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Research Article

Micropropagation and Acclimatization of Large Cardamom (*Amomum subulatum* Roxb.)

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Abstract

In vitro clonal propagation and acclimatization of the tissue culture plant of large cardamom has been established at Agriculture Research Station Pakhribas, Dhankuta, Nepal. The rhizome buds were collected from the screen house and then cultured in Murashige and Skoog (MS) medium enriched with 9 different concentrations of BAP (6-benzylaminopurine) and IBA (indole-3-butyric acid) namely 0.5 mg/L (BAP), 1.0 mg/L (BAP), 1.5 mg/L (BAP), 2.0 mg/L (BAP), 1 mg/L (IBA), 0.5 mg/L (BAP)+ 1 mg/L (IBA), 1.0 mg/L (BAP)+ 1 mg/L (IBA), 1.5 mg/L (BAP)+ 1 mg/L (IBA) and 2.0 mg/L (BAP)+ 1 mg/L (IBA). This study showed that the explants grown in MS medium supplemented with 1.0 mg / L BAP + 1.0 mg / L IBA showed the maximum root induction rate. Buds produced roots in the same medium. The roots planted in the screen house were transplanted for the hardening process. Then these hardened plants were transferred to the netted nursery for further multiplication process. This protocol developed could be used for developing the superior quality plants of large cardamom.

Keywords: Acclimatization, explants, large cardamom, micropropagation.

Introduction

Large cardamom (*Amomum subulatum* Roxb.), family member, Zingiberaceae is the main cash crop grown in the eastern hills of Nepal. It is also cultivated in other parts of Central and even to Far western region of the country. Large cardamom is successfully cultivated in Nepal, India and Bhutan. The crop is a shadow loving plant (Sciophyte) grown in regions having an average rainfall of 3000-3500 mm/year. The large cardamom is used as a spice, as well as in several Ayurvedic preparations. It consists of essential oils of 23%, has carnal, gastric, diuretic and cardiac stimulants, and is also a remedy for the throat and respiratory diseases.

In larger cardamom propagation is done through seeds, suckers and through tissue culture techniques. Virus diseases do not transmit through the seeds and therefore there are no viral diseases in the seedlings. On the other hand, if high yielding plants are collected from diseased plants, planting with suckers will provide high efficiency of the transmission of the disease from the parents to new plantation. Similarly, micropropagation technique is the suitable method to overcome the constraints of poor germination of seeds, production of disease free saplings in mass scale in a short period of time. *In vitro* micropropagation technique in agriculture has been the best proven method for rapid clonal propagation for production of healthy and disease free high yielding plants in a mass scale in a short period of time. Since, large cardamom is regarded as the major source of income and a cash crop of farmers of eastern hills of Nepal. It is necessary to have an alternative method of propagation to overcome the problem of fungal and viral diseases which has influenced for the decline of the crop area.

In this study effort was made to establish in *vitro* protocol for large cardamom. This work is obviously a first step in the advancement of large cardamom tissue culture in Nepal. It is therefore, highly desirable to standardize a methodology for efficient *in vitro* culture to provide round the year of disease-free and quality planting materials.

Materials and Methods

The healthy rhizome buds were taken from screen house of Agricultural Research Station, Pakhribas, Dhankuta, Nepal. The collected explants or buds are cut into 1.5 to 2 cm lengths together with active shoots. The explants with active buds are placed in a beaker containing detergent solution for 10 minutes. The explants were then rinsed several times with fresh tap water. Then, the explants were surface sterilized for 10 minutes with 0.2% solution of bavistin, washed with sterile distilled water and finally transferred to laminar flow.

The explants were immersed again in 70% alcohol for 30 seconds and immersed in 0.1% (w/v) mercuric chloride ($HgCl_2$) for 5 minutes in laminar flow. Finally, the explants are thoroughly rinsed 3-4 times using sterile distilled water. The explants were placed on sterile blotting paper and the explants were used for *in vitro* culture before inoculation into pre-packed sterile agar medium in culture tubes (Smith and Hamill, 1996).

Sterile explants were kept in MS medium (Murashige and Skoog, 1962) containing various concentrations and combinations of growth hormone viz 0.5 mg/L (BAP), 1.0 mg/L (BAP), 1.5 mg/L(BAP), 2.0 mg/L (BAP), 1 mg/L (IBA), 0.5 mg/L (BAP)+ 1 mg/L (IBA), 1.0 mg/L (BAP)+ 1 mg/L (IBA), 1.5 mg/L (BAP)+ 1 mg/L (IBA) and 2.0 mg/L (BAP)+ 1 mg/L (IBA). The pH of the medium was adjusted to 5.8 before autoclaving for 21 minutes at a pressure of 1.04 kg/cm^2 and 121°C temperature. All cultures were incubated for 16 hours light /8 hours dark photoperiod (under cold, white fluorescent light).There were 20 culture tubes for each experiment that was repeated 3 times in a completely randomized block design. Cultures were maintained by preparing subcultures with the same composition every four weeks.

After root extraction, the seedlings were removed from the culture tube and the roots were washed with a flow tape to remove the agar. The seedlings were then transferred to a clay pots containing sterile sandy soil. The clay pots were placed in a growth chamber at a temperature of 25°C and a relative humidity of 70-80% for 2 weeks. It was then transplanted in nursery that contained soil, sand and farm yard manure (FYM) at a ratio of 1:2:2 in weight basis under the screen house for a month for acclimatization. The parameters recorded during the study were; number of shoots, height of the shoots, number of roots and length of the roots after five months before placing the plantlets into the growth chamber. Descriptive statistical analysis mainly the mean of each trait was taken.

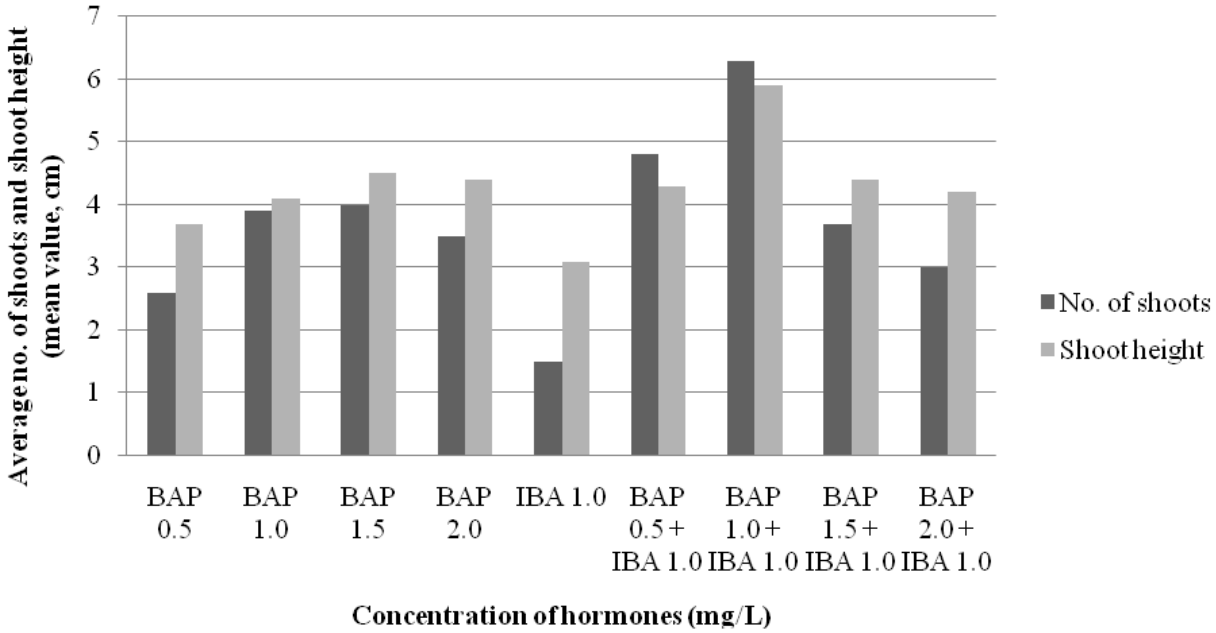

Figure 1. Effect of different concentrations of hormones on number and height of shoots of large cardamom.

Results and Discussion

The sterilized rhizome buds were placed in the culture tubes filled with the growing medium with different concentrations as well as combinations of BAP and IBA. The combination and concentrations were made to find the best combination for the multiplication of the shoots. The initial symptoms of shoot initiation and growth were after 4-5 weeks of first culture. The shoot growth and proliferation differed with the treatments. Treatment combination of MS + BAP (1.0 mg/L) + IBA (1.0 mg/L) is the most effective

condition for shoot proliferation (Figure 1 and Figure 3) though the shoot proliferation was noticed in all the treatments.

In the present study, a combination of BAP (0.5 mg/L) + IBA (1.0 mg/L) followed the best combination, and the success of micropropagation of other *Zingiberaceae* crops, such as *Zingiber officinale* (Hashim et al., 1998; Sharma and Singh, 1995; Bhat et al., 1994), *Curcuma longa* (Balachandran et al., 1990) has also been reported. In our study, under the combination of BAP (1.0 mg/L) + IBA (1.0 mg/L), the overall growths as well as average shoot length were clearly observed. Pradhan et al. (2014) also reported the maximum number of shoots was obtained on the medium containing BAP 3 mg/L + NAA 0.5 mg/L in large cardamom. Manohari et al. (2008) has reported the similar result in regeneration of small cardamom through somatic embryogenesis where shoot development was maximum from the treatment with 13.2 µM BAP + 0.5 µM NAA.

Well-developed large cardamom shoots were placed on medium provided with two different auxins; IBA and NAA for the rooting process. The concentrations used were 0.5, 1.0, 1.5, and 2.0 mg/L of both hormones (Figure 2). The growing media supplied with IBA (1.0 mg/L) was the best concentration for initiating the roots and their optimal growth. In this concentration, the average number of roots was found as 4.8 per culture and the average root length was 1.8 cm after 20 weeks of culture (Figure 2 and Figure 3). The root length was the maximum of the IBA concentration (1.5 mg/L) with an average value of 2.1 cm. Similarly, in the case of NAA, the number of roots was maximal at a concentration of 1.5 mg/L with 3.4 and a maximum root length of 1.5 cm from NAA (1.0 mg/L) (Figure 2). This result was similar to the results of Nyack et al. (1997) who found that the MS medium containing 1.0 mg/L of IBA was good for rooting in the *Praemorsa asampa*. Swar and Pant (2004) also found the best rooting on MS medium fortified with 1 ppm IBA in *Cymbidium iridiodes*. Pradhan et al. (2014) also reported the maximum number of roots was obtained on the medium containing BAP 3 mg/L + NAA 0.5 mg/L in large cardamom. Similarly, Sajina et al. (1997) reported that sprouted buds were multiplied at a rate of 5-10 shoots per culture in Murashage and Skoog medium fortified with BAP (1.0 mg/L) and IBA (0.5 mg/L).

For acclimatization process, the *in vitro* plantlets along with roots were transferred to the earthen pot in which sand, forest soil and compost were mixed at a ratio of 1:2:2 by weight. The plantlets were placed within the growing room for 2 weeks period and then transferred to the net house and screen house for further hardening process. Around 90% of the plantlets successfully survived during the acclimatization and hardening process in the screen house and net house. Finally, the plantlets were made available to the farmers for the establishment of the disease free cardamom field after 4-6 months period from Agricultural Research Station, Pakhribas.

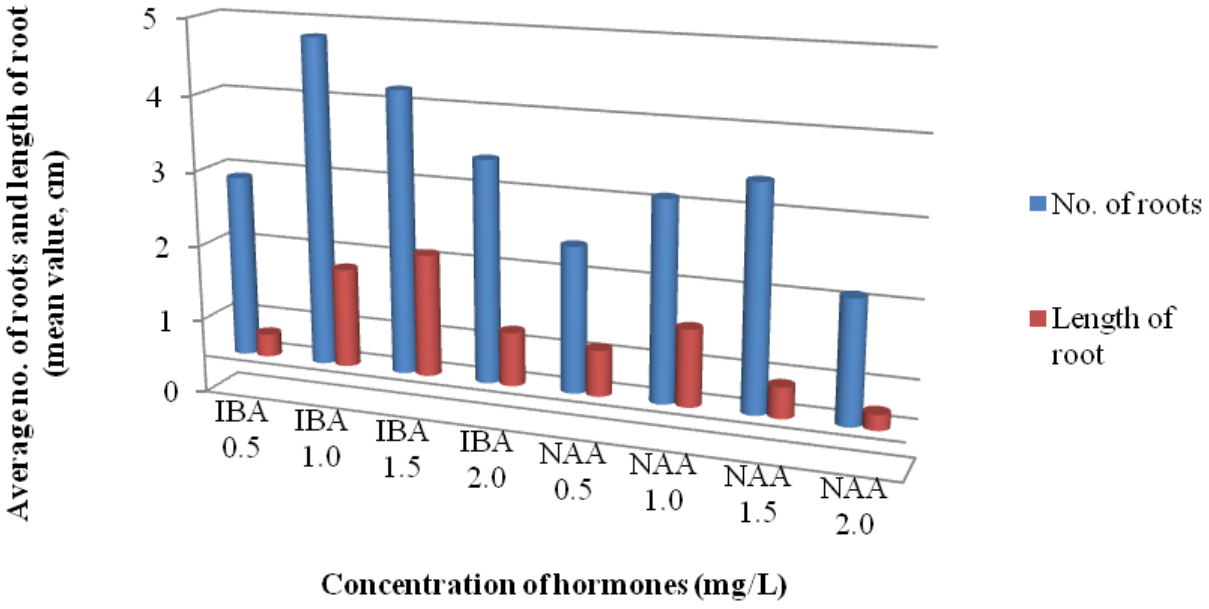

Figure 2. Effect of different concentration of hormones on number of roots and length of roots of large cardamom.

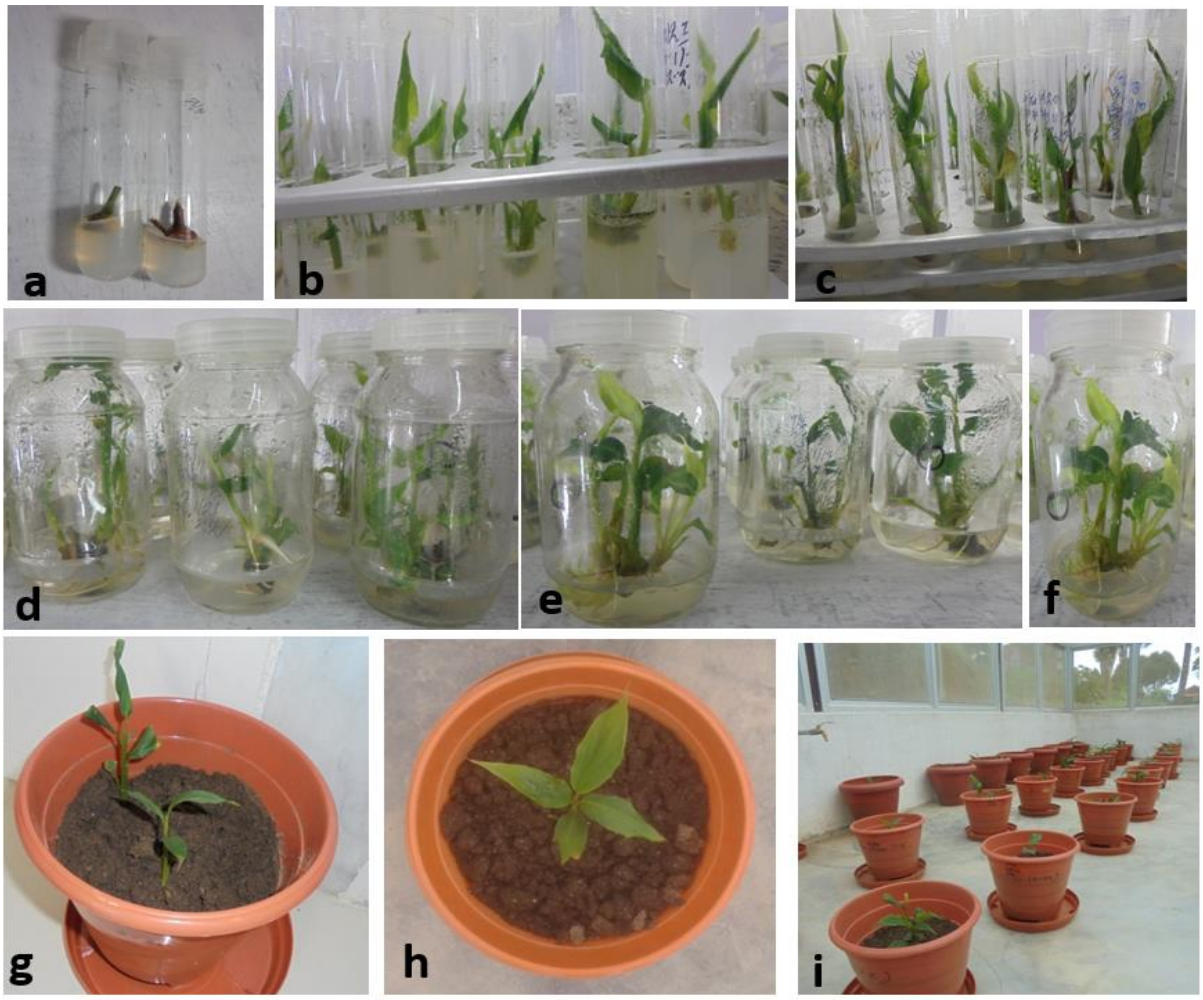

Figure 3. Different stages of micropropagation: **a.** Explant of cardamom. **b.** and **c.** Elongation of the explants. **d.** Initiation of roots. **e.** and **f.** Well developed roots from the explants. **g.** and **h.** Plantlets placed in the sterilized sand before acclimatization. **i.** Acclimatization of the plantlets under the screen house.

Conclusion

In vitro micro propagation is one of the best alternative methods of propagation for rapid clonal mass propagation for good and healthy high yielding plant with minimum disease. MS fortified with BAP (1.0 mg/L) and IBA (1.0 mg/L) was the best media for root and shoot induction. This protocol is effective for mass production and multiplication of large cardamom.

References

Balachandran, S.M., Baht, S.R., Chandel, K.P.S. 1990. *In vitro* clonal multiplication of turmeric (*Curcuma spp*.) and ginger (*Zingiber officinale* Rosc.). Plant Cell Reports. 8: 521-524.

Bhat, S.R., Chandel, K.P.S., Kacka, A. 1994. *In vitro* induction of rhizomes in ginger (*Zingiber officinale* Rosc.). Indian J. Exp. Biol. 32: 340-344.

Hashim, H.M., Ibrahim, H., Rahim, Z. 1998. Preliminary studies on some nutritional composition of the edible gingers. *In*: Advance in Biochemistry and Biotechnology in Asia and Oceania. Proceedings of the 7th Federation of Asia and Oceania Biochemists Symposium (FAOB). El-2.

Manohari, C., Backiyarani, S., Jebasingh, T., Somanath, A., Usha, R. 2008. Efficient plant regeneration in small cardamom (*Elettaria cardamomum* Maton.) through somatic embryogenesis. Indian Journal of Biotechnology. 7: 407-409.

Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Pradhan, S., Pradhan, S., Basistha, B.C., Subba, K.B. 2014. *In vitro* micropropagation of *Amomum subulatum* (zingiberaceae), a major traditional cash crop of Sikkim Himalaya. Int. J. LifeSc. Bt& Phar. Res. 3(2): 169-180.

Sajina, A., Mini, P.M., John, C.Z., Nirmalbabu, K., Ravindran, P.N., Peter, K.V. 1997. Micropropagation of large cardamom

(*Amomum subulatum* Roxb.). Journal of Spices and Aromatic Crops 6(2): 145-148.

Sharma, T.R., Singh, B.M. 1995. *In vitro* microrhizome production in *Zingiber officinales* Rosc. Plant cell Rep. 15: 274-277.

Smith, M.K., Hamill, S.D. 1996. Field evaluation of micropropagated and conventionally propagated ginger in subtropical Queensland. Australian J. Exp. Agricul. 36: 347-354.

Swar, S., Pant, B. 2004. Micropropagation of *Cymbidium iridiodes* D. Don. *In*: Proceeding 4th National Conference on Science and Technology, March 23-26, RONAST, Kathmandu, Nepal.