International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print)
Vol. 2, No. 1, p. 47-52, 2012
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Rapid *in vitro* clonal propagation of a hybrid muskmelon (*Cucumis melo* L.) cultivar from seedling explants

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Received: 04 January 2012
Revised: 16 January 2012
Accepted: 19 January 2012

Key words: Muskmelon, clonal propagation, seedling explant, leaf node.

Abstract

An investigation was undertaken to develop a reliable strategy for large scale multiplication of a hybrid cultivar of muskmelon through *in vitro* clonal propagation. Best *in vitro* seed germination was observed in MS medium containing 0.5 mg/l GA_3. Two explants excised from axenic seedlings were tested for shoot proliferation and leaf node showed better shoot proliferation than shoot tip explants. Maximum shoots (8.22±0.23) per culture were produced from leaf node explants cultured on MS medium augmented with 1.0 mg/l BA. Rooting was found best on 0.1 mg/l NAA containing medium. Rooted plantlets were acclimatized gradually and transferred to the field condition and finally, 82 % plantlets were survived well.

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Introduction

Muskmelon (*Cucumis melo* L. cv. *reticulata*) is a popular fruit plant belonging to the family of Cucurbitaceae. It is native to Persia (Iran), Armenia, and adjacent areas on the west and the east and now widely grown in the tropics, subtropics and the temperate regions of the world (Keng and Hoong, 2005). Due to having high fruit value, muskmelon has recently been introduced in Bangladesh (http://www.sdnbd.org/tomato_harvest.htm). This is an annual plant and each plant can produce an average of 5-7 fruits. The fruits being rich in vitamin B, vitamin C, calcium and β-carotene have both edible and medicinal uses also. Traditionally this plant is cultivated by seeds and the commercial growers face several problems such as high market value (150 BDT/seed) of hybrid seeds, low seed germination rate and disease susceptibility.

In this context, *in vitro* clonal propagation of hybrid plantlets could be an alternative approach for large scale cultivation of muskmelon. Keng and Hoong, (2005) used field grown nodal explants for *in vitro* propagation of muskmelon while Tarsem *et al.*, (2005) used *in vitro* derived plantlets for micropropagation of muskmelon mainly with a view to maintain a male sterile line. But the above-mentioned reports are not supported enough for large scale clonal propagation of muskmelon as these do not have a clear concept. Moreover, in Bangladesh no report has been made yet on *in vitro* clonal propagation of this fruit plant. The present study, therefore, describes the first report on rapid multiplication of homogenous plantlets of a hybrid cultivar of muskmelon via *in vitro* raised seedling explants.

Materials and methods

Hybrid seeds of muskmelon were collected from a Japanese seed company named TAKII Seed by Plant Breeding and Gene Engineering Laboratory, Rajshahi University, Bangladesh. Seeds were washed thoroughly under running tap water for 5 minutes and then washed with continuous agitation in a few drops savlon containing water for 10 minutes. Washed seeds were then treated with 0.1 % $HgCl_2$ for 8 minutes under laminar air flow cabinet to disinfect them. Finally, seeds were washed 3 to 5 times with sterile distilled water and were placed in conical flask (10-12 seeds per flask) containing MS medium (Murashige and Skoog, 1962) alone or combined with GA_3 at 0.1-2.0 mg/l. After seed germination, two different types of explants viz. leaf node and shoot tip (1-1.5 cm in length) were excised and cultured on MS basal medium containing 3 % (w/v) sucrose and 0.8 % (w/v) agar with BA (6-benzyl adenine) and Kn (6-furfuryl amino purine) alone or in combination at different concentrations (0.5-2.0 mg/l) to test the effect of plant growth regulators on *in vitro* shoot multiplication. Well formed shoots were excised from *in vitro* derived shoot clumps and then cultured on MS medium supplemented with IAA (Indol-3 acetic acid), IBA (Indol-3 butyric acid) and NAA (α-napthalene acetic acid) alone at 0.1-2.0 mg/l for adventitious root induction. Finally, rooted plantlets were transferred to small pots containing garden soil and vermicompost (2:1) and before transferring them to the experimental field plantlets were maintained carefully for almost 21 days under laboratory condition and outdoor condition.

During the investigation, the pH of the medium was adjusted 5.7±0.1 before autoclaving at 121 °C for 20 minutes at 1.2 kg/cm2 pressure. All the cultures were maintained at 25°C±2 °C (room temperature) under the cool white fluorescent lights for 16 hours photoperiod at 2000-3000 lux. Data on shoot proliferation were recorded after four weeks of culture and root induction data were recorded after three weeks of incubation.

Results and discussion

Seed germination rate along with length of germinated seedlings was markedly affected by the supplement of GA_3 (Table 1). The highest germination rate (92.31 ± 0.17 %) and the longest seedlings (5.80 ± 0.23) both were observed at 0.5 mg/l GA_3 containing MS medium (Fig. 1. A) while in MS_0 (control) medium seed germination rate and seedling length were 63.10 ± 0.36 % and 4.60 ± 0.19

respectively. These findings revealed the effect of GA_3 on breaking of seed dormancy. Many reports suggest the stimulatory effect of GA_3 on the germination of seeds and according to Diaz and Martin (1971), GA_3 is known to stimulate germination of seeds when dormancy is imposed by the different mechanisms like incomplete embryo development, mechanically resistant seed coats, presence of inhibitors and factors relating to physiological competence of the embryo axis *etc.* In another report, Nerson (2007) described that the germination ability of cucurbit seeds is related both to external and internal factors, which was also found during our investigation. Two types explant viz. leaf nodes and shoot tips having meristematic zones were excised from well developed seedlings after 20 days of seed germination and cultured on MS medium supplemented with BA and Kn alone or in different combinations. Explants responded in different manner in presence of growth regulators (Table 2). Regardless of explant type, in control treatment (MS_0) explants did not show shoot proliferation, which suggested the necessity of exogenous growth regulators for axillary shoot proliferation. Out of two cytokinins evaluated, both explants showed better responses in BA containing medium. Regarding leaf node explants, highest 93.20±0.22 % leaf node produced maximum shoots (8.22±0.23) having highest 6.37±0.25 cm length at 1.0 mg/l BA containing medium (Fig. 1. B). On the other hand, shoot tip showed maximum 88.12±0.31 % shoot proliferation with highest 6.20±0.23 number of shoots per culture and 5.47±0.25 cm average length of shoots per culture at 1.0 mg/l BA containing medium. However, shoot proliferation rate was considerably declined when explants irrespective of origin were cultured on medium containing BA and Kn.

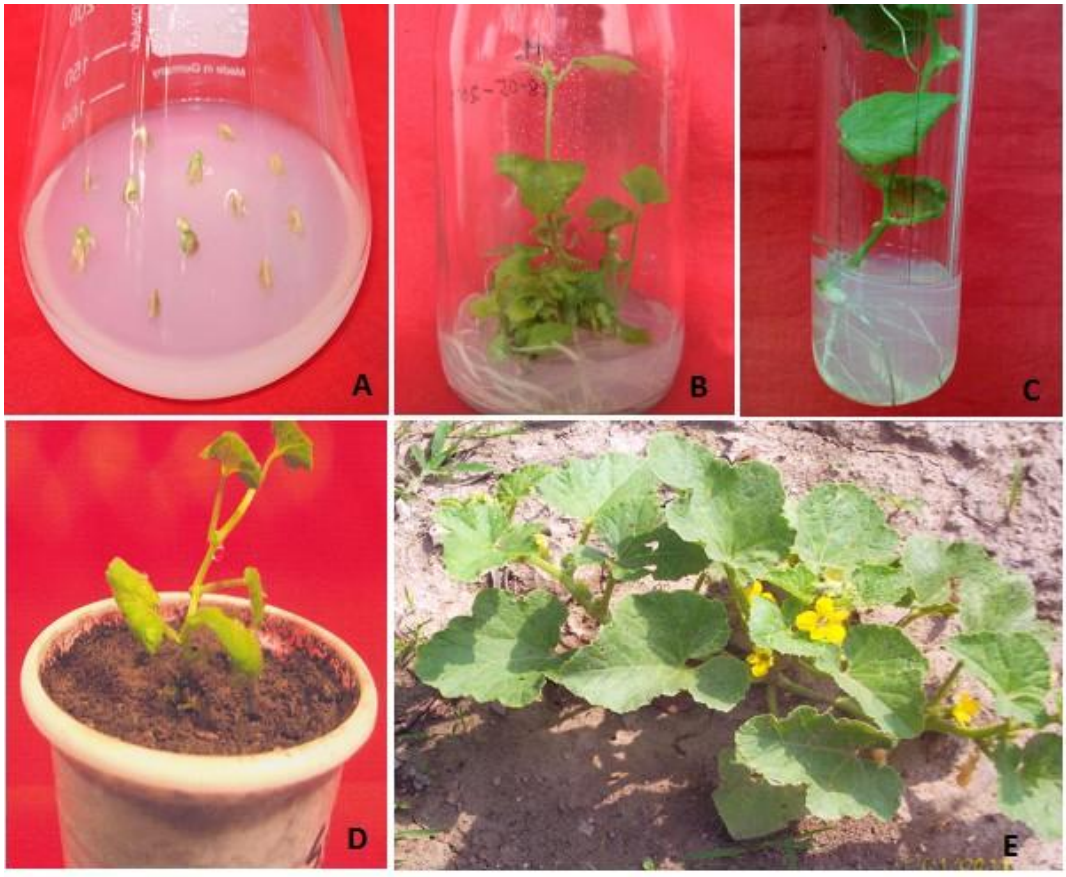

Fig. 1. In vitro clonal propagation of muskmelon (Cucumis melo L.). A. Germination of seeds on MS medium containing 0.5 mg/l GA3; B. Multiple shoot formation on MS + 1.0 mg/l BA; C. Rooting of in vitro shoots on MS +

0.1 mg/l NAA; D. A plantlet established in garden soil and vermicompost (2:1); E. Mature plant under field condition after 60 days of transplantation.

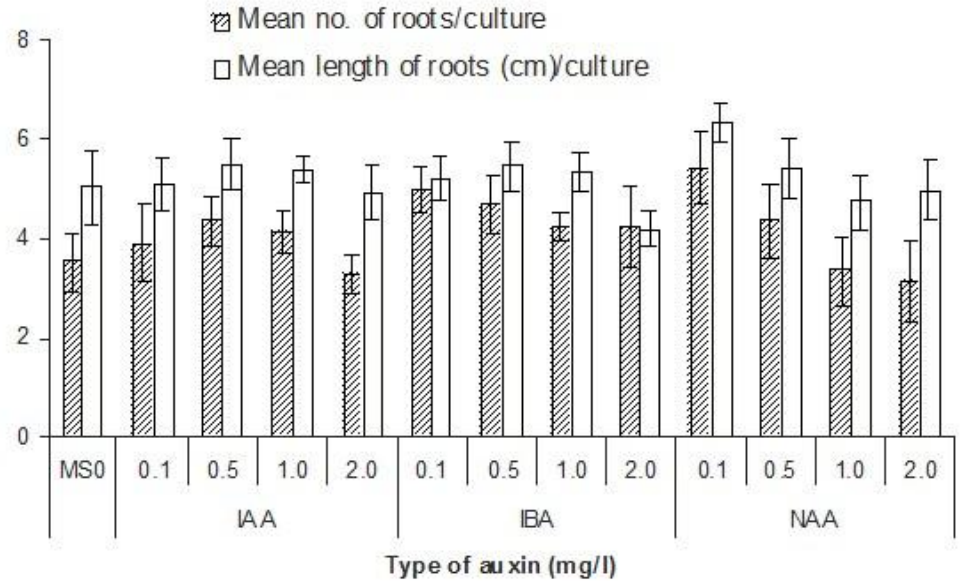

Fig. 2. Effect of three different auxins (IAA, IBA & NAA) on adventitious rooting of in vitro derived shoots of muskmelon. Each treatment consists of 12-15 replications.

Table 1. *In vitro* seed germination performance under different treatments. Data were recorded after 20 days of incubation and each treatment consists of 20 seeds and repeated thrice.

Treatment	% of seed germination (Mean ± SE)	Length of germinated seedlings (Mean ± SE)
MS_0	63.10 ± 0.36	4.60 ± 0.19
GA_3		
0.1	75.25 ± 0.45	5.11 ± 0.27
0.5	92.31 ± 0.17	5.80 ± 0.23
1.0	74.80 ± 0.80	4.91 ± 0.97
1.5	61.84 ± 0.35	4.10 ± 0.37
2.0	52.58 ± 0.17	3.72 ± 0.28

Several reports (Shalaby *et al*., 2008; Sarowar *et al*., 2003; Kumar *et al*., 2003; Hoque *et al*., 1998; Ahmad and Anis, 2005) described the effect of BA as a potent cytokinin for shoot proliferation in cucurbits, which are in agreement with the present findings. Besides, when either of the cytokinin was used in medium alone at 1.5 mg/l or higher concentration or in combinations, callus was formed at different rates (Table 2) at the basal end of the explants, which hindered both percentage of shoot proliferation and number of shoots.

Well formed shoots excised from multiple shoot clumps were cultured on full strength MS medium fortified with three different auxins (IAA, IBA and NAA) singly at different concentrations for *in vitro* root induction (Fig. 2). Out of three auxins tested, profound rooting responses were recorded in the medium containing NAA (Fig. 1. C) and the number of roots and length of roots both were highest 0.1 mg/l NAA containing medium, which followed by IBA and IAA containing medium. In control treatment (MS_0), shoots produced significant number of roots which were too thin and delicate in nature to transfer to soil. The lowest number of roots and the shortest roots were produced on medium containing 2.0 mg/l NAA and 2.0 mg/l IBA respectively. These findings indicated the superiority of NAA over IAA and IBA in adventitious root induction of *in vitro* shoots. Similar results were obtained in watermelon (Shalaby et al., 2008 and Sultana and Bari, 2003) and in cucumber (Ahmad and Anis, 2005). Plantlets

having well developed root systems were transferred to small pots containing garden soil and vermincompost (2:1) and incubated under laboratory condition for seven days (Fig. 1. D). After incubation period, plantlets were placed to outdoor condition for almost 14 days and finally transferred to experimental field condition where 82% of the micropropagted plantlets survived well and showed promising growth and development (Fig. 1. E).

Table 2. Effect of plant growth regulators on in vitro shoot proliferation from leaf node and shoot tip explants. Each treatment consists of 10 explants and repeated thrice.

Plant growth regulators (mg/l)	% of explants induced shoot proliferation		No. of shoot per explant (Mean ± SE)		Length of the longest shoot (cm.) (Mean ± SE)	
	Shoot tip	Leaf node	Shoot tip	Leaf node	Shoot tip	Leaf node
MS_0	-	-	-	-	-	-
BA						
0.5	42.11 ± 0.28	48.13 ± 0.23	2.80 ± 0.21	3.68 ± 0.21	4.23 ± 0.25	5.25 ± 0.19
1.0	88.12 ± 0.31	93.20 ± 0.22	6.20 ± 0.23	8.22 ± 0.23	5.47 ± 0.25	6.37 ± 0.25
1.5*	72.31 ± 0.25	79.12 ± 0.36	5.33 ± 0.11	6.82 ± 0.81	5.15 ± 0.23	5.30 ± 0.19
2.0**	61.42 ± 0.23	65.42 ± 0.18	4.51 ± 0.84	4.91 ± 0.90	3.31 ± 0.14	3.80 ± 0.21
Kn						
0.5	35.13 ± 0.19	37.30 ± 0.18	2.62 ± 0.76	3.11 ± 0.76	4.05 ± 0.24	4.15 ± 0.24
1.0	68.50 ± 0.31	73.42 ± 0.19	4.23 ± 0.16	4.80 ± 0.16	4.90 ± 0.26	5.05 ± 0.21
1.5*	62.31 ± 0.27	66.10 ± 0.23	3.81 ± 0.27	4.21 ± 0.27	4.72 ± 0.22	4.66 ± 0.22
2.0**	48.11 ± 0.22	51.18 ± 0.27	3.11 ± 0.14	3.90 ± 0.14	3.77 ± 0.19	4.05 ± 0.19
BA + Kn						
1.0+0.5	71.38 ± 0.29	73.51 ± 0.25	4.21 ± 0.25	4.48 ± 0.13	3.15 ± .05	3.22 ± 0.21
1.0+1.0*	66.12 ± 0.16	64.42 ± 0.21	4.25 ± 0.33	4.52 ± 0.68	3.11 ± 0.02	3.35 ± 0.29
1.0+1.5*	61.22 ± 0.34	62.10 ± 0.31	3.01± 0.23	3.22 ± 0.70	2.81 ± 0.17	3.15 ± 0.16
2.0+0.5**	52. 01 ± 0.19	55.30 ± 0.32	3.35 ± 0.23	3.48 ± 0.28	2.76 ± 0.15	2.78 ± 0.28
2.0+1.0**	46.13 ± 0.35	44.31 ± 0.25	2.88 ± 0.19	2.78 ± 0.47	2.28 ± 0.19	2.45 ± 0.15
*2.0+1.5****	31.09 ± 0.31	38.12 ± 0.19	2.11 ± 0.33	2.10 ± 0.55	2.03 ± 0.11	2.18 ± 0.14

* slight callus at the basal end of explants; ** moderate callus at the basal end of explants; *** considerable callus at the basal end of explants

In conclusion, we developed a fast and efficient micropropagation protocol using *in vitro* grown seedling derived explants of muskmelon, which allowed rapid multiplication of hybrid plantlets. We demonstrated that the high efficiency of shoot proliferation can be achieved in muskmelon cultures using BA alone while well developed adventitious roots can be formed on NAA containing MS medium. The described protocol is simple in handling, which can be used effectively for large scale micropropagation of muskmelon within a short period.

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