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Plant Regeneration in Momordica Dioica (Roxb) By Root Explant

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Abstract: *Callus cultures were established from leaf, cotyledon and root explants of Momordica dioica (Roxb.)MS basal medium containing different combinations of BA-NAA and various 20-25 days. MS basal medium fortified with 8.88 mM BA and 1.08 mM NAA. For the maintenance and proliferation of the leaf and cotyledon callus, MS medium supplemented with 8.88 mM BA and 1.08 mM NAA, was required. The root explant gave greenish yellow callus on MS basal medium with 13.32 mM BA and 2.7 mM NAA. The media supplemented with 2% of ascorbic acid proved to be significant for maintenance and proliferation of the root callus.*

Key words: *Callus cultures, Leaf, Cotyledon, Root, Momordica dioica.*

I. Introduction:

Momordica dioica (Roxb.) is medicinally and economiocally important plant, cultivated for its fruits, which is used as vegetable. It is seasonal plant with tuberous root grown during the rainy season. The plant showed antimicrobial [1], antimalarial [2], antitumor and anticancer [3] properties. Considering the medicinal importance and scarce availability of Momordica dioica, its in vitro culture was established. Callus cultures serve as a good source for the large scale production of plant secondary metabolites [4,5] Hebbel et al. [6] showed biosynthesis of steroidal alkaloids in callus cultures of Solanum xanthocarpum. Momordica dioica has other properties too, the terpenes and seroids present in root showed anticancer property [3], while alkaloids and glycosides of this plant showed antimicrobiual properties [1] Looking into the importance of plant, the callus cultures were initiated uusing leaf, cotyledon, and root explant of Momordica dioica on MS medium supplemented with various combinations of BA – NAA and concentrations of 2, 4-D.

II. Materials And Methods

The tuberous roots collected from Dr. Balasaheb Sawant Krishio Vidyapeethm Dapoli were grown in experimental green house of KET's Scientific Research Centre, Mulund. The fresh leaves, fruits and roots were used for tissue culture experiments.

The explants were surface sterilized thorougfhly using 70% alcohol, 0.1% mercuric chloride, 2% diathane Z (antifungal) and ciplox Z (antibiotic agfents). The MS (Murashige and Skoog) basal medium [7] supplemented with various combinations of BA-NAA and concentrations of 2,4-D were used for callus initiation. The cultures were incubated at 22 ± 2^0 C and 16/8 hr light/dark photoperiod. The callus was subcultured after 20-25 days. The fresh wiuight of callus was recorded and the significant difference was analyzed by Analysis of Variance (ANOVA).

III. Results

Leaf Explants: Table 1 and figures $1a_1$ to $1c_1$represent leaf explants of *Momordica dioica.* The explants were inoculated on MS basal medium supplemented with combination of (4.44 mM-17.76 mM) BA, (0.54 mM-1.08 mM) NAA and different concentrations of 2, 4 –D (0.45 mM-9 mM). The curling and bulging of leaf was observed after 8-9 days. Greenish white clump of callus was initiated after 15-20 days from inoculation (Fig. 1a,) the callus turned to friable yellowish brown mass after first subculture. MS medium fortified with (4.44mM-13.32mM) BA and 0.54 mMNAA showed gradual increase in weight of callus(Table1) While 17.16 mMBA and 0.54 NAA showed decrease in weight of callus. MS medium with 8.88 mM BA and 1.08 mM NAA showed gradual decrease in mass of callus. MS medium with 8.88 mM BA and 1.08 mM NAA (Fig $1c_1$) MS medium supplemented with various concentrations of 2-4, D was proved to be ideal for callus initiation.

Cotyledon explants: Table 2 and figures $1a_2$ to $1c_2$represent cotyledon explants of Momordica dioica. MS basal Medium supplemented with (4.44mM-17.76 mM) BA and (0.54 mM-1.08mM) NAA showed increase in callus initiation, while 17.76 mM BA and 0.54 mM NAA gave minimum callus induction (Table2). MS medium containing (4.44mM and 8.88mM NAA) and (17.76 mM BA+1.08mM NAA) showed decrease and increase in callus initiation respectively (Table2). The maximum callus initiation was observed on MS medium supplemented with 8.88 mM BA and 1.08 mM NAA (Fig $1b_2$) the maintenance of callus was protocol developed for the callus cultures can be useful in large-scale production of plant secondary metabolites.

Table 1: Induction aand maintenance of calli from leaf explants on MS basal medium supplemented with different combinations of auxin and cytokinin.

Growth regulators(mM)		% Induction	Fresh weight(gm)	Fresh weight of callus on 90th day (gm)	Growth index
NAA	BAP				
0.54	17.76	62.96	2.233+0.0749	12.405a	3.7
0.54	13.32	81.48	4.139+60.1491	19.711a	4.35
0.54	8.88	79.62	4.391+0.1584	19.091b	4.76
0.54	4.44	62.96	3.517+0.1262	13.025a	5.56
1.08	17.76	55.55	1.794+0.0647	9.442a	5.26
1.08	13.32	75.92	3.736+0.1347	12.879a	3.45
1.08	8.88	90.74	5.303+0.1911	20.396b	3.85
1.08	4.44	0	0	-	-

Culture period for initiation : 15-20 days; No of tubes : 30/ combination, no. subcultures : five culture period/ubculture : 18-20 days ; Total period (End time) 110 days approximately (3 ½ Months)216 Means followed by same letter are not significantly difgferentat p=0.05 according to ANOVA.

Table 2: Induction and maintenance of calli from cotyledon explants on MS basal medium supplemented with different combinations of auxin and cytokinin.

Growth regulators(mM)		% Induction	Fresh weight(gm)	Fresh weight of callus on 90th day (gm)	Growth index
NAA	BAP				
0.54	4.44	29.62	0.789+0.0285	26.3[a]	33.3
0.54	8.88	5.55	2.326+0.0855	31.86[b]	13.69
0.54	13.32	68.51	3.084+0.1110	30.85[a]	10
0.54	17.76	53.7	2.276+0.0819	33.98[a]	14.93
1.08	4.44	46.29	1.318+0.0512	24.87[a]	18.87
1.08	8.88	92.59	4.182+0.1493	37.01[b]	8.85
1.08	13.32	59.25	2.757+0.0974	30.63[a]	11.11
1.08	17.76	62.96	2.0848+0.1018	25.89[a]	9.09

Culurte period for initiation : 18-20 days ; No of tubes : 30/ combination; No of subcultures : Five Culture period / subculture : 18-20 days ; Total Period (End time) : 110 days approximately (3 ½ Months) Means followed by same letter are not significantly different at p=0.05 according to ANOVA.

Table 3: Induction and maintenance of calli from root explants on MS basal medium supplemented with diffierent combinations of auxin and cytokinin.

Growth regulators(mM)		% Induction	*FW on 4th sub-culturing (gm)	**FW of callus with ascorbic acid (gm)	***FW on 7th Sub-cuturing (gm)	GI
NAA	BAP					
2.70	4.44	-	-	-	-	-
2.70	8.88	40.74	3.32	3.698+0.132	10.19[a]	2.76
2.70	13.32	42.59	3.43	5.461+0.196	11.87[b]	2.2
2.70	17.76	-	-	-	-	-
5.40	4.44	-	-	-	-	-
5.40	8.88	16.66	2.81	2.997+0.107	9.65[c]	3.22

Callus cultured on *MS with BA and NAA; MS medium with BA + NAA with 2% Ascorbic acid (5^{th} subculture) *** MS medium with BA+NAA with 2% Ascorbic acid (7^{th} subculture) culture period for initiation : 20-25 days; No of tubes : 30/ combination ; No of subcultures : Five Culture period / subculture : 18-20 days ; Total Period (End time) : 110 days approximately (3 ½ Month) Means followed by same letter are not significantly different at p=0.05 according to ANOVA.

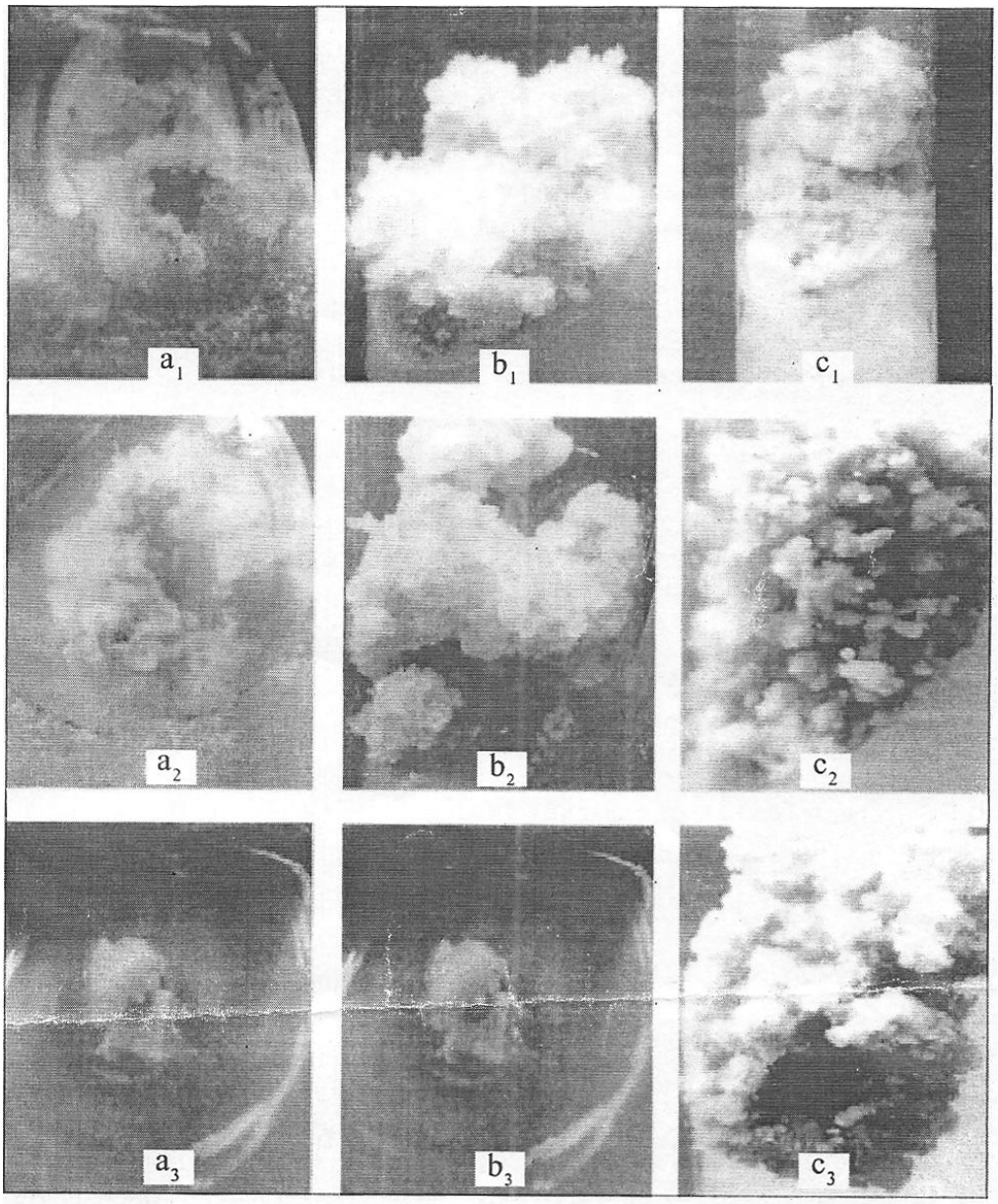

Fig 1: Callus cultures from different plant parts of Momordica silica a1a2a3 callus initiation from leaf, cotyledon and root explants respectively. $B_1b_2b_3$ leaf, cotyledon and root callus proliferation after2nd subculture $c_1c_2c_3$ callus proliferation after 4^{th} subculture from leaf, cotyledon and root explants.

Root explants: Table 3 and figures $1a_3$ to $1c_3$ represent rot explants of Momordica dioica. The tuberous roots showed yellowish green callus after 20-25 days (fig.$1a_3$) MS medium supplemented with 13.32 mM BA and 2.7 mM NAA showed comparatively maximum callus induction than 8.88 mM BA + 2.7 mM NAA and 8.88 mM BA+5.4 mM NAA (Fig 1b3) which was proved by statistical significant . After the fourth subculture the growth of callus was inhibited and it turned black. In subsequent experiments MS medium containing 8.88 mM BA+5.4 mM NAA supplemented with 2% ascorbic acid showed significant callus proliferation and maintenance (Table3, Fig.$1c_3$)

IV. Discussion

MS supplemented with different concentrations of 2, 4-D proved to be an idle media. The leaf, cotyledon and root explants are tried to initiate callus cultures on MS fortified with 0.45 mM-9mM2, 4-D but no callus initiation was observed. Irvine et al. [8] observed that basal medium supplemented with axing compounds showed initiation of callus culture from immature sugar-cane leaf tissue, while in case of Momordica dioica callus initiation was not observed on MS basal medium supplemented with various concentration of auxin (2,4-D) George and Sherrington [9] observed that most of dicotyledonous plants required both auxins and cytokinins in growth medium for callus initiation. O' Dowd et al. [10] observed that a callus culture grown on auxin reduces the yield of secondary metabolites, while callus grown on combination of auxins and cytokinins showed maximum production of secondary metabolites. Mehara and cheema [11] induced callus from Populus ciliate stems for the production of secondary metabolites on MS medium containing BA and NAA.

Study shoes that leaf, cotyledon and root of Momordica dioica gave callus cultures on MS basal medium supplemented with BA and NAA. Root explant required maximum concentration of axing (2.7mM-5.4 mM) compared to leaf and cotyledon explants. Artigas et al. [12] obtained callus cultures from *Hippeastrum vittatum* on MS medium containing BA and NAA in a ratio of 0.1:1 Similarly the leaf and cotyledon explants of *Momordica dioica* gave callus on MS medium supplemented with BA and NAA in ratio 0.1:1. Munjib et al. [13] found callus initiation from rhizome of Caladium sagitifolium on MS medium fortified with BAP-NAA. Brown and charlowood [14] established callus cultures on MS medium with 22.2 mM BA and 5.4 mM NAA from Pelargonium graviolens. Callus induction was obtained from root explant of Plumbago rosea on medium supplemented with BAP 11.10mM –NAA 1.62 mM. In case of Momordica dioica root explants, callus cultures were obtained on 13.32 mM BA and 2.7 mM NAA. The incorporation of ascorbic acid to the medium gave maintenance as well as proliferation of the callus. Skiving and Chu. [15] recorded frofuse callus from Rubus on MS medium supplemented with ascorbic acid.

The present investigation proved that the callus cultures can be successfully obtained from the leaf, cotyledon and root explants of *Momordica* dioica. MS medium supplemented with 8.88 mM BA and 1.08 mM NAA gave profuse callus from root explant. For the maintenance of the leaf and cotyledon callus, it was subcultured on MS medium supplemented with 8.88 mM BA and 1.08 mM NAA. For the maintenance of root callus ascorbic acid was added to the medium as an antioxidant. The standard protocol develpped for the callus can be uiseful in largescale production of plant secondary metabolites.

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References

[1]. Sadyojatha, A.M.and Vaidya, V.P.International seminar on recent trends in pharmaceutical Science. Ootacamund, Abstract 46(1995)
[2]. Misra, P., Pal N.L.Guru P.Y.Katiyar, J.C.and Tandon, J.S.: Intern. J.Pharmacol., 29(1) : 19-23 (1991).
[3]. Luo Lei, L., Zuguoang, Z.Y.and Huang, R.:Acta Pharmac. Sinica, 33(1) : 839-842(1998)
[4]. Tabata, M., Hiraoka, N., lkenoue, M., Sano, Y and konoshima, M.: Lloydia, 38:131-134(1975)
[5]. Wickremesinhe, E.R.M.and Arteca, R.N.Plant Cell Tissue Organ Cult, 35:181-193(1993).
[6]. Heble, M.R.Narayanswami, S and Chandha, M.S.: Sonderdruck aus Die Naturwissenschaften, 55:350-351(1968)
[7]. Murashige, T. and Skoog, F.:Physol. Plant., 15:350-351(1962)
[8]. Irvine, J.E. Fitch, M.and Moore. P.H.Plant Cell Tissue Organ Cul. 2:141-149(1983)
[9]. George, E.F. and Sherrington P.D.:Plant Propagation by Tissue Culture. Exegetics Limited, England (1984)
[10]. O' Dowd, N.A. McCauley, P.G., Wilson, G., Parnell, J.A.N., Kavanagh, T.A.K.and McConnell, D.J.:In:Biotechnology in Agriculture and Festry. 41 Medicinal and Aromatic Plants (Bajaj, Y.P.S. end), pp. 154-193(1998).
[11]. Mehra, P.N. and Cheema. G.S. : Phytomorphology, 30:336-343(1980).
[12]. Artigas-Amador, K., Pena-Garcia, E., Perezmontesion, D., Torriente-Campos, Z.and KLang C.G: Revista Del Jardin Botanioco Nacional, 19:103-111(1998)
[13]. Munjib, A., Maity. I.and Jana, B.K.: Adv.Plant Sci., 9:47-50(1996)
[14]. Brown, J.T.and Charlwood, B.V.:J.Plant Physiol., 123:409-417(1986)
[15]. Skirvin, R.M.and Chu, M.C.: Propagation of Higher Plants through Tissue Culture (Hughes, R. and Constantion, M.eds.), Technical Information Center, US Department of Energy, Springfield, VA pp.253 (1978)