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Somatic Embryogenesis in Two Varieties of Eggplant (*Solanum melongena* L.)

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Abstract: This research programme was undertaken with a view to develop an efficient and reliable method of indirect regeneration through somatic embryogenesis in two varieties of eggplant through the technique of tissue culture. For this purpose, cotyledon pairs of *Solanum melongena* were used as explants for callus induction. Three different auxins (NAA, 2, 4-D and IAA) were used singly or in combination with BAP for present investigation, as types, concentrations and combinations of growth regulators had marked influence on callus induction. Auxin/cytokinin combinations were proved more potent for callus induction as well as callus growth than that of auxin tried singly in the media. Best callus formation was observed in MS media when supplemented with 0.05 mg L^{-1} BAP + 2.0 mg L^{-1} NAA. Best calli from cotyledonary explant were subcultured on MS supplemented with different combinations and concentrations of phytohormones for somatic embryogenesis. Among the different combinations and concentrations 0.05 mg L^{-1} BAP + 2.0 mg L^{-1} NAA gave the best result on somatic embryogenesis for Loda variety. On the other hand, somatic embryos of China variety were obtained only on the media having 1.0 mg L^{-1} BAP + 0.05 mg L^{-1} GA_3. The global structure somatic embryos originated from the superficial layers of the calli, as a single structure or in a cluster. These structures could be isolated easily from parent tissues because they were floated freely in water. These floated free embryos were cultured on nutrient media again to obserb shoot primordia development and thereafter shoot development.

Key words: Eggplant, somatic embryogenesis, *Solanum melongena*

INTRODUCTION

Somatic embryogenesis offers a very fast scaling up system, specially when its possible to produce embryos in bioreactors. Callus derived plant through somatic embryogenesis will have maximum possibilities to create variability, that is somaclonal variation as cells of calli were lacking cytological uniformity (Yamada *et al*., 1967). This somaclonal variation have attracted considerable interest to the plant breeder. Such lines of produced via tissue culture with valuable agronomic characters can be easily utilized in the breeding of eggplant. It has been said that significant possibilities found in commercial cultivation of hybrid brinjal program, so it would be a valuable technique of propagation. The mass production of adventitious embryos in cell culture is still regarded by many as the ideal propagation system. The adventitious embryo is a bipolar structure that develops directly into a complete plantlet and there is no need for a separate rooting phase as with shoot culture. Somatic embryo has no food reserves, but suitable nutrients could be packed by coating or encapsulating to form some kind of artificial seeds (Murashigs, 1978). Such artificial seeds produce the plantlets directly in to the field. Unlike organogenesis somatic embryos may arise from single cells and so it is of special significance in mutagenic studies. Plant derived from asexual embryos may in some cases be free of

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viral and other pathogens. So it is also an alternative approach for the production of disease free plants. Unfortunately this production via fermentors was not simple and to day, only a few model plants are successfully produced by such technology. Several bottlenecks limit the use of this interesting technology. Therefore, there is a need to shift the focus on somatic embryogenesis in valuable crop plants like eggplant.

MATERIALS AND METHODS

This research was conducted at the Institute of Biological Sciences, University of Rajshahi in 2006.

Induction of Explant

In this experiment, two varieties of *Solanum melongena* viz. Loda and China were used. Seeds of these two varieties were cultured on MS medium for obtaining *in vitro* growing seedling from where cotyledons were collected to use as explant.

Callus Induction and Embryogenesis

The cotyledon pair of seedlings was cultured as explant on callus inducing medium for callus induction. Best calli derived from cotyledonary explants of the two varieties were subcultured separately on MS medium having different concentrations and combinations of phytohormones for somatic embryogenesis.

RESULTS AND DISCUSSION

This research was carried out to standardize the protocol for *in vitro* somatic embryogenesis in two varieties (Loda and China) of eggplant. For this purpose two different experiments were carried out to induce callus and thereafter somatic embryogenesis. Cotyledon pairs were cultured on MS medium supplemented with phytohormone for callus induction. Three different auxins (NAA, 2,4-D and IAA) were used singly or in combination with BAP for present research. Different concentrations and combinations of growth regulators had marked influence on callus induction and the result are presented in Table 1and 2. In this experiment it was found that, auxin-cytokinin combinations were proved more potent for callus induction as well as callus growth than that of auxin tried singly in the media.

Best callus formation was observed in MS medium when supplemented with 0.05 mg L^{-1} BAP + 2.0 mg L^{-1} NAA. In this combination 100% explant underwent callus formation and days to callus initiation was 9 days for Loda and 10 days for China variety. Second highest calli formation was observed when 1.0 mg L^{-1} NAA+ 0.05 mg L^{-1}BAP was used in the medium. In this combination, 100% explant also underwent callus formation and callus was initiated within 10 days of culture for Loda variety and 12 days of culture for China variety. Only in these two combinations of BAP and NAA, embryogenic calli were observed (Fig. 1A and B). But other hormonal concentration and combination of phytohormone in media failed to show embryogenic callus. These embryogenic calli of these two varieties were sub cultured separately on MS media having different concentrations and combinations of hormones to observe somatic embryogenesis and obtained results are presented in Table 2. The global structure of somatic embryos originated from the superficial layer of calli appeared as a single structure or in cluster (Fig. 1C).

In case of Loda variety all of the sub-cultured calli proliferated on the media having 2.0 mg L^{-1} NAA + 0.05 mg L^{-1} BAP, 1.0 mg L^{-1} NAA + 0.05 mg L^{-1} BAP and 1.00 mg L^{-1}BAP + 0.05 mg L^{-1} GA_3. But the degree of embryogenesis and number of embryo per callus were different from these three

Table 1: Effect of different concentrations of 2,4-D, NAA and IAA employed in MS medium on callus induction

		Varieties							
		Loda				China			
Growth regulator	Concentration of growth regulators (mg L^{-1})	Days to callus initiation	Explants responded (%)	Weight/ callus (g)	Nature of callus	Days to callus initiation	Explants responded (%)	Weight/ callus (g)	Nature of callus
2,4-D	0.01	20	21	0.28	SW	20	05	0.11	SW
	0.05	16	23	0.34	SW	17	08	0.21	SW
	0.10	13	31	0.69	SW	15	23	0.59	SW
	0.50	12	43	0.71	SW	12	41	0.69	SW
	1.00	9	86	1.03	SW	10	82	0.99	SW
	2.00	8	93	1.22	SW	9	93	1.14	SW
	3.00	8	80	1.00	SW	9	80	0.93	SW
	5.00	10	78	0.98	SW	10	75	0.90	SW
NAA	0.01	18	31	0.65	SG	20	30	0.57	SG
	0.05	16	33	0.81	SG	18	33	0.63	SG
	0.10	12	44	0.92	SG	15	44	0.74	SG
	0.50	10	60	1.06	SG	13	47	0.86	SG
	1.00	8	89	1.12	SG	12	89	1.06	SG
	2.00	7	98	1.41	SG	10	93	1.20	SG
	3.00	7	81	1.10	SG	10	81	1.00	SG
	5.00	8	79	1.00	SG	10	71	0.99	SG
IAA	0.01	23	39	0.20	HW	25	15	0.19	HW
	0.05	19	40	0.30	HW	21	26	0.29	HW
	0.10	16	46	0.35	HW	18	33	0.34	HW
	0.50	14	60	0.45	HW	16	46	0.41	HW
	1.00	13	70	0.51	HW	14	50	0.50	HW
	2.00	13	80	0.71	HW	13	60	0.65	HW
	3.00	12	83	0.73	HW	13	49	0.49	HW
	5.00	14	78	0.70	HW	14	45	0.41	H W

Here, SW = Spongy White; SG = Spongy Green; HW = Hard White

Table 2: Effect of auxin in combination with cytokinin (BAP) on callus induction

		Varieties							
		Loda				China			
Growth regulator	Concentration of growth regulators (mg L^{-1})	Days to callus initiation	Explants responded (%)	Weight/ callus (g)	Nature of callus	Days to callus initiation	Explants responded (%)	Weight/ callus (g)	Nature of callus
2,4-D+	1.0+0.05	10	60	0.93	SW	11	70	0.79	SW
BAP	2.0+0.05	9	75	1.03	SW	10	76	0.85	SW
	3.0+0.05	8	60	0.90	SW	10	65	0.81	SW
	1.0+0.10	12	58	0.89	SW	14	60	0.75	SW
	2.0+0.10	10	66	1.00	SW	14	62	0.80	SW
	3.0+0.10	9	55	0.81	SW	12	60	0.71	SW
	2.0+0.5	15	50	0.80	SW	15	55	0.65	SW
NAA+	1.00+0.05	10	100	1.00	SG	12	100	0.91	SG
BAP	2.00+0.05	9	100	1.20	SG	10	100	1.00	SG
	3.0+0.05	9	100	0.99	SG	10	100	0.90	SG
	1.0+0.10	12	100	0.98	SG	14	100	0.85	SG
	2.0+0.10	10	100	1.00	SG	12	100	0.95	SG
	3.0+0.10	10	100	0.95	SG	12	100	0.81	SG
	2.0+0.5	11	100	0.91	SG	15	100	0.90	SG
IAA+BAP	1.0+0.05	15	65	0.35	HW	16	50	0.35	HW
	2.0+0.05	14	71	0.37	HW	15	55	0.45	HW
	3.0+0.05	14	60	0.35	HW	15	39	0.34	HW

Here, SW = Spongy White; SG = Spongy Green; HW = Hard White

trails. The best performance (%) of embryogenesis of the Loda variety was recorded when the calli were subcultured on the media supplemented with 2.0 mg L^{-1} NAA + 0.05 mg L^{-1} BAP. After sub culture, somatic embryogenesis was initiated within a period of culture and the number of embryos per

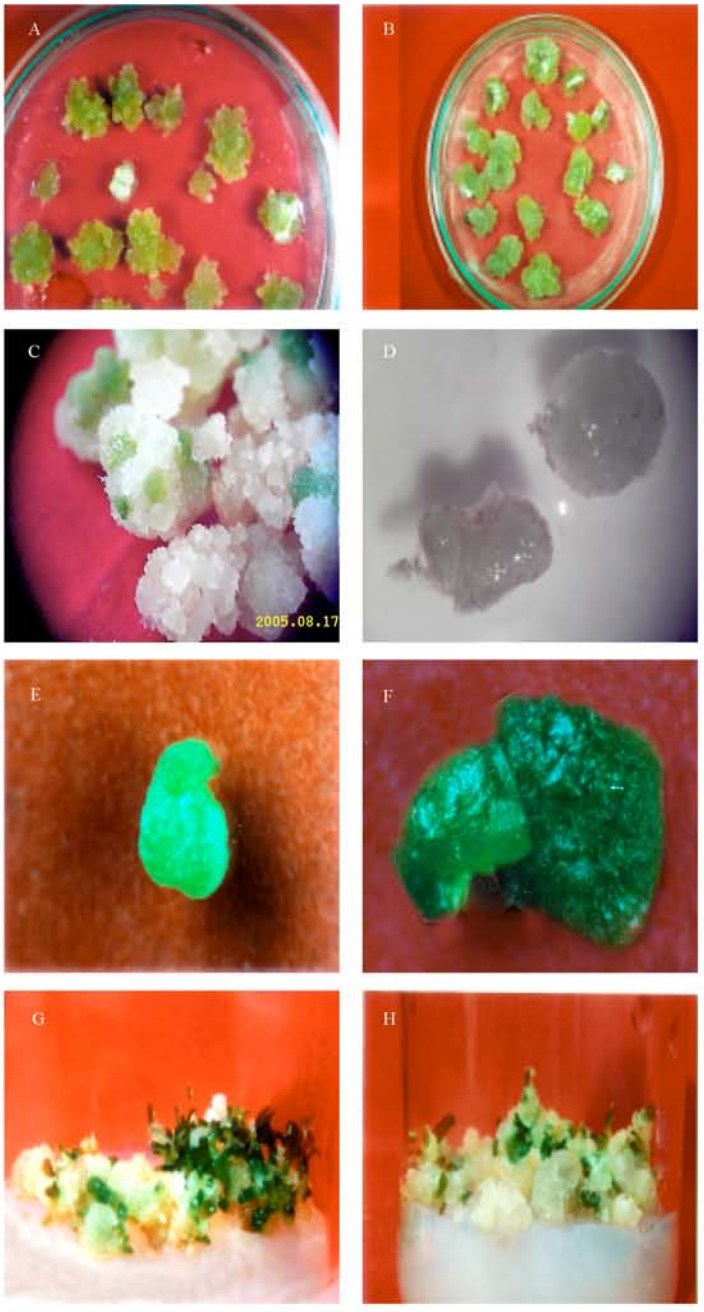

Fig. A: Embryogenic callus of Loda variety; B: Embryogenic callus of China variety; C: Somatic embryogenesis; D and E: Shap of somatic embryo; F: Development shoot primordia from somatic embryo; G: Plant regeneration from somatic embryo in China variety and H: Plant regeneration from somatic embryo in Loda variety

Table 3: Effect of sub culture on somatic embryogenesis and regeneration efficiency from unorganized calli derived from cotyledonary explants

Concentration of growth regulators (mg L^{-1})	Varieties						
	Loda				China		
Pre-culture (28 days)	Sub culture (28 days)	Days to embryo initiation	No. of embryo per callus	No. of shoots per callus	Days to embryo initiation	No. of embryo per callus	No. of shoots percallus
1.0 NAA + 0.05BAP	1.00 NAA+0.05 BAP	15-17	11.3	9	-	-	-
	2.0 NAA+0.05 BAP	10-12	19.5	14	-	-	-
	2.0 NAA+0.05 BAP	-	-	-	-	-	-
	2.0 NAA+1.0 BAP	-	-	-	-	-	-
	0.5 BAP	-	-	-	-	-	-
	1.0 BAP+0.05 GAB	10-14	21.1	13	25-28	7.1	11
	0.5 KIN	-	-	-	-	-	-
	1.0 KIN+0.05 GAB	-	-	-	-	-	-
	MS+CH	-	-	-	-	-	-
	MS + Prolin	-	-	-	-	-	-
2.0 NAA + 0.05BAP	1.0 NAA+0.05 BAP	10-12	17.1	12.1	-	-	-
	2.0 NAA+0.5 BAP	6-7	33.3	15	-	-	-
	2.0 NAA+0.5 BAP	-	-	-	-	-	-
	2.0 NAA+1.0 BAP	-	-	-	-	-	-
	0.5 BAP	-	-	-	-	-	-
	1.0 BAP+0.05 GA_3	8-9	32.5	14.3	20-22	10.9	13.2
	0.5 KIN	-	-	-	-	-	-
	1.0 KIN+0.05 GAB	-	-	-	-	-	-
	MS+CH	-	-	-	-	-	-
	MS+Prolin	-	-	-	-	-	-

Not-: determined

callus was observed 19.5 (Pre-culture on 1.0 mg L^{-1} NAA + 0.05 mg L^{-1} BAP) and 33.3 (Pre-culture on 2.0 mg L^{-1} NAA + 0.05 mg L^{-1} BAP). When the calli of Loda were subcultured on the media having 1.0 mg L^{-1} NAA + 0.05 mg L^{-1}BAP, No. of embryos per callus was 11.3 (Pre-cultured on 1.0 mg L^{-1} NAA+0.05 mg L^{-1} BAP) and 17.1 (Pre-cultured on 2.0 mg L^{-1} NAA). In case of subculture on the media having 1.0 mg L^{-1} BAP+0.05 mg L^{-1} GA_3, the embryos per callus was 21.1 (Pre-culture on 1.0 mg L^{-1} NAA) and. 32.5 (Pre-culture on 2.0 mg L^{-1} NAA).

But when the calli of China variety were subcultured embryogenesis was observed only on the media having 1.0 mg L^{-1} BAP+ 0.05 mg L^{-1} GA_3. In this treatment, days to embryo initiation were 25-28 (Pre-cultured on 1.0 mg L^{-1} NAA) and 20-25 days (pre-cultured on 2.0 mg L^{-1} NAA). The numbers of embryos per callus were 7.1 (pre-cultured on 1.0 mg L^{-1} NAA) and 10.9 (Pre-cultured on 2.0 mg L^{-1} NAA) (Table 3). Different structures (Fig. 1D and E) could be isolated easily from parent tissue because they were floated freely in the water. At first shoot primordia was developed from somatic embryos (Fig. 1F) and thereafter developed into plant (Fig. 1G and H).

Indirect regeneration through somatic embryogenesis taking mature tissue as explant are less responsive when compared with juvenile tissue (Sommer and Caldrars, 1981) and for this reason cotyledon was used as explant. Juvenile tissue such as embryo, cotyledon and various plant parts of seeding have been reported as good sources of explants by a number of workers for a number of plants (Angelini and Allavena, 1989; Manite *et al*., 1989; Agrawal *et al*., 1989; Parrott *et al*., 1989; Verhagen and Wann, 1989; Espinasse *et al*., 1989; Abarbanell and Breiman, 1989; Roy and De, 1990; Miller, 1990; Dong and Jia, 1991). A tissue culture cycle involves the establishment of a more or less dedifferentiated cell or tissue culture under defined culture conditions, proliferation for a number of cell-generations and the subsequent regeneration of plants (Scowcroft and Larkin, 1982). So firstly the process includes the induction of callus from cultured explants. Influence of auxin alone or in combination with cytokinin (BAP) on induction of callus was experimented.

Among three auxins (viz., NAA, 2,4-D and IAA), NAA was found to be the best for callus induction and for callus growth. Role of auxin alone or in combination with cytokinin, callus proliferation was well documented by other workers (Poonam *et al*., 2004; Vijendra *et al*., 2004; Sharma and Kothari 1993; Roy and De, 1990; Verhagen and Wann, 1989). Different concentrations of 2, 4-D tried with MS basal media for callus induction, which showed relatively lower percentage of induction of callus and relatively poor callus growth. But many workers observed 2,4-D as the best auxin for callus induction as common as monocot and even in dicot (Evans *et al*., 1981; Lu *et al*., 1982; Ho and Vasil, 1983; Litz, 1984; Jaiswal and Narayan, 1985; Haccius, 1978; Chee, 1990). In this investigation IAA was also less effective than NAA. But Yamada *et al*. (1967) reported the effectiveness of IAA for *Solanum* callus. Best calli from cotyledonary explant were subcultured for embryogenesis. Under the present experiment, BAP/NAA combination induced somatic embryogenesis in selected concentrations. Combinations of NAA and BAP have successfully been employed to induce plant regeneration in legume tissue (Kameya and Widholm, 1986). Nadel *et al*. (1989) observed somatic embryogenesis in celery by using 2,4-D/BAP combination which is not in agreement with the present finding. Cotyledon and hypocotyl explants of cucumber showed somatic embryogenesis on media having NAA/KIN combination (Chee, 1990). This result is also contradictary to the present investigation. Yamada *et al*. (1967) reported that IAA alone or in combination with KIN induced to form somatic embryos in *S. melongena* which is dissimilar to the present result. Development of somatic embryos was moderate and highly dependent upon the type and concentration of plant growth regulators used (Poonam *et al*., 2004). Such type of information was also proved by the present finding. The first visible indications of somatic embryhogenesis in proliferated calli were the formation of smooth and shiny globular structure on the surface of the calli. The globular structures occurred either singly or in cluster and were invariably attached to the calli. Such an observation of development of somatic embryos have been reported by Vasil *et al*. (1984) and Green and Philips (1975). At first globular somatic embryos were observed within 10-17 days of subculture and globular embryo developed into heart and round shaped. Similar result were reported as somatic embryognesis occurred after 21 days of culture by Kysely and Jacobsen (1990). After sub cultured period of 10-17 days embryos of different stages were observed on the same callus indicating that the development of somatic embryo is a non-synchronous process in differentiating callus, which is supported by Kysely and Jacobsen (1990). The upper surface of callus contained small putative embryonic tissue, which upon microscopic examination, were found to consist of cytoplasmically dense multicellular aggregates which organized into somatic embryo later. Similar observation was reported by Chee (1990).

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