomilla* by Tissue-cultured Shoot Primordia

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Shoot primordia were induced from shoot tip of the biennial *Matricaria chamomilla* L. (Compositae) by Tanaka and Ikeda's shoot primordium method. The shoot primordia in MS liquid medium supplemented with 2.0 mg/l BAP proliferated rapidly up to 30 times as large in two weeks. They regenerated rapidly plantlets if they were transplanted onto pieces of Ceramic Wool in 1/4 MS liquid medium supplemented with 1% sucrose and no phytohormone. Thus, the shoot primordia and the regenerated plantlets showed the regular chromosome number of $2n=18$.

KEY WORDS: *Matricaria chamomilla*, Compositae, biennial herb, shoot primordium, clonal propagation

Introduction

Matricaria chamomilla L. (Compositae) is a biennial herb growing in Europe. Its dried flower-head has been used for folk medicine, food and bath drug, since it contains biologically active, secondary metabolites such as sesquiterpenoids, coumarins and flavonoids.

REICHLING and BECKER (1976), REICHLING *et al.* (1984) and SZOKE *et al.* (1976, 1977, 1978) induced and propagated calli of *Matricaria chamomilla* *in vitro* for obtaining high quantity of economically important sesquiterpenoids. However, those calli were rather difficult to maintain no genetic change and vigorous propagation.

The shoot primordium method of tissue culture (TANAKA and IKEDA 1983; Patents of Canada, France, and Germany; Patent of Japan officially announced) has been being applied to various plant-species for stabilized micropropagation. The tissue-cultured shoot primordia of plants have the distinct characteristics of high stability of chromosome number, high ability of regeneration and rapid proliferation (KONDO *et al.* 1991).

Shoot primordia and plantlets of *Matricaria chamomilla* were successfully made and propagated in this paper for the first time by using TANAKA and IKEDA's shoot primordium method (1983).

Materials and Methods

Shoot apices of *Matricaria chamomilla* about 5 mm long with 3-4 true leaves were harvested and sterilized. Then, apical domes of their shoot tips with 1-2 leaf primordia were obtained

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and placed in test tubes (30 mm×200 mm, 25 ml liquid medium). The culture media used were modified Murashige and Skoog (MS) (MURASHIGE and SKOOG, 1962) and Gamborg's B5 (GAMBORG, 1968) media supplemented in combination with 1-naphtalenacetic acid (NAA) at concentrations of 0, 0.02, 0.2 or 2.0 mg/l and 6-benzylaminopurine (BAP) at concentrations of 0.2 or 2.0 mg/l to perform 16 different media. Those media were adjusted to pH 5.7-5.8. All cultures were incubated at about 22°C under 2000-9000 lux illumination by fluorescent lamp and were stirred at 2 cycles/minute on a gyrated drum 1 m in diameter for 3-4 weeks. Then, they were transplanted to fresh media for subculture at intervals of 2-3 weeks. The cultures were anatomically (FEDER and O'BRIEN, 1968) determined for true, tissue-cultured shoot primordium.

Divided shoot primordia 1-2 mm in diameter were planted into test tubes (30 mm×120 mm, 15 ml liquid medium) and were incubated at about 22°C under 3000-5000 lux light intensity for 16 hours and dark for 8 hours.

Various medium conditions were tested; sucrose at concentrations of 1, 2 and 3%, basic media of MS, 1/2MS and 1/4MS, and combination of NAA at concentrations of 0, 0.02 and 0.2 mg/l and BAP at concentrations of 0 and 0.02 mg/l. They were either of liquid or 0.8% agar plate or Ceramic Wool (SHIN-NIHON KAGAKU).

Masses of shoot primordia 0.1 g fresh-weight were planted into test tubes (25 mm×150 mm, 15 ml liquid medium) and their fresh weights were periodically measured for growth rate. Number of shoot primordia per mg was calculated by number of shoot primordia/fresh weight of aggregated shoot primordia about 2 mm in diameter.

Results and Discussion

Four weeks after the inoculation, some masses of shoot primordia 3-5 mm in diameter were induced in nine different media for the primary culture. After three months, those shoot primordia were continuously subcultured in MS supplemented either with 2.0 mg/l BAP or with 2.0 mg/l BAP and 0.2 mg/l NAA (Table 1). A yellow to yellowish green-colored mass of shoot primordia subcultured in MS supplemented with 2.0 mg/l BAP and 0.2 mg/l NAA contained some friable calli, while the other masses subcultured in MS supplemented with 2.0 mg/l BAP were quite uniform as the "standard shoot primordia" 200-500 μ m in diameter, which exhibited 15-20 domes (Fig. 1A). An instance of the cross-section of the mass of shoot primordia (Fig. 1B) showed a maturely developed dome called the "secondary shoot primordium" and a few young secondary shoot primordia around the base of the dome. Those young secondary shoot primordia occurred rapidly from the primary shoot primordia. These phenomena were exactly the same as those of *Haplopappus gracilis* observed by TANAKA and IKEDA (1983).

The shoot primordia of *Matricaria chamomilla* subcultured for four months maintained stability of their chromosome number of $2n=18$ which was the same as that of the parental plant-mass (Table 2).

Using Ceramic Wool and 1/4MS supplemented with 1% sucrose and no phytohormone (Table 3), average four shoots with roots were regenerated from every mass of shoot primordia 1.5 mm in diameter (approximately 1 mg fresh weight) (Fig. 2). Those plantlets were morpho-

Table 1. Induction of shoot primordia from shoot tips of *Matricaria chamomilla* in MS media modified with combination of NAA and BAP

NAA mg/l	BAP mg/l			
	0.2		2.0	
0.00	S+C ¹⁾	1/4 ²⁾	S+SP	1/4
	C	1/4		
	D	2/4	D	3/4
0.02	C	1/4	S+C	2/4
	D	3/4	D	2/4
0.20	S+C	1/4	SP+C	1/4
	C	1/4	C	1/4
	D	2/4	D	2/4
2.00	C	1/4		
	D	3/4	D	4/4

¹⁾ Formation of: S = shoots, SP = shoot primordia, C = calli and D = dead

²⁾ Number of shoot tips differentiated/Number of shoot tips planted

Table 2. Chromosome counts in the parental plant-mass, shoot primordia and regenerated plantlets in *Matricaria chamomilla*

Cell source	Total cells observed	Number of cells and (%)	
		2n = 18 (2X)	2n = 36 (4X)
Root tips of parental plant-mass	100	100 (100)	0 (0)
Shoot primordia	156	155 (99.4)	1 (0.6)
Root tips of regenerated plants	100	100 (100)	0 (0)

Fig. 1. Mass of shoot primordia of *Matricaria chamomilla*. A. Surface view, Bar = 1 mm. B. Cross section, Bar = 100 μ m.

logically normal and contained the regular chromosome number of $2n=18$ (Table 2).

The shoot primordium extract of *Matricaria chamomilla* contained the major chemical components of the above-ground portion of the parental plant-mass such as chamazulene and bisabolol. However, those chemical components were not quantitatively measured and compared between the tissue-cultured shoot primordia and the parental plant mass.

Proliferation of shoot primordia of *Matricaria chamomilla* was shown in Fig. 3. At fifteen days period after transplantation the mass of shoot primordia grew up to average 3.1 g fresh weight and a few parts of it got colored brown. Moreover, at 35-40 days period most parts of it got colored brown. Continued subculturing at intervals of 14 days, the shoot primordia proliferated rapidly and grew up to 900 times as heavy in fresh weight as ones at intervals of a month. At fourteen days period the density of shoot primordia of 7.4/mg fresh weight promoted 0.54 shoot-regeneration rate by a shoot primordium. Thus, it was simply calculated that every mass of shoot primordia might produce 486 ($=900 \times 0.54$) plantlets.

Thus, the combination of the shoot primordium method and the regeneration method described above must perform rapid mass-clonal propagation of *M. chamomilla*. The tissue-cultured shoot primordia seem to be hardly mutated with respect to chromosome number and external morphology for at least five years. The further chemical analysis is necessary to clarify that ability of the biosynthesis of the tissue-cultured shoot primordia of *M. chamomilla* extends over a long culture-period.

Table 3. Effects of basic media, phytohormone and base layers on plant regeneration from shoot primordia of *Matricaria chamomilla* after 12 weeks culture

Base layer	BAP mg/l	NAA mg/l	Basic media					
			1/4MS		1/2MS		MS	
CW	0.00	0.00	4.0 ¹⁾	+++ ²⁾	9.5 ¹⁾	++ ²⁾	8.0 ¹⁾	± ²⁾
CW	0.00	0.02	3.0	+++	6.0	++	8.0	±
CW	0.00	0.20	3.0	+++	5.0	+++	14.0	+
CW	0.02	0.00	8.5	+++	10.0	+++	9.5	-
CW	0.02	0.02	4.0	+++	9.5	++	7.5	-
CW	0.02	0.20	2.5	+++	2.5	+++	5.5	±
Agar	0.00	0.00	7.5	+++	8.0	±	10.0	-
Agar	0.00	0.02	8.5	+++	9.0	±	8.5	-
Agar	0.00	0.20	2.0	++	5.0	-	3.0	-
Agar	0.02	0.00	11.0	+	8.5	++	9.5	-
Agar	0.02	0.02	8.5	+	4.0	±	6.5	-
Agar	0.02	0.20	0.0	-	3.5	±	6.0	-

CW = Ceramic Wool

¹⁾ Average of: Number of regenerated shoots/Number of shoot primordia per (1.5mm)

²⁾ Rates of regenerated shoots forming roots; +++: all, ++: most, +: half, ±: a few, and -: zero.

Fig. 2. Regeneration on Ceramic Wool (A and B, Bar=1 cm) and acclimatization (C, Bar=2 cm) of plantlets of *Matricaria chamomilla*.

Fig. 3. Growth of mass of shoot primordia of *Matricaria chamomilla*.

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組織培養苗条原基によるカミツレの急速大量増殖

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セスキテルペノイドの安定大量生産を目的に, 苗条原基法を使ってカミツレの遺伝的に安定したクローンの急速大量増殖を試みた。減菌下で, 3-4枚の本葉を持った5mmほどの茎頂から, 1-2個の葉原基を持った茎頂ドームを取り出した。2.0 mg/l BAP添加MS液体培地(30 mm×200 mm試験管, 25 ml/液体培地, pH 5.7-5.8), 22℃, 2,000-9,000 lux, 回転培養装置(直径1 m, 毎分2回転)で, 茎頂ドームを3-4週間培養して, 苗条原基を得た。そして同じ組成の新鮮な培地に2-3週間毎に植え継いで, 同条件で, 継代培養を続けたところ, 安定して生長を続け, 15-20個のドームをもつ, 直径200-500 μmの標準的苗条原基集塊となった。一方, 植物ホルモン無添加でショ糖濃度1%の4倍希釈MS(1/4 MS)セラミック・ウール支持培地に, 直径1.5 mmほどの苗条原基集塊を移植すると, 平均4シュートが現れ, 発根して, 容易に小植物体に再分化した。

この苗条原基は, 培地を換えずに培養を続けると, 14日間の培養で30倍の重さに増殖し, 平均生重量3.1 gとなった。このとき苗条原基集塊の一部は茶色を呈したが, さらに35-40日後には殆どの部分が茶色となった。14日毎に培地を交換して継代培養を続けると, 1ヶ月で生産量にして900倍までに急速大量増殖した。14日目で, 1 mg生重量あたり平均7.4個の苗条原基密度が, 1苗条原基あたり0.54シュート形成に関与しているところから, 単純計算して, 1苗条原基集塊あたり $900 \times 0.54 = 486$ 本の小植物体を形成することになる。得られた植物体は, 外部形態的に親植物と変らなかった。親植物, 4ヶ月間継代培養を続けた苗条原基, 再分化植物体で観察した体細胞のほとんどすべてで, 染色体数 $2n=18$ を得た。従って, カミツレの急速大量増殖には, 苗条原基法と再分化法の両用が効率的であることが分った。