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In vitro Plantlets Regeneration from Nodal Segments of Musk Melon (*Cucumis melo* L.)

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Abstract: *In vitro* plantlets of musk melon cv. Honey Dew (*Cucumis melo* L.) could be established from the nodal segments of mature field grown plants. Multiple shoots were induced from the nodal segments on MS medium supplemented with 8.0 mg L^{-1} BA. The apical shoots collected from the *in vitro* multiple shoots were used as explants for subsequent multiple shoots induction on MS medium supplemented with 0.5 mg L^{-1} BA. NAA was not required together with the presence of BA for the induction of multiple shoots. All the *in vitro* shoots produced roots when transferred to MS medium without any plant growth regulator. The plantlets with well-developed roots were successfully transplanted to soil after acclimatization process with 97% of the plantlets survived after two weeks and 70% of them produced flowers after four weeks of transplanting to soil.

Key words: BA, NAA, multiple shoots, musk melon, *Cucumis melo*

INTRODUCTION

Musk melon cv. Honey Dew (*Cucumis melo* L.), belonging to the family cucurbitaceae, is a popular fruit believed to be originated from Iran. It is now widely grown in the tropics, subtropics and the temperate regions. In Malaysia, they are commercially planted as one of the economic fruit crops in the east coast states of Malaysia[1].

There are various varieties or cultivars of musk melons and each can be differentiated from the other based on individual fruit shape, size, color of the fruit pulp and fruit skin sculpture. Each tree can produce an average of 4-5 fruits with each fruit weight ranging from one to 4 kg. The fruits provide a valuable alternative source of vitamin C, calcium and β-carotein.

Musk melon is conventionally propagated by seeds. Low seed germination, expensive seeds and disease susceptibility are the major problems faced by the commercial growers. The seedlings are very susceptible to fungal and bacterial rots that are mainly due to *Alternaria tenuis, Erwinia* sp., *Penicillium* sp., *Cladosporium cucumerinum, Fusarium* spp. and *Rhizopus* stolonifer. *In vitro* propagation of plantlets could hence be an alternative method for overcoming these constraints. Dong and Jia[2] had successfully produced *in vitro* plantlets of watermelons using callus derived from cotyledon. Ahad *et al.*[3] reported complete plant regeneration of watermelons from the immature and mature embryo axis. Sultana *et al.*[4] produced *in vitro* plantlets of watermelons from the callus culture of leaf segments. According to Bayliss[5], plantlets regeneration from callus often resulted in aneuploidy and polyploidy. While plantlets regenerated from shoot meristem, apical shoot explants and nodal segments were usually homogenous and true-to-type. Hence, in our study, nodal segments were used for clonal propagation of musk melon cv Honey Dew with the aim to produce homogenous plantlets.

MATERIALS AND METHODS

Nodal segments were collected from field grown plants and washed thoroughly under running tap water. They were then surface sterilized with 10% (v/v) Clorox® added with three drops of Tween 20 with continuous agitation for 10 min at the first stage and followed with rinsing three times with sterile distilled water. The nodal segments were again surface-sterilized with 5% (v/v) Clorox® added with three drops of Tween 20 for 5 min at the second stage and followed with rinsing three times with sterile distilled water. The cleansed nodal segments were inoculated on basic Murashige and Skoog[6] medium (MS). After one week, the aseptic nodal segments were cultured on MS medium supplemented with BA (0, 2, 4, 6, 8 and 10 mg L^{-1}) for induction of multiple shoots formation. Three nodal segments were culture in each 150 mL jam jar fitted with plastic cap and 10 jam jars were used for each BA concentration. The experiment was carried out using Complete Randomized Design. The number of shoots produced from each nodal segment was determined after three weeks of culture.

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To investigate the effect of NAA with the present of BA on multiple shoot formation of the musk melon, the nodal segments were cultured on MS medium + 8 mg L^{-1} BA (the optimum amount of BA for multiple shoot formation) supplemented with NAA (0, 2, 4, 6, 8 and 10 mg L^{-1}). The number of shoots formed from each explant was recorded after three weeks of culture.

For continuous production of multiple shoots, the apical shoots of the *in vitro* multiple shoots produced on MS plus 8.0 mg L^{-1} BA were cultured on MS medium supplemented with lower concentration of BA (0, 0.5, 1.0, 1.5, 2.0 mg L^{-1}). Three apical shoots were cultured in each 250 mL jam jar and 10 jam jars were used for each culture medium. The number of shoots formed from each apical shoot was determined after 3 weeks of culture.

All the data were analyzed using one-way analysis of variance (ANOVA) and comparison of mean by Tukey test at p=0.05.

Individual shoot was separated from the multiple shoots and cultured on MS medium without any growth regulator for rooting. Two shoots were cultured in each 250 mL jam jar and 30 jam jars were used for the rooting experiment. The number of rooted shoots was recorded after two weeks of culture. Rooted shoots were then removed from the culture medium and washed with running tap water to remove traces of agar. They were transferred to Jiffy-7 that were placed in a plastic container and covered with punched holes transparent plastic sheet. After two weeks, the acclimatized *in vitro* plantlets were transferred to pots containing top soil : organic soil mixture (2:1).

The pH of all media was adjusted to 5.7 - 5.8 before the addition of 7.5 g L^{-1} of agar (Chile Algas) and autoclaved for 15 min at 1.5 kg cm^2 pressure at 121°C. The culture vessels containing the explants or plantlets were placed in a culture room maintained at 25±2°C under continuous cool white fluorescent lights with a photon flux density of 2500 - 3000 Lux.

RESULTS AND DISCUSSION

Induction of multiple shoots: All the nodal segments (100%) produced multiple shoots when they were cultured on MS medium supplemented with 2 - 10 mg L^{-1} N^6-benzyl adenine (BA) after three weeks of culture. The number of shoots induced from each nodal segment increased as the amount of BA added into MS medium increased. MS medium supplemented with 8 or 10 mg L^{-1} BA induced the most number of shoots with an average of 20 shoots induced from each nodal segment. No multiple shoot was produced from the nodal segment when they were cultured on basic MS medium (Table 1) but some of the nodal segments produced roots instead of shoots.

Fig. 1: Short and normal multiple shoots of musk melon induced from normal segments on MS medium supplemented with 8.0 mg L^{-1} BA.

Table 1: Effect of BA at different concentration supplemented in MS medium on multiple shoots formation from the nodal segments of musk melon after three weeks of culture

MS medium + BA (mg L^{-1})	Number of shoots per nodal segment
0	1.0e
2	3.8d
4	7.6c
6	12.3b
8	19.4a
10	20.3a

Mean number of shoots followed by the same alphabet was not significantly different at p=0.05 (Tukey Test)

Table 2: Effect of cytokinin (BA) with auxin (NAA) supplemented in MS medium on multiple shoot formation from the nodal segments of musk melon after three weeks of culture

MS medium + growth regulators		
BA (mg L^{-1})	NAA (mg L^{-1})	Number of shoot per nodal segment
8	0	17.9a
8	2	2.3b
8	4	1.8bc
8	6	1.3c
8	8	1.1c
8	10	1.1c

Mean number of shoots followed by the same alphabet was not significantly different at p=0.05 (Tukey Test)

The multiple shoots formed from the nodal segments cultured on MS + 8 mg L^{-1} BA or 10 mg L^{-1} BA were short but normal (Fig. 1). *In vitro* organogenesis generally required the action of growth regulators auxin and cytokinin in a certain ratio depending on the plant species. High cytokinin to auxin ratio generally induced multiple shoots formation while high auxin to cytokinin ratio usually induced rhyzogenesis[7].

Experimental results showed that the addition of 1-naphthylacetic acid (NAA) into the MS medium supplemented with 8 mg L^{-1} BA did not help to induce more multiple shoots formation from the nodal segments of musk melon. Only the nodal segments that

Fig. 2: The formation of calluses on the nodal segments of musk melon on MS medium supplemented with 8 mg L^{-1} BA and 2.0 mg L^{-1} NAA after 6 weeks of culture

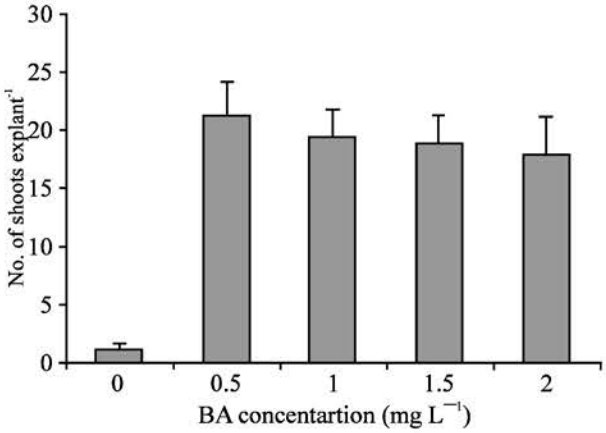

Fig. 3: Effect of BA supplemented into the MS medium on multiple shoot firmation of apical shoot of musk melon

were cultured on MS medium supplemented with 8 mg L^{-1} without NAA produced the most multiple shoots with an average of 17.9 shoots per nodal segments (Table 2). This indicated that NAA, an auxin, was not necessary for shoot proliferation of musk melon. Hence MS medium supplemented with 8 mg L^{-1} BA was considered as shoot proliferation medium. For musk melon, only BA, a cytokinin, was needed for the induction of multiple shoots formation from the nodal segments. The addition of only BA into the culture medium was also found to be effective for *in vitro* shoot multiplication of *Alpinia purpurata*[8]. According to *Yang et al.*[9] BA was found to be more effective than kinetin and 2-isopentyl adenine (2iP) for shoot proliferation of *Stevia rebaudiana*. However, some other plant species required more than one type of

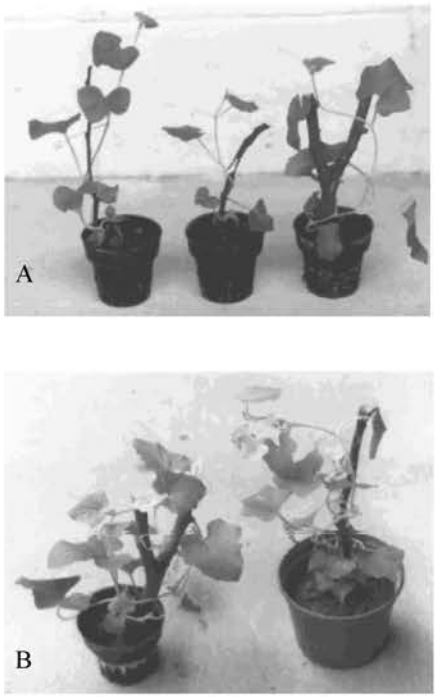

Fig. 4: Micropropagated plantlets of musk melons after transferred to soil. A, two weeks old plantlets. B, The plantlets produced flowers after 4 weeks transferred to soil

cytokinin for shoot proliferation such as *Tectona grandis*[10].

The presence of auxin (NAA) in the proliferation medium instead induced the formation of friable calluses (Fig. 2) from the nodal segments of musk melon. The same phenomenon was happened for *Kaempferia galangal*[11] and *Daura metel*[12] and calluses were formed when the explants of these plants were cultured on culture media that were supplemented with auxin (2,4-D) and BA. On the other hand, Sultana *et al.*[3] reported that MS medium supplemented with 1.0 mg L^{-1} BA + 0.2 mg L^{-1} NAA was found to be the best medium for the production of multiple shoots in watermelon. Hoque *et al.*[13] found that a combination of 1.5 mg L^{-1} BA and 0.1 mgL^{-1} NAA was more suitable for adventitious multiple shoot formation in teastle grourd (*Citrullus vulgaris* Schrad.).

To mass produce the *in vitro* shoots, it could be easily carried out by culturing the apical shoots derived from the multiple shoot stocks cultured on MS + 8.0 mg L^{-1} BA on to MS medium supplemented with 0.5 mg L^{-1} BA. This medium (MS + 0.5 mg L^{-1} BA) could induce the formation of an average 21±1.3 shoots from each apical shoot explant. The results showed that the addition of BA more than 0.5 mg L^{-1} until 2.0 mg L^{-1} did not significantly

increase the number of multiple shoots formed for the purpose of mass shoot propagation (Fig. 3). This also indicated that the *in vitro* apical shoots of musk melon were suitable and easily mass propagated in MS medium with the aid of very much lower concentration of cytokinin (0.5 mg L^{-1} BA).

Rooting of micro-shoots: Since the nodal segments could produce roots when they were cultured on the basic MS medium, all the individual shoots separated from the multiple shoot stocks were directly cultured on MS medium without the addition of any growth regulator. All the shoots (100%) produced roots after inoculating onto the basic MS medium for two weeks. The same finding was observed in *Cymbopogon nardus* L. whereby all the shoots produced roots after transferred to MS medium free of growth regulators[14] . After two weeks acclimatized in a container covered with punched holes plastic sheets, the plantlets with well-developed roots were successfully transplanted to pots containing top soil plus dark organic soils in the ratio of 2:1. Ninety-seven percent of the acclimatized plantlets survived after transferred to pots and 70% of the tissue cultured plantlets produced flowers after four weeks transferred to the soil (Fig. 4). All the *in vitro* plantlets produced normal fruits similar to the seed derived plants. Hence, this indicated micropropagation technique could be used as an alternative method for mass production of musk melon seedlings and would be able to solve the limited supply of plant materials for commercial planting of musk melons.

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