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REGENERATION OF *CUCUMIS MELO* L. THROUGH SOMATIC EMBRYOGENESIS AND ORGANOGENESIS FROM CULTURED EMBRYONIC AXES

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Summary

Embryonic axes of four genotypes of *Cucumis melo* were cultured on different fortifications of MS medium to assess their *in vitro* response. *In vitro* morphogenesis (somatic embryogenesis and organogenesis) leading to plantlet regeneration varied considerably influenced by genotypes and culture medium. Among various medium tested MSD.5B (MS + 1.0mg.l^{-1} 2,4-D + 0.5mg.l^{-1} BA) supported maximum direct somatic embryogenesis (23.91%) and average formation of somatic embryo(s) per explant (27.80) whereas, MS2D.5B (MS + 2.0mg.l^{-1} 2,4-D + 0.5mg.l^{-1} BA) was found superior for induction of indirect somatic embryogenesis (21.32%). Inoculation medium MS2NB (MS + 2.0mg.l^{-1} NAA + 1.0mg.l^{-1} BA) proved superior for initiation of direct (26.59%) and indirect (21.05%) organogenesis as well as regeneration of plantlets (154.41%) with direct organogenesis. MS medium fortified with 0.5 mg.l^{-1} NAA + 0.5 mg.l^{-1} BA + 0.5 mg.l^{-1} Kn proved superior for plant regeneration *via* somatic embryogenesis (41.58%) and indirect organogenesis (121.76%). Among genotypes, Pusa Madhuras was found to be superior for most *in vitro* traits followed by Local Durgapura Madhu and RM-50 for all attributes investigated.

सारांश

खरबूजा कों चार जननद्रव्यों का भ्रूणीय तरूण कों लेकर पात्रे तकनीक से विभिन्न रूपान्तरित एम.एस. पोषपदार्थ पर संबर्धित होने का ऑकलन किया गया। पात्रे तकनीक (वर्धीय भ्रूण व अंग) में पौधो का पुनर्जनन जननद्रंव्य एवं संवर्धन पोष पदार्थ के परीक्षण में एम. एस. डी. 5 बी (एम. एस. + 1.0 मि. ग्रा. एल0.1 2, 4–डी + 0.5 मिग्रा. एल. 1 बीए.) सें सबसे ज्यादा प्रत्यप्स कायिक भ्रूणीय विकास हुआ (23.91 प्रतिशत) तथा औसत कायिक भ्रूण प्रति पौधे (27.80) रहा जबकि एम0 एस. 2 डी 5 बी (एम.एस. + 2.0 मिग्रा. एल–1 2 , 4– डी + 0.5 मि.ग्रा. ए$^{-1}$ बी.ए.) सें अप्रत्यक्ष कयिक भ्रूण उत्तम (21.32 प्रतिशत) रहा। प्रवेश संबर्ध एम0 एस0 2 एन 0 बी (एम.एस. + 2.0 मिग्रा. एल–1 एन.ए.ए. + 1.0 मि.ग्रा. एल0^{-1} बी.ए.) सें प्रत्यक्ष (22.59 प्रतिशत तथा अप्रत्यक्ष (21.05 प्रति प्रतिशत) अंगीय विकास उत्तम रहा। तथा अप्रत्यक्ष पौध पुर्नजनन (154.41 प्रतिशत) भी ज्यादा रहा। रूपान्तरित एम.एस. पोष पदार्थ 0.5 मि.ग्रा. एल $^{-1}$ एन.ए.ए. + 0.5 मि. ग्रा. एल$^{-1}$ बी.ए. + 0.5 मि. ग्रा. एल$^{-1}$ कें. एल सें कायिक भ्रूण सें पौध विकास ज्यादा (41.58 प्रतिशत) रहा तथा अप्रत्यक्ष रूप सें ॲगरूि विकास (121.76 प्रतिशत) भी अच्छा रहा। जनद्रव्यों में प्रजाति पूसा मधुरस पात्रे तकनीक हेतु सबसे उत्तम गुणों वाला पाया गया तथा उसके बाद प्रजाति स्थानिय दुर्गापुर मखु व आर.एम. 50 कों सभी परीक्षित गुणों कें लिए पाया गया।

Introduction

At present, melon is beeing targeted for tailoring transgenic plants with increased sugar content and fruit shelf-life, resistance to diseases and insects, tolerance to drought, heat and soil toxicities and genetic yield potentials. The efficient *in vitro* regeneration procedure *via* somatic embryogenesis is prerequisite to obtain transgenic plants expressing desired genes.

In muskmelon, diverse explants, such as, cotyledons (Oridate and Oosawa, 1986; Branchard and Chateau, 1988; Dirks and van Buggenum,1989; Niedz *et al.,* 1989; Tabei *et al*., 1991; Gray *et al*., 1993; Aldelderg *et al.,* 1994; Yadav *et al.,* 1996; Guis *et al.,* 1997; Rhimi *et al.,* 2006), hypocotyls (Kathal *et al*., 1986), leaves (Moreno *et al.,* 1985; Kathal *et al*., 1988;; Stipp *et al.,* 2001), roots (Kathal *et al*., 1994) and protoplasts (Li *et al*., 1990; Debeaujon and Branchard, 1992) have been used efficiently to produce regenerable cultures *via* organogenesis and/or embryogenesis. However, very limited *in vitro* work has been documented for embryo culture. Embryonic axes are quick and convenient to obtain from the seed and it is possible to homogenize the physiological state of the tissue in terms of its age, i.e. days after pollination. However, embryonic axes have previously been used for regeneration of plantlets in other cucurbits, i.e. watermelon (Ahad *et al*., 1994) and in Teasle gourd (Hoque *et al*., 2007).

Although melon genotypes have been regenerated successfully *in vitro* throughout the world, results of

this type of research in varieties cultivated in India are very limited, as the response to *in vitro* culture depends upon the genotype (Kathal *et al.*, 1986; Tabei *et al.*, 1992; Molina and Nuez, 1995; Yadav *et al.*, 1996; Rhimi *et al.*, 2006). Hence, it is necessary to engender a precise methodology for the genotype of interest. Moreover, *in vitro* morphogenesis influenced by different plant growth regulators added into culture media (Oridate and Oosawa, 1986; Branchard and Chateau, 1988; Tabei *et al.*, 1991; Gray *et al.*, 1993; Guis *et al.*, 1997). However, a systematic study on the effects of combinations of plant growth regulators on morphogenesis is still insufficient, which may overlook the potential combinations of certain plant growth regulators that are more suitable. Present study has been conducted to develop a potential system of *in vitro* regeneration from embryonic axes *via* somatic embryogenesis and to understand the possible role of plant growth regulators in morphogenic pathway.

Materials and Methods

Two different fortifications of basal media *viz*: MS (Murashige and Skoog, 1962) and B_5 (Gamborg's, 1968) with four cultivars of *Cucumis melo* RM 50, Durgapura Madhu, Pusa Madhuras and a 'Local' cultivar collected from Mandsaur MP were assessed to find out better *in vitro* response. During the preliminary investigation, MS basal medium proved more responsive as compared to B_5 medium. Hence, in subsequent experiments MS basal medium was used. During the preliminary experiment MS basal media was fortified with three different auxins, namely: 2,4-D, NAA and 2,4,5 T and three varied cytokinins *viz*: BAP, kinetin and TDZ in varying concentrations for embryonic axis culture. During preliminary experiments, it was observed that an auxin or a cytokinin alone is not adequate for inducing morphogenesis in higher frequencies. Furthermore, auxin 2,4,5-T and cytokinin TDZ responded scantily during initial experiments. Consequently, for concluding experiment basal MS medium was amended with different concentrations of BAP and Kn in combination with NAA and 2,4-D in varying concentrations. Apart from MS basal macro and micro salts, vitamins, all the media were supplemented with 30g.l^{-1} sucrose, 7.5g.l^{-1} agar and pH was adjusted to 5.8 ± 0.1.

For obtaining embryonic axes, seeds were washed with 2% Tween 20 (v/v) for 15-20 minutes, washed thoroughly with running tap water for 30 minutes, a treatment with 70% (v/v) ethanol for 1 minute, treatment of 2% Bavistin® for five minutes 0.2% $HgCl_2$ for seven minutes followed by 4 -5 rinse with sterile double distilled water and were soaked for 24 hours in sterilized double distilled water. Embryonic axis explants were obtained from pre-soaked seeds. Cultures were incubated under complete darkness at $25 \pm 2°C$ for one week. Later *in vitro* cultured explants were subjected to 12 photoperiod regime at 60 m mol m^{-2} s^{-1} of white cool fluorescent tubes.

Figure 1. Plant regeneration from embryonic axis in muskmelon: A. Cultured embryonic axes after 4 -7 days in culture; B. Cultured embryonic axes after 10-14 days in culture; C-D. Initiation of somatic embryo; E. Germination of somatic embryo; F. Regeneration of plantlets from somatic embryo; G. Initiation of direct organogenesis; H-I. Multiple plantlet regeneration *via* direct organogeensis J. indirect organogenesis; K. Multiple plant regeneration *via* indirect organogenesis and L. Elongated shootlets after 35-40 days in culture.

After 4 -5 weeks of initial culturing, somatic embryoids and calli were transferred to MS regeneration medium fortified with different concentrations and combinations of plant growth regulators (BAP, NAA and Kn alone as well as in combinations), 20g.l^{-1} sucrose and 7.5g.l^{-1} agar. However, in case cultures forming organ directly on explant surface were sub-cultured on same initial medium for regeneration of plantlets. Cultures were kept at 25 ± 2^{o}C and 12 hr photoperiod.

Once root formation was unattained on regeneration medium, plantlets were subsequently transferred to MS rooting medium supplemented with different concentrations of IBA, 15g.l^{-1} sucrose and 7.5g.l^{-1} agar. For regeneration and rooting, reduced level of sucrose was used on the basis of work conducted by various scientists as well as preliminary experiments conducted in this laboratory.

The experiment was laid out in factorial Completely Randomized Design. Experiment in two replications cultivars and different culture media combinations as two factors. Per replication approximately 100-120 embryonic axis were excised and cultured on each media. The arc-sine transformation was made before the analysis of data, since all data were in percentage. In cases, where values exceeded more than 100% *Log* transformation was made before analysis.

Results and Discussions

Embryonic axes of four muskmelon genotypes were cultured on different fortifications of MS medium. During the present investigation, cultured embryonic axes followed either direct or indirect pathways of plantlet regeneration depending upon the nature of different plant growth regulators added in basal culture medium. When plantlets regenerated on explant surface without callus formation referred as direct somatic embryogenesis or organogenesis and plantlets originated *via* callus formation as indirect somatic embryogenesis or organogenesis. The first response of all the embryonic axes cultures was similar after 3-5 days independent from explant and culture media. All explants became swollen and no callus proliferation was evident during first few days. After 7 days of culture, callus formation was usually observed from embryonic axis (Fig.1 A-B). Embryoid formation started after approximately 10 days from

Table 1. Direct and indirect somatic embryos induction from embryonic axes cultured on different fortification of MS media

Culture Media ↓	Direct somatic embryogenesis (%)					Indirect somatic embryogenesis (%)					Average number of somatic embryos per explants				
Genotypes→	Pusa Madhuras	RM-50	Durgapura Madhu	Local	Mean	Pusa Madhuras	RM-50	Durgapura Madhu	Local	Mean	Pusa Madhuras	RM-50	Durgapura Madhu	Local	Mean
MS.5D.5B	18.32	9.68	13.75	16.27	14.51^{d}	16.21	7.64	8.45	12.36	11.17^{g}	16.79	9.98	11.56	14.39	13.18^{f}
MSD.5B	28.45	18.39	21.87	26.91	23.91^{a}	22.87	15.95	17.78	19.91	19.13^{b}	31.94	23.37	26.41	29.49	27.80^{a}
MS2D.5B	25.89	16.57	18.98	21.06	20.63^{b}	25.91	15.74	20.25	23.36	21.32^{a}	28.12	21.46	22.34	24.67	24.15^{b}
MS3D.5B	8.95	3.81	6.13	8.12	6.75^{i}	6.13	2.54	3.26	5.21	4.29^{j}	20.17	11.54	15.83	18.31	16.46^{e}
MS5D.5B	7.02	2.14	3.25	5.75	4.54^{j}	4.18	1.67	2.01	2.55	2.60^{k}	6.45	2.34	3.62	5.13	4.39^{k}
MSDB	14.73	6.67	10.41	11.39	10.80^{g}	12.35	4.11	6.86	10.16	8.37^{i}	14.32	5.91	8.36	11.64	10.06^{g}
MS2DB	20.11	12.84	14.57	18.2	16.43^{c}	17.23	9.24	12.75	15.39	13.65^{e}	23.51	18.22	20.57	21.08	20.85^{c}
MS.5N.5B	11.22	4.92	6.07	8.67	7.72^{h}	12.88	8.36	9.32	11.04	10.40^{h}	11.28	5.36	7.85	9.92	8.60^{h}
MSN.5B	13.69	8.14	9.58	11.83	10.81^{g}	18.32	12.97	15.07	16.25	15.65^{d}	24.62	15.77	18.14	22.32	20.21^{c}
MS2N.5B	10.91	6.28	7.15	8.56	8.23^{h}	14.11	10.23	9.78	12.62	11.69^{fg}	22.75	11.48	16.65	20.49	17.84^{d}
MS3N.5B	3.47	1.65	2.39	3.18	2.67^{k}	3.24	1.85	2.46	3.37	2.73^{k}	16.37	3.21	6.98	13.53	10.02^{g}
MS5N.5B	2.95	1.25	1.99	2.61	2.20^{k}	2.95	1.25	2.04	2.32	2.14^{k}	2.68	1.45	2.66	3.29	2.52^{l}
MSNB	14.75	8.46	11.63	14.03	12.22^{f}	18.25	11.46	13.69	17.78	15.30^{d}	6.83	1.67	3.23	4.75	4.12^{k}
MS2NB	16.84	10.38	13.45	15.86	14.13^{e}	20.39	12.65	15.89	18.11	16.76^{c}	12.21	4.12	6.18	7.33	7.46^{i}
MS.5D.5Kn	10.86	5.75	7.56	9.77	8.49^{h}	6.83	3.12	4.13	4.45	4.63^{j}	8.39	3.06	4.54	6.43	5.61^{j}
MSD.5Kn	16.28	11.17	12.46	14.39	13.58^{e}	14.36	10.56	11.61	12.89	12.36^{f}	10.64	3.34	7.72	8.43	7.53^{i}
MS2D.5Kn	14.49	9.64	11.71	12.37	12.05^{f}	12.75	7.44	9.27	10.67	10.03^{h}	12.75	4.65	8.39	9.91	8.93^{h}
MS3D.5Kn	5.76	2.34	4.63	5.03	4.44^{j}	3.54	1.25	1.98	2.85	2.41^{k}	4.58	1.87	2.25	3.86	3.14^{l}
MS5D.5Kn	4.25	1.98	2.14	3.39	2.94^{k}	3.12	1.34	2.03	2.38	2.22^{k}	3.15	1.35	2.04	2.98	2.38^{l}
MSDKn	8.79	4.26	6.51	7.05	6.65^{i}	5.26	2.59	4.35	4.91	4.28^{j}	3.62	1.36	2.51	3.24	2.68^{l}
Mean	12.89^{a}	7.32^{d}	9.31^{c}	11.22^{b}		12.04^{a}	7.10^{d}	8.65^{c}	10.43^{b}		14.06^{a}	6.31^{d}	9.89^{c}	12.06^{b}	
CD 0.05															
Genotypes					0.39					0.31					0.41
Media					0.87					0.70					0.91
G x M					1.74					1.39					1.82

Figures in parenthesis are transformed values (Arc-sine transformation).
Values within column followed by different letters are significantly different at 5% probability level.

Table 2. Effect of different plant growth regulators on plantlet regeneration from cultured embryonic axes *via* direct and indirect somatic embryogenesis.

Culture Media ↓ Genotype →	Plant regeneration (%)				
	Pusa Madhuras	RM-50	Durgapur Madhu	Local	Mean
MS.1B	25.2	14.28	13.64	20.81	18.48^{i}
MS.2B	26.02	17.41	17.94	22.06	20.86^{g}
MS.5B	38.24	23.23	28.32	34.49	31.07^{c}
MSB	38.52	25.41	30.31	32.43	31.67^{c}
MS2B	30.01	22.12	25.43	29.56	27.53^{d}
MS.1N	17.61	14.50	15.43	15.86	15.85^{k}
MS.2N	18.49	11.87	13.23	15.26	14.71^{l}
MS.5N	23.45	16.25	18.63	21.39	19.93^{h}
MSN	21.22	15.42	16.72	18.65	18.00^{i}
MS2N	19.89	13.26	15.44	17.98	16.64^{j}
MS.1Kn	10.03	7.09	7.54	9.13	8.45^{n}
MS.2Kn	13.34	4.71	7.01	9.40	8.62^{n}
MS.5Kn	16.90	13.45	15.32	16.12	15.45^{kl}
MSKn	16.08	7.25	10.39	14.33	12.01^{n}
MS2Kn	15.25	7.89	12.23	13.25	16.16^{ik}
MS.2B.5N	10.01	6.32	7.15	7.89	7.84^{n}
MS.5B.5N	43.65	27.12	36.6	40.32	36.92^{b}
MSB.5N	45.14	29.03	33.67	39.46	36.83^{b}
MS2B.5N	35.53	18.32	23.46	29.45	26.69^{d}
MS.5BN	28.08	12.06	18.27	22.58	20.25^{gh}
MS.5B2N	9.96	6.14	7.36	7.69	7.79^{n}
MS.5N.5Kn	28.24	18.26	21.34	24.04	22.97^{f}
MSNKn	31.64	22.67	26.06	25.09	26.37^{e}
MS.5N.5B.5Kn	49.59	32.04	38.56	46.13	41.58^{a}
Mean	25.63^{a}	16.09^{d}	19.17^{c}	22.22^{b}	
CD 0.05					
Genotypes					0.34
Media					0.84
G x M					1.67

Figures in parenthesis are transformed values (Arc-sine transformation).

Values within column followed by different letters are significantly different at 5% probability level.

initial culturing however, in indirect embryogenesis it started approximately after 15 days (Fig.1C). The embryoid like structures were rounded with irregular outlines usually appeared in clusters (Fig.1D). Such somatic embryos germinated after transfer into regeneration medium (Fig.1 E-F). In direct organogenesis, adventitious structures developed on explant surface (Fig.1G-I). Gradually, these adventitious structures developed into multiple shoots (Fig.1H-I). In indirect organogenesis, shootlets developed from the meristems arising on the surface of the callus (Fig.1 J-K). Similar response was addressed by Kathal *et al*. (1986) for hypocotyl culture of melon.

Shoot formation started approximately 15 days from initial culturing and duration varied from culture to culture and in a few cases shoots initiated after 35 days. Most of the calli, after prolonged culturing on the induction media initiated plantlets. However, transfer into regeneration medium allowed higher plant formation and growth rate (Fig.1L). Various shoot forming calli were able to produce one or many plantlets at a time. Complete plantlets regenerated *via* embryogenesis and shoots developed *via* organogenesis were also counted as regenerated plantlets since they gave rise to complete plantlets after rhizogenesis on rooting medium**.**

The analysis of variance (Table 1-5) revealed highly significant ($p < 0.01$) differences between the response of genotypes, nutrient media as well as their interactions in terms of overall direct and indirect somatic embryogenesis, average somatic embryo formation, direct and indirect organogenesis and plantlet regeneration. It indicates the presence of the considerable amount of variability amongst the genotypes and culture media combinations as well as their interactions.

Effects of different culture media combinations on somatic embryogenesis are presented in Table 1. Results revealed that auxin 2,4-D induced callus in higher frequencies at concentration ranging from 1.0-2.0mg.l^{-1}. The size of callus enlarged with an increased level of 2,4-D up to 3.0mg.l^{-1}. Beyond this concentration the calli were turned into dark black colour and cell mortality was observed from cultured tissues. Direct somatic embryogenesis as well as average number of somatic embryo per explant in higher proportion were achieved on culture medium containing relatively higher proportion of 2,4-D (1.0mg.l^{-1}) in combination with a lower BA (0.5mg.l^{-1}), while higher indirect somatic embryogenesis was observed with even higher 2,4-D (2.0mg.l^{-1}) in combination with lower BA (0.5mg.l^{-1}). This revealed that a combination of higher proportion of 2,4-D with BA is necessarily required for induction of somatic embryogenesis. Moreover, for indirect somatic embryogenesis, two-fold 2,4-D required as compared to direct somatic embryogenesis. The results are in accordance with the findings of Branchard and Chateau (1988), Guis *et al*. (1997), Stipp *et al*. (2001) and Rhimi *et al*. (2006).

Effects of different culture media combinations on organogenesis are presented in Table 2. Presence of NAA and BA in culture medium supported the

Table 3. Number of direct and indirect organs forming embryonic axes cultured on different fortification of MS media

Culture Media ↓	Direct organogenesis					Indirect organogenesis				
Genotypes →	Pusa Madhuras	RM-50	Durgapura Madhu	Local	Mean	Pusa Madhuras	RM-50	Durgapura Madhu	Local	Mean
MS.5D.5B	12.67	7.11	10.12	11.27	10.29^{h}	17.94	13.67	15.75	7.26	13.66^{de}
MSD.5B	21.58	14.26	16.13	17.68	17.41^{e}	22.75	14.23	18.35	20.44	18.94^{c}
MS2D.5B	18.39	12.37	15.08	16.95	15.70^{f}	22.07	13.93	17.14	19.74	18.22^{c}
MS3D.5B	4.48	2.16	3.24	3.57	3.36^{lm}	6.15	2.06	3.24	4.56	4.00^{j}
MS5D.5B	3.25	1.73	2.18	2.73	2.47^{n}	3.98	1.54	2.86	3.35	2.93^{kl}
MSDB	8.52	5.34	6.15	7.65	6.92^{ij}	14.45	8.67	9.89	11.01	11.01^{fg}
MS2DB	9.83	3.45	5.53	7.26	6.52^{j}	15.79	10.23	12.37	14.49	13.22^{e}
MS.5N.5B	17.66	11.01	13.76	15.65	14.52^{g}	14.37	8.13	10.75	11.56	11.20^{f}
MSN.5B	23.56	18.34	19.28	21.39	20.64^{d}	17.25	13.17	15.05	6.76	13.06^{e}
MS2N.5B	25.45	19.06	19.87	23.78	22.04^{c}	15.99	10.78	12.85	14.97	13.65^{e}
MS3N.5B	6.23	3.01	3.84	5.14	4.56^{k}	4.68	1.95	2.84	3.28	3.19^{jk}
MS5N.5B	4.17	1.85	2.76	3.06	2.96^{m}	2.97	1.21	1.85	2.16	2.05^{l}
MSNB	26.94	21.93	23.61	24.77	24.31^{b}	23.12	16.45	19.67	20.89	20.03^{b}
MS2NB	28.75	23.49	26.43	27.68	26.59^{a}	24.42	17.45	20.47	21.84	21.05^{a}
MS.5D.5Kn	8.64	4.32	4.57	7.39	6.23^{j}	11.34	7.76	8.71	10.67	9.62^{h}
MSD.5Kn	13.28	7.14	8.36	10.45	9.81^{h}	18.69	10.79	11.88	16.35	14.43^{d}
MS2D.5Kn	9.11	5.66	5.79	8.23	7.20^{i}	13.87	7.13	8.36	11.26	10.16^{gh}
MS3D.5Kn	3.85	1.78	2.05	2.97	2.66^{mn}	4.36	1.45	2.64	3.95	3.10^{k}
MS5D.5Kn	2.98	1.57	1.98	2.14	2.17^{n}	3.11	1.27	1.53	2.86	2.19^{l}
MSDKn	5.46	2.35	2.82	4.69	3.83^{l}	6.45	4.78	4.67	5.39	5.32^{i}
Mean	12.74^{a}	8.40^{d}	9.68^{c}	11.22^{b}		13.19^{a}	8.33^{d}	10.04^{c}	10.64^{b}	
CD 0.05										
Genotypes					0.32					0.42
Media					0.72					0.94
G x M					1.43					1.88

Figures in parenthesis are transformed values (Arc-sine transformation).
Values within column followed by different letters are significantly different at 5% probability level.

formation of organs directly as well as indirectly. However, media with higher levels of auxins (~5mg.l^{-1}) performed lowly for direct and indirect organogenesis. Maximum plantlet realization from direct organogenesis in the range of 114-154% was achieved on culture medium with 1.0mg.l^{-1} NAA and 0.5-1.0mg.l^{-1} BAP. However, an increase in auxin concentration of 5.0mg.l^{-1} drastically reduced the plantlet regeneration to as low as 6%. Cytokinins are known to promote bud formation in several plant species including melon (Ezura *et al.*, 1992; Deng *et al.*, 1996; Verma *et al.*, 2005). However, the requirement for exogenous auxin and cytokinin in the process varies with the tissue system, apparently depending on the endogenous levels of the hormones present in the tissue (Norstrog, 1970). Moreover, the formations of adventitious organs depend on the reactivation of genes concerned with the organogenic phase of development.

When cultures did not form plantlet on induction medium they were transferred to regeneration medium and medium MS.5N.5B.5Kn proved to be the most responsive for the regeneration of plantlets (121.76%) out of 24 regeneration media tested. Maximum plantlets regeneration on media supplemented with two cytokinins and one auxin, i.e. 0.5 mg.l^{-1} each of BAP, kinetin and NAA as compared to other media with a growth regulator in isolation suggests that plantlet formation is determined by quantitative interaction, i.e. ratios rather than absolute concentration of substances participating in growth and development.

Considerable variability was observed among four genotypic for *in vitro* response (Table 1-5). Genotype Pusa Madhuras was found to be consistently superior as compared to other three genotypes for all *in vitro* attributes investigated. Genotypic differences for *in vitro* response have also been reported in several other investigations on muskmelon for various explant cultures (Kathal *et al.,* 1986; Tabei *et al.,* 1991; Molina and Nuez, 1995; Yadav *et al.,* 1996; Rhimi *et al.,* 2006).

During the present investigation, interactions of genotype with culture medium also varied considerably. Significant interactions of all cultivars

Table 4. Effect of plant growth regulators on regeneration *via* direct organogenesis from cultured embryonic axes.

Culture Media ↓ Genotypes→	Plant regeneration (%)				
	Pusa Madhuras	RM-50	Durgapur a Madhu	Local	Mean
MS.5D.5B	105.38	89.79	93.28	100.99	97.36[h]
MSD.5B	123.48	103.85	110.57	118.32	114.06[d]
MS2D.5B	117.11	99.23	101.65	109.21	106.80[f]
MS3D.5B	14.13	7.49	10.47	12.75	11.21[p]
MS5D.5B	8.17	4.67	6.42	7.32	6.65[s]
MSDB	77.22	53.96	60.15	68.78	65.03[l]
MS2DB	89.45	65.39	72.47	79.05	76.59[k]
MS.5N.5B	111.39	92.78	97.54	106.04	101.94[g]
MSN.5B	128.49	110.15	115.37	121.69	118.93[c]
MS2N.5B	120.44	99.17	105.55	114.32	109.87[e]
MS3N.5B	23.13	8.17	12.25	21.49	16.26[n]
MS5N.5B	8.09	3.99	6.47	7.32	6.47[t]
MSNB	144.46	118.25	121.37	135.15	129.81[b]
MS2NB	173.01	132.56	149.39	162.67	154.41[a]
MS.5D.5Kn	59.79	42.63	48.38	53.75	51.13[m]
MSD.5Kn	94.63	80.18	85.39	89.25	87.36[i]
MS2D.5Kn	84.45	70.65	75.23	80.92	77.81[j]
MS3D.5Kn	12.91	3.99	6.79	8.98	8.17[q]
MS5D.5Kn	10.69	3.89	6.85	7.61	7.26[r]
MSDKn	23.17	9.45	12.54	19.49	16.16[o]
Mean	76.48[a]	60.01[d]	64.90[c]	71.26[b]	
CD 0.05					
Genotypes					0.03
Media					0.07
G x M					0.14

Figures in parenthesis are transformed values (Log transformation).

Values within column followed by different letters are significantly different at 5% probability level.

with medium reveals that in addition to varying response of genotype for culture medium, specific genotype does not necessarily respond in the similar manner to each of culture media tested.

During present study, usually explants exhibiting higher morphogenic calli formation also regenerated more plantlets. However, the process of morphogenesis was not a predication of higher regeneration frequencies. For embryonic axis culture, single cultivars, culture medium or their interaction exhibiting higher morphogenic callus formation may regenerate lesser plants as compared with lower morphogenic calli. Such deviations occurred because single morphogenic calli produced none or many plantlets (up to 173 for cultivar Pusa Madhuras cultured on medium MS2NB (Fig.1 H) *via* direct organogenesis. In addition, not all the shootlets were established as complete plants. Some times shoot growth retarded after initiation and not all the shoots were able to produce roots even on rooting medium.

Table 5. Effect of plant growth regulators on regeneration of plantlets *via* indirect organogenesis from cultured embryonic axes.

Culture Media↓ Genotypes→	Plant regeneration (%)				
	Pusa Madhuras	RM-50	Durgapur a Madhu	Local	Mean
MS.1B	78.73	67.45	71.98	75.32	73.37[l]
MS.2B	82.91	70.13	74.32	79.69	76.76[j]
MS.5B	105.65	89.98	97.41	101.45	98.62[e]
MSB	108.43	94.11	98.95	104.83	101.58[d]
MS2B	95.43	76.39	84.43	90.61	86.72[g]
MS.1N	64.02	51.62	55.76	59.48	57.72[q]
MS.2N	66.87	53.43	56.24	61.12	59.42[p]
MS.5N	78.25	61.01	62.85	76.48	69.65[m]
MSN	73.98	54.45	61.74	71.21	65.35[n]
MS2N	68.73	61.62	60.49	65.16	64.00[o]
MS.1Kn	41.31	32.76	34.62	38.74	36.86[v]
MS.2Kn	45.19	34.98	38.38	40.79	39.84[u]
MS.5Kn	53.56	43.67	46.54	49.41	48.30[r]
MSKn	52.63	41.82	40.06	43.54	44.5[s]
MS2Kn	49.06	37.48	38.12	41.68	41.59[t]
MS.2B.5N	38.82	27.61	31.68	33.25	32.84[w]
MS.5B.5N	112.23	96.43	101.22	105.96	103.96[c]
MSB.5N	118.75	103.18	108.32	114.67	111.23[b]
MS2B.5N	101.66	86.42	93.16	97.42	94.67[f]
MS.5BN	86.41	74.42	81.45	83.26	81.39[h]
MS.5B2N	34.58	25.37	28.24	31.85	30.01[x]
MS.5N.5Kn	89.12	65.45	71.96	75.85	75.60[k]
MSNKn	93.41	68.73	72.81	78.34	78.32[i]
MS5N.5B.Kn	128.69	115.46	118.87	124.02	121.76[a]
Mean	77.85[a]	63.92[d]	67.90[c]	72.67[b]	
CD 0.05					
Genotypes					0.03
Media					0.06
G x M					NS

Figures in parenthesis are transformed values (Log transformation).

Values within column followed by different letters are significantly different at 5% probability level.

In conclusion, it was revealed that under appropriate conditions embryonic axes cultures of melon as in number of other vegetable crops gave rise to higher number of shoots *via* organogenesis as well as somatic embryogenesis, which could be useful in rapid propagation of elite melon cultivars. In addition, the embryogenic frequency and the conversion rate of embryos into plantlets will be more comprehensible for gene transfer studies using *Agrobacterium*-mediated or particle bombardment methods in melon.

References

Ahad A, Islam R, Hossain M, Khalekuzzaman M and Joarder OI (1994). Plant regeneration from immature and mature embryo axes of watermelon. *Plant Tissue Cult.* 4: 39-44.

Aldelberg JW, Rhodes BB, Skorupska HT and Bridges WC (1994). Explant origin affects the frequency of tetraploid plants from tissue culture of melon. *Horti. Sci.* 29: 689-692.

Branchard M and Chateau M (1988). Obention de plantes de melon (*Cucumis melo* L.) par embryogenesis somatique. *Comptes Rendus Academic Science Paris* 307: 777-780.

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.

Deng XD, Gene Y, Lu Z, Gu A and Zhu B (1996). Effect of explants and culture factors on induction of adventitious shoots in Hami muskmelon (*Cucumis melo* L.). *Acta Horticulture Sinica* 23:57-61.

Ezura H, Amagai H, Yoshioka K and Oosawa K (1992). Efficient production of tetraploid melon (*Cucumis melo* L.) by somatic embryogenesis. *Jap. J. Breed.* 42: 137-144.

Gamborg O L, Miller RA and Ojima K (1968). A nutrients requirement of suspension cultures of soybean root cells. *Exp. Cell Res.* 50: 151-158.

Gray DJ, McColley DW and Comptom M E (1993). High frequency somatic embryogenesis from quiescent seed cotyledons of *Cucumis melo* cultivars. *J. American Soc. Hort. Sci.* 118: 425-432.

Guis M, Latche A, Pech JC and Roustan JP (1997). An efficient method for the production of diploid Cantaloupe Charentais Melon (*Cucumis melo* L. var. cantalupensis) by somatic embryogenesis. *Scientia Horticult.* 69: 199-206.

Hoque A, Hossain M, Alam S, Arima S and Islam R (2007). Adventitious shoot regeneration from immature embryo explant obtained from female x female *Momodica dioica*. *Plant Tissue Cult. & Biotech.* 17: 29-36.

Kathal R, Bhatnagar SP and Bhojawani SS (1986). Regeneration of shoots from hypocotyls callus of *Cucumis melo L* cv. Pusa Sharbati. *J.Plant physiol.* 126: 59-62.

Kathal R, Bhatnagar SP, and Bhojwani SS (1988). Regeneration of plant from leaf explants of *Cucumis melo* cvltivar Pusa Sharbati. *Plant Cell Rep.* 7: 449-451.

Kathal R, Bhatnagar SP, and Bhojwani SS (1994). Plant regeneration from the callus derived from roots explants of *Cucumis melo* cvltivar Pusa Sharbati. *Plant Sci.* 96: 137-142.

Li R, Sun Y, Chang L and Li X (1990). Plant regeneration from cotyledon protoplasts of Xinjiang muskmelon. *Plant Cell Rep.* 9: 119-203.

Molina R V and Nuez F (1995). Characterization and classification of different genotype in a population of *Cucumis melo* based on their ability to regenerate shoots from leaf explants. *Plant Cell Tiss. Org. Cult.* 43: 249-257.

Murashige T and Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiol.* 15: 473-497.

Niedz RP, Smith SS, Dunbar KB, Stephens CT and Murakishi HH (1989). Factors influencing shoot regeneration from cotyledonary explants of *Cucumis melo* L. *Plant Cell Tissue Org. Cult.* 18: 313-319.

Norstog K (1970). Induction of embryo like structures by kinetin in cultured barley embryos. *Dev. Biol.* 23:665-670.

Oridate T and Oosawa K (1986). Somatic embryogenesis and plant regeneration from suspension callus culture in melon (*Cucumis melo* L.). *Jap. J. Breed.* 36: 424-428.

Rhimi N, Ben F and Boussaid M (2006). Plant regeneration *via* somatic embryogenesis from *in vitro* tissue culture in two Tunisian *Cucumis melo* cultivars Maazoun and beji. *Plant Cell Tiss.Org. Cult.* 84: 215-219.

Stipp LCL, Mendes BMJ, Piedade SMDS and Rodriguez APM (2001). *In vitro* morphogenesis of *Cucumis melo* var. inodorus. *Plant Cell Tiss. Org. Cult.* 65: 81-89.

Tabei Y, Kanno T and Nishio T (1991). Regulation of organogenesis and somatic embryogenesis by auxin in melon, *Cucumis melo* L. *Plant Cell Reports* 10: 225-229.

Tabei Y, Kanno T and Nishio T (1992). Shoot regeneration from cotyledonary protoplast of melon (*Cucumis* melo L. cultivar *Charentais*) *J Japan. Soc. Horti. Sci.* 61: 317-322.

Verma D, Yadav RC, Yadav NR, Chowdhury VK and Chowdhury, JB (2005). High frequency plant regeneration from cotyledon explants of muskmelon (*Cucumis melo* L.). *Physiology and Molecular Biology of Plants* 11:135-139.

Yadav RC, Mohamed Salesh, TS and Grumet R (1996). High frequency shoot regeneration from leaf explants of muskmelon. *Plant Cell Tiss. Org. Cult.* 45: 207-214.