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Somatic embryogenesis and plantlet regeneration from embryogenic suspension culture in muskmelon

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ABSTRACT

A rapidly and maintainable embryogenic suspension culture of *Cucumis melo* was accomplished by transferring embryogenic callus, obtained from mature cotyledon and hypocotyl explants in liquid medium. The cultures obtained were flooded with clumps of globular embryos with very little non-embryogenic tissues. The number and size of somatic embryos/clump was measured to quantify growth of embryogenic tissues under given conditions. The suspensions were sub-cultured every 15 days by adding 10 ml of the old suspension to 40 ml of fresh medium. Initiation and proliferation of such embryogenic suspension cultures depend upon the genotype and various exogenous plant growth regulators fortified to the culture medium at different levels. Culture medium MS2D (MS + 2.0 mg l^{-1} 2,4-D) proved superior for callus induction from cultured mature cotyledon and hypocotyl segments. For the establishment of suspension cultures, the liquid MS medium fortified with 2.0 mg l^{-1} 2,4-D and 0.5 mg l^{-1} BAP was found to be the most effective. For subsequent subcuturing, reduced level of 2,4-D at 1.0 mg l^{-1} in combination with 0.5 mg l^{-1} BAP promoted faster development of somatic embryos. Frequent and efficient plantlet regeneration occurred on MS medium with 0.5 mg l^{-1} each of NAA, BAP and kinetin. Among the four genotypes tested Pusa Madhuras followed by Local cultivar responded better as compared to other genotypes. Regenerated plants were found to be phenotypically normal and true-to-the-type.

Key words: *Cucumis melo*, cotyledon, hypocotyl, suspension culture, somatic embryogenesis.

INTRODUCTION

Muskmelon (*Cucumis melo* L.) is an important vegetable crop is widely cultivated throughout the world. This species has recently been the target for genetic engineering efforts to speed the development of improved cultivars with higher sugar content, increased shelf-life, increased resistance to diseases and pests with greater tolerance to drought, heat and soil toxicities. Developing a resistant variety through conventional breeding is tedious and time consuming and non-availability of suitable resistance sources adds to the breeder's impediment. Unconventional means, such as *in vitro* culture and genetic manipulation can however, circumvent the whole process in a short span of time. In melon, like other crop species, efficient and high frequently plant regeneration through embryogenesis is imperative for crop improvement based on genetic transformation.

Ever since the first report of somatic embryo induction from mature seed derived suspension culture of muskmelon by Oridate and Ooswa (13), several attempts have been made to increase the efficiency of somatic embryogenesis from diverse explant cultures (Bairwa *et al*., 2; Bairwa *et al*., 1, 3; Tabei *et al*., 16; Debeaujon and Branchard, 4; Gray *et al*., 7). However, only in a few instances successful plant regeneration was achieved from melon suspension cultures (Oridate and Ooswa, 13; Kageyama *et al*., 9). In the present investigation efforts were develop a reliable and efficient plant regeneration protocol for muskmelon genotypes by manipulating plant growth regulators and culture conditions.

MATERIALS AND METHODS

Four cultivars of *Cucumis melo* were selected for the study. Cultivars RM50 and Durgapura Madhu were procured from Agricultural Research Station of Rajasthan Agricultural University, Durgapura, cultivar Pusa Madhuras from IARI, New Delhi and Local were collected from Mandsaur, Madhya Pradesh.

To begin with a preliminary experiment, varied fortifications of two basal media, *viz*., MS (Murashige and Skoog, 11) and B_5 (Gamborg *et al*., 6) were compared for better *in vitro* response. During the preliminary investigations, MS basal medium was found to be more responsive as compared to B_5 medium. Consequently, MS was used as basal medium for all the experimentations. Apart from MS basal micro- and macro-salts, B_5 vitamins, and agar, three auxins, namely, 2, 4-D, NAA and 2, 4, 5-T and four cytokinins, *viz*., BAP, kinetin, TDZ and

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zeatin in varying concentrations were added to fortify MS medium for initiation of embryogenic callus and suspension cultures from mature cotyledon and hypocotyl explants. During the initial experiment, it was observed that an auxin as well as a cytokinin alone were not adequate for inducing embryogenic calli and embryogenic suspension cultures. Basal MS medium was used after fortifying it with varying concentrations and combinations of plant growth regulators (BAP and Kn in combination with NAA and 2,4-D) and 30.0 g l^{-1} sucrose. For preparation of semi-solid culture media 7.5 g l^{-1} agar was incorporated. Readymade MS basal medium, plant growth regulators and other ingredients were procured from Hi-Media® Laboratories, Mumbai.

Prior to explant isolation, mature seeds were washed with 2% Tween 20 (v/v) for 15-20 min. and under the running tap water for 10 min. followed by a series of treatments with 70% (v/v) ethanol for 1 min., 2% Bavistin® (a benzimidazole fungicide; BASF, Germany) for 5 min. and 0.2% $HgCl_2$ for also for 5 min. Mature cotyledon and hypocotyl explants were obtained from 3-4-day-old water soaked seeds. Cultured 100 mm × 17 mm glass petri dishes with 7-8 pieces of explant were incubated sealed with Parafilm® at 25 ± 2°C under complete darkness for 1 week. Later the cultured petridishes were subjected to 16 h photoperiod regime of 2,000 lux luminance provided with cool white fluorescent lamps.

For raising suspension cultures three to four week-old embryogenic calli (~2.0 g fresh mass) obtained from mature cotyledon and hypocotyl cultures were transferred to 250 ml Erlenmeyer flasks containing 50 ml of MS liquid medium of same constituents. Callus pieces were strained through a stainless steel mesh (1 mm) placed on the top of a funnel. The cultures were agitated on a horizontal shaker (140 rpm) at 25° ± 2°C under the complete darkness. The suspensions were subcultured every 15 day by adding 10 ml of the old suspension to 40 ml of fresh medium. Relative growth rate was calculated on the basis of increment in fresh weight after culturing embryogenic calli on different liquid media after 4 weeks. Cell cultures were examined microscopically within 15 to 30 days for somatic embryo induction for determination of developmental stages.

Plantlet regeneration was obtained from 4-6 week-old 2.5 to 5 mm Ø cell clumps/embryoids obtained from suspension cultures on MS regeneration medium fortified with different concentrations and combinations of BA, kinetin and NAA alone as well as combination in addition to 20 g l^{-1} sucrose and 7.5 g l^{-1} agar. Each treatment consisted of two replications. Frequency of plantlet regeneration was calculated as percentage of cell clumps with plantlets from total cell clumps plated. The regenerated plantlets were transferred to MS medium supplemented with 1.0 mg l^{-1} for rooting IBA and 15 g l^{-1} sucrose.

Rooted plants were thoroughly washed with running tap water to remove the adhering agar and were planted in 2.5 cm root trainers filled with 1:1:1 sand, soil and FYM sterilized mixture under 30° ± 2°C and 60 ± 5% RH for 15-20 days in a glasshouse for acclimatization before transfer to field.

RESULTS AND DISCUSSION

The initial response of cultured mature cotyledon and hypocotyl explants was similar after 3-5 days and 5-7 days of culture, callus initiated from the cut edges on cotyledons whereas, from hypocotyl segments it started from the wounded ends. The induced calli were light to moderate yellow or greenish in colour, friable to compact and small to medium in size. Compact calli embedded with green nodular structures were not found suitable for initiating suspension cultures and 21-day-old, green/yellow friable calli were used to establish the suspension cultures. It was observed that in liquid culture, compact calli could not disintegrate to form cell suspension. Tanikawa *et al*. (17) and Tiwari *et al*. (19) have reported similar response for onion callus in liquid medium. On the other hand, friable calli when placed in liquid medium were easily broken and dispersed into clumps of 1.2-3.0 mm. Further agitation fragmented these clumps into small cell aggregates (Fig.1 A-B). Genotype Pusa Madhuras followed by Local responded better as compared to rest of the two genotypes forming higher cell clump leading to embryoidogenesis. Callus lines were selected for improved friability and/or regenerability for their use in initiating cell suspension cultures. Most cells from the suspension cultures, when observed under the microscope revealed undifferentiated nature consisting of meristem-like cells with dense cytoplasm and prominent nuclei.

Suspension cultures raised from embryogenic callus produced embryoids that enlarged and germinated after maturation (Fig.1 C-D). Another developmental pathway followed by the cultured embryogenic cultures was the proliferation of multiple embryoids from primary embryoids (Fig.1 E-G). The clumps of developing globular embryos were characteristically yellow-green and varied 1.2 to 3 mm in size. Individual clumps were multi-lobed with each lobe representing an early-stage globular embryo (Fig.1 E-F). Embryos were apparently attached at their bases to the cultures and phenotypic appearance indicated that the embryos arose from the surface of primary embryos. This was similar to the findings of Oridate and Oosawa (13), and Kageyama *et*

al. (9) for muskmelon, and Tiwari *et al*. (19) for onion. Proliferation of primary embryos gave rise to concentric layering of the proliferating secondary embryos until the clumps enlarged and seceded into the liquid culture medium. Some embryoids turned into multi-polar structures, *i.e.*, exhibiting multiple gammogenesis from single embryoid (Fig.1 H-J). A 3-4 week incubation period was necessary to determine which developmental pathway is being followed by the suspension cultures.

For initiating suspension cultures, inoculation of ~ 2.0 g embryogenic calli per 250 ml medium was found to be beneficial for sustainably proliferating embryogenic suspension cultures. Higher response from low explant inoculation frequency during the present experiment is in accordance with the reports of wild cotton (Finer *et al*., 5) and onion (Tiwari *et al*., 19). After 5-6-weeks, embryogenic cultures with 2-8 mm in diameter clumps were placed in fresh liquid medium. The inoculum frequency was kept uniform and many suspension cultures could be initiated using the tissue obtained even from a single initial culture. In the event of higher inoculation frequency, medium replenished partially and initiated non-embryogenic cell suspension cultures.

The auxin 2,4-D has proven extremely useful for initiating embryogenic cultures (Smith and Street, 15 ; Vasil and Vasil, 20). By and large, elimination or change of auxin concentration is a necessary procedure for somatic embryo development. The effect of various auxins at varying levels on initiation of suspension culture is presented in Table 1. Although, at lower levels all auxins have been found to initiate embryoid proliferation, such embryos fail to produce normal plants. At levels higher than 4.0 mg l^{-1} of 2,4-D and NAA, growth of the embryogenic cells in suspension stopped. On the other hand, at lower concentrations of 2,4,5-T a low-activity auxin promoted moderate embryo proliferation.

The culture medium containing 2,4-D is known for the production of friable callus that in turn initiate loose cell aggregates. In contrast, culture media containing NAA produced fast growing compact callus, but failed to initiate cell suspensions. For the initiation of cell suspension from the hypocotyl segments the response of auxins was reverse as NAA favoured the culture of single cells and 2,4-D showed early toxicity to cultures. After initiating two types of cell suspensions from mature cotyledons and hypocotyls it was possible to distinguish the origin of cultures. Cell suspension initiated from hypocotyls directly produced chlorophyllous cells with rapid growth rate (Fig.1B), while from mature cotyledons (Fig. 1A) were yellowish with a slower growth rate.

As compared to the suspension cultures raised from mature cotyledons, the initiation and growth response was totally different with all the culture media combinations. 2,4-D was found to be deleterious for the initiation of suspension cultures from hypocotyls. Even after 3-4 sub-cultures, cell growth declined and suspension became necrotic. Several sub-cultures with medium containing higher concentrations of 2,4-D declined cell growth and caused plasmolysis of the cells, due to the gradual increase in the absolute concentration of 2,4-D per cell (Kageyama *et al*., 9; Oridate and Oosawa,13; Tewes, 28; Tiwari *et al*., 19). The initiation response of cell cultures were found to be auxin-specific. Growth regulator NAA showed beneficial effect on the cell growth on suspension cultures raised from hypocotyls. This finding was in accordance to Jelaska (8) who found IBA and NAA beneficial for embryogenesis in pumpkin.

The effect of cytokinin (alone) on growth of the melon embryogenic suspension is presented in Table 2. The lower level of cytokinins (0.1 mg l^{-1}) resulted slight stimulatory effect on growth of embryogenic tissues. On the other hand, enhanced levels of cytokinin resulted in initiation of non-embryogenic cultures. Culture media containing only cytokinins have been found to be ineffective for growth and development of suspension cultures of melon since rapid growth and development of embryogenic cultures can be achieved without their presence. This phenomenon is in accordance with the findings of Ranch *et al*. (14) in soybean and Tiwari *et al*. (19) in onion. However, in a number of other species cytokinins have been found important for induction of somatic embryogenesis (Matsuoka and Hinata, 10; Nagarajun *et al*., 12).

Since fortification of culture media with auxins and cytokinins in isolation did not support the initiation of suspension cultures in melon, various combinations of auxin and cytokinin were tested subsequently (Table 3). For the establishment of suspension cultures, the liquid medium containing 2,4-D (2.0 mg l^{-1}) in combination with lower concentration of BAP (0.5 mg l^{-1}) have been found to be the most effective. During initial two sub-cultures this medium gave rise to loose, friable cell aggregates from the cultured embryogenic calli, which formed a fine and light cream cell suspension. For subsequent sub-cultures, 2,4-D concentration was reduced to 1.0 mg l^{-1}, which supported faster development of embryos/clump. In present experiment, presence of 2,4-D and BAP in medium was important for somatic embryogenesis in cell suspension cultures of melon. This is in accordance with the findings of Oridate and Oosawa (13), Kageyama *et al*. (9) for melon, and Tiwari *et al*. (19) for onion.

Table 1. Effect of different auxins in varying concentrations on growth of embryogenic muskmelon suspension cultures.

Culture medium	Auxin	Conc. (mg l^{-1})	Embryogenic suspension culture							
			Pusa Madhuras		RM-50		Durgapura Madhu		Local	
			Increment in weight (g)	RG (%)	Increment in weight (g)	RG (%)	Increment in weight (g)	RG (%)	Increment in weight (g)	RG (%)
Control	-	-	2.00 ± 0.12	100	2.00 ± 0.11	100	2.00 ± 0.12	100	2.00 ± 0.12	100
MS5D	2,4-D	0.5	2.12 ± 0.24	106	2.04 ± 0.12	102	2.04 ± 0.12	102	2.08 ± 0.12	104
MSD		1.0	2.80 ± 0.36	140	2.56 ± 0.22	128	2.60 ± 0.24	130	2.72 ± 0.22	136
MS2D		2.0	6.12 ± 0.60	306	5.44 ± 0.56	272	5.68 ± 0.54	284	5.92 ± 0.62	296
MS3D		3.0	5.68 ± 0.56	284	4.96 ± 0.52	248	5.12 ± 0.56	256	5.44 ± 0.56	272
MS4D		4.0	4.92 ± 0.48	246	4.48 ± 0.48	224	4.60 ± 0.52	230	4.72 ± 0.52	236
MS5D		5.0	CM	-	CM	-	CM	-	CM	-
MS.5N	NAA	0.5	2.24 ± 0.22	112	2.08 ± 0.12	104	2.08 ± 0.13	104	2.24 ± 0.24	112
MSN		1.0	2.76 ± 0.24	138	2.36 ± 0.22	118	2.44 ± 0.24	106	2.60 ± 0.26	130
MS2N		2.0	4.84 ± 0.38	242	4.48 ± 0.58	224	4.64 ± 0.52	232	4.76 ± 0.52	238
MS3N		3.0	4.52 ± 0.32	226	4.04 ± 0.44	202	4.08 ± 0.50	204	4.32 ± 0.46	216
MS4N		4.0	3.12 ± 0.28	156	2.76 ± 0.24	138	2.80 ± 0.30	140	2.96 ± 0.30	148
MS5N		5.0	2.36 ± 0.20	118	2.28 ± 0.22	114	2.28 ± 0.26	114	2.36 ± 0.24	118
MS.5T	2,4,5-T	0.5	2.24 ± 0.18	112	2.16 ± 0.18	108	2.08 ± 0.11	104	2.20 ± 0.22	110
MST		1.0	2.56 ± 0.24	128	2.32 ± 0.20	116	2.24 ± 0.16	112	2.24 ± 0.12	112
MS2T		2.0	4.48 ± 0.30	224	4.08 ± 0.44	204	4.20 ± 0.42	210	4.32 ± 0.44	216
MS3T		3.0	4.68 ± 0.36	234	4.24 ± 0.48	212	4.40 ± 0.48	220	4.52 ± 0.46	226
MS4T		4.0	2.72 ± 0.28	136	2.44 ± 0.18	122	2.56 ± 0.22	128	2.60 ± 0.22	130
MS5T		5.0	2.32 ± 0.22	116	2.20 ± 0.14	110	2.24 ± 0.18	112	2.32 ± 0.18	116

RG: Relative growth

Table 2. Effect of different cytokinins on growth of embryogenic muskmelon suspension cultures.

Culture medium	Cytokinin	Conc. (mg l^{-1})	Pusa Madhuras		RM-50		Durgapura Madhu		Local	
			Increment in weight (g)	RG (%)	Increment in weight (g)	RG (%)	Increment in weight (g)	RG (%)	Increment in weight (g)	RG (%)
	Control	-	2.00 ± 0.11	100	2.00 ± 0.13	100	2.00 ± 0.11	100	2.00 (g)	100
MS.1B	BAP	0.1	2.36 ± 0.12	118	2.12 ± 0.14	106	2.20 ± 0.14	110	2.28 ± 0.12	114
MS.5B		0.5	2.76 ± 0.18	138	2.60 ± 0.22	130	2.64 ± 0.20	132	2.72 ± 0.28	136
MSB		1.0	2.88 ± 0.22	144	2.60 ± 0.25	130	2.68 ± 0.32	134	2.80 ± 0.30	140
MS2B		2.0	3.16 ± 0.34	158	2.68 ± 0.34	134	2.84 ± 0.38	142	2.96 ± 0.38	148
MS3B		3.0	CC	-	CC	-	CC	-	CC	-
MS.1Kn	Kn	0.1	2.28 ± 0.12	114	2.16 ± 0.12	108	2.24 ± 0.12	112	2.24 ± 0.12	112
MS.5Kn		0.5	2.56 ± 0.18	128	2.40 ± 0.32	120	2.52 ± 0.22	126	2.44 ± 0.16	122
MSKn		1.0	2.68 ± 0.22	134	2.52 ± 0.22	126	2.60 ± 0.24	130	2.52 ± 0.20	126
MS2Kn		2.0	2.96 ± 0.34	148	2.72 ± 0.32	136	2.76 ± 0.32	138	2.88 ± 0.34	144
MS3Kn		3.0	CC	-	CC	-	CC	-	CC	-
MS.1Td	TDZ	0.1	2.56 ± 0.22	128	2.40 ± 0.13	120	2.48 ± 0.24	124	2.52 ± 0.22	126
MS.5Td		0.5	2.68 ± 0.24	134	2.56 ± 0. 24	128	2.25 ± 0.26	126	2.60 ± 0.26	130
MSTd		1.0	2.44 ± 0.26	122	2.32 ± 0.22	116	2.24 ± 0.18	112	2.48 ± 0.22	124
MS2Td		2.0	CC	-	CC	-	CC	-	CC	-
MS.1Z	Zeatin	0.1	2.32 ± 0.24	116	2.08 ± 0.11	104	2.12 ± 0.11	106	2.24 ± 0.26	112
MS.5Z		0.5	2.64 ± 0.32	132	2.32 ± 0.22	116	2.24 ± 0.16	112	2.48 ± 0.28	124
MSZ		1.0	CC	-	CC	-	CC	-		-

RG: Relative growth

Table 3. Combined effects of cytokinins and auxins on proliferation of embryogenic suspension cultures.

Culture medium	Plant growth regulators (mg l^{-1})				Pusa Madhuras		RM-50		Durgapura Madhu		Local	
	2,4-D	NAA	BAP	Kn	Increment in weight (g)	RG (%)	Increment in weight (g)	RG (%)	Increment in weight (g)	RG (%)	Increment in weight (g)	RG (%)
Control					2.00 ± 0.13	100	2.00 ± 0.11	100	2.00 ± 0.12	100	2.00 ± 0.08	100
MS.5D.5B	0.5	-	0.5	-	3.16 ± 0.32	158	2.84 ± 0.24	142	2.88 ± 0.14	144	3.00 ± 0.20	150
MSD.5B	1.0	-	0.5	-	5.44 ± 0.54	272	5.00 ± 0.66	250	5.12 ± 0.64	256	5.28 ± 0.52	264
MS2D.5B	2.0	-	0.5	-	6.48 ± 0.72	324	6.16 ± 0.78	308	6.32 ± 0.68	316	6.36 ± 0.60	318
MS3D.5B	3.0	-	0.5	-	5.96 ± 0.64	298	5.60± 0.58	280	5.68 ± 0.58	284	5.84 ± 0.58	292
MS5D.5B	5.0	-	0.5	-	CM	-	CM	-	CM	-	CM	-
MSDB	1.0	-	1.0	-	4.80 ± 0.46	240	4.48 ± 0.48	224	4.56 ± 0.44	228	4.68± 0.25	234
MS2DB	2.0	-	1.0	-	6.16 ± 0.68	308	5.72 ± 0.58	286	5.80 ± 0.50	290	5.96 ± 0.58	298
MS.5N.5B	-	0.5	0.5	-	2.40 ± 0.14	120	2.24 ± 0.12	112	2.28 ± 0.16	114	2.36 ± 0.12	118
MSN.5B	-	1.0	0.5	-	3.56 ± 0.30	178	2.80 ± 0.26	140	3.28 ± 0.28	164	3.40 ± 0.36	170
MS2N.5B	-	2.0	0.5	-	5.24 ± 0.56	262	5.00 ± 0.54	250	5.04 ± 0.46	252	5.12 ± 0.58	156
MS3N.5B	-	3.0	0.5	-	4.80 ± 0.38	240	4.52 ± 0.44	226	4.56 ± 0.42	228	4.68 ± 0.47	234
MS5N.5B	-	5.0	0.5	-	2.16 ± 0.12	108	2.04 ± 0.12	102	2.04 ± 0.05	102	2.08 ± 0.17	104
MSNB	-	1.0	1.0	-	4.80 ± 0.38	240	4.64 ± 0.46	232	4.68 ± 0.52	234	4.76 ± 0.48	238
MS2NB	-	2.0	1.0	-	5.84 ± 0.60	292	5.64 ± 0.62	282	5.68 ± 0.58	284	5.80 ± 0.62	290
MS.5D.5Kn	0.5	-	-	0.5	2.28 ± 0.12	114	2.08 ± 0.11	104	2.12 ± 0.12	106	2.24 ± 0.12	112
MSD.5Kn	1.0	-	-	0.5	2.48 ± 0.24	124	2.28 ± 0.12	114	2.32 ± 0.14	116	2.40 ± 0.16	120
MS2D.5Kn	2.0	-	-	0.5	5.16 ± 0.62	258	4.64 ± 0.46	232	4.84 ± 0.46	242	5.04 ± 0.46	252
MS3D.5Kn	3.0	-	-	0.5	4.68 ± 0.46	234	4.24 ± 0.34	212	4.44 ± 0.38	222	4.60 ± 0.42	230
MS5D.5Kn	5.0	-	-	0.5	2.12 ± 0.12	106	2.04 ± 0.13	102	2.04 ± 0.10	102	2.08 ± 0.12	104
MSDKn	1.0	-	-	1.0	3.84 ± 0.30	192	3.68 ± 0.28	184	3.72 ± 0.32	186	3.80 ± 0.32	190

RG: Relative growth

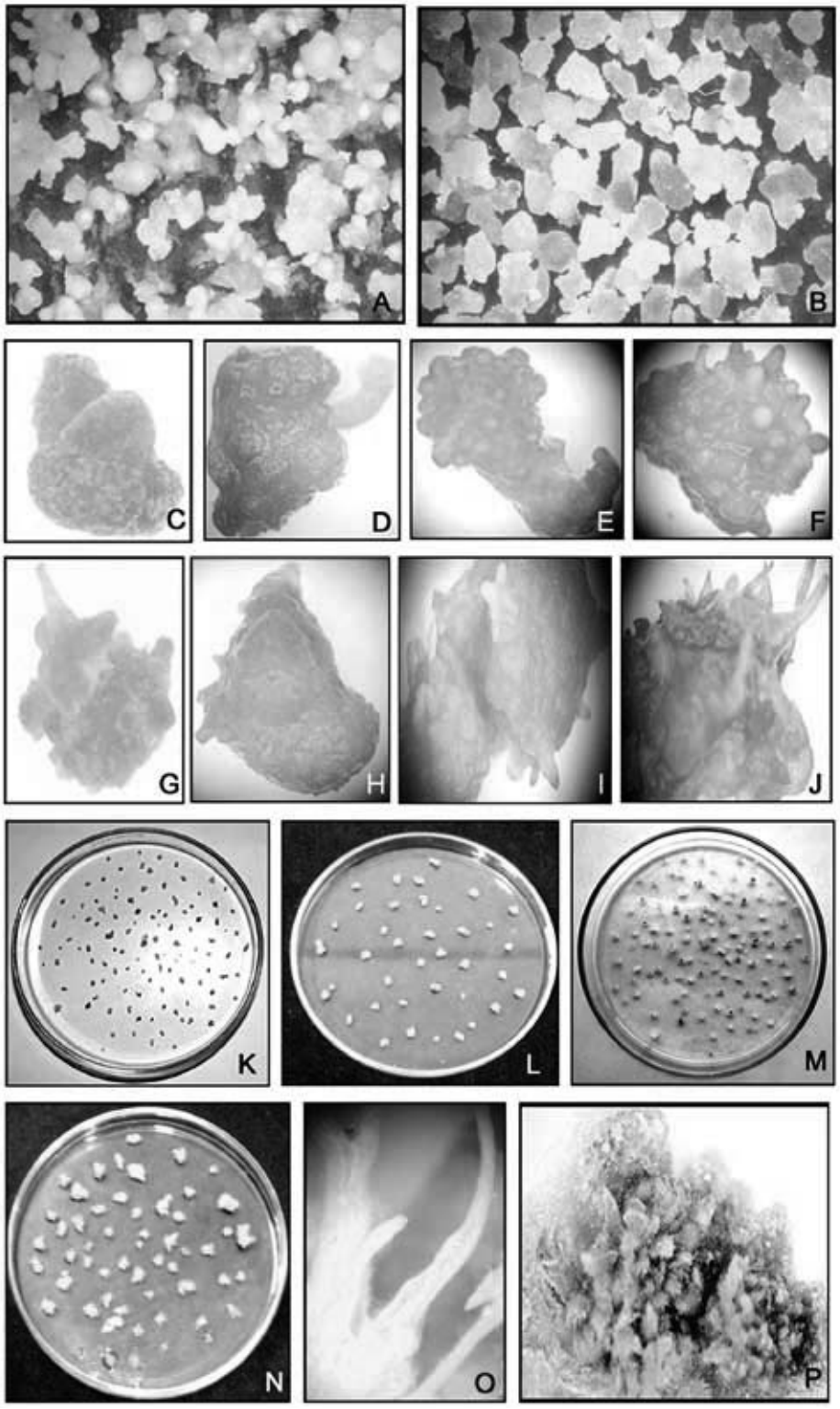

Fig 1. Suspension culture of muskmelon : A. Initiation of cell clumps and embryoid formation from suspension culture raised from embryogenic calli of hypocotyl; B. Initiation of cell clumps and embryoid formation from suspension culture raised from embryogenic calli of mature embryos; C. Typical bipolar embryoid; D-E. Germination of embryoid; F-G. Multiple embryoid formation; H. Germination of multiple embryoids; I-J. Typical multi-polar embryoid; K-N. Germination of multi-polar embryoid; O-P. Regenerated shoot with rootlets.

Regeneration of plantlets require the addition of plant growth regulators for onion suspension culture (Tanikawa *et al*., 17). Growth regulator 2,4-D used in melon tissue culture do not support whole plants regeneration in liquid cultures. During present experimentation, combinations of 0.5-1.0 mg l^{-1} cytokinin with 0.5 mg l^{-1} auxin supported plantlet regeneration (Table 4). MS basal medium supplemented with two cytokinin and one auxin, *i.e.*, 0.5 mg l^{-1} each of BAP, kinetin and NAA regenerated plantlets in higher frequencies. This is contradicting with the findings of Kageyama *et al*. (9), and Oridate and Oosawa (13) as they recovered plantlets in higher frequencies on hormone-free medium in melon cell suspension cultures. After 4 to 6 weeks of plating of the cell clumps/embryoids on various

Table 4. Effects of plant growth regulators on the regeneration on plantlets from embryoids/cell clumps.

Culture medium	Plant growth regulators (mg l^{-1})			Plantlet regeneration (%)				
	BAP	NAA	Kinetin	Pusa Madhuras	RM-50	Durgapura Madhu	Local	Mean
MS.1B	0.1	-	-	66.46	61.54	63.74	64.21	63.99^{g}
MS.2B	0.2	-	-	67.34	60.79	63.67	65.29	64.27^{g}
MS.5B	0.5	-	-	79.61	71.34	77.86	77.31	76.53^{c}
MSB	1.0	-	-	78.89	72.16	75.75	77.34	76.04^{c}
MS2B	2.0	-	-	73.34	66.18	68.79	71.63	69.99^{e}
MS.1N	-	0.1	-	37.44	30.31	33.46	36.84	34.51^{k}
MS.2N	-	0.2	-	48.78	40.31	42.64	45.87	44.40^{j}
MS.5N	-	0.5	-	63.23	57.49	58.87	59.79	59.85^{h}
MSN	-	1.0	-	54.11	48.37	50.61	53.24	51.58^{i}
MS2N	-	2.0	-	54.02	45.67	50.12	53.08	50.72^{i}
MS.1Kn	-	-	0.1	26.45	18.67	22.31	24.87	23.08^{n}
MS.2Kn	-	-	0.2	24.13	20.47	21.64	23.44	22.42^{n}
MS.5Kn	-	-	0.5	33.67	26.85	29.47	31.02	30.25^{l}
MSKn	-	-	1.0	28.51	21.48	23.67	25.75	24.85^{m}
MS2Kn	-	-	2.0	27.12	21.02	23.11	25.31	24.14^{m}
MS.2B.5N	0.2	0.5	-	23.12	15.64	17.89	20.78	19.36^{o}
MS.5B.5N	0.5	0.5	-	80.15	72.49	74.37	77.64	76.16^{c}
MSB.5N	1.0	0.5	-	87.26	78.64	81.29	85.47	83.17^{b}
MS2B.5N	2.0	0.5	-	77.22	70.64	72.84	75.31	74.00^{d}
MS.5BN	0.5	1.0	-	68.49	61.97	63.48	67.84	65.45^{g}
MS.5B2N	0.5	2.0	-	21.34	15.97	17.34	19.57	18.56^{o}
MS.5N.5Kn	0.5	-	0.5	71.56	64.71	65.62	68.41	67.58^{f}
MSNKn		1.0	1.0	73.64	65.45	67.48	70.34	69.23^{e}
MS.5B.5N.5Kn	0.5	0.5	0.5	91.34	83.67	84.12	87.59	86.68^{a}
$CD_{0.05}$								
Genotype								0.36
Medium								0.88
G × M								1.77

Figures in percentage are transformed values (Arc-sine transformation).
Values within column followed by different letters are significantly different at 1% probability level.

regeneration media, shoots and roots emerged (Fig. 1O-P). More than 98% regenerants were rooted when they were transferred into MS rooting medium fortified with 1.0 mg l^{-1} IBA, 20 g l^{-1} sucrose and 7.5 g l^{-1} agar. Phenotypically normal plants were obtained after hardening in greenhouse. Genotype Pusa Madhuras followed by the 'Local' produced higher shootlets as compared to other genotypes. Tiwari *et al*. (19) have also reported strong genotypic effects in shoot regeneration from suspension culture in *Allium cepa*.

Present investigation demonstrates that the successful plant regeneration from suspension cultures would be useful for mutant cell selection in melon against various biotic and abiotic stresses. The suspension cultures will be a good source for the isolation of totipotent protoplasts for potential use in genetic manipulations.

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REFERENCES

1. Bairwa S.K., Kushwah S.S., Tripathi, M.K., Baghel, B.S. and Tiwari, S. 2010a. Regeneration of *Cucumis melo* L. through somatic embryogenesis and organogenesis from cultured embryonic axes. *Veg. Sci*. **37**: 14-20.

2. Bairwa S.K., Tripathi, M.K., Kushwah, S.S., Baghel, B.S. and Tiwari, S. 2008. Effect of genotypes and plant growth regulators on regulation of *in vitro* morphogenesis in muskmelon (*Cucumis melo* L.) from cultured mature cotyledons. *Plant Cell Biotech. Mol. Biol*. **9**: 45-60.

3. Bairwa S.K., Tripathi, M.K., Kushwah, S.S., Baghel, B.S. and Tiwari, S. 2010. Induction of somatic embryogenesis and organogenesis from hypocotyl of muskmelon (*Cucumis melo* L.). *J. Agric. Tech.* **6**: 687-702.

4. Debeaujon, I. and Branchard, M. 1992. Induction of somatic embryogenesis and cauliogensis from cotyledon and leaf protoplast-derived colonies of melon (*Cucumis melo* L.). *Plant Cell Rep*. **12**: 37-40.

5. Finer, J.J., Reilley, A.A. and Smith, R.H. 1987. Establishment of embryogenic suspension cultures of a wild relative of cotton (*Gossypium klotzschianum* Anderss.). *In Vitro Cell Dev. Biol*. **23**: 717-22.

6. Gamborg, O.L., Miller, R.A. and Ojima, K. 1968. A nutrients requirement of suspension cultures of soybean root cells. *Exp. Cell Res.* **50**: 151-58.

7. Gray, D.J., McColley, D.W. and Comptom, M.E. 1993. High frequency somatic embryogenesis from quiescent seed cotyledons of *Cucumis melo* cultivars. *J. American Soc. Hort. Sci*. **118**: 425-32.

8. Jelaska, S. 1974. Embryogenesis and organogenesis in pumpkin explants. *Physiol. Plant*. **31**: 257-61.

9. Kageyama, K., Kazunori Y. and Shiggetoshi, M. 1991. Somatic embryogenesis in suspension culture of mature seed of *Cucumis melo* L. *Japanese J. Breed*. **41**: 273-78.

10. Matsuoka, H. and Hinata, K. 1979. NAA-induced organogenesis and embryogenesis in hypocotyl callus of *Solanum melongena* L. *J. Exp. Bot*. **30**: 33-39.

11. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiol*. **15**: 473-97.

12. Nagarajun, P., McKenzie, J.S. and Walton, P.D. 1986. Embryogenesis and plant regeneration of *Medicago* spp. in tissue culture. *Plant Cell Rep.* **5**: 77-80.

13. Oridate, T. and Oosawa, K. 1986. Somatic embryogenesis and plant regeneration from suspension callus culture in melon (*Cucumis melo* L.). *Japanese J. Breed*. **36**: 424-28.

14. Ranch, J.P., Oglesby, L. and Zielinski, A.C. 1985. Plant regeneration from embryo-derived tissue cultures of soybean. *In Vitro Cell Dev. Biol*. **21**: 653-58.

15. Smith, S.M. and Street, H.E. 1974. The decline of embryogenic potential as callus and suspension cultures of carrot (*Dacus carota* L.) is serially subcultured. *Ann. Bot*. **38**: 223-41.

16. Tabei, Y., Kanno, T. and Nishio, T. 1991. Regulation of organogenesis and somatic embryogenesis by auxin in melon, *Cucumis melo* L. *Plant Cell Rep.* **10**: 225-29.

17. Tanikawa, T., Takagi, M. and Ichii, M. 1996. Plant regeneration from suspension cultures of onion (*Allium cepa* L.). *Plant Tissue Cult. Lett.* **13**: 259-64.

18. Tewes, A. 1984. High yield isolation and recovery of protoplasts from suspension cultures of tomato (*L. esculentum*). *Z. Pflanzenphysiol.* **113**: 141-50.

19. Tiwari, S., Tripathi, M.K., Khare U.K. and Rana, R. 2007. Plant regeneration through embryogenic suspension cultures of onion (*Allium cepa* L.). *Indian J. Biotechnol*. **6**: 100-106.

20. Vasil, V. and Vasil, I.K. 1981. Somatic embryogenesis and plant regeneration from suspension cultures of pearl millet (*Pennisetum americanum*). *Ann. Bot*. **47**: 669-78.

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