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Rapid *in vitro* clonal propagation of two hybrid muskmelon cultivars and their field evaluation in agro climatic condition of Bangladesh

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Abstract

An attempt was made to develop a large scale production of planting materials of two Japanese hybrid cultivars of muskmelon through micro-propagation. Highest percentage of seed germination was found in MS medium supplemented with 0.2 mg/l GA_3. In the present work, shoot tip explant of two muskmelon cultivars viz. Sweet Gold and Midori excised from axenic seedlings were cultured for shoot proliferation. The maximum efficiency of shoot multiplication was recorded in MS medium supplemented with 2.0 mg/l BA and maximum number and length of shoots were achieved in the same concentration of BA. The highest percentage of *in vitro* grown cultured shoots was rooted when they were cultured in MS rooting medium containing 0.5 mg/l NAA for each cultivar. Successfully acclimatized plantlets were transplanted to the field and 80% plantlets survived. Field grown plantlets showed wide ranges of variations between the two sources of explants for different agronomic characters such as plant length, number of leaves/plant, number of fruits/plant and average weight/fruit.

Key words: Micro-propagation, Seedling explants, Shoot multiplication, Adventitious rooting, Plantlets regeneration, Field evaluation, Hybrid muskmelon.

Introduction

Muskmelon (*Cucumis melo* L.) is the most popular and high valued fruit in different countries in the world and belongs to the family Cucurbitaceae. Immature melons are used fresh in salads, cooked (soup, stew, curry and stir-fry) or pickled. Mature fruit eaten fresh as a dessert, canned or used for syrup or jam. The fruits can be used as a cooling light cleanser or moisturizers for the skin. They are also used as a first aid treatment for burns and abrasions (Kirkbride, 1993; Vallone *et al.*, 2013).

Muskmelon is conventionally propagated by seeds and for better production of muskmelon every year hues amount of hybrid seeds are imported from abroad. But the commercial growers face several problems such as high market value of F_1 hybrid seeds, low seed germination rate and disease susceptibility, conventional plant breeding and cultivation practices for commercial production of muskmelon have some limitations to achieve success in several agronomic traits. Recent discoveries in applied genetics and plant biotechnology developed some methods for crop improvement (Rahman *et al.*, 2012). In this context, *in vitro* clonal propagation of two exotic muskmelon cultivars and their field evaluation could be an alternative approach for overcoming these constrains and large scale cultivation of muskmelon in agro-climatic condition of Bangladesh (Banu, 2011). The present study describes rapid multiplication of homogeneous plantlets of two exotic cultivars of muskmelon via *in vitro* derived seedling explants and their field evaluation in agro-climatic condition of Bangladesh.

Materials and Methods

The F_1 hybrid seeds of muskmelon plants were the source of experimental materials. The seeds of two hybrid muskmelon cultivars viz. Sweet Gold and Midori were imported from Japanese seed company named TAKII. The shoot tips having meristematic zone was excised from the well germinated seedlings after three weeks of seed germination and were used as explants for the establishment of primary *in vitro* cultures. The F_1 seeds of muskmelon were washed thoroughly under running tap water for a few minutes to

reduce the dust and surface contaminants and then the seeds were surface sterilized with 2-3 drops of tween-80 and a few drops of savlon (ACI Pharma, Bangladesh) for about 5-9 minutes with constant shaking and washed with distilled water for complete removal of sterilizing agents. Surface disinfections of seeds were done with 0.1% $HgCl_2$ solution by gently shaking for 3-8 minutes. After exposure the sterilant, the seeds were washed in several times with double distilled water to remove all traces of $HgCl_2$ (Bhojwani and Razdan, 1983).

Sterile seeds were cultured on MS0 and MS medium supplemented with different concentration of GA_3. After seed germination, shoot tips (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 KIN (6-furfuryl amino purine) alone or in combination with NAA (a-naphthalene acetic acid) or IBA (Indole-3 butyric acid) to test the effect of plant growth regulators on *in vitro* shoot multiplication (Bhojwani and Razdan, 1983). Well formed shoots were excised from *in vitro* derived shoot clumps and then cultured on MS medium supplemented with IBA (Indol-3 butyric acid) and NAA (α-napthalene acetic acid) alone 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/cm^2 pressure. All the cultures were maintained at $25^0C \pm 2^0C$ (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 (Banu, 2011).

Results and Discussion

Seed germination rate along with length of germinated seedlings were markedly affected by the supplement of GA_3. The highest germination rate (90.36 ±0.12%) and longest seedlings (5.95 ±0.47cm) both were observed at 0.2 mg/l GA_3 containing MS medium in Sweet Gold cultivar (Figure 1 A). 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.

Shoot tips from well developed seedlings were cultured on MS (Murashige and Skoog, 1962) medium supplemented with auxin (NAA, IBA) and cytokinin (BA, KIN) in different concentrations and combinations. The efficient shoot multiplication from the shoot tip explants was induced in MS medium supplemented with 2.0 mg/l BA after 30 days of culture. The highest means of micro-shoots/explant was obtained in MS medium containing 2.0 mg/l BA and 90% of explants proliferated with 9.83 ± 0.32 shoots for Sweet Gold (Table 2). Several reports (Shalaby *et al.*, 2008; Sarowar *et al.*, 2003; Kumar *et al.*, 2003; Hoque *et al.*, 1998; Ahmed and Anis, 2005; Rahman *et al.*, 2012) described the effect of BA as a potent cytokinin for shoot proliferation in cucurbits, which are in agreement with the present findings.

Well formed shoots excised from the multiple shoot clumps were cultured on MS medium fortified with two different auxins (NAA and IBA) singly at different concentrations for *in vitro* root induction (Table 2). Among the two types of auxins used, NAA was found to be the best for root induction. 100% micro-cuttings were found rooted when the medium contained 0.5 mg/l NAA and 7.45±0.48 roots/shoot, 9.65±0.15 cm average length of roots were observed after 30 days of culture. The findings are in agreement with those observed in *Caphaelis ipecacuanha* (Jha and Jha, 1989), *Plantago ovata* (Wakhlu and Barna, 1989), *Rehum emodi* (Lal and Ahuja, 1989), *Ruscus hypophyllum* (Jha and Sen, 1985) and some other plant species. The lowest number of roots and the shortest roots were produced on medium containing 2.0 mg/l IBA and 2.0 mg/l NAA respectively.

These findings indicated the superiority of NAA over IBA in adventitious root induction of *in vitro* shoots. Similar results were obtained in

Watermelon (Shalaby *et al.*, 2008; Sultana and Bari, 2003) and in cucumber (Ahmed and Anis, 2005). Plantlets of both the cultivars having well developed root systems were successfully transferred to small pots containing garden soil and vermicompost (2:1) and incubated under laboratory condition for seven days. After incubation period, *in vitro* regenerated plantlets were placed to outdoor condition for almost 14 days and finally transferred to experimental field condition. Only about 80% of the micropropagated plantlets could tolerate transplantation shock and evaluated well under *ex vitro* environment or field condition and showed promising growth and development.

Statistical analysis and subsequent *t*-Test indicates that the calculated *t*-value of both cultivars was significant at 5% probability level. So the cultivars were significantly different from each other. In the present investigation, morphological characters of tissue culture derived plants were observed at different growing stages. In respect to different growth parameters cultivar Sweet Gold was found to produce higher plant length, number of fruits/plant and average weight/fruit.

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.

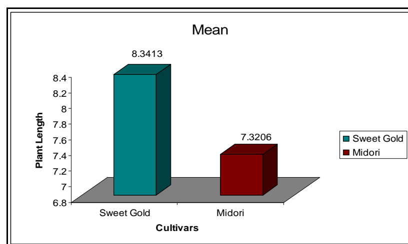
Treatments (mg/l)	Cultivars	Time taken to germination (in days)	% of seed germination (Mean±SE)	Length of germinated seedlings (in cm) (Mean±SE)
MS0	Sweet Gold	6	64.15 ± 0.23	4.70 ± 0.52
	Medori	7	62.14 ± 0.54	4.60 ± 0.23
GA_3				
0.2	Sweet Gold	4	90.36 ± 0.12	5.95 ± 0.47
	Medori	5	85.34 ± 0.51	5.11 ± 0.46
0.5	Sweet Gold	7	70.80 ± 0.46	4.72 ± 0.24
	Medori	7	65.28 ± 0.33	4.95 ± 0.56
1.0	Sweet Gold	8	50.31 ± 0.36	3.16 ± 0.33
	Medori	9	46.26 ± 0.38	4.70 ± 0.37

Table (2): Effect of different concentrations of cytokinin (BA and KIN) singly or in combination with NAA and IBA in MS medium on shoot multiplication from *in vitro* grown shoot tip explants of two muskmelon cultivars. Each treatment consisted of 10 explants. Data were recorded after 30 days of culture.

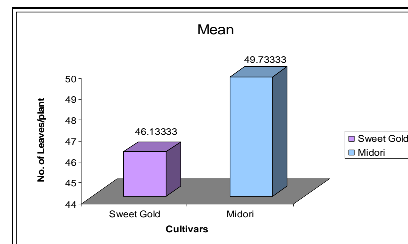
Treatments (mg/l)	Cultivars	% of explants induced micro-shoot proliferation	No. of micro-shoots/explant (Mean± SE)	Length of the longest shoot (in cm) (Mean± SE)
BA	Sweet Gold	30	3.58±0.11	6.25±0.35
0.5	Medori	40	6.15±0.24	7.15±0.22
2.0	Sweet Gold	90	9.83±0.32	10.10±0.47
	Medori	80	6.45±0.27	8.65±0.47
KIN	Sweet Gold	20	4.62±0.76	6.15±0.24
0.5	Medori	20	6.45±0.26	6.61±0.11
2.0	Sweet Gold	60	7.54±0.27	7.86±0.22
	Medori	60	4.35±0.25	6.15±0.22
BA+NAA	Sweet Gold	50	6.23±0.62	6.02±0.19
1.0+0.5	Medori	40	5.65±0.31	6.10±0.02
2.0+0.5	Sweet Gold	80	8.85±0.39	7.80±0.18
	Medori	60	6.94±0.21	6.99±0.13
BA+IBA	Sweet Gold	30	6.31±0.53	6.64±0.36
1.0+0.5	Medori	50	6.66±0.71	5.60±0.22
2.0+0.5	Sweet Gold	70	8.70±0.50	7.65±0.27
	Medori	70	7.08±0.24	6.65±0.20
KIN+NAA	Sweet Gold	20	6.21±0.26	5.63±0.47
1.0+0.5	Medori	20	6.35±0.33	6.20±0.54
2.0+0.5	Sweet Gold	70	7.80±0.39	7.10±0.18
	Medori	60	7.80±0.27	5.80±0.52
KIN+IBA	Sweet Gold	50	6.56±0.56	6.10±0.27
1.0+0.5	Medori	50	6.85±0.27	4.10±0.67
2.0+0.5	Sweet Gold	80	8.75±0.37	7.9±0.19
	Medori	80	7.08±0.38	7.65±0.37

Table (3): Effect of MS0 and varied concentrations of auxin (IBA and NAA) on adventitious root formation from the *in vitro* grown micro-cuttings cultured on MS medium. There were 10 micro-cuttings in each treatment and data were collected after 30 days of culture.

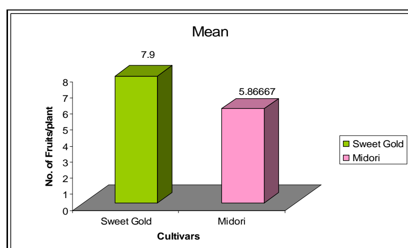
Cultivars	Treatments	% of micro-cutting rooted	Number of roots/microcutting (Mean±SE)	Length of the longest root (in cm) (Mean±SE)
Sweet Gold	MS_0	50	6.10±0.13	7.05±0.35
	IBA			
	0.2	70	3.82±0.16	6.03±0.17
	0.5	80	5.14±0.57	8.84±0.18
	1.0	70	4.37±0.25	7.34±0.45
	NAA			
	0.2	80	5.64±0.41	7.43±0.03
	0.5	100	7.45±0.48	9.65±0.15
	1.0	70	4.23±0.18	8.32±0.23
Midori	MS_0	50	5.26±0.12	6.66±0.22
	IBA			
	0.2	50	3.31±0.11	5.34±0.33
	0.5	60	4.16±0.29	6.11±0.21
	1.0	70	4.35±0.29	7.73±0.54
	NAA			
	0.2	70	4.65±0.55	6.10±0.15
	0.5	90	5.69±0.51	8.24±0.38
	1.0	70	3.50±0.25	7.87±0.12



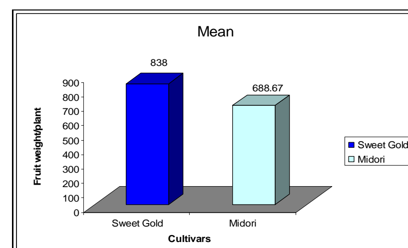
(a) Comparison of plant length (feet) at 60th day after plantation of tissue culture derived plants in the field between two muskmelon cultivars.



(b) Comparison of number of leaves/plant at 60th day after plantation of tissue culture derived plants in the field between two muskmelon cultivars.



(c) Comparison of number of fruits/plant at 70th day after plantation of tissue culture derived plants in the field between two muskmelon cultivars.



(d) Comparison of average weight/fruit (g) at 90th day after plantation of tissue culture derived plants in the field between two muskmelon cultivars.

Figure 1 (a-d). Field performances of micropropagated plantlets.

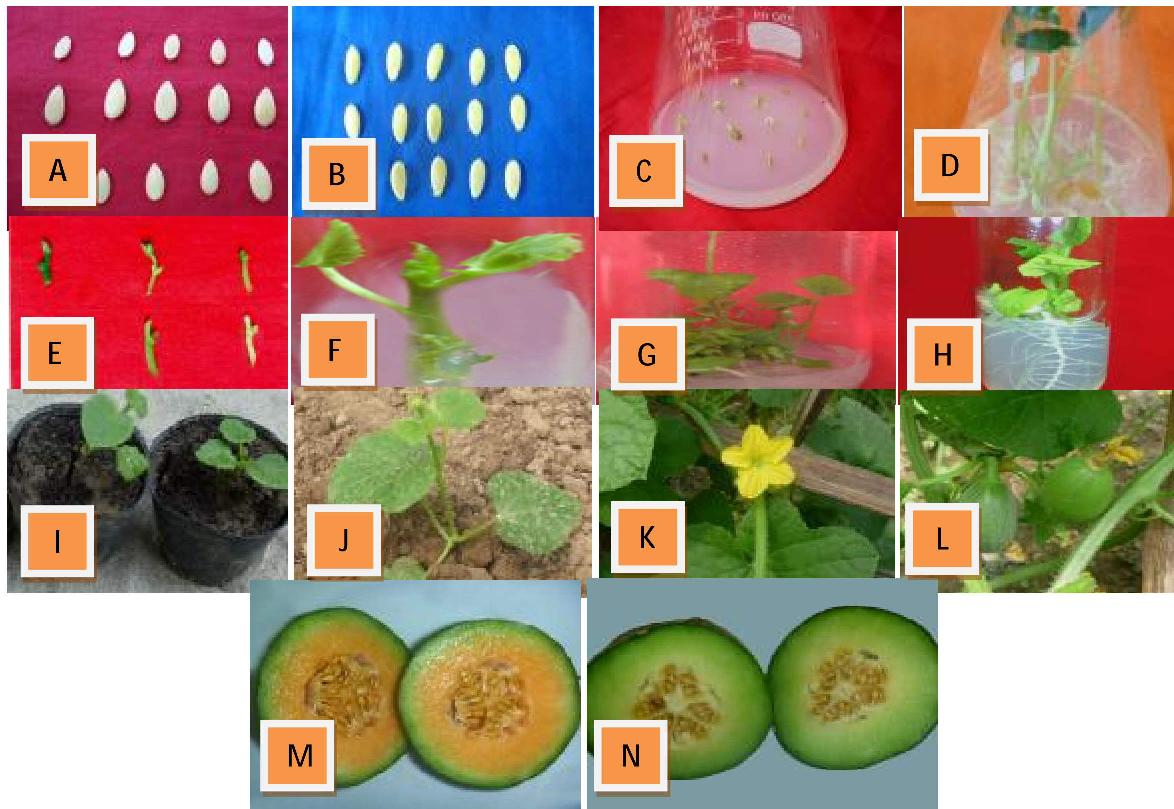


Figure (2): Hybrid seeds of Muskmelon, CV. Sweet Gold (A) and Midori (B). The germination of seeds on MS medium containing 0.2 mg/l GA_3 after 7 days of inoculation (C). *In vitro* grown germinated seedlings after 21 days of seed culture (D). Shoot tip excised from *in vitro* grown seedling (E). Primary establishedment of shoot tip explants in MS_0 medium after 7 days of culture (F). Multiple shoot proliferation from shoot tip explants on MS + 2.0 mg/l BA (G). Formation and development of adventitious root from *in vitro* micro-cuttings on 0.5 mg/l NAA supplemented MS medium (H). Rooted Plantlets transplantation on thump pot under *ex vitro* condition (I). Plantlets transferred in field after 20 days intervals (J). Flowering stage (K) and immature fruits (L) of muskmelon plants in a field. The inner part of a sliced ripen fruit of Muskmelon, cultivar Sweet Gold (M) and Midori (N).

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