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Induction of somatic embryogenesis and organogenesis from hypocotyl of muskmelon (*Cucumis melo* L.)

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Hypocotyls of four genotypes of *Cucumis melo* were cultured on different fortifications of MS medium to assess their *in vitro* response. Higher *in vitro* morphogenesis (somatic embryogenesis and/or embryogenesis) leading to plantlet regeneration varied considerably due to genotypes and inoculation medium. Culture medium MSD.5B (MS + 1. 0 mg. l^{-1} 2,4-D + 0. 5 mg. l^{-1} BA + 30.0 g. l^{-1} sucrose + 7.5 g.l^{-1} agar) induced direct somatic embryogenesis (21.77%) in higher frequencies. Average number of somatic embryo(s) per explant in higher number (s) (25.16) was induced by induction medium MS2D.5B (MS + 2. 0 mg. l^{-1} 2, 4-D + 0. 5 mg. l^{-1} BA + 30.0 g. l^{-1} sucrose + 7.5 g.l^{-1} agar). Culture medium MS2NB (MS + 2. 0 mg. l^{-1} NAA + 1.0 mg. l^{-1} BA + 30.0 g. l^{-1} sucrose + 7.5 g. l^{-1} agar) proved superior for induction of indirect somatic embryogenesis (18.83%), direct (24.04%) and indirect (17.83%) organogenesis as well as regeneration of plantlets (151.40%) *via* direct organogenesis. Regeneration medium MS.5N.5B.5Kn (MS + 0.5mg. l^{-1} NAA+ 0.5mg. l^{-1} BA + 0. 5 mg. l^{-1} Kn + 20.0 g.l^{-1} sucrose + 7.5 g.l^{-1} agar) was proved superior for plantlet production *via* somatic embryogenesis (36.90%) and indirect organogenesis (117.45%). Among the cultivars, Pusa Madhuras was found significantly superior followed by Local, Durgapura Madhu and RM-50 for the most of the culture phases.

Key words: muskmelon, hypocotyls, callus, morphogenesis, somatic embryogenesis, organogenesis and plantlet regeneration.

Introduction

Efficient *in vitro* plant regeneration systems are critical for many purposes including plant transformation in muskmelon. This species recently has been targeted for genetic engineering efforts to speed the development of improved

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cultivars with higher sugar content and genetic yield potential, increased shelf-life of fruit, increased resistance to diseases and insects and greater tolerance to drought, heat and soil toxicities of cultivated muskmelon. The efficient *in vitro* regeneration procedures are required to obtain transgenic plants expressing potentially useful engineered genes. Furthermore, before the transformation of *Cucumis,* to cater to the regional needs and to address the acclimatization issue, responding indigenous cultivars need to be identified. Although, all plant cells are derived from the fertilized egg cell and contain identical information, callus derived from somatic cells varies in competence to express totipotency i.e. their genetic ability to produce plants. In muskmelon, various explants including hypocotyl have been used efficiently to produce regenerable cultures via organogenesis and/or embryogenesis. Hypocotyl is one of the tissues of choice for plant genetic manipulations (Liu *et al*., 1996) in view of the fact this tissue is amenable to *in vitro* techniques in addition to contributing substantially to the plantlet biomass. Hypocotyl is 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 seed germination. Melon hypocotyls have previously been used for regeneration of plantlets via organogenesis (Moreno *et al*., 1985; Kathal *et al*., 1986) and via somatic embryogenesis (Moreno *et al.,* 1985).

The 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 depend on 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 genotypes of interest. Recently, the improvement of *in vitro* morphogenesis by adding various combinations of plant growth regulators to media has been reported (Oridate and Oosawa, 1986; Branchard and Chateau, 1988; Tabei *et al.,* 1991; Gray *et al.,* 1993; Guis *et al*., 1997). However, a systematic experiment on the effects of combinations of plant growth regulators on morphogenesis are not still enough, which may overlook the potential combinations of certain plant growth regulators that are more suitable for induction of *in vitro* morphogenesis. This paper will report the induction of morphogenesis (somatic embryogenesis and /or organogenesis) by searching *in vitro* responding genotypes and appropriate culture medium. Tombinations ensuing an effectual protocol for regeneration of muskmelon varieties already under cultivation in Madhya Pradesh and adjoining areas by means of the culture of hypocotyls.

Materials and methods

The present investigation was carried out at the Horticultural Biotechnology Laboratory, KNK College of Horticulture, Mandsaur, Jawaharlal Nehru Agricultural University, Jabalpur (M. P.) during the session 2007-008. Four cultivars of *Cucumis melo* were selected for the study of hypocotyl culture. Cultivars RM 50 and Durgapura Madhu were procured from Agriculture Research Station, Durgapura, Rajasthan Agriculture University, Rajasthan, Pusa Madhuras from IARI, New Delhi and 'Local' cultivar was collected from local sources, Mandsaur (M.P., India).

Culture media

To begin with a preliminary experiment, two different fortifications of basal media *viz*: MS (Murashige and Skoog, 1962) and B_5 (Gamborg *et al*., 1968) were tested to find out better *in vitro* response. During the preliminary investigations, MS basal medium was found more responsive than B_5 medium (data not presented). Hence, for later experimentations basal MS medium was used. Apart from MS basal micro and macro salts, vitamins, and agar powder, three different auxins, namely: 2, 4-D, NAA and 2, 4, 5 T (alone) and three diverse cytokinins *viz*: BAP, kinetin and TDZ (alone) in varying concentrations were added to fortify MS media for culturing the hypocotyls in preliminary experiments. During preliminary experiments, it was observed that an auxin as well as 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. Therefore, for final experiment basal MS medium was fortified with different concentrations and combinations of plant growth regulators (BAP and Kn in combination with NAA and 2, 4-D), 30.0 g l^{-1} sucrose and 7.5 g l^{-1} agar powder. Warm culture media, still in liquid state was poured into baby food bottles (50-60 ml / bottle) and culture tubes (15-20 ml / tube) followed by autoclaving at 121^{o}C under 15 psi pressure for 20 min after adjusting the pH to 5.6 ± 0.1 with 1 N KOH. However, in case of pouring in petridishes, autoclaved warm culture media was poured into pre-sterilized 100 x 17 mm glass petridishes (25-30 ml / dish) under aseptic conditions of laminar flow clean air cabinet. Readymade MS basal medium, plant growth regulators and other ingredients were procured from Hi-media Laboratories, Mumbai, India.

Surface sterilization of seed

Seed were washed with 2% Tween 20 (v/v) (a commercial detergent) for 15-20 minutes and then washed thoroughly with running tap water for 30

minutes to remove dirt and residues followed by a treatment with 70% (v/v) ethanol for 1 minute. Then seed were subjected to treatment of 2% Bavistin® (BASF, Germany) for five minutes followed by 0.2% $HgCl_2$ for seven minutes. Finally seed were rinsed 4-5 times with sterile double distilled water and inoculated in culture tubes containing agar gelled water (7.5 $g.l^{-1}$ agar) and kept under diffused luminance of 16 μ mol m^{-2} s^{-1} provided with white fluorescent lamps.

Hypocotyl excision and plating technique

Hypocotyl explants were obtained from 3-4 days old germinated seeds. In 100 x 17 mm glass petridishe, 7-8 pieces of hypocotyls were plated.

Culture conditions

Petridishes and culture tubes containing cultures sealed with Lab film (Parafilm®) were incubated under complete darkness at 25 ± 2°C for one week. Later *in vitro* cultured explants were subjected to photoperiod regime of 12 hours light and 12 hours dark cycle at an intensity of 1200-lux luminance provided by white cool fluorescent tubes.

Regeneration of plantlets

After 4-5 weeks of initial culturing, somatic embryos (induced directly on explant surface or indirectly *via* callus formation) and calli obtained from indirect organogenesis (*via* callus formation) were transferred onto fortified semi-solid MS regeneration medium supplemented with different concentrations and combinations of plant growth regulators (BAP, NAA and Kn alone as well as in combinations), 20 g l^{-1} sucrose and 7.5 gl^{-1} agar. However, in case of direct organogenesis (explants showing sign of organ formation directly on explant surface) was subcultured on same medium (initial medium) for regeneration of plantlets. Cultured baby food bottles /culture tubes were subjected to 25±2°C temperature and photoperiod regimes of 60 μ mol m^{-2} s^{-1} luminance provided by cool fluorescent tubes for 12 hr.

In vitro rooting of regenerants

When root formation was not obtained on regeneration medium, plantlets were subsequently transferred to MS rooting medium supplemented with different concentrations of IBA, 15.0 g l^{-1} sucrose and 7.5 g 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 on the basis of preliminary experiments conducted in this laboratory.

Experimental design and analysis of data

The experiment was laid out in factorial Completely Randomized Design. Experiment had two factors, first - four cultivars and second- twenty/ twenty-four different culture media combinations. Design was used to find out the significance of genotype, culture medium combination and their interactive effect. Each treatment was consisting of two replications. Per replication approximately 100-120 explants 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. The data were analyzed as per method suggested by Snedecor and Cochran (1967).

Results and discussion

Hypocotyls of four muskmelon genotypes were cultured on different fortifications of MS medium. In this study, cultured hypocotyls followed either direct or indirect pathway of plant regeneration depending upon the nature of different culture media combinations. In direct approach, plantlets were regenerated on explant surface directly without callus formation (*via* direct somatic embryogenesis or organogenesis) and in indirect mode; plantlets were originated *via* callus formation (either indirect somatic embryogenesis or organogenesis). The first response of cultured hypocotyls was similar after 3-5 days and mostly independent from explant and culture media. All explants became swollen and no callus proliferation was evident during first few days. After 5-7 days of culture, callus initiation started from the wounded ends and spread towards the middle region of the tissue segment (Fig. 1A-B). In direct embryogenesis, embryo like structures initiated directly on the surface of cultured explants. Embryoid formation started after approximately 7 days from initial culturing (Fig. 1C). The embryoid like structures was rounded with irregular outlines usually appeared in clusters (Fig. 1C). These somatic embryos germinated after transferring into regeneration medium (Fig. 1D). In indirect embryogenesis, embryoid formation started approximately 10 days from initial culturing on callus surface. However, the duration varied from culture to culture and in a few cases embryoids developed after 35 days of apparently undifferentiated growth. In direct organogenesis, adventitious structures were developed on explant surface (Fig. 1E-F). Adventitious formation started approximately 7-10 days from initial culturing. However, the

duration varied from culture to culture and in a few cases adventitious formed after 28 days. With time, these adventitious structures formed multiple shoots (Fig. 1G). In indirect organogenesis, shootlets developed from the nodules arising on the surface of the callus (Fig. 1H-J). Similar response was addressed by Kathal *et al*. (1986) for hypocotyl culture of melon. Shoot formation started approximately 10 days from initial culturing. However, the duration varied from culture to culture and in a few cases shoots formed after 45 days. Most of the calli, after prolonged culturing on the induction media gave rise to plants. However, transfer into regeneration medium allowed higher plant formation and growth rate (Fig. 1K-L). 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 counted as regenerated plantlets. Regenerated shoots alone were also counted as plantlets since they gave rise to complete plants after rhizogenesis on rooting medium**.**

Some of the most intriguing questions faced by us concern the conditions that reprogramme hitherto differentiated or otherwise committed cells into forming adventitious structures in culture and that determine the path taken, embryogenic or organogenic, shoot or root. By varying growth regulator levels and types, one can determine the route of morphogenesis *in vitro*. The Plant growth regulators were compared with each other in combinations only and the effect of a cytokinin or an auxin per se was not investigated in the work being reported. This was so because during the course of preliminary investigation (data not presented), it was observed that both cytokinins as well as auxin were essential for increased morphogenesis but they were most effective when used in combination, and that the type and pace of callus growth was dependent on their ration. Similar responses were also reported by Moreno *et al.* (1985); Yadav *et al*. (1996); Guis *et al*. (1997); Stipp *et al.* (2001); Verma *et al*. (2005) and Rhimi *et al.* (2006) for diverse explants cultures of melon.

The analysis of variance presented in Table 1-5 revealed that there were highly significant ($p<0.01$) differences between the response of genotypes, nutrient media as well as their interactions in terms of overall somatic embryogenesis (direct and indirect), average number (s) of somatic embryos, organogenesis (direct and indirect) and plantlet regeneration (*via* somatic embryogenesis and organogenesis). It indicates the presence of the considerable amount of variability amongst the genotypes and culture medium as well as their interaction. Furthermore, although the genotypes varied highly significantly ($p<0.01$) for the induction of somatic embryogenesis, organogenesis and plantlet production in response to incubation on different plant growth regulators regimes, non-significant interactions were found between genotypes and nutrient media for regeneration of plantlets *via* indirect organogenesis.

In terms of induction of somatic embryogenesis *in vitro*, culture medium MSD.5B (21.77%) was found significantly superior for direct somatic embryo induction followed by nutrient media MS2D.5B (18.76%) and MS2DB (14.97%). Next better performance was shown by the culture media MS.5D.5B (12.70%) and MSD.5Kn (11.51%). Remaining culture media combinations had low direct somatic embryos inducing ability including culture media MS5N.5B (1.55%) and MS3N.5B (1.90%), which had significantly minimum counts (Table 1). Maximum somatic embryos were induced from callus mass by culture media MS2NB (18.83%) followed by MSNB (16.39%) and MS2D.5B (15.64%). The lowest performance was exhibited by a group of four culture media, namely: MS5D.5Kn (1.67%), MS3D.5Kn (1.69%), MS3N.5B (1.74%) and MS5N.5B (1.81%) (Table 1). Nutrient media combinations MS2D.5B (25.16) followed by MSD.5B (21.64) and MS2DB (18.89) were found to be more responsive for initiation of somatic embryos per explant in higher numbers. The lowest performance was exhibited by a group of three culture media, namely: MS5D.5Kn (1.67), MS5N.5B (1.93) and MSDKn (1.98). Remaining culture medium performed intermediately (Table 1).

During present investigations, auxin 2, 4-D induced callus in higher frequencies at concentration ranging from 1.0-3.0 mg l^{-1}. The size of callus enlarged with an increased level of 2, 4-D up to 3.0 mg.l^{-1}. Beyond this concentration the calli were turned into dark black colour and cell mortality was observed from cultured tissues. Higher proportion of direct somatic embryogenesis and average number of somatic embryo per explant were achieved on culture medium containing relatively higher concentration of 2, 4-D (1.0-2.0 mg.l^{-1}) in combination with a lower concentration of BA (0.5-1.0 mg.l^{-1}). A higher concentration of NAA (2.0 mg.l^{-1}) combined with a lower concentration of BA (1.0 mg.l^{-1}) promoted indirect somatic embryogenesis. This revealed that a combination of higher proportion of auxin to cytokinin is necessarily required for embryogenesis. These 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), since they suggested that a higher auxin concentration in combination with a lower concentration of a cytokinin followed by sub-culturing in hormone free medium enhances embryogenesis *in vitro* in melon.

In terms of organogenic response, nutrient medium MS2NB (24.04%) was found more responsive followed by culture media MSNB (21.28%), MSN.5B (19.37%) and MS2N.5B (18.06%) for formation of organs directly from cultured hypocotyls. The lowest performance was shown by nutrient media MS.5N.5B (4.79%) followed by MS5D.5B (4.89%) and MS5D.5Kn (5.18%) (Table 3). Culture media MS2NB (17.83%) followed by MSD.5B

(16.74%) and MS2D.5B (14.39%) were found more responsive for induction of indirect organogenesis. The lowest performance was exhibited by a group of four culture media, *viz*: MS5N.5B (1.19%), MS5D.5Kn (1.37%), MS5D.5B (1.61%) and MS3D.5Kn (1.68%) (Table 3).

Promotion of bud formation by cytokinin occurs 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. During present investigations, ratio of organ formation directly as well as indirectly in higher frequencies was achieved on culture medium fortified with higher concentrations of NAA (2.0 $mg.l^{-1}$) instead of 2,4-D in combination with a lower concentration of BA (1.0 $mg.l^{-1}$). In such cases, the both cut ends of hypocotyl are so profused that the entire surface of the cutting has covered with shoot buds. Similar response was reported by Kathal *et al*. (1986) for hypocotyl culture of melon.

For the regeneration of plantlets, culture medium MS.5N.5B.5Kn was proved superior *via* somatic embryogenesis (36.90%) as well as indirect organogenesis (117.45%) followed by regeneration medium MSB.5N. The lowest performance was exhibited by nutrient medium MS.5B2N *via* somatic embryogenesis (5.48%) and indirect organogenesis (26.91%) (Tables 3 and 5). Maximum plantlets regenerated *via* somatic embryogenesis and indirect organogenesis 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 medium with a cytokinin (alone) even at the concentration of 1.0 $mg.l^{-1}$. Thus, it was concluded that plantlet formation is determined by quantitative interaction, i.e. ratios rather than absolute concentration of substances participating in growth and development. From direct organogenesis maximum plantlet regeneration was achieved on regeneration medium MS2NB (151.40%) followed by culture media MSNB (127.16%) and MSN.5B (116.06%). The lowest performance was exhibited by culture media MS5N.5B (4.79%) followed by MS5D.5B (4.89%) (Table 4). This revealed that a combination of higher proportion of auxin (NAA) to cytokinin (BA) is necessarily required for higher degree of plantlet production.

In terms of the genotypic response to *in vitro* culture considerable variability was observed among four cultivars (Table 1-5). Genotype Pusa Madhuras was found consistently superior to others for all attributes investigated. Genotype Pusa Madhuras induced direct (10.62%) and indirect

(10.61%) somatic embryogenesis including average number (11.95) of somatic embryos per explant in higher frequencies. Moreover, direct (10.70%) and indirect (10.13%) organogenesis and plant regeneration *via* somatic embryogenesis (18.73%) as well as by means of direct (74.22%) and indirect (74.31%) organogenesis in higher frequencies was exhibited by the same genotype. The lowest performance was exhibited by genotype RM50. However, genotypes Local and Durgapura Madhu performed in between them. In various other investigations on muskmelon genotypic differences have also been observed for various explant cultures (Kathal *et al.,* 1986; Tabei *et al.,* 1991; Molina and Nuez, 1995; Yadav *et al.,* 1996 and Rhimi *et.al.,* 2006).

During present investigations interactions of genotype with culture medium also varied considerably. Cultivars either on MSD.5B or MS2NB or in both media induced more than 15 per cent somatic embryos (either directly or indirectly). Interactions of all cultivars with medium MS2D.5B and MSD.5B also exhibited similar results for average number (s) of somatic embryo(s) per explant. Organogenesis (direct or indirect) influenced by interaction of genotypes with culture media. Culture medium MS2NB or MSNB or both proved superior for induction of direct and indirect organogenesis as well as regeneration of plantlets *via* direct organogenesis for the most of the genotypes. This trend was also followed by various cultivars on different media for plantlet regeneration via indirect organogenesis, where, culture media MS.5B.5Kn.5N or MSB.5N or both regenerated more than 100 per cent regenerants. For other parameters, genotypes x medium interactions have shown to be differing in similar manner. This 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.

Although, shoots and roots or shoots, roots and somatic embryos have been observed on the same tissue, it is generally considered that the process leading to their appearance are mutually exclusive i.e. cells either committed to the organogenic or to the embryogenic pathway. In present investigations, plantlets were regenerated via somatic embryogenesis as well as organogenesis depending upon the cultivar and different culture media combinations (relative concentrations and proportions of an auxin to a cytokinin). Shoots were formed directly from explant surface (direct organogenesis) in higher frequencies as compared to indirect organogenesis (from callus mass) and somatic embryogenesis. Similar findings were documented by Gray *et al*. (1993); Guis *et al.* (1997) and Rhimi *et al*. (2006) for cotyledon culture in muskmelon.

During present study, usually explant cultures exhibiting higher number of morphogenic calli regenerated more plantlets. However, the process of morphogenesis was not a predication of higher regeneration frequencies. For

hypocotyl 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 as a single morphogenic calli produced none or many plantlets (up to 172 for cultivar Pusa Madhuras cultured on medium MS2NB (Fig. 1G) *via* direct organogenesis). In addition, not all the shootlets developed into complete plants i.e. shoots with roots. Some times shoot could not develop after initiation, in a few cases they were deformed and not all the shoots developed without roots were able to produce roots even on rooting medium.

In conclusion, it was shown that under appropriate conditions hypocotyl 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. Further, the embryogenic frequency and the conversion rate of embryos into plantlets obtained by our process are higher which will be more accessible for *Agrobacterium*-mediated or particle bombardment transformation to be more successful by giving rise to more transformed plants in a short time, avoiding somaclonal variation.

Table 2. Effect of different plant growth regulators on regeneration of plantlets *via* somatic embryogenesis (direct and indirect) from cultured hypocotyls.

Culture Media	Plant regeneration (%)				
	Genotypes				Mean
	Pusa *Madhuras*	RM-50	Durgapura Madhu	Local	
MS.1B	14.26	6.72	9.61	10.09	**10.17**^{j}
MS.2B	15.43	24.54	31.85	34.29	**26.53**^{d}
MS.5B	31.53	14.28	20.95	25.26	**23.01**^{e}
MSB	32.92	20.22	26.43	29.65	**27.31**^{c}
MS2B	28.14	19.32	22.24	24.01	**23.43**^{e}
MS.1N	10.04	9.11	9.32	9.49	**9.49**^{jk}
MS.2N	10.22	8.24	8.56	9.14	**9.04**^{l}
MS.5N	12.37	6.47	6.49	9.62	**8.74**^{l}
MSN	13.31	9.28	14.13	19.09	**13.95**^{i}
MS2N	11.43	5.36	5.89	6.78	**7.37**^{m}
MS.1Kn	8.17	7.49	7.78	7.83	**7.82**^{m}
MS.2Kn	8.32	7.35	7.84	8.07	**7.90**^{m}
MS.5Kn	9.91	8.75	8.89	9.27	**9.21**^{kl}
MSKn	8.45	8.31	8.75	9.13	**8.66**^{l}
MS2Kn	9.06	8.16	8.47	8.95	**8.66**^{l}
MS.2B.5N	6.71	4.25	5.12	7.49	**5.89**^{n}
MS.5B.5N	35.26	17.59	27.23	31.04	**27.78**^{c}
MSB.5N	38.28	22.43	31.24	35.04	**31.75**^{b}
MS2B.5N	28.37	17.61	22.34	23.43	**22.94**^{e}
MS.5BN	21.32	10.82	14.35	16.98	**15.87**^{h}
MS.5B2N	6.16	5.12	5.23	5.42	**5.48**^{n}
MS.5N.5Kn	21.72	13.51	14.05	17.98	**16.82**^{g}
MSNKn	23.48	13.82	17.46	20.84	**18.90**^{f}
MS.5N.5B.5Kn	44.56	27.22	35.25	40.56	**36.90**^{a}
Mean	**18.73**^{a}	**12.33**^{d}	**15.39**^{c}	**17.48**^{b}	
CD 0.05					
Genotypes					**0.30**
Media					**0.74**
G x M					**1.48**

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

Table 4. Effect of different plant growth regulators on regeneration of plantlets *via* direct organogenesis from cultured hypocotyls.

Culture Media	Plant regeneration (%)				
	Genotypes				Mean
	Pusa Madhuras	RM-50	Durgapura Madhu	Local	
MS.5D.5B	103.25	85.63	91.45	98.17	**94.63^{g}**
MSD.5B	120.14	101.01	106.32	115.42	**110.72^{d}**
MS2D.5B	115.37	97.98	99.91	106.09	**104.84^{e}**
MS3D.5B	12.23	5.47	8.23	10.56	**9.12^{p}**
MS5D.5B	6.42	3.25	4.46	5.43	**4.89^{s}**
MSDB	75.25	51.07	57.46	65.34	**62.28^{l}**
MS2DB	87.48	62.67	71.13	77.07	**74.59^{j}**
MS.5N.5B	108.11	98.79	95.67	104.32	**10.72^{f}**
MSN.5B	125.75	106.91	112.48	119.11	**116.06^{c}**
MS2N.5B	118.06	94.49	102.36	11.24	**81.54^{i}**
MS3N.5B	21.49	6.23	10.09	18.43	**14.06^{n}**
MS5N.5B	6.49	2.21	4.32	6.13	**4.79^{t}**
MSNB	142.34	115.17	118.89	132.24	**127.16^{b}**
MS2NB	172.08	129.48	145.67	158.36	**151.40^{a}**
MS.5D.5Kn	56.49	39.39	45.25	50.49	**47.91^{m}**
MSD.5Kn	91.32	76.47	75.99	85.64	**82.36^{h}**
MS2D.5Kn	81.22	67.99	71.46	75.32	**74.00^{k}**
MS3D.5Kn	10.45	2.75	4.49	6.16	**5.96^{g}**
MS5D.5Kn	8.97	2.45	4.14	5.17	**5.18^{r}**
MSDKn	21.46	6.87	8.32	18.32	**13.74^{o}**
Mean	**74.22^{a}**	**57.81^{d}**	**61.90^{c}**	**63.45^{b}**	
CD 0.05					
Genotypes					**0.03**
Media					**0.06**
G x M					**0.12**

Figures in parenthesis are transformed values (Log transformation).
Values within column followed by different letters are significantly different at 1% probability level.

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

Culture Media	Plant regeneration (%)				
	Genotypes				
	Pusa Madhuras	RM-50	Durgapura Madhu	Local	Mean
MS.1B	75.69	64.23	68.42	72.81	**70.29^{l}**
MS.2B	80.43	68.02	71.23	76.15	**73.96^{j}**
MS.5B	102.22	85.41	93.86	97.43	**94.73^{e}**
MSB	104.32	91.05	94.25	102.64	**98.07^{d}**
MS2B	91.78	72.32	81.26	88.27	**83.41^{g}**
MS.1N	60.59	48.19	56.68	57.96	**55.86^{q}**
MS.2N	62.83	50.76	57.12	59.03	**57.44^{p}**
MS.5N	75.36	57.21	59.44	71.25	**65.82^{m}**
MSN	71.14	51.66	58.39	65.42	**61.65^{n}**
MS2N	64.97	53.41	58.35	61.78	**59.63^{o}**
MS.1Kn	38.09	29.76	31.96	35.22	**33.76^{v}**
MS.2Kn	43.21	31.28	35.21	37.41	**36.78^{u}**
MS.5Kn	50.37	41.64	44.59	46.34	**45.74^{r}**
MSKn	48.91	36.02	38.13	41.52	**41.15^{s}**
MS2Kn	45.72	34.89	37.52	39.65	**39.45^{t}**
MS.2B.5N	29.41	23.94	27.45	28.17	**27.24^{w}**
MS.5B.5N	108.96	93.78	97.29	101.25	**100.32^{c}**
MSB.5N	114.05	98.86	103.41	111.85	**107.04^{b}**
MS2B.5N	97.33	81.58	90.76	94.53	**91.05^{f}**
MS.5BN	85.69	71.42	78.54	80.89	**79.14^{h}**
MS.5B2N	32.21	22.34	24.31	28.77	**26.91^{x}**
MS.5N.5Kn	85.44	62.79	68.66	72.62	**72.38^{k}**
MSNKn	91.05	65.48	69.85	75.15	**75.38^{i}**
MS.5N.5B.5Kn	123.61	112.23	115.49	118.46	**117.45^{a}**
Mean	**74.31^{a}**	**60.34^{d}**	**65.09^{c}**	**69.36^{b}**	
CD 0.05					
Genotypes					**0.02**
Media					**0.05**
G x M					**NS**

Figures in parenthesis are transformed values (Log transformation).
Values within column followed by different letters are significantly different at 1% probability level.

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Table 1. Number of direct and indirect somatic and average number (s) of somatic embryo(s) inducing hypocotyls fortifications of MS media

Culture Media ▼ Genotypes ▶	Direct somatic embryogenesis (%)					Indirect somatic embryogenesis (%)					Average number of somatic embryos per explants				
	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	15.75	7.23	13.69	14.13	12.70^{d}	14.78	5.55	6.16	10.47	9.24^{g}	14.24	7.45	9.45	12.17	10.83^{g}
MSD.5B	25.37	16.25	22.25	23.19	21.77^{a}	17.59	10.37	12.25	16.01	14.00^{c}	25.13	19.77	20.17	21.47	21.64^{b}
MS2D.5B	23.13	14.37	19.18	18.37	18.76^{b}	26.07	9.47	11.47	15.56	15.64^{e}	28.37	20.45	24.45	27.35	25.16^{a}
MS3D.5B	5.98	2.25	2.15	3.45	3.21^{k}	4.45	2.21	2.24	3.23	3.03^{i}	18.48	9.34	13.54	16.54	14.48^{f}
MS5D.5B	5.55	1.89	4.39	3.25	3.77^{jk}	2.79	1.45	1.75	1.47	1.87^{kl}	4.14	1.45	2.79	3.24	2.91^{kl}
MSDB	12.21	4.39	8.48	8.39	8.37^{h}	9.97	3.39	4.47	8.9	4.29^{i}	12.11	3.29	6.49	8.96	7.71^{h}
MS2DB	17.47	10.07	15.76	16.56	14.97^{c}	15.45	6.62	10.37	13.24	11.42^{f}	21.37	16.14	18.57	19.47	18.89^{c}
MS.5N.5B	5.99	3.67	6.36	6.24	6.57^{i}	10.08	5.55	6.49	9.54	8.02^{h}	8.15	3.32	6.43	6.97	6.22^{i}
MSN.5B	11.78	6.02	7.91	8.21	8.48^{gh}	15.49	10.79	13.17	14.02	13.37^{e}	22.22	12.53	16.54	16.37	15.92^{d}
MS2N.5B	8.15	4.37	5.45	6.39	6.09^{i}	12.47	8.04	7.16	10.47	9.54^{g}	20.37	9.33	14.34	18.54	15.65^{e}
MS3N.5B	1.99	1.35	1.99	2.25	1.90^{m}	1.27	1.5	2.04	2.14	1.74^{j}	14.37	2.47	5.05	11.11	8.25^{h}
MS5N.5B	2.03	1.05	1.35	1.78	1.55^{m}	2.45	1.15	1.75	1.87	1.18^{j}	1.98	1.02	1.98	2.75	1.93^{m}
MSNB	11.27	5.45	9.23	11.98	9.48^{fg}	20.47	13.47	15.25	16.37	16.39^{b}	4.73	1.49	2.94	3.99	3.29^{k}
MS2NB	14.13	8.39	11.37	13.62	11.88^{de}	23.45	13.24	18.17	20.45	18.83^{a}	10.89	3.07	4.57	5.45	6.00^{i}
MS.5D.5Kn	8.37	3.27	5.89	7.56	6.27^{i}	4.49	2.25	2.25	2.34	2.83^{jk}	6.47	2.98	2.69	4.79	4.23^{j}
MSD.5Kn	14.25	9.45	10.17	12.17	11.51^{e}	12.79	8.57	9.67	10.45	10.47^{f}	8.45	3.04	5.45	6.69	5.91^{j}
MS2D.5Kn	12.67	7.05	9.47	10.47	9.92^{f}	10.01	5.49	6.49	8.97	7.74^{h}	10.07	2.67	6.07	7.27	6.52^{i}
MS3D.5Kn	3.39	1.99	2.23	4.39	3.00^{kl}	2.22	1.02	1.54	1.98	1.69^{j}	2.79	1.5	1.95	2.85	2.27^{lm}
MS5D.5Kn	2.17	1.73	1.73	3.78	2.11^{lm}	1.89	1.37	1.97	1.45	1.67^{j}	2.24	1.22	1.45	1.73	1.67^{m}
MSDKn	6.65	3.87	2.25	5.13	4.48^{j}	4.02	1.5	3.24	3.29	3.01^{j}	2.34	1.29	1.67	2.6	1.98^{m}
Mean	**10.62^{a}**	**5.71^{d}**	**8.07^{c}**	**8.97^{b}**		**10.61^{a}**	**5.70^{d}**	**6.90^{c}**	**8.61^{b}**		**11.95^{a}**	**6.19^{d}**	**8.33^{c}**	**10.02^{b}**	
CD0.05															
Genotypes					**0.45**					**0.38**					**0.31**
Media					**1.01**					**0.85**					**0.69**
G x M					**2.02**					**1.70**					**1.39**

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

Table 3. Number of direct and indirect organs forming hypocotyls cultured on different fortifications of MS media.

Culture Media ▼ Genotypes►	Direct organogenesis (%)					Indirect organogenesis (%)				
	Pusa Madhuras	RM-50	Durgapura Madhu	Local	Mean	Pusa Madhuras	RM-50	Durgapura Madhu	Local	Mean
MS.5D.5B	10.04	5.05	8.87	9.47	**8.36^{g}**	15.37	11.93	12.14	11.12	**12.64^{d}**
MSD.5B	18.57	11.17	14.47	15.15	**14.84^{e}**	20.95	14.06	17.56	14.39	**16.74^{b}**
MS2D.5B	16.37	10.56	13.78	14.49	**13.80^{e}**	19.37	11.67	15.01	11.49	**14.39^{c}**
MS3D.5B	2.98	1.98	2.69	2.75	**2.60^{jk}**	4.45	1.9	2.95	1.99	**2.82^{hi}**
MS5D.5B	2.32	1.11	1.99	2.13	**1.89^{k}**	2.17	1.37	1.45	1.45	**1.61^{j}**
MSDB	6.45	3.45	4.17	5.56	**4.91^{h}**	11.54	6.26	6.37	6.23	**7.60^{f}**
MS2DB	7.01	2.75	3.78	5.34	**4.72^{h}**	12.47	8.45	10.07	1.87	**8.22^{f}**
MS.5N.5B	15.18	8.47	10.54	12.17	**11.59^{f}**	12.5	6.11	8.45	6.07	**8.28^{f}**
MSN.5B	23.24	16.54	16.25	21.45	**19.37^{c}**	16.79	10.47	13.43	11.95	**13.16^{d}**
MS2N.5B	20.93	15.95	16.2	19.17	**18.06^{d}**	13.54	8.45	10.04	8.45	**10.12^{e}**
MS3N.5B	4.49	2.17	2.73	3.69	**3.27^{ij}**	2.79	1.37	2.45	1.25	**1.97^{ij}**
MS5N.5B	3.27	1.45	1.04	2.79	**2.14^{k}**	1.24	1.2	1.25	1.05	**1.19^{j}**
MSBN	24.79	19.18	19.15	21.98	**21.28^{b}**	2.25	13.15	16.13	12.37	**10.98^{e}**
MS2NB	26.98	21.37	21.94	25.85	**24.04^{a}**	21.95	15.17	18.71	15.47	**17.83^{a}**
MS.5D.5Kn	6.16	2.25	2.25	5.45	**4.03^{hi}**	8.49	5.24	6.54	5.43	**6.43^{g}**
MSD.5Kn	10.54	4.79	5.67	8.17	**7.27^{g}**	16.25	8.63	8.13	8.39	**10.35^{e}**
MS2D.5Kn	6.69	3.45	3.45	6.84	**5.11^{h}**	11.95	5.45	6.37	5.49	**7.32^{fg}**
MS3D.5Kn	2.23	1.25	1.37	2.05	**1.73^{k}**	2.17	1.32	1.99	1.25	**1.68^{j}**
MS5D.5Kn	1.99	1.12	1.02	1.75	**1.47^{k}**	1.99	1.07	1.32	1.11	**1.37^{j}**
MSDKn	3.82	2.04	2.13	3.29	**2.82^{j}**	4.43	3.25	3.25	3.39	**3.58^{h}**
Mean	**10.70^{a}**	**6.81^{d}**	**6.67^{c}**	**9.48^{b}**		**10.13^{a}**	**6.83^{c}**	**8.18^{b}**	**6.51^{c}**	
CD 0.05										
Genotypes					**0.51**					**0.45**
Media					**1.15**					**1.01**
G x M					**2.29**					**2.02**

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