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SOMATIC ORGANOGENESIS/EMBRYOGENESIS FROM *IN VITRO* CULTURES OF MUSSAENDA *(MUSSAENDA ERYTHROPHYLLA* SCHUM & THONN.)

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Mussaenda erythrophylla Schum & Thonn. is propagated by vegetative means; cutting and layering being commonly employed. As such, true-to-type nature with respect to the colour of the foliaceous sepals exist among the clonal progenies. Being an ornamental plant, variability in colour/type will have economic relevance. *In vitro* regeneration of plants *via* callus-mediated somatic organogenesis is useful in inducing genetic variability or to recover pre-existing natural genetic variability (Larkin and Scowcroft, 1981). The present paper reports on a preliminary attempt to standardise the techniques for callus-mediated somatic organogenesis and embryogenesis in mussaenda.

Materials and Methods

Shoot apices as well as segments of leaves, internodes and ovary wall were used as the explants. They were agitated in a solution of 50 mg/l ascorbic acid for 30 min, followed by surface sterilisation with mercuric chloride solution (0.1 % for 15 min). The apices were then rinsed (at least five times) with sterile water and trimmed to 1.0 cm long segments, aseptically, after which they were inoculated into the different media. The MS medium (Murashige and Skoog, 1962) was used as the basal medium.

For somatic organogenesis, callus induction was attempted with media containing combinations of NAA (1 to 8 mg/l) and kinetin (1 to 4 mg/l), NAA (1 to 8 mg/l) and BA (1 to 8 mg/l) and BA (1 to 4 mg/l) and IAA (1 to 8 mg/l) and BA (1 to 4 mg/l). For somatic embryoid induction, media supplemented with either 2, 4-D (0.1 to 2 mg/l) or combinations of 2, 4-D (0.1 to 2 mg/l) and kinetin (1 to 4 mg/l) were used.

Growth of the callus was assessed based on visual rating (with score 1 to the smallest and score 4 to the largest callus). The mean score was expressed as the growth score. Callus index (CI) was worked out by multiplying per cent explants initiating callus with the growth score. The CI values gave estimates of the overall effectiveness of the treatments to induce and support the growth of callus.

Combinations of kinetin (0.1 to 1 mg/l) and BA (0.1 to 1 mg/l); kinetin (1 to 4 mg/l) and NAA (0 to 8 mg/l) and BA (1 to 4 mg/l) and NAA (0 to 2 mg/l)

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were tried to induce shoot/root regeneration from callus. Callus from the first subculture was used for this. For inducing the development of somatic embryoids, the calli from the induction medium were transferred to media containing combinations of BA (0 to 1 mg/l) and kinetin (0 to 1 mg/l).

The pH of the media was adjusted to 5.7. Semi-solid media (30 ml. containing 0.6% agar in 100 ml conical flasks) were used for the studies. All the chemicals were of analytical grade. Cultures were incubated at 26 ± 2°C with a 16 h photoperiod (1000 lux) applied by cool white flourescent tubes.

Results and Discussion

Somatic organogenesis

Callus induction

Attempts on callus-mediated somatic organogenesis from various explants of mussaenda indicated shoot apices, leaf segments of ovary wall as suitable for callus production. Majority of the treatments were effective in inducing profused callus growth from the shoot apices (Table 1). Sixteen treatments recorded 100 per cent callus initiation and the remaining 0 to 66.7 per cent. The kinetin/NAA combination 1+2 mg/l registered the maximum callus index (CI = 367) and per cent cultures initiating callus (100). Six treatments recorded CI values above 100.

In the case of explants from the leaves, callus index values of 200 and above coupled with 100 per cent callus initiation were recorded by four treatments (Table 1). The maximum values for callus index (CI=267) and per cent cultures initiating callus (100) were registered by kinetin/NAA combination 4+4 mg/l.

All the 12 treatments tried were effective in inducing profuse callusing from segments of ovary wall (Table 1). Except one, all the treatments registered 66.7 to 100per cent cultures initiating callus. In five treatments, the CI values were 200 or above. Kinetin/NAA combinations 1+2 mg/l and 2+4 mg/l were the two very effective treatments, recording CI values of 400 and 100 per cent callus initiation.

Segments of ovary wall yielded the maximum callusing. Among the combinations of auxins and cytokinins, those involving NAA and kinetin gave the maximum response in the different explants tried. The difference in the physiological condition of the explants like level of endogenous phytohormones, nutrients' metabolites etc. might have been responsible for this variation. Quantitative interactions between diverse growth factors may have decisive role in organogenesis (Skoog and Miller, 1957).

Callus initiation started in about 10 days, in all the cases. It was preceded by swelling at the cut end of the shoot apices and swelling all over the segments of

ovary wall. In the case of leaves, callus initiation started along the veins. In all the cases, the entire explant was finally covered by white or light cream, friable to slightly compact callus which turned light green at times.

Callus differentiation

Instances of shoot regeneration from the callus were observed though at a rather low frequency and after long period of culture. Three out of the 46 treatments tried were effective in inducing shoot regeneration. The per cent cultures initiating shoot regeneration was 33.3 in the three treatments. The effective treatments were BA/kinetin combinations 0.5+0.3 mg/l (two shoots per culture), 0.5+0.5 mg/l (five shoots per culture) and BA 2 mg/l (four shoots per culture). The white or cream coloured callus, in about 30 days of culture, organised into meristematic protuberences. Greening of the callus was simultaneously initiated as a result of chlorophyll synthesis. Greening at a few points became more intense and shoot differentiation was initiated from such points. The shoot regeneration process was slow and the new shoots formed were tiny with their apices surrounded by miniature green leaves. Shoots were formed only from the upper surface of the callus. Root formation was not observed in the cultures.

Compared to shoot differentiation, rhizogenesis was more frequent. Nine out of the 58 treatments tried were effective in inducing moderate to profuse root regeneration from the callus (Table 2). In seven out of the nine effective treatments, 66.7 per cent or more cultures exhibited root initiation, Maximum number of roots was produced at the kinetin/NAA combination 2 + 8 mg/l. Among the effective treatments were kinetin/NAA combinations 4+4 mg/l (9.67 roots), 2+2 mg/l (8.67 roots) and BA/NAA combination 1 + 2 mg/1 (6.50 roots), The number of roots produced by the various treatments ranged from 2.00 to 13.33. The roots were formad from all over the surface of the callus. They were initiated after about 60 days of culture as white outgrowths from the callus and rapidly elongated with tufts of snow-white root hairs. Some of the roots turned light green due to the presence of chlorophyll. The roots in contact with the medium exhibited a faster rate of growth with the production of primary and secondary branches.

The results indicate the regenerative potential of mussaenda callus. Further improvement in the frequency of regeneration and growth of the shoots formed, may be possible. Plantlet regeneration from the callus enables generation of desirable variants (Larkin and Scowcroft, 1981). Differentiation of roots was more frequent than that of shoots. The roots exhibited a fast rate of growth, producing primary and secondary branches.

Somatic embryogenesis

Induction

Callus initiation from shoot apices was effected by seven of the sixteen treatments tried (Table 3). Maximum callus index (CI = 367) and 100 percent callus initiation were recorded by 2,4-D/kinetin combination 2+1 mg/l.

Table 1

Effect of different treatments on the production and growth of calli from shoot apex, ovary wall segment and leaf segment cultures of mussaenda (with respect to somatic organogenesis)

Basal medium : MS

Treatment (Concentration in ppm)				Shoot apex		Leaf segment		Ovary wall	
				Cultures initiating callus (%)	Callus index (CI)	Cultures initiating callus (%)	Callus index (CI)	Cultures initiating callus (%)	Callus index (CI)
	1		2	3	4	5	6	7	8
kinetin	1	+ NAA	1	33.3	33.3	66.7	66.7	100.0	100.0
	1	+	2	100.0	367.0	100.0	200.0	100.0	400.0
	1	+	4	33.3	93.0	33.3	33.3	66.7	66.7
	1	+	8	66.7	66.7	0	0	100.0	100.0
	2	+	1	0	0	33.3	33.3	33.3	33.3
	2	+	2	100.0	233.3	100.0	167.0	100.0	300.0
	2	+	4	100.0	133.0	100.0	133.0	100.0	400.0
	2	4	8	100.0	167.0	100.0	200.0	100.0	167.0
	4	+	1	66.7	66.7	66.7	100.0	66.7	66.7
	4	+	2	66.7	100.0	66.7	66.7	100.0	200.0
	4	4	4	100.0	267.0	100.0	267.0	100.0	267.0
	4	+	8	100.0	267.0	100.0	200.0	100.0	133.0
BA	1	4 NAA	1	33.3	33.3	—	—	—	—
	1	4	2	66.7	166.8	—	—	—	—
	1	4	4	0	0	—	—	—	—
	1	+	8	33.3	66.6	—	—	—	—
	2	4	1	0	0	—	—	—	—
	2	+	2	100.0	233.0	—	—	—	—
	2	+	4	66.7	66.7	—	—	—	—
	2	+	8	66.7	166.8	—	—	—	—
	4	+	1	0	0	—	—	—	—
	4	+	2	0	0	—	—	—	—
	4	+	4	100 0	167.0	—	—	—	—
	4	+	8	100.0	233 0	—	—	—	—
BA	1	+ IAA	1	33.3	33.0	—	—	—	—
	1	4	2	66.7	100.0	—	—	—	—
	1	+	4	100.0	100.0	—	—	—	—
	1	4	8	100.7	133.0	—	—	—	—

1	2	3	4	5	6	7	8
2 +	1	33.0	33.3	—	—	—	—
2 +	2	66.7	66.7	—	—	—	—
2 +	4	100.0	100.0	—	—	—	—
2 +	8	100.0	1670	—	—	—	—
4 +	1	100.0	100.0	—	—	—	—
4 +	2	0	0	—	—	—	—
4 +	4	100.0	133.0	—	—	—	—
4 +	8	100.0	100.0	—	—	—	—

Average of three observations 0 : No response
Culture period : four weeks — : No treatment

Twelve out of the 16 treatments effected 66.7 per cent or more callus initiation from the leaf explants (Table 3). However, only three treatments registered CI values of 166.8 or above. Maximum CI value (CI = 233) and 100 per cent callus initiation were observed in the case of 2, 4-D/kinetin combination 1+1 mg/l.

Development

Globular structures resembling somatic embryoids were formed after 70 to 73 days of culture in BA/kinetin combination 0.5 + 0.5 mg/l and 1.1+0.5 mg/1. These structures then exhibited simultaneous root and shoot development. The frequency of development of the structures was low and was observed in 26.7 per cent cultures of BA/kinetin combination 0.5 + 0.5 mg/l and in 6.7 per cent cultures of BA/kinetin combination 1+0.5 mg/l. Finally, 4.5 shoots per culture in the former and 2.0 roots per culture in the latter were seen formed. In both the cases, tufts of snow-white miniature roots were formed at the base of the shoots. The morphology and growth rate of the roots were strikingly different from those observed in somatic organogenesis. Proliferation of roots/shoots was absent in other parts of the callus. These observations indicate that the plantlets resulted from somatic embryogenasis. However, histological examination needs to be done to ascertain their status.

Summary

Ovary wall was identified as the best source of explant for callus production, registering a callus index value of 400, when cultured on MS medium supplemented with NAA 2 mg/l+ kinetin 1 mg/l or NAA 4 mg/l + kinetin 2 mg/l. Shoot regeneration from the callus occurred at a frequency of 33.3 per cent on MS medium supplemented with BA 2 mg/l or BA/kinetin combinations 0.5 + 0.5 mg/l or

Table 2

Effect of combinations of NAA and kinetin, and NAA and BA on somatic organogenesis (root differentiation) from the calli of mussaenda

Basal medium : MS

Treatment (Concentration in ppm)					Cultures initiating roots	Roots per culture
kinetin	1.0	+	NAA	0	0	0
	1.0	+		0.5	0	0
	1.0	+		1.0	0	0
	1.0	+		2.0	0	0
	1.0	+		4.0	66.7	6.50
	1.0	+		9.0	0	0
	2.0	+		0	0	0
	2.0	+		0.5	0	0
	2.0	+		1.0	0	0
	2.0	+		2.0	100.0	8.67
	2.0	+		4.0	0	0
	2.0	+		8.0	100.0	13.33
	4.0	+		0	0	0
	4.0	+		0.5	0	0
	4.0	+		1.0	33.3	3.00
	4.0	+		2.0	0	0
	4.0	+		4.0	100.0	6.67
	4.0	+		8.0	100.0	9.67
BA	1.0	+	NAA	0	0	0
	1.0	+		0.5	0	0
	1.0	+		1.0	33.3	2.00
	1.0	+		2.0	66.7	6.50
	2.0	+		0	0	0
	2.0	+		0.5	0	0
	2.0	+		1.0	0	0
	2.0	+		2.0	100.0	3.00
	4.0	+		0	0	0
	4.0	+		05	0	0
	4.0	+		1.0	0	0
	4.0	+		1.0	0	0

Average of three observations

Table 3

Effect of different treatments on the induction of somatie embryoids (callus induction) from shoot apex and leaf segment cultures of mussaenda

Basal medium : MS

Treatment (Concentration in ppm)		Shoot apex culture		Leaf segment culture	
		Cultures initiating callus (%)	Callus index (CI)	Cultures initiating callus (%)	Callus index (CI)
2,4-D	0.1	66.7	66.7	66.7	100.0
	0.5	1.00.0	100.0	66.7	66.7
	1.0	33.3	33.3	66.7	66.7
	2.0	0	0	0	0
2,4-D	0.1 + kinetin 1.0	66.7	66.7	66.7	66.7
	0.1 + 2.0	100.0	100.0	100.0	100.0
	0.1 + 4.0	66.7	66.7	33.3	33.3
	0.5 + 1.0	100.0	100.0	66.7	66.7
	0.5 + 2.0	33.3	33.3	66.7	66.7
	0.5 + 4.0	0	0	33.3	33.3
	1.0 + 1.0	66.7	200.0	100.0	233.0
	1.0 + 2.0	100.0	167.0	100.0	100.0
	1.0 + 4.0	100.0	180.0	33.0	33.0
	2.0 + 1.0	100.0	367.0	66.7	166.8
	2.0 + 2.0	66.7	166.8	10.00	133.0
	2.0 + 4.0	100.0	100.0	66.7	166.8

Average of three observations Culture period : four weeks

0.5 + 0.3 mg/1. Root regeneration was observed at a frequency of 66.7 per cent, with 13.33 roots per culture on MS medium containing kinetin NAA combination 2+8 mg/l after 60 days of culture. Globular structures resembling somatic embryoids having simultaneous root and shoot development were observed when callus from the induction medium (MS + 2,4-D 2 mg/l + kinetin 1 mg/l) was transferred to MS medium containing BA/kinetin combinations 0.5 + 0.5 mg/l or 1 + 0.5 mg/l, after 70 to 73 days of culture. About 4.5 shoots with tufts of miniature roots were formed per culture in the best treatment.

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