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Acclimatization of the Tissue culture Plantlets of *Amomum subulatum* Roxb in Different medium Substrates

Ratnesh Kumar Singh* and T. Roy**

ABSTRACT: *The successful acclimatization of the tissue culture plant of large cardamom has been established. The ex-agar well rooted plantlets has been taken for the Ex- Vitro acclimatization. The plantlets has been transplanted in portray filled with different substrate medium which are kept in different conditions in poly tent, relative humidity (50% - 80%), temperature (25 to 35 degree centigrade), Different prophylactic treatment has been given to avoid any occurrence of any fungal attack. Observation of all the parameters has been done for one month.*

Result showed that, In case of Primary hardening the maximum survival rate of 98% has been noticed in Trail (T4), in standard condition of , (Substrate – coco peat 50%+ Rice husk 40%+ Perlite 10%), relative humidity under the tent is 80% to 90%, temperature ranging from 25 to 35 degree centigrade, under dull light intensity.

In secondary hardening the maximum survival rate of 90% has been noticed in the Trail (T3). (Substrate – Vermicompost 80%+ soil 10%+ coco peat 10%), relative humidity 50% to 60 % , temperature ranging from 25 to 40 degree , under 50% shade net condition.

Key Words: *acclimatization, Ex- Vito, Amomum subulatum roxb*

INTRODUCTION

Large Cardamom (*Amomum subulatum Roxb* is a perennial herb with subterranean rhizomes and 50-140 aerial leafy shoots. Each shoot has height of 1.7 to 2.6 mtr and possess 9 to 13 leaves in each tiller. Leaves are glabrous on both sides with a prominent mid-rib. Inflorescence is a condensed spike with yellowish perianth. Each spike has 10-15 fruits. Fruit is round or oval shape, capsule with reddish brown color. Each capsule is trilocular with many seeds.

Large Cardamom is cultivated in the Sub-Himalayan region of North Eastern India, Nepal and Bhutan. It is grown in cold humid conditions under shade of trees at an altitude between 800-2000 meters above MSL. With an average precipitation of 3000-3500 mm spread over about 200 days and with temperature ranging from 6-30 degree C.

It is used as a flavourant in dishes like Pulavu, Biriyani and meat preparations. It is an ingredient in curry powder and spice masala mixtures and is also used in Ayurvedic and Unani medicines. It has applications in flavoring cola, biscuits, liquors (Spices Board of India). The three main varieties of large cardamom cultivated are Ramsey, Sawney and Golsey. Ramsey is suitable for cultivation above 1500 m; its foliage is green to light green with leafy stem appearing maroon. Flowers are small and yellowish in colour, while the colour of the raw capsule is maroon. Sawney grows best within the altitudes of 1000–1500 m. These varieties of plants are tall arobust with dark green leaves and greenish to purple stem. It usually flowers in May and has yellowish flowers and maroon coloured capsules. Golsey grows best in altitudes slightly below 1000 m. It has deep green foliage and a greenish coloured stem. The fruits are oval in shape. Other varieties also cultivated are Bebo, Bharlangey and Ramla. Besides these, there are several sub-varieties or strains, which are named in the local dialect of Lepchas, Bhutia and Nepalese in the cardamom growing areas of Sikkim and adjoining areas.

MATERIAL & METHOD

The fresh meristem buds were collected & washed under running tap water for one hour & after the

* Technical Director, Shree Ganesh Biotech India Ltd., Kolkatta, West Bengal, *E-mail: rat_bio@yahoo.co.in*

** Department of Botany, Vinobha Bhave University, Hazzaribag, Jharkhand.

removal of all the soil debris the buds were trimmed and then dipped in antifungal solution for one hour. Again the buds were washed with deterrent solution & dipped in 70% alcohol for 60 sec, finally the buds were taken inside the laminar flow bench for further treatment with 10% sodium hypochlorite solution for 10 minutes & after many wash with sterile water finally the buds were inoculated in the MS medium with 1mg/l BAP for further growth.

After the successful establishment of the cultures, the cultures were transferred to the fresh medium with same composition for the further multiplication & once the enough stock built up , the elongated plants were transferred to the rooting medium MS + 0.5 mg/ l NAA. The cultures were incubated in 25 degree centigrade for 12 hour light period. After three weeks the most of the plantlets were rooted. Finally the rooted plantlets had been taken outside the lab for their hardening.

The Ex-Agar plantlets had been taken & finally washed properly to remove all the traces of the Agar, ultimately it was dipped in antifungal solution for some time before inoculation in to the portray. We have taken it for two stage hardening. Primary hardening & Secondary hardening as advocated by earlier by (Mengesha. A *et al*, 2013).

In case of the Primary Hardening in (Table 1)

The various substrate were used (Vermicompost, Cocopeat, Rice husk, Perlite) in single or in combination in different percentage & filled in 98 cavity portray & after transplanting the plants the portrays had been kept in the Poly Tunnel with standard condition of , relative humidity varying from 50% to 90%, temperature ranging from 25 to 35 degree centigrade . The observation was done for one month. All the experiments were conducted under polythene tunnel to maintain the humidity. In case of T1 the observation was done in open Tunnel to reduce the humidity. The prophylactic spray of antifungal spray was applied on weekly basis. The first opening of the tunnel was done after two weeks' time for irrigation & antifungal spray. Then after, every day for one hours the tunnel was being open for the gradual exposure of the plants to external environment & finally the duration of opening of the tunnel was gradually extended up to full day,as observed earlier that the plant species need gradual changes in environmental condition to avoid desiccation loses and photo inhibition as stated earlier by (Pospisilova *et al*, 1999).

The Growth regulator 1mg /l , IAA was sprayed to the plants at the time of transplantation for stimulation for the healthy root growth; that the acclimatization of the plants can be improved by the use of the hormonal stimulation for the root development as stated earlier by (Van. Telgan *et al*. 1992, Diaz- Prez *et al.* 1919a).

RESULT & DISCUSSION

In our Observation the Trail 4 (Coco peat 50%+ Rice Husk 40%+ Perlite 10%), gave the 90% of survival plants with healthy growth. The optimum light intensity & healthy growth was observed with 140 micron Plastic sheet, as stated earlier by (Chaudhary M.T & Bora. A, 2009), The temperature noticed which were ranging from 25 degree centigrade to 35 degree centigrade. The maximum mortality was noticed because of the fungal attack.

In case for Secondary hardening in (Table 2)

The secondary hardening was conducted in 50% net Shade as the survival rate was zero in case of full sun as observed earlier by (Rodrigues. V.H.P *et al.*, 2005). The substrate was filled in black color poly bags, Again the same substrate were mixed in different proportion to find the best one. The Relative humidity was noticed less because of the open net condition that was 50 to 60 % & temperature were ranging from 25 to 35 degree centigrade. The Five trials had been made, the most suited result came with trail no- T3

(Vermicompost 80%+soil 10%+cocopeat 10%), that was 90% survival rate with 10 % mortality. The maximum mortality was noticed in case of T5, mainly due to fungal attack & root rot. From our study it is

Table 1
Primary Hardening of the Tissue culture plants of Large Cardamom

Trails	*Substrate*	*Percentage*	*Relative Humidity*	*Temperature Degree cent*	*Duration*	*Mortality %*	*% survival*
T1	Vermicompost	100	50 to 60	25 to 35	1 month	90	10
T2	Vermicompost	100	80 to 90	25 to 35	1 month	70	30
T3	Vermi + Cocopeat	40 : 60	80 to 90	25 to 35	1 month	50	50
T4	Cocopet+ Ricehusk+ Perlite	50 : 40: 10	80 to 90	25 to 35	1 month	10	90
T5	Vermi+ sand+ cocpeat	60 : 20: 20	80 t0 90	25 to 35	1 month	80	20

Tabe 2
Secondary Hardening of the Tissue culture Plant of the large cardamom

Trails	*Substrate*	*Percentage*	*Relative Humidity*	*Temperature Degree cent*	*Duration*	*Mortality %*	*% survival*
T1	Soil+ sand+ vermicompost	50:25:25	50 to 60	25 to 35	1 month	20	80
T2	Rice husk+ sand+ vermicompost	40:30:30	50 to 60	25 to 35	1 month	30	70
T3	Vermicompost+ soil+cocpeat	80: 10: 10	50 to 60	25 to 35	1 month	10	90
T4	Vermicpost+ cocpeat+sand	30:10: 60	50 to 60	25 to 35	1 month	30	70
T5	Sand + soil	80 : 20	50 to 60	25 to 35	1 month	60	40

concluded that the large cardamom can be well acclimatized with two stage hardening that is Primary and secondary hardening. The Protocol can be applied for the mass production of the healthy plantlets with minimum mortality loses.

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