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Full Length Research Paper

Minimizing the hyperhydricity associated with *in vitro* growth and development of watermelon by modifying the culture conditions

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Hyperhydricity or glassiness is considered as a frequent problem associated with the *in vitro* growth and development of watermelon (*Citrulus lanatus*, cv. Giza 1). Explants were cultured on MS (Murashige and Skoog, 1962) medium containing 6-bensyladenine (BA), kinetin (Kin) or thidiazuron (N-phenyl N 1,2,4-chlorophenoxyacetic acid) (TDZ) each applied at 0, 0.25, 0.5, 1, 2 and 4 mg L^{-1}). The highest number of regenerating shoots per explants MS medium containing 0.5 mg/l TDZ proved to be the most efficient in the induction of adventitious organogenesis, while BA applied at 2 mg L^{-1} was the most efficient in axillary shoot proliferation. Increase in BA concentration (above 2 mg L^{-1}) gave multiple shoots and led to more pronounced hyperhydricity, while the use of 1 mg L^{-1} facilitated single-shoot growth with relatively lower incidence of hyperhydricity. Substitution of agar (10 g L^{-1}) with gelrite (4 g L^{-1}) in the medium as well as improving vessel aeration reduced occurrence of hyperhydricity. Provision for aeration was relatively superior to other approaches, but it led to faster dehydration of medium and limited growth of cultures. Rooted shoots produced on half strength MS medium with aeration, and 0.5 mg L^{-1} indole 3-butyric acid (IBA) were transferred to pots and acclimatized in green-house with a survival rate of 80%.

Key words: Aeration, gelling agents, hyperhydricity, *in vitro*, rooting, plant growth regulators, ventilation, watermelon.

INTRODUCTION

Watermelon is an important cucurbitaceous crop. It is grown worldwide and ranked sixth in the world production of fruit crops (Shalaby et al., 2008). Watermelon is one of the most important vegetable crops cultivated in many parts of the tropic and sub-tropic regions. The cultivated area with watermelon is ranked the second behind tomato and world production ranks third in metric tons among vegetables (Choi et al., 1994). *Citrullus lanatus* Thunb is an annual vegetable crop with sweet taste and it is often consumed as a cool dessert. The species originated in tropical Africa (Anghel and Rosu, 1985). Excessive seed number in watermelon fruit is fast becoming unacceptable in international markets. Plant regeneration of watermelon via organogenesis in cotyledonary tissue cultures (Anghel and Rosu, 1985; Compton and Gray, 1993a, b; Dong and Jia, 1991; Srivastava et al., 1989), via shoot-tip culture (Compton and Gray, 1993a, b) has been reported. Murashige and Skoog (MS) medium containing 1, 2.25 and 5.5 mg L^{-1} 6-bensyladenine (BA) showed the highest percentage of explants with shoots (Shalaby et al., 2008). Explants on medium supplemented with 1.0 mg L^{-1} of BA produced the most axillary shoots with the watermelon genotypes Beyazkış and Karakış, while the genotype Surme produced the most axillary shoots with 0.5 mg L^{-1} of BA (Oumus et al., 2011). A physiological condition known as vitrification (synonymous with glassiness or hyperhyrdic transformation) is a serious problem associated with plant

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micropropagation (Narayanaswamy, 1994).

Liquid as well as semi-solid media used in tissue cultures can induce the physiological disorder known as hyperhydricity (vitrification). Vitrified plants have water-soaked translucent stems and leaves that are elongated and concave (Debergh, 1983). The vitrification phenomenon could be minimized or avoided by improved aeration and use of different gelling agents (Mackay and Kitto, 1987). Hyperhydrated plants are enlarged, thick, translucent, and brittle. This physiological malformation is associated with chlorophyll deficiency, poor lignifications, and excessive hydration of tissues which results in poor regeneration of normal and mature plants (Sreedhar et al., 2009). In closed culture vessels, hyperhydricity affected as much as 84% of the newly formed shoots on media gelled with gelrite (Ivanova and Van Staden, 2010). Despite the extensive studies on the *in vitro* growth and development of watermelon, there are no efforts to minimize or avoid the associated hyperhydricity. In this work, we tested the hypothesis that vessel aeration as well as plant growth regulators and gelling agent (type and concentration) might nullify or even minimize the adverse harmful effects originated from the hyperhydricity of *in vitro* grown watermelon. Therefore, this investigation aimed mainly to minimize or avoid hyperhydricity, and partially to increase survival, multiplication and adaptation of *in vitro* propagated watermelon.

MATERIALS AND METHODS

Plant material and surface sterilization of the seeds

Watermelon (*Citrulus lanatus* cv. Giza 1) mature seeds were used as explant source for establishment of aseptic culture *in vitro* in this study. They were obtained from Genetic Engineering and Biotechnology Research Institute (GEBRI), Menofyia Univ., Sadat City. Seeds were soaked under aseptic conditions in 10, 20, 30 or 40% ethanol for 1 min and then soaked in 5, 10, 15 or 20% Clorox (commercial solution with 5.25 % sodium hypochlorite) for 10 min. In all cases, one drop of tween 20 (polyoxyethylene sorbitan monolaurate) was added as a wetting agent. After sterilization, the explants were rinsed several times in sterilized water to remove all traces of chlorine. Sterilized seeds were placed in half-strength MS medium (Murashige and Skoog, 1962). The pH was adjusted to 5.7 prior to the addition of agar at 5 g/l. The medium was distributed in aliquots of 50 ml into the culture jars of 325 ml. The jars were capped with polypropylene closure, and then autoclaved at 121°C for 15 min.

Effect of cytokinin type and concentration on axillary shoot proliferation

This experiment was carried out to propagate watermelon via axillary shoot-tip cultures. Shoot-tips (2 cm long) were harvested from germinated seeds after 7 to 8 days. Explants were cultured in glass jars containing 25 ml of MS medium. The culture medium was treated with BA, kinetin (KN) or thidiazuron (N-phenyl N 1,2,4 - chlorophenoxyacetic acid) (TDZ); each applied at 0.0, 0.25, 0.5, 1, 2 or 4mg L^{-1}. Two explants were cultured in each jar, and each treatment included ten jars (replicates). The following parameters were determined after one month of culture: shoot number, shoot length and leaf number.

Effect of auxin type and concentration

Established shoot tip explants were transferred and recultured on MS medium containing 2 mg L^{-1} BA, 30 gL^{-1} sucrose and 7 gL^{-1} agar. The culture medium was supplemented with different concentrations of α-naphthaleneacetic acid (NAA) or indole-3-acetic acid (IAA) at 0.1, 0.2 or 0.5 mg L^{-1} for proliferation. The culture medium of each treatment was distributed in culture jars and incubated as mentioned above. Each treatment contained ten replicates and each replicate included one cluster of three shoots. The following parameters were determined after one month of culture: shoot number, shoot length and leaf number.

Effect of cytokinin type and concentration on adventitious shoot regeneration from cotyledon explants

Cotyledons were cut transversally into two halves, proximal and distal, and these were used as explants. Explants were cultured in 250 ml glass jars containing 25 ml of MS medium that contained 30 g L^{-1} sucrose and 8 g L^{-1} agar. The culture medium was supplemented with BA, KN or TDZ, each at 0.0, 0.25, 0.5, 1, 2 or 4 mg L^{-1}. After one month of culture, shoot number, shoot length and leaf number were determined.

Effect of aeration on shoot development and hyperhydricity

There were two treatments; one contained screw caps of jars with an opening and plugged with a cotton plug and the other had normal screw caps. Shoots harvested from healthy stock cultures were transferred to two types of vessels each containing 25 ml of MS medium supplemented with 2 mg L^{-1} BA, 30 g L^{-1} sucrose and 7g L^{-1} agar. Shoot number, shoot length, leaf number and shoot fresh weight were determined after 25 days of culture.

Effect of type and concentration of gelling agent on shoot development and hyperhydricity

Two types of gelling agent (gelrite and agar) were used. Gelrite was added as 2, 3 or 4 g L^{-1}, while agar was used as 6, 8 or 10 g L^{-1}. The culture medium of each treatment (MS medium) was supplemented with 2 mg L^{-1}BA and 30 g L^{-1} sucrose. The following parameters were determined after 25 days of incubation; shoot number, shoot length, leaf number, shoot fresh weight and the degree of hyperhydricity.

In vitro rooting and *ex vitro* adaptation

Effect of aeration, type and concentration of auxins on root formation

4 to 5 cm long excised cultured shoots were transferred and cultured onto MS medium that contained 30 g L^{-1} sucrose and 8 g L^{-1} agar. The medium was supplemented with 0.0, 0.25, 0.5, 0.75 or 1 mg L^{-1} NAA or IBA. Each treatment containing three explants was replicated ten times. All treatments were incubated for root development under both aerated and non-aerated conditions as mentioned above. After 25 days, root number and root length were determined.

Effect of aeration and MS salt-strength on root formation

Survived and established shoot explants were transferred and recultured on MS medium of different strengths (full, half and

Table 1. Germination and cleaned of watermelon seeds as affected by ethanol concentration and the further disinfection by clorox and ethanol.

Ethanol concentration (1 min)	Number of germinating seed			Cleaned		
	Clorox concentration (10 min)		Mean (B)	Clorox concentration (10 min)		Mean (B)
	5%	10%		5%	10%	
10%	10.00	9.80	9.90	5.00	6.00	5.50
20%	8.80	7.80	8.30	6.00	8.80	7.40
30%	7.8	7.20	7.50	7.60	10.00	8.80
40%	5.80	5.00	5.40	7.40	10.00	8.70
Mean	8.10	7.45		6.50	8.70	
L.S.D at 5 % A		0.2327			0.2187	
B		0.3290			0.3093	
A x B		0.4653			NS	

quarter strengths). All rooting media were supplemented with 30 g L^{-1} sucrose, 0.5 mg L^{-1} IBA and 6 g L^{-1} agar. Each treatment included ten replicates (ten jars) and each replicate contained three shoot explants. All treatments were incubated for root development, under aerated and non-aerated conditions as mentioned above. After 25 days, root number, root length and rate of root formation were determined.

Effect of *in vitro* aeration and strength of MS medium on *ex vitro* adaptation

Rooted shoots that originated from different rooting treatments (full-, half- and quarter-strength MS medium in combination with aerated and non-aerated conditions) were transferred to the *ex vitro* planting medium (mixture of peat moss and perlite, 1:1 v/v). The plants were cultured in pots (6 cm diameter) and light-exposed at 4,000 lux. After three months from transplanting in greenhouse, the rate of survival was determined.

Statistical analysis and culture conditions

In all the experiments, cultured jars were incubated for four weeks at 25°C, 16 h day/8 h night photoperiod, and light intensity of 1,500 lux was provided by white fluorescent tubes. Each treatment of proliferation included ten replicates (ten jars) and each replicate (jar) contained one cluster of three shoots. All experiments were done in controlled conditions. Data were averaged and statistically analyzed by using one- and two-way analysis of variance. Data presented in the form of percentage were subjected to arcsine transformation prior to statistical analysis. The Fisher's least significant difference test (LSD test at 5%) was used for means separation (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Surface sterilization of the seeds

Preliminary experiment indicated that ethanol concentration as well as clorox concentration influenced both watermelon germination and cleaned (Table 1). Clorox was a more efficient microbicidal agent than ethanol. Soaking seeds in ethanol (10%) for 1 min and clorox (5%) for 10 min resulted in microbial-free explants. 30% ethanol (for 1 min) and 10 % clorox (for 10 min) resulted in the highest cleaned. The explants remained green and showed healthy growth and proliferation of axillary shoots. These results disagree with some others (Nasr et al., 2004) but mostly agree with those of Vasudevan et al. (2007). This could be attributed to the microbicidal action of the disinfectants on the cytoplasmic membrane and/or the innermost parts of the microbial cell. Russel et al. (1982) stated that clorox is a powerful antimicrobial agent that has bactericidal, fungicidal and sporicidal properties, while ethanol acts only as bactericidal and believed to potentiate the sporicidal activity of hypochlorites. However, it was found that sterilization of watermelon seeds required removing seed coat, surface sterilization, and finally washing all traces of chlorine (Shalaby et al., 2008). Results obtained from this experiment reveal that the best sterilization method includes immersing seeds in ethanol (10%) for 1 min and clorox (5%) for 10 min.

Effect of cytokinin type and concentration on axillary shoot formation

The culture media containing 1 or 2 mg L^{-1}BA produced the highest shoot number per explant (10.0 and 11.2, respectively), whereas 1, 2 or 4 mg L-1 TDZ and the control resulted in the lowest shoot number (Table 2). Increase in BA concentration significantly increased shoot number per explant, but the production mainly included small undefined shoots and bulbous structures. BA resulted in more shoot production (Figure 1) compared to Kin and TDZ. Significantly highest leaf number per explant was produced in the case of explants cultured on MS medium supplemented with 2 mg L^{-1} BA. All TDZ concentrations and control gave the lowest leaf number per explant. 0.5 mg L^{-1} KN and the control resulted in significantly increasing shoot length compared to cytokinin concentrations. The effect of cytokinin level on the *in vitro* shoot growth and development has been

Table 2. Effect of cytokinin type (BA, Kin, and TDZ) and concentration on *in vitro* shoot growth and development of watermelon.

Cytokinin	Concentration (mg L^{-1})	Shoot number	Leave number	Shoot length (cm)
	0.00	3.00	7.20	5.52
	0.25	5.60	13.00	1.86
	0.50	6.40	18.60	2.38
BA	1.00	10.00	26.60	1.56
	2.00	11.20	31.40	1.52
	4.00	8.20	20.00	1.38
	0.25	9.60	18.20	4.48
	0.50	7.60	15.00	4.80
Kin	1.00	4.60	16.40	4.70
	2.00	5.00	15.60	3.08
	4.00	5.20	16.40	4.40
	0.25	9.20	9.20	0.50
	0.50	4.20	4.20	0.52
TDZ	1.00	2.80	2.80	0.50
	2.00	1.40	1.40	0.50
	4.00	1.00	1.20	0.28
LSD at Level 5%		2.19	5.82	0.62

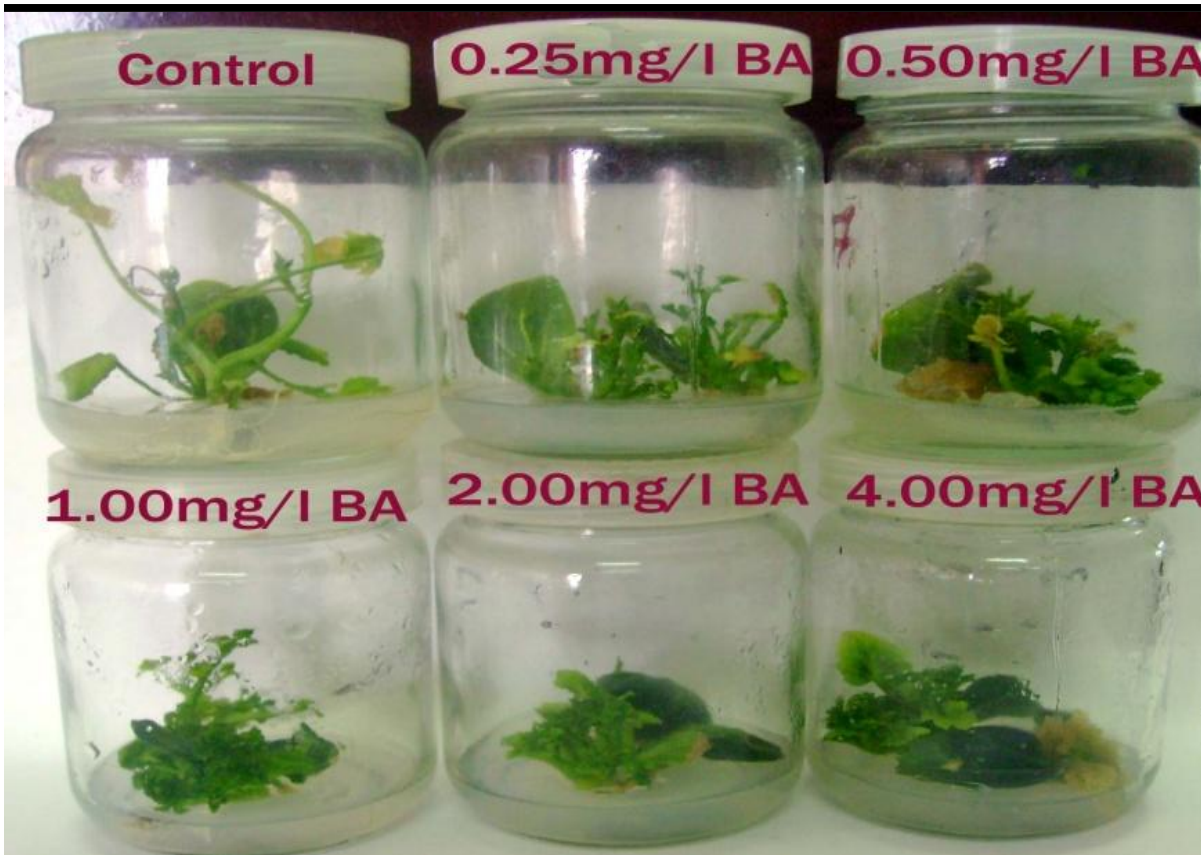

Figure 1. Effect of different concentration of BA on *in vitro* growth and development of watermelon shoot.

Table 3. Effect of auxins type (NAA, IAA) and concentration on the *in vitro* shoot growth and development of watermelon **.**

Auxin	Concentration (mg L^{-1})	Shoot number	Leaf number	Shoot length (cm)
NAA	0.0	24.40	90.00	3.60
	0.1	19.80	73.00	4.64
	0.2	17.40	59.60	4.44
	0.5	10.60	43.60	3.90
IAA	0.1	6.40	30.20	3.40
	0.2	8.80	36.00	3.78
	0.5	9.00	50.20	3.16
LSD at level 5%		3.46	12.13	0.59

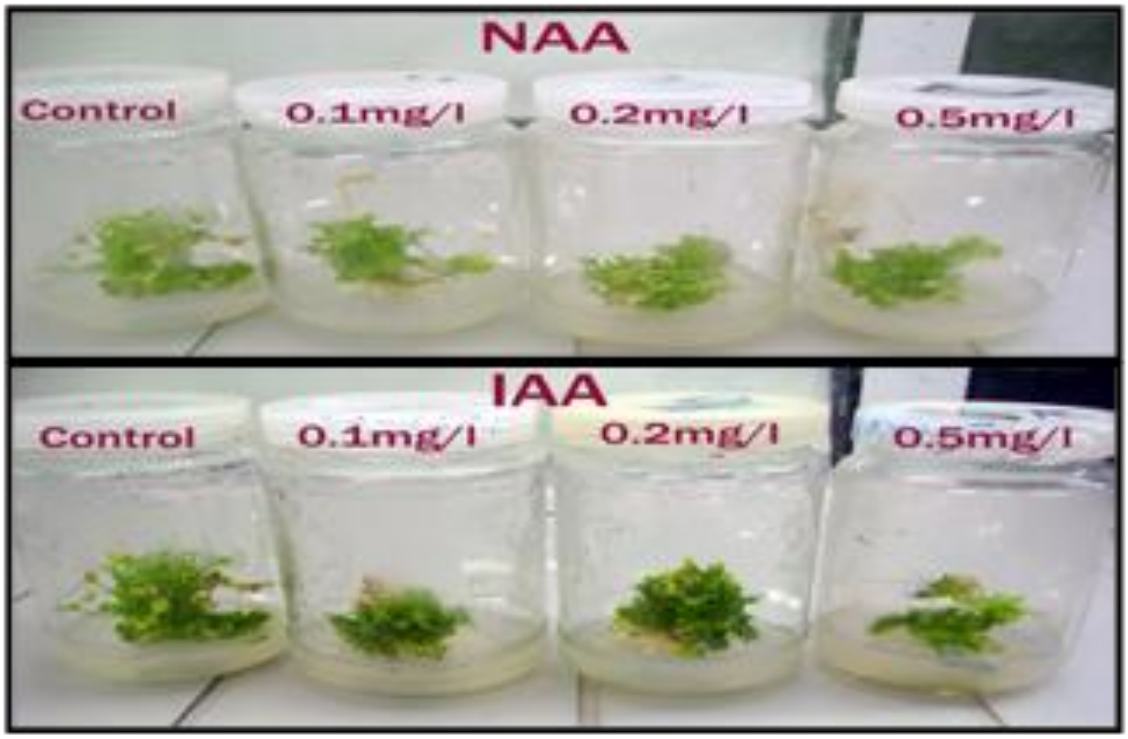

Figure 2. Effect of auxin type (NAA and IAA) and concentration on the *in vitro* growth and development of watermelon shoot.

already reported (Youssef, 1994; Arafa et al., 1999; Ebrahim, 2004). This increase could be ascribed to a stimulatory effect on cell division and enlargement. Explants on medium supplemented with 1.0 mg L^{-1} of BA produced the most axillary shoots with the watermelon genotypes Beyazkış and Karakış, while the genotype Surme produced the most axillary shoots with 0.5 mg L^{-1} of BA (Oumus et al., 2011).

Effect of auxin type and concentration

Data showed significant effects of NAA and IAA on the *in vitro* shoot growth and development of watermelon (Table 3, Figure 2). The highest shoot and leaf numbers per explant were recorded in the culture medium without auxins, whereas the medium that contained 0.1 mg L^{-1} NAA gave the longest shoot). Also, the data revealed that NAA at 0.2 mg L^{-1} was more effective than IAA applied at the same concentration. Similar results were reported by Song et al. (1988) in watermelon. Nasr et al. (2004) reported that MS medium supplemented with 1.0 mg L^{-1}BA and 0.5 mg L^{-1} IBA was the best treatment for both shoot and root proliferation in watermelon. Trigiano and Gray, (1996) mentioned that auxins play an important role in many developmental processes, including cell

Table 4. Effect of cytokinin type and concentration on adventitious shoot formation from cotyledon explants of watermelon cultured *in vitro.*

Cytokinin type	Concentration (mg L^{-1})	Shoot number /explant	Leaf number /explant	Shoot length (cm)
	0.00	0.00	0.00	0.00
	0.25	0.00	0.00	0.00
	0.50	0.00	0.00	0.00
BA	1.00	2.00	3.80	1.00
	2.00	0.00	0.00	0.00
	4.00	0.00	0.00	0.00
	0.25	3.00	2.10	0.50
	0.50	4.20	6.00	1.20
TDZ	1.00	2.80	2.80	0.50
	2.00	2.00	2.00	0.50
	4.00	0.00	0.00	0.00
	0.25	0.00	0.00	0.00
	0.50	0.00	0.00	0.00
KN	1.00	0.00	0.00	0.00
	2.00	0.00	0.00	0.00
	4.00	0.00	0.00	0.00
LSD at 5%		1.39	1.92	0.07

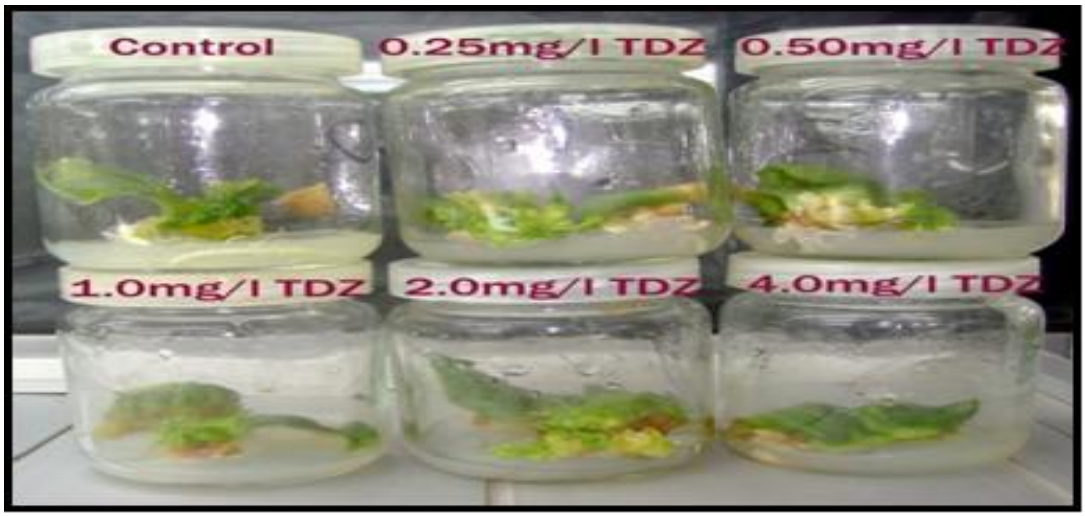

Figure 3. Effect of different concentrations of TDZ on adventitious shoot formation from cotyledon explants of watermelon cultured *in vitro*.

elongation and swelling of tissue, apical dominance, adventitious root formation and somatic embryogenesis.

Effect of cytokinin type and concentration on adventitious shoot regeneration from cotyledon explants

Highest shoot and leaf numbers per explants and shoot length were obtained in the culture medium supplemented with 0.5 mg L^{-1} TDZ. TDZ was found to be best for induction of adventitious regeneration, followed by BA. Adventitious regeneration was not achieved in any of media containing KN. As regards BA, it induced development of adventitious shoots only at 1.0 mg L^{-1}, while TDZ was effective within broad range of concentrations (0.25 to 2.0 mg L^{-1}) (Table 4).

Application of BA led to thicker, stunted and vitrified shoots, whereas shoots that originated from TDZ-supplemented media were highly elongated and easily handled (Figure 3). TDZ has been reported to stimulate organogenesis in several crops (Zhang et al., 2001; Fratini and Ruiz, 2002), as well as in watermelon (Compton and

Table 5. Effect of aeration on shoot growth and development, and hyperhydricity criteria of watermelon cultured *in vitro.*

Treatment	Shoot number	Leaf number	Shoot length (cm)	Fresh weight/ shoot	General appearance	Reduction in medium weight
With aeration	8.60	50.40	8.20	5.43	Healthy	High
Without aeration	5.80	37.00	4.06	8.01	Hyperhydric (vitrified)	Partial (low)
LSD at level 5%	2.04	9.56	0.46	1.20		

Figure 4. Effect of aeration on development and hyperhydricity of watermelon cultured *in vitro*.

Gray, 1993a, b).

Effect of aeration on *in vitro* shoot development and hyperhydricity

The effect of aeration on *in vitro* development and hyperhydricity of watermelon is presented in Table 5. The fresh weight was significantly low in treatment with aeration. This indicates that healthier culture has less water retention. Compared to non-aerated conditions, aeration provided the higher shoots number, leaf number and shoot length of cultures as shown in Figure 4. The reduction in the medium weight, on account of evaporative loss, was negligible in vessels with normal caps, while the reverse was true in the vessels with aeration. Such reduction seemed to depend on the cap opening area as well as the type of closure material. These results agree with Murphy et al. (1998), who found that improving aeration significantly reduced hyperhydricity and water condensation, and led to faster medium desiccation, affecting growth and necessitating frequent transfers. Thomas et al. (2000) found that improving vessel aeration using caps with an opening covered with cotton bunk or cellulose nitrate filter individually helped in reducing hyperhydricity to some extent. Lai et al. (2005) showed that hermitically sealed culture vessels resulted in high occurrence of hyperhydricity. It was also indicated that aeration had influenced the growth of *in vitro* plantlets and microstructures of *Artemisia annua* leaves (Yann et al., 2010).

Effect of type and concentration of gelling agent on shoot development and hyperhydricity

Agar and gelrite are natural polysaccharides with high capability of gelation. Their gels combine with water and absorb other compounds (Ebrahim and Ibrahim, 2000). Agar is the most frequently used for solidification of plant culture media because of its desirable characteristics such as clarity, stability, resistance to metabolism during use, and its being inert (Ibrahim, 1994). Gelrite, the alternative gelling agent, is increasingly used because it forms clear gels and contains no contaminants (Pierik, 1987). The present data demonstrate that proliferation rate and growth were influenced by both type and the

Table 6. Effect of gelling agent type and level on shoot growth and development, and hyperhydricity criteria of watermelon cultured *in vitro*.

elling agent type	Concentration (g L^{-1})	Shoot number	Leaf number	Shoot length (cm)	Shoot Fresh weight (g)	Hyperhydricity degree
Gelrite	2.00	12.40	35.60	6.50	12.10	+++
	3.00	7.60	18.80	3.40	3.98	++
	4.00	3.80	8.40	3.04	3.06	+
Agar	6.00	9.40	45.20	5.36	12.78	+++
	8.00	8.80	35.80	5.00	5.68	+
	10.00	5.20	13.60	4.600	4.35	-
LSD at level 5%		1.39	6.06	0.72	1.60	

- = No, hyperhydricity(vitrification); + = low hyperhydricity (vitrification); ++ = moderate hyperhydricity (vitrification); +++ = Sever hyperhydricity (vitrification).

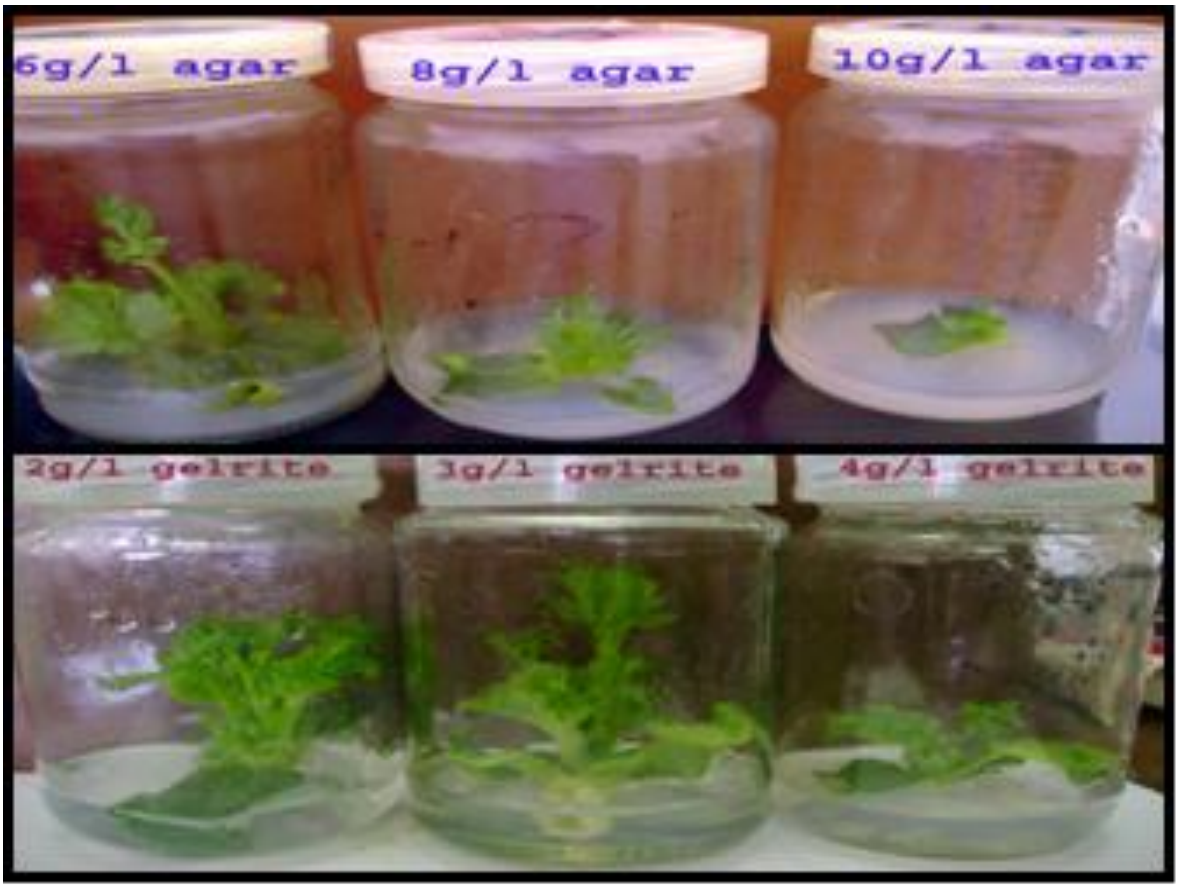

Figure 5. Effect of type and concentration of gelling agent on development and hyperhydricity of watermelon cultured *in vitro.*

concentration of gelling agent. Gelrite gave better values than agar (Table 6). Comparing gelrite and agar with MS medium containing 2 mg L^{-1} BA, 2 g L^{-1} gelrite appeared superior in growth response (12.4), but led to high hyperhydricity, while the use of agar at 10 g/l led to the control of hyperhydricity, but led to reduction of shoot number (5.2). It was clear that using agar at 6 g L^{-1} or gelrite at 2 g L^{-1} gave the highest shoot weight (12.78 and 12.1 g, respectively) compared with other concentrations of both agar and gelrite (Figure 5). Generally, it could be concluded that using gelrite at 2 g L^{-1} or agar at 6 g L^{-1} gave the highest number of shoots and weight. These results were confirmed by Turner and Singha (1988), who reported that the highest rate of shoot proliferation

Table 7. Effect of aeration and auxin (IBA & NAA) levels on root growth criteria of watermelon cultured *in vitro.*

Auxin type	Concentration (mg L^{-1}) (B)	Aeration (A)					
		Root number			Root length (cm)		
		Without	With	Mean (B)	Without	With	Mean(B)
IBA	0.00	1.20	1.40	1.30	6.40	7.86	7.13
	0.25	1.40	2.20	1.80	2.00	3.50	2.80
	0.50	5.60	6.60	6.10	7.10	9.30	5.00
	0.75	4.00	5.20	4.60	4.14	4.94	2.40
	1.00	3.60	3.80	3.70	6.00	4.60	0.00
	1.25	2.40	3.00	2.70	4.06	3.80	0.00
NAA	0.25	7.00	8.40	7.70	1.00	1.22	0.00
	0.50	5.80	7.00	6.40	0.98	1.16	0.00
	0.75	3.80	3.80	3.80	0.57	0.50	0.00
	1.00	3.00	3.60	3.30	0.58	0.50	0.00
	1.25	2.00	2.60	2.30	2.06	0.47	0.00
Mean (A)		3.61	4.32		3.17	3.44	
LSD at level 5% A			0.24			0.30	
B			0.57			0.72	
Ax B			0.81			1.02	

occurred at 0.2% gelrite higher than that obtained at 0.6% phyta agar. Similar results were reported by Zimmerman and Robacker (1988) in cotton culture and Klimaszeqska (1989) in immature zygotic embryos of hybrid Irach. This observation could be attributed to the difference between both gelling agents in most characteristics mentioned above. Moreover, the completely solidification medium was obtained at different conditions for different gelling agents (5 g L^{-1} and pH 5.7 for agar, 1.5 g L^{-1} and pH 5.2 for Gelrite). Also, both agents were only semi-solidified at the lower concentration (data not shown). These differences not only affect the medium pH but also the studied parameters. Among the solidified media, lowering the concentration of agar below 8 g L^{-1} or raising the concentration of gelrite above 3 g L^{-1} did not significantly affect most studied parameters especially shoot weight and length of shoots (Table 6). Likewise, the *in vitro* growth and development were adversely affected when the concentration of gelling agent was too high (above the limiting values). In this regard, agar should be used at 0.6 to 0.8% (Debergh, 1983), while 0.2% gelrite was recommended (Pierik, 1987). It is also indicated that adding gelling agent decreased vitrification and insured obtaining healthy vigorous plantlets. In the liquid medium, vitrification was clearly shown and led to pronounced elongation followed by translucence (increase of FW, DW ratio) and eventually necrosis. Furthermore, the non-vitrified plantlets were dark green (higher chlorophyll content) and did not branch, while the vitrified ones were light green (low chlorophyll content). Cowpea cultivars showed maximum mean number of shoots per explant in gelrite compared to agar gelled medium (Aasim et al., 2009).

In vitro rooting and *ex vitro* adaptation of plants

Effect of aeration, type and concentration of auxins on root formation

Aeration and auxin concentration as well as their interactions influenced both division and elongation of the roots of watermelon cultured *in vitro* (Table 7, Figure 6). Aeration increased root number significantly, while the root length seemed not to be affected. Rooting medium containing 0.5 mg L^{-1} IBA induced the highest number of root number (6.1), whereas the longest roots (7.13 cm) were observed in auxin-free MS medium. It was evident that root number and length were superior (8.4 and 9.3 cm, respectively) in comparison with non-aerated media of the same hormonal composition. These findings agree with those observed in *Caphaelis ipecacuanha* and *Plantago ovata* (Nasr et al., 2004). The best rooting medium for watermelon micropropagation was half-MS + 0.3 mg L^{-1} IBA (Niu-Shanshan et al., 2006). Shalaby et al. (2008) reported successful rooting of elongated shoots on MS medium containing 1.2 mg L^{-1} NAA. Adventitious shoots of *Colocynthis citrullus* elongated on medium containing 0.1 mg L^{-1} BA were successfully rooted on hormone-free MS medium (Ntui et al., 2009). Rooting rate was 100% when shoots from the 2nd subculture were cultured on medium with 1 mg L^{-1} IBA (Khatun et al., 2010).

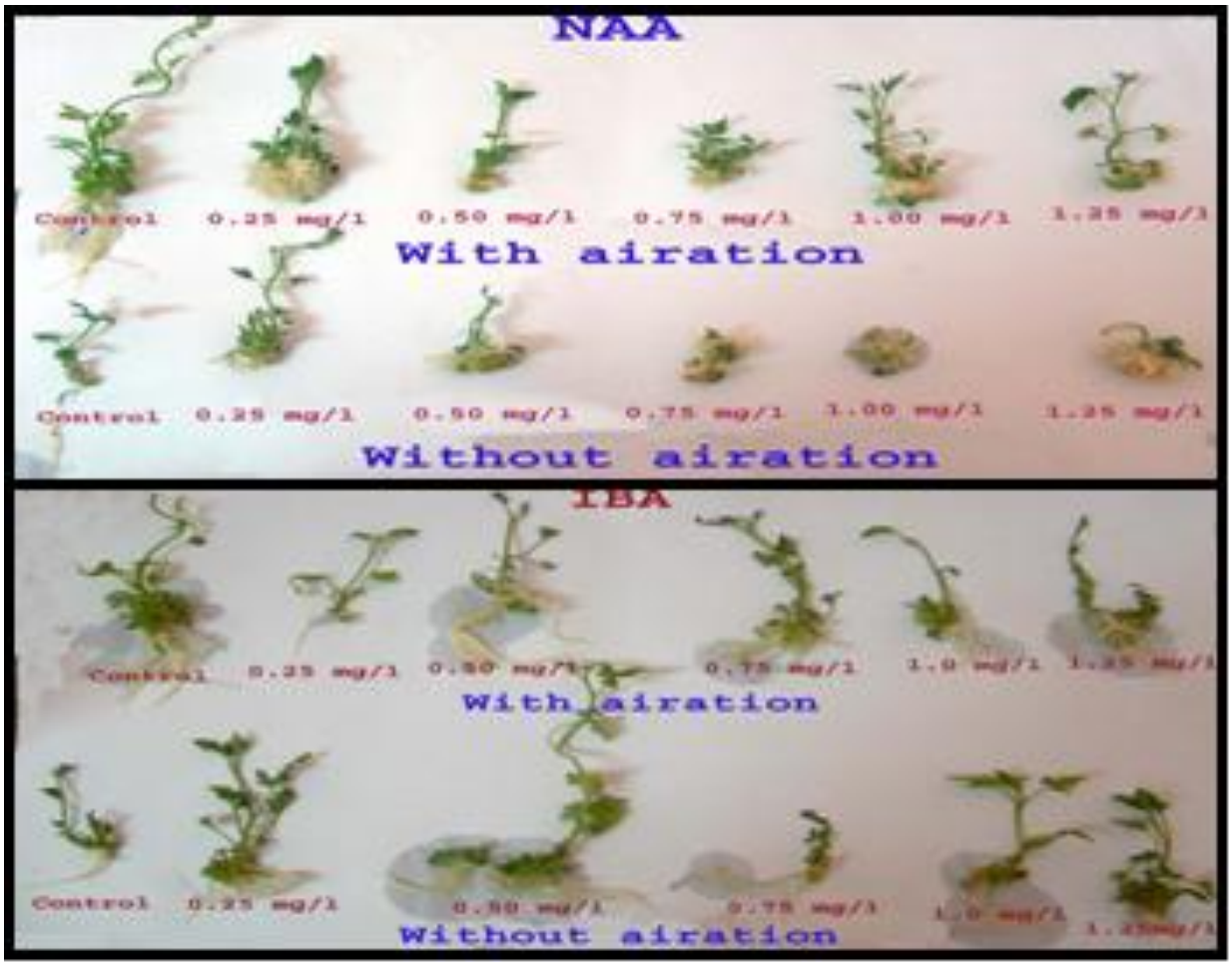

Figure 6. Effect of aeration and concentration of auxins (NAA, IBA) on number and length of roots in watermelon cultured *in vitro.*

Table 8. Effect of aeration and MS salt-strength on root formation of watermelon cultured *in vitro*.

MS salt-strength	Aeration (A)								
	Root number			Root length (cm)			Root formation (%)		
	Without	With	Mean (B)	Without	With	Mean (B)	Without	With	Mean (B)
One quarter	1.80	3.20	2.50	5.20	5.78	5.49	95	100	97.5
One half	7.20	7.40	7.30	7.20	8.50	7.85	100	100	100
Full strength	5.60	6.40	6.00	6.60	6.86	6.73	100	100	100
Mean (A)	4.86	5.66		6.33	7.04		98.33	100	
L.S.D at 5%									
A		0.37		0.59		0.72			
B		0.45			1.02				
Ax B		0.64							

Effect of aeration and MS salt-strength on root formation

Due to incidence of vitrification during explant subculturing especially in the liquid multiplication medium, this experiment was performed on three rooting media of different strengths: quarter-, half- and full-strength MS medium (no BA, pH 5.7, 6 g L^{-1} agar). Most of the employed media

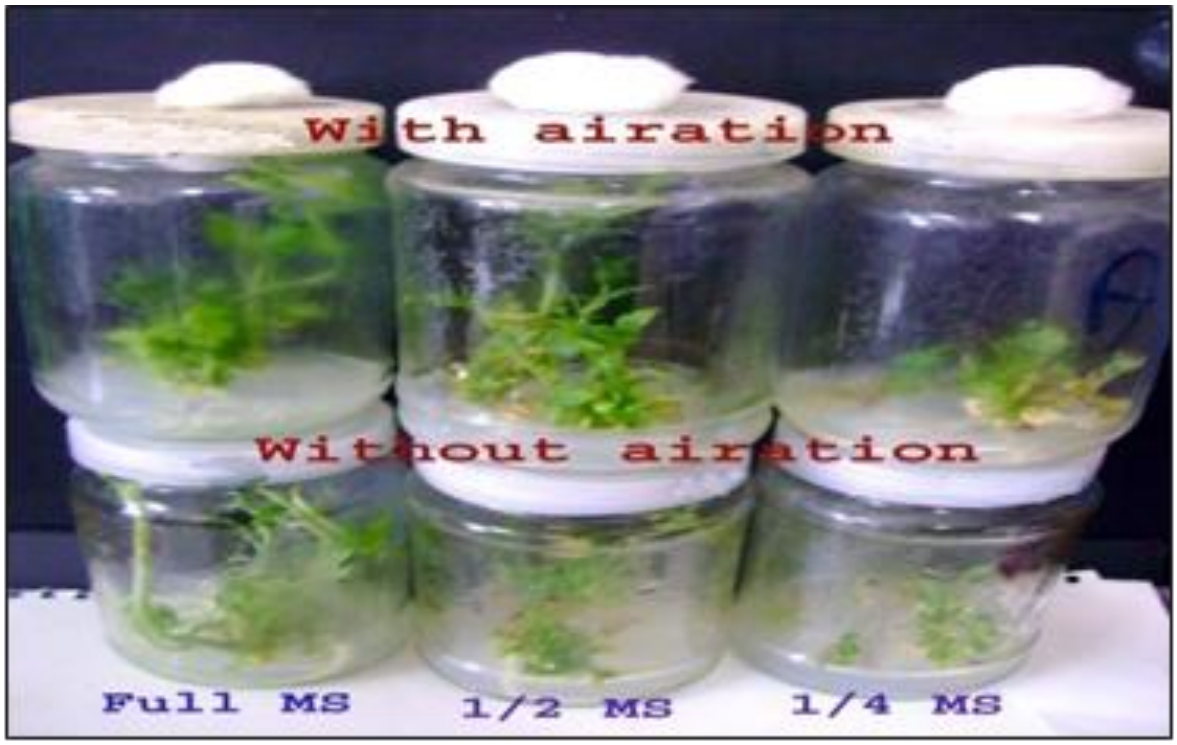

Figure 7. Effect of aeration and strength of MS medium on *in vitro* rooting of watermelon.

Table 9. Effect of *in vitro* aeration and MS salt-strength on the *ex vitro* adaptation and survival of watermelon.

MS salt strength	Number of initial *ex vitro* plantlets used for hardening-off	Aeration			
		Without		With	
		Number of adapted plantlet	Survival (%)	Number of adapted plantlet	Survival (%)
One quarter	50	16	32	29	58
One half	50	31	62	40	80
Full	50	25	50	36	72

induced high rooting response (100%) with significant differences in root number and length of roots (Table 8). In all media, aeration evidently increased root number and length, whereas the rooting rate was not significantly affected. Rooting achieved in half-strength MS medium led to development of roots of the highest number (7.3) and length as well (7.85 cm). The superior values of root number and length (7.4 and 8.5 cm, respectively) were achieved in the aerated rooting medium containing half MS salt-strength (Figure 7). These findings agree with those observed in *Maranta* (Ebrahim and Ibrahim, 2000; Tascan et al., 2010). According to Niu-Shanshan et al. (2006), the best rooting medium for watermelon was half-strength MS medium supplemented with 0.3 mg L^{-1} IBA, while Shalaby et al. (2008) found that elongated shoots rooted best on MS medium containing 1.2 mg L^{-1}NAA. Adventitious shoots of *Colocynthis citrullus* elongated on medium containing 0.1 mg L^{-1}BA were successfully rooted on hormone-free MS medium (Ntui et al., 2009).

Effect of *in vitro* aeration and strength of MS medium on *ex vitro* adaptation

In this experiment, plantlets produced from quarter-, half- and full-strength MS medium were hardened *ex vitro*. Plantlets rooted in aerated (ventilated) half-strength MS medium supplemented with 0.5 mg L^{-1} IBA recorded the highest value of *ex vitro* survival (80%) (Table 9, Figure 8). The results obtained agree with those obtained by Dong and Jia (1991) on watermelon. The regenerated shoots, of squash plants, rooted well on half-strength MS medium supplemented with IBA and NAA applied at 0.1 mg L^{-1} (Islam et al., 1992). According to Ren-Chun Mei et al. (2000), shoots of watermelon, can be rooted easily in half- strength MS medium supplemented with 0.1 mg L^{-1}

Figure 8. Greenhouse-grown watermelon plants that originated from *in vitro* shoots rooted on aerated MS medium.

in MS medium containing 1.2 mg L^{-1} NAA. Rooted plants were successfully acclimatized and gradually hardened-off to green-house conditions and subsequently established in soil with a survival rate of 80% (Shalaby et al., 2008).

Conclusion

It was concluded that hyperhydricity can be controlled effectively with satisfactory growth on MS medium by adopting a combination of low BA (1 mg L^{-1}), agar gelling and vessel aeration. Hyperhydric stock cultures could be salvaged or reverted to give normal growth by employing this approach coupled with better aeration and selective use of shoot tip segments. Rooted shoots produced from one month-old cultures, initiated on half strength MS medium with aeration and 0.5 mg L^{-1} IBA were transferred to pots after *in vitro* hardening. The produced plantlets were acclimatized and subsequently transferred to the field.

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