

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.607.292>

## Identification of the Suitable Hardening Protocol and Hardening Medium in Micropropagation of Gerbera (*Gerbera jamesonii* Bolus)

Vivek Kumar Singh<sup>1\*</sup>, V.M. Prasad<sup>1</sup>, Supriya Kumari<sup>2</sup>, Preeti Rajoria<sup>3</sup> and Pragati Misra<sup>3</sup>

<sup>1</sup>Department of Horticulture, Naini Agricultural Institute, Faculty of Agriculture, SHUATS, Allahabad 211007, India

<sup>2</sup>Department of Microbiology and Fermentation Technology, <sup>3</sup>Tissue Culture Lab, Department of Molecular and Cellular Engineering, Jacob Institute of Biotechnology and Bioengineering, Faculty of Engineering and Technology, SHUATS, Allahabad 211007, India

\*Corresponding author

### ABSTRACT

#### Keywords

Gerbera, Micropropagation, Rooting, Hardening and medium.

#### Article Info

Accepted:  
23 June 2017  
Available Online:  
10 July 2017

An experiment was employed to identify the suitable rooting hormone and a potential hardening medium for gerbera micropropagation techniques. IBA (Indole Butyric Acid) and GA<sub>3</sub> (Gibberellic Acid) are taken for the study of rooting and Sand, Cocopeat, Vermicompost and Vermiculite selected alone or in combinations for the study of hardening in gerbera. Different parameters on root induction were taken similarly morphological and biochemical parameters were studied in hardened plants of gerbera. On the basis of different parameter it is concluded that higher rate of IBA (3 ppm and 4 ppm) lower concentration of GA<sub>3</sub> was found good but overall performance of IBA was best. In the hardening process among the different growing medium were tested. The combined use of Sand, Cocopeat, vermicompost and vermiculite gives excellent result but alone they were failed to give desirable result.

### Introduction

Gerbera are ornamental decorative garden plant (Kanwar and Kumar, 2008). Gerbera (*Gerbera jamesonii*) also known as Transvaal, African or Barbeton Daisy. It has massive demand in the floral industry as a cut flower as well as potted plant due to its beauty and long vase life which ranked at fifth only after rose, carnation, chrysanthemum, and tulip, in the global cut flower trade (Bhatia *et al.*, 2009). It is a perennial herb belongs to the Asteraceae family. Cultivation of gerbera for cut flower purpose gives high return in polyhouse and shade house (Singh *et al.*, 2014). In India, commercial production of

gerberas is centered around Pune and Bangalore, parts of Sikkim, Nagaland, Meghalaya and Uttarakhand.

Micropropagation is the only viable alternative for large-scale multiplication of gerbera. This method is free of seasonal bonds and enables manifold multiplication of the selected plants. The other advantages are product uniformity, disease-free plants, easy exchange of germplasm and planting material (Murashige *et al.*, 1974). Acclimatization is an important step in micropropagation. It is an adaptation process to the natural environment

for various plant species which has undergone growth and development process in vitro (Preece and Sutter, 1991). Most species grown in vitro require an acclimatization process in order to ensure that sufficient number of plants able to survive and grow vigorously when transferred to soil. The benefit of any micropropagation system can only be realized by the successful transfer of plantlets from tissue culture system to the natural environment found ex-vitro. Morphological characters could clearly be seen when in vitro plantlets were successfully transferred to soil through acclimatization process (Mohammed and Vidavar, 1990). The selection of suitable substrates or media can be a major decision for acclimatization.

Coco peat is coir fiber pith that is having coconut husk as its base. It is a soil conditioner and easily be mixed with other growing media. Its uniqueness is that it can hold 8-10 times of water of its own weight and hydrophilic by nature. It is best for commercial and home gardening application. It provides breathing space, *i.e.* letting in and letting out of air for a root which helps better growth. It also encourages favourable micro organisms around the root zone and its slow degradation level is universally accepted. (Nguyen Van Son, 2007).

Vermiculite is common ingredient in growing medium. The origin of vermiculite is from mineral called mica. Mica is heated to 18000F, which causes it to expand like an accordion. Spaces made by the expansion result in good water holding ability and aeration. It is a light weight material and can be easily compressed. Vermiculite has a good cation exchange capacity and a neutral to slightly alkaline pH (6.3-7.8). It also provides small amounts of calcium, magnesium, and potassium for plants use (Singh, 2012). Sand consist small rock grains in the range of 0.05 - 2.0 mm in diameter in size, formed as a result of weathering of various rocks. Its mineral

composition depends upon the type of parent rock. Quartz sand, generally used for propagation purposes, consists chiefly of silica complex. Sand used in plastering is satisfactory for rooting of cuttings (Sadhu 1989). It does not retain moisture unlike other media, hence needs frequent watering. It is enough to retain some moisture around the cutting, yet coarse enough to allow water to drain through it. It is used mostly in combination with organic material (Nguyen Van Son, 2007).

Vermicomposts are peat-like materials with high porosity, aeration, drainage, and water-holding capacity. They have a vast surface area, providing strong absorbability and retention of nutrients. Vermicomposts contain nutrients in forms that are readily taken up by the plants.

Uses of Vermicompost as substrates of growing media have a great potential in the horticultural and agricultural plants. Vermicomposts, whether used as soil additives or as components of horticultural media, improved seed germination and enhanced rates of seedling growth and development (Atiyeh *et al.*, 2000).

Keeping all facts above mentioned, in the present study we have elucidated the most efficient protocol for acclimatization of Gerbera plantlets regenerated from tissue culture technique system.

## **Materials and Methods**

All the experiments were done at Experimental Field, Department of Horticulture, Naini Agricultural Institute, Faculty of Agriculture and Tissue Culture Lab, Molecular and Cellular Engineering, Jacob Institute of Biotechnology and Bioengineering, Faculty of Engineering and Technology, SHUATS, Allahabad in the year 2015-16.

### **Source of *in vitro* plantlets**

The explants such as Gerbera flower buds and flower stalks were procured from Floriculture Unit, Department of Horticulture, Naini Agricultural Institute, Faculty of Agriculture SHUATS, Allahabad, and run through the an efficient micropropagation protocol which was optimized by the author.

Before transferring the in rooting medium microshoots were separated from adhering callus and old leaves are sniped off than placed in half strength ms medium (Murashige and Skoog, 1962) supplemented with various concentrations of IBA and GA<sub>3</sub>.

### **Transplantation of *in Vitro* Plantlets to Various Hardening Media**

All selected plantlets for hardening were of the same age, height and leaves number and size. Plantlets were removed carefully from the culture vessels and the roots were rinsed with sterile distilled water to remove excess agar from the roots. Plantlets were transferred to various growth substrates such as Sand, Cocopeat, Vermicompost and Vermiculite.

The plantlets were planted in small pots with size 80x60 mm. The plantlets were first kept in the culture room at 25±1 °C under 16 hours light and 8 hours dark for 3 weeks before being transferred to the green house. Initially, the plant pots were covered with plastic covers with small holes to allow adaptation and adjustment process of plantlets to the new environment. All plantlets were watered every day. After 3 weeks in the culture room, plantlets were ready for the next step of growth and transferred to the green house. Plantlets were transferred to 10 different scheme of growth medium.

### **After care of transplanted plantlets**

Hoagland solution (Epstein, 1972) was prepared by diluting concentrated stock

solution. Stock solution was prepared such that they do not react themselves. Plants were irrigated with the double distilled water regularly and once in two days with half strengths Hoagland solution for adequate nutrients supply. Physiological and Biochemical observation like survival of plants, plant height, number of leaves and chlorophyll content (Hiscox and Isralesham, 1979) recorded and results were analyzed on effect hardening medium on rooted gerbera plantlets.

### **Results and Discussion**

Rooting in gerbera micro-shoots were employed in half strength ms medium containing various levels of IBA and GA<sub>3</sub>. Among the two rooting hormones were tested IBA was found better in terms of number of roots, longest root and weight of root while GA<sub>3</sub> found good in terms of maximum shoots forms roots and minimum days taken for root initiation. Among the Different levels of IBA, 4 ppm found good as it produces root in 22 days, maximum number of roots (5.0), highest length of root (71.1 mm) and maximum weight of root (7.2) followed by 3 ppm of IBA. While lower concentration of IBA 1 ppm gives poor results among all concentration of IBA were tested. In different levels of GA<sub>3</sub>, 2 ppm was found good in terms of percentage of shoots form roots (65 %), length of longest root (22.5 mm) weight of root (4.2) followed by 3 ppm of GA<sub>3</sub>. Hardening of *in vitro* raised plants (Table 1). Out of the Four potting mixture, the highest survival percentage (90.00 %) was observed in combination of Sand: Vermicompost: Cocopeat: Vermiculite (1:1:1:1) medium followed by Cocopeat: Vermicompost: Vermiculite (1:1:1) mixture and sand mixture gave poor result in plant survival (30.3) 30 days after transplanting. Minimum number of new leaf per plantlets (1.5) was also found in sand medium while maximum numbers of newly emerged leaves (5.5) was recorded in

Vermicompost: Cocopeat: Vermiculite (1:1:1) followed by 5.2 in Sand: Vermicompost: Cocopeat: Vermiculite (1:1:1:1) medium. The highest plant height (78.0 mm) was recorded in Cocopeat: Vermicompost: Vermiculite (1:1:1) mixture, followed by (77.2 mm) in Sand: Vermicompost: Cocopeat: Vermiculite (1:1:1:1) mixture and minimum (62.0 mm) was in Sand. days for new leaf initiation was minimum 5 days and new leaf formation 94 % in Cocopeat: Vermicompost: Vermiculite (1:1:1) whereas sand medium took maximum number of days (15) for new leaf initiation and minimum 58 % plants shows new leaf formation in Sand: Vermiculite (1:1). Total plant survival in growing field was 96 % in Sand: Vermicompost: Cocopeat: Vermiculite (1:1:1:1) followed by 93 % in Sand: Vermicompost: Vermiculite (1:1:1) minimum was 71 % found in sand. Highest total chlorophyll content (12.79 mg/gm) was found in Cocopeat: Vermicompost: Vermiculite (1:1:1) followed by 12.78 in Sand: Vermicompost: Cocopeat: Vermiculite (1:1:1:1) while minimum was 12.09 in sand.

The process of rooting is of great importance because a good quality root system is essential for successful acclimatization of the plantlets (Yang, 2010). Among the different levels of IBA it is observed that the higher level of IBA (3 and 4 ppm) along with half strength ms medium is good and found suitable for root induction in gerbera. This finding is close to (Kanwar and Kumar 2006). Rooting was achieved in all the concentration of GA<sub>3</sub>, the reason for this could be the fact that the GA<sub>3</sub> and sucrose are known to induce roots (Calamar and De Klerk, 2002). Lower concentration of GA<sub>3</sub> produces good quality roots increasing in GA<sub>3</sub> concentration gives poor results than the lower once, similar finding were recorded by Ranasinghe *et al.*, 2006 in Gerbera. Overall among the GA<sub>3</sub> and

IBA, IBA was found excellent and produces good quality roots in gerbera plantlets. The maximum number of roots per micro-shoot was recorded on half MS medium supplemented with IBA the roots were short and swollen, brownish in colour and were without root hairs in GA<sub>3</sub>. However, rooting was delayed and number of roots was reduced when high dose of both the auxins were used in combination.

Findings indicated that sand alone did not found a better hardening medium for the *in vitro* raised plantlets of gerbera in comparison to the other medium were tested. It may be due to high aeration rate and unavailability of nutrients to the plants (Nguyen Van Son, 2007) (Table 2).

The water holding capacity of the medium per unit volume was reported to be more in coco peat (Martyr, 1981), This could have caused rotting of the plantlets as it had high water holding capacity up to 90 per cent. It is might be minimize Superiority of coco peat. On the other hand, evapo-transpiration was recorded higher with vermiculite because of higher hydraulic conductivity (Maloupa *et al.*, 1993). This indicates that, along with good water holding capacity vermiculite also has good aeration. Vermicompost is found good in availability of nutrients to the plants but leads to microbial contamination while it used alone. It holds nutrients readily taken up by plants and improves the growth rate of plants in seedlings (Atiyeh *et al.*, 2000). The combined use of above medium proved its superiority as a hardening medium for establishment of Gerbera plantlets because of optimum conditions like sand provided good aeration, cocopeat gives higher water holding capacity while vermiculite utilized for both and vermicompost supplied nutrients.

**Table.1** *In vitro* rooting in gerbera plantlets as influenced by IBA (Indole Butyric Acid) and GA<sub>3</sub> (Gibberellic Acid)

Treatments	Days to roots initiation	No of roots per Shoots	Shoots forms roots (%)	Length longest root (mm)	Weight of fresh roots (mg)	Nature of roots		
						Color	texture	Branching habit
Half MS Basal	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	--	--	--
Half MS + 1 IBA	30.33	3.70	39.66	29.23	4.76	Light	Thin	Partial Branched
Half MS + 2 IBA	25.33	4.43	50.66	40.16	5.63	Light	Thin	Branched
Half MS + 3 IBA	22.00	4.86	75.33	70.66	6.83	Light	Thin	Branched
Half MS + 4 IBA	21.66	4.93	71.00	71.16	7.16	Light	Thin	Branched
Half MS + 0.5 GA <sub>3</sub>	24.66	1.83	50.33	15.63	3.06	Light	Thick	Branched
Half MS + 1.0 GA <sub>3</sub>	25.00	2.56	65.66	26.10	4.73	Brownish	Thick	Partial Branched
Half MS + 2 GA <sub>3</sub>	23.00	2.03	64.00	22.53	4.23	Brownish	Thick	Un-branched
Half MS + 3 GA <sub>3</sub>	22.33	2.23	59.00	16.63	3.76	Brownish	Thick	Un-branched
CD <sub>(0.05)</sub> at 5 %								
Level of Significance	<b>1.51</b>	<b>0.10</b>	<b>2.03</b>	<b>0.28</b>	<b>0.20</b>			

**Table.2** Hardening of rooted gerbera plantlets as influenced by different hardening medium

Treatments	Survival % at 7 days	Survival % at 15 days	Survival % at 30 days	No of leaves per plantlet	New leaf formation (%)	Days to leaf formation	Plant height (mm)	Survival in field (30 DAP)	Total chlorophyll content (mg/gm FW)
Cocopeat	74.36	66.70	60.76	3.50	69.60	11.66	63.40	71.66	12.39
Cocopeat: Vermiculite (1:1)	68.00	66.40	63.83	3.83	61.66	7.00	68.23	75.33	12.73
Cocopeat: Sand (1:1)	67.33	64.26	60.50	3.16	65.00	11.33	63.46	81.33	12.70
Cocopeat: Vermicompost (1:1)	74.33	62.76	61.33	3.73	65.66	7.33	67.76	86.00	12.71
Sand	63.16	44.16	31.33	1.50	59.66	15.33	62.10	70.66	12.09
Sand: Vermicompost (1:1)	66.50	63.16	51.00	2.33	73.00	8.00	65.53	78.33	12.28
Sand: Vermiculite (1:1)	70.33	65.33	59.66	2.16	58.33	10.33	65.13	83.66	12.65
Vermiculite: Vermicompost (1:1)	80.16	75.80	72.00	4.33	77.00	8.33	74.36	88.33	12.60
Vermicompost	68.33	65.00	60.00	3.33	65.66	12.33	63.46	91.00	12.60
Cocopeat: Vermicompost: Sand (1:1:1)	81.33	76.50	74.33	5.16	77.60	7.33	75.33	90.33	12.75
Cocopeat: Vermicompost: Vermiculite (1:1:1)	96.33	90.00	85.80	5.50	93.66	5.33	78.23	92.33	12.79
Sand: Vermicompost: Vermiculite (1:1:1)	94.50	88.50	85.50	4.83	91.33	8.66	73.40	92.66	12.76
Sand: Vermicompost: Cocopeat: Vermiculite (1:1:1:1)	95.16	92.66	90.50	5.33	83.66	6.33	77.73	95.66	12.75
CD <sub>(0.05)</sub> at 5 % Level of Significance	<b>2.25</b>	<b>2.41</b>	<b>2.10</b>	<b>0.66</b>	<b>2.31</b>	<b>2.11</b>	<b>0.51</b>	<b>2.35</b>	<b>0.13</b>



**Fig.** A Showing multiple shooting taken for study, B. Showing root initiation, C. Rooted and fully developed plantlets, D. Single rooted plantlet before hardening, E. Hardened plant 30 days old & F. One month old acclimatized plant in the net house condition



Previous researchers also found good results with combination of different hardening medium (Vadawale *et al.*, 2006; Chabukswar and Deodhar, 2005). Thus the present study describes an efficient protocol for rooting and hardening of gerbera is of great importance. These findings proved that to be higher dose of IBA (2-4 ppm) is suitable in root induction and combined use of Sand, Vermicompost, Cocopeat and Vermiculite as growing medium can be successfully employed for hardening of micropropagated gerbera. It could be helpful for the commercial cultivation of Gerbera.

### Acknowledgement

Authors are heartily thankful to the Prof. Jonathan A. Lal Dean, Jacob Institute of Biotechnology and Bioengineering, Faculty of Engineering and Technology, SHUATS, for providing all the essential facilities and moral support to conduct the whole research program and to obtain its significant findings.

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#### **How to cite this article:**

Vivek Kumar Singh, V.M. Prasad, Supriya Kumari, Preeti Rajoria and Pragati Misra. 2017. Identification of the Suitable Hardening Protocol and Hardening Medium in Micropropagation of Gerbera (*Gerbera jamesonii* Bolus). *Int.J.Curr.Microbiol.App.Sci.* 6(7): 2476-2484. doi: <https://doi.org/10.20546/ijcmas.2017.607.292>