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Studies on standardization of acclimatization of micropropagated bitter gourd (*Momordica charantia*) plants

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ABSTRACT

Tissue culture has tremendous impact on the crop improvement programme for rapid multiplication in different vegetable crops. However, large scale mortality of plantlets raised through tissue culture occurs during acclimatization phase (Stage IV), i.e. glasshouse and later at field transfer. Micropropagated plants are generally susceptible to rapid dessication when exposed to relative humidity and therefore require proper acclimatization procedure. The ultimate success of micropropagation on a commercial scale depends on the ability to transfer plants out of culture on a large scale, at low cost and with high survival rates. Therefore, acclimatization of micropropagated plants corresponds to a transition period when roots become adapted to a substrate with less available nutrients, and to an autotrophic condition. To ameliorate this problem in bitter gourd (*Momordica charantia* L.), standardization of acclimatization is very important which is the main objective of this study. The microshoot survival was maximum with glass jar with PP cap in the shoot tip of DBGy 201 with 65.61% while maximum number of leaves/plantlet was highest in the apical meristem of Pusa Vishesh (8.07) and the plant height was also observed to be maximum when glass jar with PP cap was used being highest in the shoot tip of DBGy 201 (15.02 cm). For acclimatization the glass jar with PP caps was found to be the best in respect to establishment in comparison to plastic pots.

Key words: Acclimatization, Bitter gourd, Hardening

Momordica charantia L. is a tropical and subtropical vine of the family Cucurbitaceae, widely grown for edible fruit, which is among the most bitter of all fruits. Despite its unflattering appearance and bitter flavour, it is one of most nutritious gourds. Presence of alkaloids like momordicine and cucurbitacins proved to be highly effective components not only as antidiabetic, but also medicine for jaundice and leprosy and also as an antiseptic. Bitter gourd is rich source of nutrients (Xiang *et al.* 2000) and ranks first among cucurbits for its nutritive value (Miniraj *et al.* 1993). Studies on standardization of protocol for rapid *in vitro* multiplication in bitter gourd has been done for the multiplication of gynoecious line and other traits. Very little study has been done on the acclimatization which is the IV stage of micropropagation. Micropropagated plants are adversely affected by water stress, either due to low absorption capacity of their roots or due to stomata deficient regulation of water loss (Bonga 1977, Flick *et al.* 1983). Acclimatization of micropropagated plants corresponds to a transition period when roots become adapted to a substrate with less available nutrients, and to an autotrophic condition. Micropropagated plants develop under high moisture and low lighting conditions, often with low lignifications levels decreased functionality of the root systems that cause low survival rates to weaning. The ultimate success of micropropagation on a commercial scale depends on the ability to transfer plants out of culture on a large scale, at low cost and with high survival rates. Acclimatization is an essential and an important stage which cannot be neglected as it is the final stage of plant establishment. Therefore standardization of acclimatization is very important which is the objective of this study.

MATERIALS AND METHODS

Two monoecious bitter gourd cultivars, Pusa Do Mausami and Pusa Vishesh and one gynoecious line DBGy 201 were selected as the experimental material for the present study. Vigorous, disease and pest free plants were selected as mother/stock plants from the net house of Vegetable Farm, Indian Agricultural Research Institute, New Delhi, for the experiment. Tender axillary buds (nodal segment) and apical meristems derived from healthy shoots were used as explants. Explants were cultured on MS (Murashige and Skoog 1962) media fortified with various concentrations and combinations of BAP, Kn, IAA and NAA for culture initiation, BAP, NAA, IBA and GA_3 for multiple shoot regeneration. For root induction, elongated shoots were cultured on half-strength of MS media

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supplemented with various concentrations of NAA, IBA, IAA and GA_3. After the initiation of roots the microshoots were taken out from the rooting medium and washed with distilled water slowly for several times to remove the media sticking to it. The washed plants are then kept in test tube filled with distilled water for a week for proper hardening. The water is to be changed at regular intervals and finally in the hardening media. For standardization of acclimatization hardening media filled in jam bottles and plastic pots were used for the comparative study. For acclimatization, agro-peat + vermiculite (3:1, v/v) were used as hardening medium with the addition of one fourth of nutrient solution. For hardening the *in vitro* raised plantlets, autoclaved media filled jam bottles are used and then caps are to be closely tight. After a week the caps are to be loosened slowly. The loosened caps are to be gradually removed and intermittently the nutrient solution was given. Plants after being well established are to be transferred to the polyhouse for further growth. Results were observed at regular intervals and tabulated. The experiments were laid out in three factorial completely randomized designs (CRD) with three replications. The percentage data were subjected to arc sin transformation before analysis.

RESULTS AND DISCUSSION

Hardening or stage IV is the last step of micropropagation which is very crucial and critical stage for plant establishment. The ultimate success of micropropagation on a commercial scale depends on the ability to transfer plants out of culture on a large scale, at low cost and with high survival rates. For standardisation of acclimatization two types were used to compare the efficacy for its sustenance. Well-rooted plantlets were gradually hardened in different phases; *in vitro* hardening, *ex vitro* hardening in plastic pots and in glass jars with pp caps in culture room and preparing them ready for field transfer as complete photoautotrophic plants. The well-rooted plants (4-5 cm) were gently removed from the vessels, washed initially to remove adhered traces of the depleted medium and then washed for 5-10 min in autoclaved distilled water (Thiruvengadam *et al*. 2006). In the present study, glass jar with PP caps were found to be comparatively better than plastic pots for hardening in respect to survival percentage, number of leaves/plantlet and plant height. From the Table 1, it was found that the survival was maximum with glass jar with PP cap in the shoot tip of DBGy 201 with 65.61% followed by Pusa Vishesh (65.42%) and Pusa Do Mausami (63.55%).Using nodal as explants, the survival of the plants followed the same pattern as above while using plastic pots, DBGy 201 was observed with 57.46% followed by Pusa Vishesh 57.37%. Explant and treatment were found significant while their interactions were found non-significant. Maximum number of leaves per plantlet was highest in the apical meristem of Pusa Vishesh (8.07, Table 2) followed by the nodal segment of Pusa Vishesh (7.94). Among the plastic pots treatment, Pusa Vishesh showed maximum leaves both in the apical and nodal segment (5.94 and 5.74). In this only, treatment was found to be significant. During acclimatization, proper plant growth is very crucial as they are to be prepared for the field transfer. Weak and lanky plants are during acclimatization leads to mortality of the plants during field transfer. The plant height was also observed to be maximum when glass jar with PP cap was used being highest in the shoot tip of DBGy 201 (15.02 cm) followed by Pusa Do Mausami (14.42 cm, Table 3). Apical meristem of Pusa Vishesh was observed to have with maximum height of 12.25 cm in case of plastic pot. Explant, variety and treatment were found to be significant and

Table 1 Effect of different acclimatization methods on survival (%) using apical and node as explant

Treatment	Explant							
	Apical				Node			
	Pusa Do Mausami	Pusa Vishesh	DBGy-201	Mean	Pusa Do Mausami	Pusa Vishesh	DBGy 201	Mean
Glass jar with PP cap (Soil + perlite)	79.60 (63.55)	82.23 (65.42)	82.49 (65.61)	81.44 (64.85)	77.90 (61.87)	80.09 (63.43)	80.56 (63.77)	79.52 (63.02)
Plastic pots with polythene cover (Soil + perlite)	69.58 (57.03)	70.14 (57.37)	70.28 (57.46)	70.00 (57.29)	65.13 (53.68)	67.15 (54.91)	67.64 (55.21)	66.64 (54.60)
Mean	74.59 (60.19)	76.19 (61.24)	76.39 (61.37)	75.72 (60.93)	71.51 (57.63)	73.62 (58.99)	74.10 (59.31)	73.08 (58.64)
CD (P=0.05)								
Explant (E)			1.146					
Variety (V)			NS					
E × V			NS					
Treatments (T)			2.213					
E × T			NS					
V × T			NS					
E × V × T			NS					

Table 2 Effect of different acclimatization methods on number of leaves per plantlet

Treatment	Explant							
	Apical				Node			
	Pusa Do Mausami	Pusa Vishesh	DBGy-201	Mean	Pusa Do Mausami	Pusa Vishesh	DBGy 201	Mean
Glass jar with PP cap (Soil + perlite)	7.62	8.07	7.68	7.79	7.51	7.94	7.60	7.68
Plastic pots with polythene cover (Soil + perlite)	5.36	5.94	5.47	5.59	5.11	5.74	5.45	5.43
Mean	6.49	7.01	6.57	6.69	6.31	6.84	6.53	6.56
CD (P=0.05)								
Explant (E)			NS					
Variety (V)			NS					
E × V			NS					
Treatments (T)			0.757					
E × T			NS					
V × T			NS					
E × V × T			NS					

Table 3 Effect of different acclimatization methods on plant height (cm)

Treatment	Explant							
	Apical				Node			
	Pusa Do Mausami	Pusa Vishesh	DBGy-201	Mean	Pusa Do Mausami	Pusa Vishesh	DBGy 201	Mean
Glass jar with PP cap (Soil + perlite)	14.42	13.92	15.02	14.45	13.14	12.82	14.00	13.32
Plastic pots with polythene cover (Soil + perlite)	11.97	12.25	12.03	12.08	11.99	10.77	10.86	11.21
Mean	13.20	13.09	13.53	13.27	12.57	11.80	12.43	12.26
CD (P=0.05)								
Explant (E)			0.416					
Variety (V)			0.513					
E × V			NS					
Treatments (T)			0.668					
E × T			NS					
V × T			0.668					
E × V × T			NS					

among interactions variety × treatment were found to be significant. So it was clearly found and observed that acclimatization of micropropagated plants of bitter gourd that glass jar with PP caps was far better in comparision to plastic pots. Both the jar and the substrate used for hardening the plants during acclimatization phase are the key factors that can be manipulated to optimize plant growth, since the substrate may define the patterns of drainage and development of new roots. During successful acclimatization, relative humidity levels gradually decreases from the high humidly of *in vitro* culture vessels to the lower humidity of greenhouse or field conditions (Preece and Sutter 1991, Read and Fellman 1985). The high success in glass jar might be due to high moisture retention and also due to constant maintenance of relative humidity (RH) level compared to other strategies. Furthermore, it is also speculated that in the glass jar, the leaves are not in contact with vessel surface. While hardening in plastic pots with polythene covering, the microbial infection was observed as leaves were touching the polythene cover. Also there was constant loss of moisture from the pots, which resulted in high mortality of the plants due to desiccation. The glass being transparent also allow early hardening, since the leaves expanded in area and also were shown to develop cuticle earlier as compared to other strategies. Superiority of glass jars over pots has been proved earlier also in many crops (Parathasarathy and Nagaraju 1999, Bhatia 2007 in gerbera, Kumar 2005 in chrysanthemum and Jyothi 2007 in tuberose).

Bitter gourd is a significant crop for the adaptation and application of tissue culture and biotechnology for various purposes like cultivar improvement, for the extraction and production of steroids, etc. Efficient protocol has been developed for acclimatization of bitter gourd through micropropagation which has paved the way for easy and economical establishment of plants with reduced mortality. The present investigation has come out with an efficient protocol and the outcome of the experiment can further be used for the establishment of micropropagated plants of bitter gourd. For acclimatization the glass jar with PP caps was found to be the best in respect to establishment in comparision to plastic pots.

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