P-164

Callus Induction by In Vitro Culture of Rosmarinus officinalis L.

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로즈마리 기내배양에 의한 Callus 형성

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Objectives

Rosemary (Rosmarinus officinalis) is a perennial woody sub-shrub native to the Mediterranean. The leaves are a powerful source of natural antioxidant, used as raw material in traditional medicine, perfumery, and food industry. This is a preliminary study in order to determine the optimal technique for callus induction through tissue culture.

Materials and methods

○ Materials

Plant materials were collected from the well-grown two-year-old R. officinalis plants which were cultured in the greenhouse. The collected young shoots(5-7cm) were washed under tap water and then held in the distilled water for about one hour. The shoot-tips were removed, and use the young stems and leaves as the materials which were dipped into 70% ethyl alcohol for 1 min, 0.5% sodium hypochlorite supplemented with several drops of Tween-20 for 15 min and rinsed with autoclaved distilled water three times, 5 min each. The sterilization of the plant materials was finished in the clean bench.

○ Methods

Leaf segments(0.8-1cm) and stems(1-1.5cm) were cultured in MS medium with 7 g.L-1agar and 30 g.L-1sucrose supplemented with different concentrations of BA and NAA or 2,4-D(under light or dark at 25±1℃) for callus induction. And the pH of the medium was adjusted to 5.8, 16/8h photoperiod for the cultures under light. After 8 weeks, did the first subculture of the induced callus to 0.2 mg.L-1 NAA and 2.0 mg.L-1 BA. The next subcultures were done interval 3 weeks.

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Results

Small callus were initiated after 10 days. In addition, the colors and growth states of callus in either light and dark or from leaf segments and stems were different. Green or light green under light while pale green or non-green under dark, and compact callus from leaves, growing slowly while the water-soaked callus from stems, easy browning. The NAA and BA treatments were much better than 2,4-D and BA treatments. And the result of callus induction under light was better than dark. The best result for callus induction were obtained at concentrations of 1.0 mg.L-1 NAA. Especially, the treatments 1.0 mg.L-1 NAA combined either with 1.0 mg.L-1 BA or not, were efficient for callus induction both from leaf segments and stems under light. And the 4.0 mg.L-1 2,4-D and 0.5 mg.L-1 BA treatment were better for stems under light, meanwhile it was also available for callus induction from leaf segments.

Table1. Effects of plant growth regulators on different explants for callus induction and proliferation of R.officinalis, after 8 weeks.

Treatment ($mg.L^{-1}$)		Survival rate(%) (light / dark)		Callus proliferation (%) (light / dark)		Result of callus induction (light / dark)[a]	
BA	NAA	Leaf	Stem	Leaf	Stem	Leaf	Stem
	0	0/0	0/0	0/0	0/0	—/—	—/—
0	1.0	98/96	96/97	99/98	98/95	+++/++	+++/+++
	3.0	60/18	36/12	61/0	37/33	+/—	—/—
	0	0/0	0/0	0/0	0/0	—/—	—/—
0.5	1.0	80/62	37/49	82/67	43/35	+/+	++/+
	3.0	87/0	33/0	87/0	70/0	++/—	—/—
	0	0/0	0/0	0/0	0/0	—/—	—/—
1.0	1.0	100/99	98/90	100/96	90/89	+++/++	+++/++
	3.0	80/67	72/63	89/17	78/17	++/+	++/+
BA	2,4-D						
	0	0/0	0/0	0/0	0/0	—/—	—/—
0	2.0	67/0	33/0	17/0	17/0	+/—	—/—
	4.0	0/0	0/0	0/0	0/0	—/—	—/—
	5.0	0/0	0/0	0/0	0/0	—/—	—/—
	0	0/0	0/0	0/0	0/0	—/—	—/—
0.5	2.0	50/53	41/0	61/32	33/0	+/+	+/—
	4.0	87/70	78/46	87/45	98/61	++/+	++/+
	5.0	0/0	0/0	0/0	0/0	—/—	—/—
	0	0/0	0/0	0/0	0/0	—/—	—/—
1.0	2.0	0/0	0/0	0/0	0/0	—/—	—/—
	4.0	65/0	31/0	18/0	13/0	+/—	+/—
	5.0	53/0	30/0	18/0	17/0	+/—	—/—

a —, no callus induced; +, a little callus induced but very small; ++, small pale green callus induced; +++, large pale green or green callus induced.