

**Adventitious shoot regeneration via direct organogenesis from the leaf explant of *Melastoma malabatricum* Linn.**

Bimal Kumar Ghimire<sup>a</sup>, Eun Soo Seong<sup>b</sup>, Nam Jun Kim<sup>b</sup>, In Seong Hwang<sup>b</sup>, Jung Dae Lim<sup>c</sup>, Myong Jo Kim<sup>b</sup>, Balkrishna Ghimire<sup>b</sup>, ChangYeon Yu<sup>b</sup>, Ill Min Chung<sup>a\*</sup>

<sup>a</sup>*Department of Applied Life Science, Konkuk University, Seoul 143-701, SouthKorea*

<sup>b</sup>*Bioherb Research Institute, Kangwon National University, Chunchon 200-701, SouthKorea*

<sup>c</sup>*Department of Herbal Medicine Resource, Kangwon National University*

**Objectives**

An efficient protocol is developed for inducing direct shoot organogenesis from leaf explants of *M. malabatricum*. The effect of PGRs on shoot regeneration and the effect of basal media, carbon sources, which are responsible for shoot formation in regeneration of adventitious shoots from leaf explants of *M. malabatricum* were also discussed. To assess genetic stability of adventitious shoots we evaluated the ploidy level using flow cytometry analysis.

**Materials and Methods**

Plant Material: Mature *M. malabatricum* seeds were agitated in 70 % ethanol for 1min and surface sterilized with an using a 2.0% sodium hypochlorite solution containing 0.02% tween 20 for 15min, subsequently rinsed six times in sterile distilled water and inoculated aseptically on 15ml MS medium supplemented with 3% sucrose and 0.8% agar in culture petridishes. The sterilized explants were cultured in sterile culture petridishes. The seedlings were transferred petridishes containing 50ml solid MS medium and 4-week-old leaf explants measuring was used as sources of explants.

**Results**

Among the two cytokinins tested (BA and TDZ), TDZ in combination with NAA into MS medium induced significantly more shoots per explants than BA. Maximum regeneration occurred on medium containing 1 mg/l NAA and 1 mg/l TDZ. On this medium  $78.00 \pm 0.58$  % of cultures exhibited regeneration with  $11.67 \pm 3.05$  shoots per explant. Highest frequency of shoot induction was observed, when the leaves explants were cultured in the presence of 3% sucrose. The presence of petiole with the lamina attached affected shoot regeneration, number of shoot per explants and % of shoot regeneration.

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\*Corresponding author: Ill Min Chung E-mail : imcim@konkuk.ac.kr Tel : 02-450-3730

Table 1. Effect of different PGRs on direct regeneration from leaves explants of *Melastoma malabathricum* observed after 4 weeks.

Plant growth regulator(mg/l)			Number of shoots/explant	Shoot height	Number of root/explant	% of shoot regeneration	Root length
NAA	BA	TDZ					
0.0	0.0		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.1	0.1		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.1	1.0		7.67 ± 1.52	3.50 ± 0.50 <sup>e</sup>	0.00 ± 0.00	46.70 ± 1.52	0.00 ± 0.00
0.1	2.0		9.00 ± 1.00	5.00 ± 1.00	0.00 <sup>a</sup>	50.00 ± 1.00	0.00 ± 0.00
1.0	0.1		0.00 <sup>a</sup>	0.00 <sup>a</sup>	6.67 ± 1.53 <sup>d</sup>	0.00 <sup>a</sup>	7.00 ± 1.00
1.0	1.0		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1.0	2.0		2.00 ± 1.73	3.67 ± 1.53	0.00 ± 0.00	46.70 ± 1.53	0.00 ± 0.00
2.0	0.1		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2.0	1.0		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2.0	2.0		1.33 ± 0.58	1.50 ± 0.50	2.33 ± 1.53	4.30 ± 2.08	4.00 ± 2.00
0.0		0.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.1		0.1	8.00 ± 1.00	5.00 ± 1.00	0.00 ± 0.00	33.33 ± 2.52	0.00 ± 0.00
0.1		1.0	12.67 ± 2.08	3.00 ± 1.00	0.00 ± 0.00	50.00 ± 2.00	0.00 ± 0.00
0.1		2.0	8.00 ± 1.00	2.50 ± 0.50	0.00 ± 0.00	70.7 ± 0.58	0.00 ± 0.00
1.0		0.1	1.33 ± 0.58	2.33 ± 0.58	0.00 ± 0.00	56.70 ± 2.52	0.00 ± 0.00
1.0		1.0	11.67 ± 3.05	6.33 ± 1.53	0.00 ± 0.00	78.00 ± 0.58	0.00 ± 0.00
1.0		2.0	9.33 ± 1.53	2.67 ± 1.53	0.00 ± 0.00	75.60 ± 0.58	0.00 ± 0.00
2.0		0.1	9.33 ± 1.53	5.33 ± 2.08	2.33 ± 1.53	50.00 ± 1.00	5.50 ± 0.50
2.0		1.0	9.43 ± 1.00	2.50 ± 0.50	0.00 ± 0.00	63.30 ± 1.53	0.00 ± 0.00
2.0		2.0	11.00 ± 1.73	6.00 ± 2.00	0.00 ± 0.00	57.67 ± 2.08	0.00 ± 0.00

Table 2. Effect of explant type on shoot regeneration in *Melastoma malabathricum* after 4 weeks in culture.

Leaf parts	No. of shoot/explant	% of shoot induction
Lamina	8.00 ± 2.00	18.89 ± 1.92
Lamina + petiole	14.67 ± 2.52	76.7 ± 0.58

Table 3. Effect of carbohydrate source and concentration on the number of shoots per explant of *Melastoma malabathricum*.

Carbohydrate source	Conc. (%)	Number of shoot/explant	Shoot length (mm)	% of shoot regeneration
Sucrose	1	2.00 ± 1.00	2.53 ± 0.45	36.67 ± 2.77
	2	4.00 ± 1.00	2.37 ± 0.31	60.00 ± 1.08
	3	15.33 ± 1.53	6.27 ± 0.86	80.00 ± 2.00
	4	4.33 ± 2.52	3.20 ± 0.90	30.07 ± 1.06
Maltose	1	7.00 ± 1.00	2.40 ± 0.26	40.00 ± 1.18
	2	7.67 ± 1.53	3.03 ± 0.21	45.33 ± 3.58
	3	10.67 ± 1.53	3.63 ± 0.40	48.45 ± 1.50
	4	4.00 ± 1.00	1.83 ± 0.31	46.67 ± 2.50
Fructose	1	8.00 ± 1.65	2.90 ± 0.40	38.90 ± 1.52
	2	6.00 ± 2.00	2.97 ± 0.50	36.67 ± 2.08
	3	4.00 ± 1.00	1.70 ± 0.53	32.00 ± 1.53
	4	7.33 ± 2.08	3.67 ± 0.64	22.00 ± 2.00