



## Standardization of media mixture for hardening of *in vitro* plantlets of *Dendrobium* cv. Sonia-17

S. Muna, S. Beura and S.K. Patra\*

Department of Floriculture and Landscaping, College of Agriculture,  
Orissa University of Agriculture and Technology, Bhubaneswar-751 003, India.

Received: 03-09-2015

Accepted: 14-01-2016

DOI: 10.18805/asd.v35i1.9319

### ABSTRACT

Experiment was carried out to standardize media mixture for hardening of *in vitro* plantlets of *Dendrobium* hybrid Sonia-17 during the year 2013-2014. The results revealed that three pieces coconut husk tied with the ex-agar plantlets and planted in portray containing cocopeat significantly enhanced the formation of new leaves/plantlet (5.3), number of roots/plantlets (7.5), length of the root (2.5cm) and percentage of survival (96.67%) of the plantlets.

**Key words:** Ex-agar, Hardening, Orchid, Portray, Survival.

Orchids, the most beautiful flowers in God's creation, comprise a unique group of plants. They are most pampered of the plants and occupy top position among all the flowering plants valued for cut flower production and as potted plants. They are known for their longer lasting and bewitchingly beautiful flowers which fetch a very high price in the international market. Generally, orchids are propagated both asexually and sexually. But the traditional asexual propagation is extremely slow which can give rise to 2-4 plants per year (Nasiruddin *et al.*, 2003). Micropropagation of orchids is the most frequently used convenient technique for their exploitation as major trade in developed countries (Sagawa and Kunisaki, 1982). It is the only method currently available which initially starts in early 1960. Because commercially mass propagation is possible by producing millions of plantlets using tissue culture techniques. Orchids can be rapidly propagated through tissue culture techniques by using shoot tips (Saiprasad *et al.*, 2002), leaf and stem nodes (Pathania *et al.*, 1998). Due to difficulties in protocorm regeneration and plantlet formation, all procedures mentioned above have become inadequate for meeting commercial need for vegetative propagation. Some of these methods gave a lot of protocorms but they develop slowly or poorly to vital plants. Large-scale multiplication of orchids, especially rare hybrids and endangered species using tissue culture techniques has helped orchids occupy a position as one of the top ten cut flowers. Orchid propagation by seed caused to the production of heterozygous plants. Thus, *in vitro* proliferation is a suitable alternative procedure for propagation of orchids. Different protocols have been established for micropropagation of orchids species through *in vitro* culture of various parts consisting shoot tip, root tip, stem, leaf, node, bud, inflorescence and rhizome as well somatic lls and thin embryo, callus and thin cell layer (Park

*et al.*, 2000, Ket *et al.*, 2004, Kalimuthu *et al.*, 2006). The success of tissue culture depends on the sustainability of the *in vitro* produced plants under the natural conditions. Under *in vitro* conditions, the plantlets are heterotrophs. So they have to be gradually converted into autotrophs. The prehardening and hardening are the process for making the *in vitro* raised plantlets into autotrophs and adapt them to the outside environment (Beura, 1998).

The present experiment was carried out at Department of Floriculture and Landscaping, College of Agriculture, OUAT, Bhubaneswar to standardize media mixture for hardening of the *in vitro* raised plantlets of *Dendrobium* cv. Sonia-17.

The well developed seedlings of 2.5-3 cm height were taken and thoroughly washed with tap water to remove adhering medium completely without causing any damage to the roots. The media mixture were prepared with charcoal and cocopeat in equal proportions and filled in the portrays. The components of the media were sterilized with 0.2% bavistin for 30 minutes before used. The seedling were planted carefully in the respective medium so that the roots of seedlings passed through the media and kept under the green house. The half MS liquid medium was fertigated to the seedlings interval of two days up to two weeks and then one fourth MS liquid medium was applied at an interval of two days the next two weeks. Then irrigation was given with the drippers present in green house. For conducting the experiment total eight treatments replicated thrice with 10 plants per replication were taken. The observations were recorded on plant height, number of leaf, number of root/plant, length of root.

The data recorded from the experiments were analyzed following the method of Gomez and Gomez (1984)

\*Corresponding author's e-mail: sailendri\_patra@yahoo.co.in.

using one way ANOVA in completely Randomized Design with 3 replications each consisting of 30 pots per replication.

The success of tissue culture depends on the sustainability of the *in vitro* produced plants under the natural conditions. Under *in vitro* conditions, the plantlets are heterotrophs. So they have to be gradually converted into autotrophs. The prehardening and hardening are the process for making the *in vitro* raised plantlets into autotrophs and adapt them to the outside environment (Beura, 1998). The existing rooted plantlets of *Dendrobium Sonia* -17 varying from height 2.5-3 cm were taken for hardening in 8 different combinations of media i.e. 1. cocopeat, 2. Cocopeat+ 1 pieces coconut husk, 3. Cocopeat + 2 pieces coconut husk 4. Cocopeat + 3 pieces coconut husk, 5. Cocopeat + charcoal (1:1), 6. Cocopeat + charcoal +1 pieces coconut husk, 7. Cocopeat +charcoal+2 pieces coconut husk, 8. Cocopeat +charcoal+3 pieces coconut husk in portrays (Table 1). The plants were hardened up to 60 days. The half MS liquid medium was fertigated to the seedlings at an interval of two days up to two weeks and then one fourth MS liquid medium was applied at an interval of two days for the next two weeks. Different observations such as plant height, leaf number, leaf length and percentage of survival have been recorded after 60 days of hardening. The data indicated (Table 2) very specific results that the plantlets tied with 3 pieces coconut husk and planted in portray containing cocopeat media combinations significantly increase plant height, leaf number,



**Plate 1:** Hardening of plantlets tied with coconut husk and planted in portrays containing cocopeat:charcoal in 1:1 ratio

leaf length and percentage of survival after 60 days of hardening followed by the plantlets tied with 2 pieces coconut husk and planted in portray containing cocopeat and charcoal in 1:1 ratio (Plate 1) for plant height, leaf number, leaf length concerned. The results of the present investigation are in partially agreement with that of Sharma (1995) who reported that the media combination comprising of brick:charcoal:tree fern: bark pieces : leaf mould: dry sphagnum moss 1:1:1:1:2 in the ratio was considered suitable for maximum survival of *Dendrobium* and chrysanthemum seedlings. Indhumati *et al.*, (2003) reported that charcoal+brick+cocopeat in the ratio 1:1:1 was found suitable for establishment of plantlets of *Dendrobium* Hybrid Soniabrick pieces :moss: charcoal pieces (1:1:1:1) proved to be suitable for the establishment of *in vitro* rooted plantlets of *Sonia* -17.

## CONCLUSION

The work on *in vitro* culture of *Dendrobium* spp cv. *Sonia*-17 has given very promising results in respect of hardening of *in vitro* plantlets. The tissue cultured plantlets of *Dendrobium* *Sonia*-17 tied with 3 pieces coconut husk and planting in portrays containing cocopeat was found to be most suitable for hardening.

**TABLE 1:** Effect of different composition of potting media on hardening and survivability percentage of the seedling after 60 days of hardening

| Treatment      | Media composition                    |
|----------------|--------------------------------------|
| T <sub>1</sub> | Cocopeat                             |
| T <sub>2</sub> | Cocopeat+1 pc coconut husk           |
| T <sub>3</sub> | Cocopeat +2pc coconut husk           |
| T <sub>4</sub> | Cocopeat + 3pc coconut husk          |
| T <sub>5</sub> | Cocopeat + charcoal (1:1)            |
| T <sub>6</sub> | Cocopeat +charcoal +1pc coconut husk |
| T <sub>7</sub> | Cocopeat +charcoal +2pc coconut husk |
| T <sub>8</sub> | Cocopeat +charcoal +3pc coconut husk |

**TABLE 2:** Standardization of media mixture for hardening of *in vitro* plantlets of *Dendrobium* hybrid *Sonia*-17

| Treatments                                            | Plant height | No of leaf | No of root/plant | Length of root | Survival %   |
|-------------------------------------------------------|--------------|------------|------------------|----------------|--------------|
| T <sub>1</sub> - Cocopeat                             | 6.67         | 4.17       | 6.33             | 1.83           | 53.33(43.22) |
| T <sub>2</sub> - Cocopeat+1 pc coconut husk           | 19.67        | 4.60       | 7.03             | 2.07           | 56.66(44.32) |
| T <sub>3</sub> - Cocopeat +2pc coconut husk           | 21.67        | 5.10       | 7.23             | 2.37           | 76.66(51.48) |
| T <sub>4</sub> - Cocopeat + 3pc coconut husk          | 27.33        | 5.30       | 7.50             | 2.50           | 96.67(79.37) |
| T <sub>5</sub> - Cocopeat + charcoal (1:1)            | 8.33         | 4.17       | 6.43             | 1.83           | 53.33(43.22) |
| T <sub>6</sub> - Cocopeat +charcoal +1pc coconut husk | 18.00        | 4.60       | 6.93             | 2.17           | 56.66(44.32) |
| T <sub>7</sub> - Cocopeat +charcoal +2pc coconut husk | 19.67        | 4.87       | 7.06             | 2.27           | 60.00(45.42) |
| T <sub>8</sub> - Cocopeat +charcoal +3pc coconut husk | 21.67        | 4.93       | 7.16             | 2.43           | 66.66(47.73) |
| SE(m)                                                 | 0.42         | 0.12       | 0.03             | 0.04           | 1.90         |
| CD%                                                   | 1.28         | 0.37       | 0.11             | 0.11           | 5.76         |

(Fig.in parenthesis indicate angular/arsenic value)

**REFERENCES**

- Gomez, A. A. and K. A. Gomez (1984). *Statistical Procedures for Agricultural Research*. John Wiley and Sons, New York.
- Beura, S. (1998). *In vitro* multiplication, agrobacterium mediated transformation and post. Harvest handling of spiking gladiolus. Ph. D. Thesis.
- Indhumati, K., Kannan M., Jawaharlal, M. and Amarnath V.(2003). Standardization of prehardening and hardening techniques for *in vitro* derived plantlets of *Dendrobium* Orchid Hybrid Sonia-17. *Journal of Ornamental Horticulture*, **6**:212-216.
- Kalimuthu, K., Senthikumar, R., and Vijayakumar, S. (2006). *In vitro* micropropagation of Orchid, *Oncidium* sp. (dancing doll). *African Jour. Of Biotechnology*. **6** :1171-1174.
- Ket, N.V., Hahn, E.J., Park, S.Y., Chakrabarty, D., Paek, K.Y.E. (2004). Micropropagation of an endangered orchid *Anoectochilm formosanns*, *Biological Plantarum* (Czech Republic). **48**: 339-344.
- Nasiruddin, K.M., Begum, R. and Yasmin, S. (2003). Protocorm like bodies and plantlet regeneration from *Dendrobium formosum* leaf callus. *Asian Journal of Plant Sciences* .**2**:955-957.
- Panthania, N.S; Shegal, O.P., Debojit, P., Dutta, B.S and Paul, D. (1998). Studies on micropropagation in *Dendrobium* cv Sonia. *J.Orchid Soc.* **12** : 35-38.
- Park, S.Y., Murthy, H.N., and Paek, K.Y. (2000) *In-vitro* seed germination of *Calanthesieboldii*, an endangered Orchid species. *Journal of Plant Biology*. **43**:158-161.
- Saiprasad, G.V.S., Raghuveer, P., Khetrapal, S., Chandra, R. (2002). Effect of various growth regulators on the production of protocorm like bodies in three orchid genera. *Indian Journal Plant Physiology*. **7**: 35-39.
- Sagawa, Y. and Kunisakhi, J.T. (1982) . Clonal propagation of orchids by tissue culture. In (A., Fujiwara) *Plant Tissue Culture* Maruzen ,Tokyo. 683-688.
- Sharma, J. and Chauhan, Y.S. (1995). Establishment of *in vitro* raised seedlings of *Dendrobium* and *Paphiopedilum*. *Journal of the Orchid Society of India*, **9**:37-41.