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DIRECT MULTIPLE SHOOTS PROLIFERATION OF MUSKMELON (*CUCUMIS MELO* (L.) FROM SHOOT TIP EXPLANTS

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ABSTRACT

In vitro plantlet regeneration has been obtained from 20-25days old seedling shoot tip segments of *Cucumis melo* (L.). We were examined using various phyto hormones individually and in combination on Murashige and Skoog (MS) semi solid medium supplemented with BAP (1.0-3.0 mg/L) Kn (1.0-3.0 mg/L),IAA (0.5 mg/L)+ BAP (1.0-3.0 mg/L) and IAA (0.5 mg/L)+Kn (1.0-3.0 mg/L) for multiple shoots proliferation IAA (0.5 mg/L)+BAP (2.0 mg/L) was proved to be best for induction of shoots for shoot tip explants. All regenerated plantlets were rooted on MS medium supplemented with (1.0 mg/L) IAA the regenerated plants grew normally in the green house.

KEY WORDS

Cucumis melo (L.), shoot tip, *In vitro* Regeneration,

ABBREVIATIONS

BAP, 6-Benzyl amino purine; Kin, Kinetin IAA, Indole acetic acid MS, Murashige and Skoog.

INTRODUCTION

Cucurbitaceae are an economically important family of plants, with species commercially cultivated in tropical and subtropical regions. Species such as muskmelon, watermelon, melon, cucumber and squash are widely cultivated in all regions of India. Among cucurbit species muskmelon is the most cultivated genera in India. *Cucumis melo* L. is rich in phosphorus, potassium and oxalic acid and is popularly used in salads. Its seeds are diuretic, tonic and refrigerant. The odorous principle of *Cucumis melo* L. is extractable with alcohol and is used in certain bouquet perfumes (Pandey 2000). A good micropropagation protocol could reduce the cost of hybrid seed production, which can account for 30% of the total seedling cost. The commercial application of *in vitro* techniques in cucurbitaceous taxa has been well demonstrated and the regeneration of plants has been reported from excised cotyledons (Sing *et al.*1996, Drink and Buggenum 1989) and leaf explants (Kathal *et al.* 1988, Mishra and Bhatnagar 1995; Stipp *et al*. 2001). Regeneration by organogenesis or somatic embryogenesis has been described on a wide range of *Cucumis melo* L. cultivars (Guis *et al.* 2000; Akasaka-Kennedy *et al.* 2004 and Tabit *et.,al.* 1991) and investigations have been focused on the effect of plant genotype, growth regulation and explant source. Besides growth regulators, seedling age at the time of explant preparation (Srivastava *et al.,* 1989; Compton and Gray, 1994), explant dissection method (Compton and Gray, 1993; Compton, 2000), and culture conditions (Choi *et al.,* 1994) also influence adventitious shoot development. The development of tissue culture systems, for commercially important Brazilian cucurbit species, is restricted to the analysis of *in vitro* morphogenesis of *Cucumis melo* var. *inodorus* (Stipp *et al.,* 2001).

The regeneration response is genotype dependent (Galperin *et al.* 2003) and the presence of meristematic protuberances that fail to develop into elongated normal shoots has frequently been observed (Liborio Stipp *et al.*, 2001). The present communication describes *in vitro* multiple shoot regeneration from shoot tip explants, rooting and the successful greenhouse establishment of muskmelon.

MATERIAL AND METHOD

Aseptic seed germination explants preparation and culture conditions:

Seeds of Musk melon (*Cucumis melo*) were obtained from the Agriculture Research Station Warangal India, Seeds were surface sterilized in 70% (v/v) ethanol and seeds were surface sterilized with 0.1%(w/v) mercuric chloride ($HgCl_2$) for 5 minutes and then rinsed thoroughly with sterile double distilled water 3-4 time. The seeds were germinated on MS **(**Murashige and Skoog 1962) basal medium with 3% (w/v) sucrose 4 weeks old seedlings served as the source of shoot explants.(Fig-1a)

Figure1
Direct shoot proliferation of shoot tip culture in muskmelon (*Cucumis melo* L.)

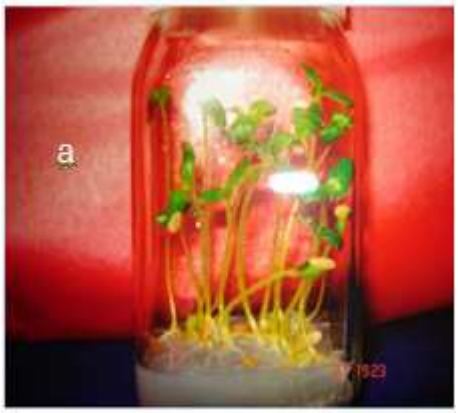

Figure -1a
in vitro seedling after 30 days of seed culture

Plant Regeneration:

Shoot tips measuring 4-6 mm in size were excised aseptically and inoculated on MS medium containing 3% w/v sucrose with various concentrations of cytokinin BAP(1.0-3.0 mg/L), Kn (1.0-3.0 mg/L) alone and also in combination with auxin IAA (0.5mg/L) + BAP (1.0-3.0 mg/L), and IAA (0.5mg/L) + Kn (1.0-3.0 mg/L) (Table-1) the pH of the media was adjusted to 5.8 $\pm$ 1 with I N HCl or 1N NaOH solidified with 0.8% difco-bacto agar and autoclaved at 121^{o}C at psi for 15-20 minutes. Single explants was inoculated in each culture tube and incubated at 25 $\pm$ 2^{o}C under white fluorescent light of 40-60 µ mol m^{-2} s^{-1} intensity for 16 hrs light /8 hrs dark period. Every two weeks the explants were transferred to fresh medium. The number of shoots produced was counted 6 weeks after culture. Isolated single shoots after reaching 5 centimeters in size were transferred to MS medium (Murashige and Skoog 1962) supplemented with (0.2 mg/L) IAA for rooting. Plantlets were transferred to the greenhouse for acclimatization and growth.

RESULTS AND DISCUSSION

Multiple shoot buds proliferation was observed within 15-20 days of culture from the cut ends of shoot tips. The data on *in vitro* regeneration was presented in (Table 1).

Table-1

Effect of BAP, Kn, IAA+BAP and IAA+Kn on direct shoot induction from shoot tip explants on MS medium in Cucumis melo L.

Hormone conc. (mg/L)	% of cultures responding	Average number of shoots/ explants (S.E)*	Hormone conc. (mg/L)	% of cultures responding	Average number of shoots/ explants (S.E)*
BAP			IAA + BAP		
1.0	50.0	2.0 ± 0.25	0.5 + 1.0	53.0	3.6 ± 0.48
1.5	56.0	3.3 ± 0.25	0.5 + 1.5	58.0	4.0 ± 0.75
2.0	60.0	4.0 ± 0.27	0.5 + 2.0	68.0	4.8 ± 0.36
2.5	45.0	3.8 ± 0.38	0.5 + 2.5	55.0	4.2 ± 0.32
3.0	43.0	2.7 ± 0.32	0.5 + 3.0	53.0	3.8 ± 0.42
Kn			IAA + Kn		
1.0	52.0	2.7 ± 0.43	0.5 + 1.0	48.0	3.2 ± 0.42
1.5	50.0	3.2 ± 0.34	0.5 + 1.5	52.0	3.0 ± 0.32
2.0	53.0	3.8 ± 0.35	0.5 + 2.0	58.0	3.3 ± 0.42
2.5	48.0	3.4 ± 0.22	0.5 + 2.5	50.0	2.8 ± 0.32
3.0	46.0	3.0 ± 0.45	0.5 + 3.0	40.0	2.6 ± 0.32

**Mean ± Standard Error.*

Effect of BAP and Kn:

Table 1 represents, direct regeneration of shoot tip explants to various concentration of cytokinins such as BAP and Kn alone in BAP (1.0 -3.0 mg /L) and Kn (1.0-3.0 mg/L) was studied on direct multiple shoot bud induction. Direct adventitious shoot regeneration on MS medium containing various results (Table-1) Highest responding cultures with maximum frequency of multiple shoot bud induction was observed at (2.0 mg/L) BAP (4.0+ 0.35 shoots/explants) (Fig –I b) followed by 2.5 and 3.0 mg/L BAP, produced (3.8±0.38 and 2.7±0.32 shoots/explants)with 45 and 43% cultures were responded. The numbers of shoots were considerably reduced, when BAP concentration was increased. Kn was less responsive compared to BAP in inducing shoot buds from the explant with 1.0 mg/L Kn the shoot tip explants produced (2.7±0.43 shoots/explants) and 52% culture responded. 2.0 mg/L Kn was more responsive in inducing maximum number of shoots (3.6±0.32 shoots/explants) with greater frequency (53%) Kn at 2.5 and 3.0 mg/L Produced (3.4±0.62 and 3.0± 0.45 shoots/explants) with 48 and 46% cultures responded

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Figure – 1b
Direct shoots on (2.0mg/L) BAP after three weeks

Effect of IAA + BAP and IAA + Kn:

When the auxin was taken in combination with IAA (0.5 mg/L) + BAP (1.0 -3.0 mg/L) and IAA 0.5 mg/L + Kn (1.0-3.0 mg/L) (Table-1) in combination produced shoots from the explants. At 0.5 mg/L IAA with 1.0 mg/L BAP 53% cultures responded with (3.6 ± 0.48 shoots /explants) maximum number of shoots (4.8± 0.36 shoots/explant) (Fig –I c) with greater

Figure – 1c
Multiple shoots on IAA(0.5mg/L)+(2.0mg/L) BAP after six weeks

Frequency 68% were produced at (2.0 mg/L) BAP + IAA (0.5 mg/L) (Fig –I d) As the concentration of BAP was increased from 2.5 mg/L to 3.0 mg/L the number of shoots were considerably reduced (Table -1).

Figure – 1d
Direct shoots formation on IAA(0.5mg/L)+Kn (2.0mg/L) after eight weeks

IAA + Kn was less responsive compared to IAA + BAP in including shoot buds from the explants (Table -1) with (1.0 mg/L) Kn and (0.5 mg/L) IAA produced (3.2±0.42 shoots/explants) with 48 % cultures responded. At 2.0 mg/L Kn was more responsive in inducing maximum number of shoots (3.3± 0.42 shoots) with greater frequency (58%) Kn at 2.5 and 3.0 mg/L produced (2.8+ 0.32 and 2.6+ 0.32 shoots/explants) with 50% and 40% cultures were responded . To find out the efficiency of auxin cytokinin combination the shoot tip explants were cultured on MS medium supplemented with IAA (0.5 mg/L) in combination with various concentration of BAP /Kn (1.0 -3.0 mg/L). Direct shoot bud

proliferation was found in all the concentrations and combinations of phyto hormones used.

***In vitro* rooting:**

The micro-shoots were cultured on MS medium containing 3% sucrose supplemented with different concentrations of IBA/IAA. Profusely branched rooting was found on MS medium fortified with 2.0mg/L IBA , followed by 2.0mg/L IAA on comparing to more number of roots per shoot at 2.0mg/L IAA.. Regenerated rooted plants from shoot tips were transplanted in pots containing sterilized soil for acclimatization and transplanted in the field to observe their fertility status (Table-2).

Table – 2

in vitro rooting ability of Shoot tip regenerated shoots on MS media fortified with various concentration of IBA and IAA in Cucumis melo L.

Growth regulators (mg/L)	% of cultures responding	Average number of roots / shoots (SE)*
IBA		
0.5	72	4.0 ± 0.2
1.0	80	8.0 ± 0.4
1.5	70	9.2 ± 0.5
2.0	70	3.0 ± 0.3
IAA		
0.5	60	2.0 ± 0.6
1.0	72	5.0 ± 0.7
1.5	78	8.4 ± 0.3
2.0	56	4.8 ± 0.4

*Mean ± Standard Error.

DISCUSSION

We were successful in regeneration of plants from, shoot tip culture on MS medium fortified with different concentrations of cytokinin ie BAP /Kn individually and also in combinationwith (0.5 mg/L) IAA. Maximum number of shoot buds were induced at (2.0 mg/L) BAP in comparison to Kn. Kn as a role growth regulators with low levels of auxin (0.5 mg/L) were added to the medium containing BAP/Kn . It was interesting to find that the shoot induction was enhanced in all the concentrations of cytokinin tested. However the shoot buds proliferation was found to be more on (0.5 mg/L) IAA in combination with BAP/Kn and this might be IAA have triggered the action of BAP/Kn in a proper way for inducing more number of shoots per explants but the combination of IAA + BAP induced higher number of plantlet regeneration among all hormonal combinations and concentrations used.

The present findings from *Cucumis melo* L demonstrate the possibility of the *in vitro* propagation of cucurbits through shoot tip explants to obtain plantlets with uniform growth characteristics of the mother plant, direct regeneration is essential. Literature on cucurbits indicates a low rate of regeneration and survival of plants with abnormalities such as premature flowering (Gambley and Dodd 1990). Previous works on *in vitro* propagation of cucumber from shoot tips indicate low shoot regeneration frequencies (Handley and Chambliss 1979, Vasudavan *et., al.* 2004). However in the present study it was possible to obtain 100% shoot regeneration response from shoot tip explants of *Cucumis melo.* Regeneration from cotyledon, sections of hypocotyls and apical buds with varying regeneration frequency has been reported by Gambley and Dodd (1991). Similarly Hoque *et al.* (2005) have reported the high frequency of plant regeneration on MS

medium containing (2.0 mg/L) BAP in combination with (0.5 mg/L) IAA from cotyledon derived callus in *Momordica dioica.* They have also found the maximum number of shoots per explants on BAP compared to Kn. Essentially to both auxin cytokinin combination for inducing shoot organogenesis has been reported in leaf culture of *Cicer ariutum* (Arockia swamy *et al.* 2000) of the cytokinin used BAP proved as most effective than Kn in inducing shoots, the same finding were recorded in *Capsicum* spp (Phillips and Hubsten berger 1985).

Our results show enhanced shoot formation by proliferation of cotyledon and hypocotyls on a medium fortified with cytokinin and auxins. The fortification of cytokinin for multiple shoot induction at lower concentrations has also been reported (Kathal *et al.* 1988; Singh *et al.* 1996). It is concluded that the manipulation of culture conditions using various combinations and concentrations of growth hormones and other adjuvants can provide a reproducible protocol and reduce the high costs of hybrid seed production. It is concluded that the procedure described here appear to be adapted for large clonal propagation of *Cucumis melo in vitro* and could be used for reducing the high cost of hybrid seed production.

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