



Research Journal of
**Medicinal
Plant**

ISSN 1819-3455



Academic
Journals Inc.

www.academicjournals.com



Research Article

Influence of Plant Growth Regulators and Media strength on *In vitro* Propagation of *Amomum subulatum* Roxb

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Abstract

Background and Objective: Micro-propagation technique significantly improves the quality of the potentially important crops. In this study *in vitro* propagation of *Amomum subulatum* through sprouting bud was carried out to develop a suitable protocol for development of superior quality plantlets. **Materials and Methods:** Sprouting buds were sterilized and cultured into MS medium supplemented with different plant growth regulators. The data presented were average of the triplicate with standard deviation. **Results:** Augmentation of Murashige and Skoog (M.S.) medium with 2.0 mg L⁻¹ BAP (6-benzylaminopurine) recorded the highest number of shoot proliferation (8.07 ± 0.06 shoots/explant) followed by AS 3 (1.5 mg L⁻¹ BAP) with 6.23 ± 0.02 shoots/explant, AS 10 with 5.12 ± 0.02 and AS 7 (0.8 mg L⁻¹ BAP + 0.2 mg L⁻¹ IAA) with 5.09 ± 0.09 shoots. M.S. media AsR5 supplemented with 1 mg L⁻¹ IBA + 0.2 mg L⁻¹ IAA has highest root proliferation with 7.79 ± 0.11 roots/shoot. Media AsR4 (0.8 mg L⁻¹ IBA + 0.2 mg L⁻¹ IAA) has 60.06 ± 0.15 root/shoot followed by media AsR3 (0.6 mg L⁻¹ IBA + 0.2 mg L⁻¹ IAA) with 4.14 ± 0.04 roots/shoot. The maximum percentage of acclimatization, hardening and rhizomes production of *in vitro* derived plants in shade net house conditions was between 75-90%. **Conclusion:** The present study revealed that, highest growth of plantlets in terms of shoots developed/explant and number of leaves developed/shoots was achieved in MS media having BAP with the concentration of 2.0 mg L⁻¹. Likewise, 1.0 mg L⁻¹ BAP with 0.2 mg L⁻¹ of IAA also exhibit positive impact on root proliferation and root length. The protocol developed could be used for developing the superior quality of plants.

Key words: Growth regulators, tissue culture, plantlet, amomum, shoot proliferation, root proliferation

Citation: Arvind Kumar Bhandari, Mamta Baunthiyal, Vinod Kumar Bisht, Jagdish Chandra Kaim and Bir Singh Negi, 2017. Influence of plant growth regulator and medium strength on *in vitro* propagation of *Amomum subulatum* Roxb. Res. J. Med. Plants, 11: 160-166.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Amomum subulatum Roxb. (family: *Zingiberaceae*) is a spice grown commercially in India, Nepal, China and Bhutan in moist deciduous and semi-evergreen forests^{1,2}. It was also reported to have medicinal properties such as carminative, stomachic, diuretic, cardiac stimulant². Due to the economic potential of the crop, it was considered as boon to the growers of the Himalaya. Baunthiyal *et al.*³, reported that, the state of Sikkim and the Darjeeling district of West Bengal are the leading areas for its production in India. Being a cash crop of the state of Sikkim, it was registered as a geographical indication (GI-376) for the state. Realizing the economic potential of crop and presence of preferable habitat, its cultivation has also been started in the state of Uttarakhand, India¹. Multiplication of *A. subulatum* was generally carried out through sucker multiplication which may cause transmission of disease from mother plant to daughter plant and also deteriorate quality and quantity of the yield as there was no genetic variation. The other way of multiplication is through producing seedlings through seeds. But, in *A. subulatum* the germination of seeds is very poor and also takes very long time⁴. Different media and plant growth hormones were used for producing superior quality of planting material of *A. subulatum* through *in vitro* propagation technique^{5,6}. Again strength of growth media also influences the proliferation of shootlets and rootlets^{7,8}. The climatic parameters such as humidity, temperature, moisture in substrate etc. also plays vital role in survivability and growth of the plantlets.

To fetch good price from cultivation of *A. subulatum*, cultivation of superior quality of plantlets is prerequisite. Thus, to propagate desired taxon, independent of season, germination hurdles, barriers in reproducing the plant species and to produce quality planting material, *in vitro* regeneration is an efficient technique. Therefore, the present study was design to develop a protocol for raising superior quality plantlets of *A. subulatum* via *in vitro* technique.

MATERIALS AND METHODS

Collection of explant samples: The *in vitro* propagation study was carried out during the year 2016. Young explant of *A. subulatum* was collected from the nursery of Herbal Research and Development Institute, Mandal, Gopeshwar (Chamoli) Uttarakhand. The plant was identified by Botanical Survey of India, Dehradun, Uttarakhand, India with an Accession number 117262.

Surface sterilization: The explant plants were washed thoroughly under running tap water and the leaves and rhizome were trimmed off from the plant. Shoot pieces were excised from the bud and kept under running tap water for 30 min. Explant were disinfected by washing several times with sterile distilled water, followed by dipping in 70% ethanol (analytical grade) (v/v) for 10 sec, then immersing in a solution of 0.5% (v/v) sodium hypochlorite containing 1 drop of Tween-20 for 5 min and the step repeated three times. All chemicals used were of analytical grade. The explants were then taken to the laminar airflow chamber. Final washing step carried out in a laminar flow cabinet consisted of 3 rinses of 5 min each with sterile distilled water. Under aseptic conditions the explants were again treated with mercuric chloride (0.1%) for 3 min followed by thorough washing in sterile distilled water. The explants were trimmed to the appropriate size, making them ready for inoculation.

Culture media and conditions: Throughout the study, Murashige and Skoog (MS) culture media were semi solidified with using 0.7% (w/v) agar and containing 3% sucrose. The pH of the medium was adjusted to 5.8 and then autoclaved for 20 min at 121 °C (Model: Equitron #7433FA, Make: Medica Instruments, India). Cultures of all treatment were maintained under light condition 16 h day⁻¹ photoperiod at intensity of 3000 lux from cool white light of fluorescent lamp. The cultures were maintained at 25 ± 1 °C. Three replicate of each treatment were followed.

Multiplication stage: MS medium was supplemented with different concentrations of BAP, NAA (α -naphthaleneacetic acid), IAA (indole-3-acetic acid) and Kin (kinetin) to examine the effects on augmentation of shootlets multiplication. The designed experiment consisted of 14 treatments as shown in the Table 1. The parameters such as number of shoot lets/bud, shoot length, number of leaves/shoot were recorded with an interval of 4, 8 and 12 weeks of expansion.

Rooting stage: Shoot lets of 4-5 cm in size produced from *in vitro* multiplication were separated and cultured on solidified MS medium. MS nutrient media were augmented with different concentration of IBA alone and in combination of different concentration of IBA (indole-3-butyric acid) and IAA (indole-3-acetic acid). Moreover, different types of nutrient media viz. MS, NN, B5 and Heller's media at full or half strength were also used to examine the rootlets formation. The parameters such as number of roots/shoot and root length were recorded after 4 weeks of transplantation.

Table 1: Effect of different growth regulators with M.S. medium on shootlets multiplication of *Amomum subulatum* after 4 weeks

| Codes | M.S. medium supplemented with growth regulator | Number of shoots/explant | Number of leaves/shoots | Shoot length (cm) |
|-------|---|--------------------------|-------------------------|-------------------|
| AC | Control/free of growth regulators | 0.97±0.15* | 1.08±0.07 | 1.84±0.02 |
| AS1 | 0.5 mg L ⁻¹ BAP | 3.16±0.08 | 6.52±0.02 | 2.82±0.02 |
| AS2 | 1.0 mg L ⁻¹ BAP | 5.02±0.06 | 8.09±0.08 | 3.21±0.01 |
| AS3 | 1.5 mg L ⁻¹ BAP | 6.23±0.02 | 10.54±0.04 | 4.12±0.02 |
| AS4 | 2.0 mg L ⁻¹ BAP | 8.07±0.06 | 12.08±0.07 | 4.41±0.03 |
| AS5 | 0.4 mg L ⁻¹ BAP+0.2 mg L ⁻¹ NAA | 3.07±0.06 | 7.08±0.07 | 2.12±0.02 |
| AS6 | 0.6 mg L ⁻¹ BAP+0.2 mg L ⁻¹ NAA | 4.09±0.07 | 8.44±0.03 | 3.52±0.02 |
| AS7 | 0.8 mg L ⁻¹ BAP+0.2 mg L ⁻¹ NAA | 5.09±0.09 | 9.12±0.02 | 4.08±0.07 |
| AS8 | 0.4 mg L ⁻¹ BAP+0.2 mg L ⁻¹ IAA | 3.45±0.04 | 3.08±0.07 | 2.57±0.02 |
| AS9 | 0.6 mg L ⁻¹ BAP+0.2 mg L ⁻¹ IAA | 4.16±0.13 | 5.22±0.02 | 3.48±0.02 |
| AS10 | 0.8 mg L ⁻¹ BAP+0.2 mg L ⁻¹ IAA | 5.12±0.02 | 6.08±0.07 | 3.72±0.02 |
| AS11 | 1 mg L ⁻¹ Kn.+0.5 mg L ⁻¹ NAA | 2.08±0.07 | 5.12±0.02 | 2.05±0.06 |
| AS12 | 2 mg L ⁻¹ Kn.+0.5 mg L ⁻¹ NAA | 3.45±0.04 | 6.51±0.01 | 3.12±0.02 |
| AS13 | 3 mg L ⁻¹ Kn.+0.5 mg L ⁻¹ NAA | 4.08±0.02 | 7.09±0.08 | 4.09±0.08 |
| AS14 | 4 mg L ⁻¹ Kn.+0.5 mg L ⁻¹ NAA | 4.52±0.02 | 8.51±0.01 | 4.12±0.02 |

*± Standard Deviation (SD)

Hardening and *ex-vitro* acclimatization: *In vitro* grown plantlets were thoroughly washed with tap water to remove residual agar from roots. Then were immersed in 0.2% aqueous solution of Bavistin (fungicide) for 15-20 min and washed thoroughly with tap water. Thereafter, the plantlets were transplanted in pots filled with different substrate such as peatmoss, Peatmoss: Sand (1:1), Peatmoss: Vermiculite (1:1) and Soil: Sand: Cow dung (1:1:1) for acclimatization. The plantlets transplanted in different media were then covered with transparent polyethylene bags to maintain high humidity around the plantlets. After 1 week these plantlets were transferred to shade house for hardening. Survivability, height and number of leaves/plantlets place for acclimatization under shade net house conditions was also recorded.

The data presented were average of the triplicate with standard deviation.

RESULTS AND DISCUSSION

The effect of different growth regulators on shoot let multiplication, number of leaves/shoot and shoot length were presented in Table 1. Media AS 4 showed the highest number of shoot proliferation followed by AS 3, AS 10 and AS 7 (Fig. 1). The other treatments showed shoot proliferation of less than 5 leaves/explant. Sajina *et al.*⁹, also reported that, MS medium having BAP has positive impact of shoot proliferation.

Furthermore the result for shoot proliferation in present study was in agreement with the findings of Shukla *et al.*¹⁰, where medium containing BAP and NAA exhibit highest number of shoot proliferation. Likewise, MS media AS 4 showed the highest number of leaves/shoots followed by

AS 3 leaves/shoot and AS 7. Shukla *et al.*¹⁰, also reported that the media containing BAP produced the higher number of leaves in *Curcuma angustifolia*. Superior effects of BAP for shoot multiplication had been reported among various medicinal plants like *Tanacetum cinerarrifolium*¹¹ and *Spilanthes acmella*¹². Rajore and Batra⁵ used MS supplemented with 3.0 mg L⁻¹ IBA for rooting of *in vitro* propagated shootlets of *J. curcas*. However, Pradhan *et al.*¹³, found that MS media with different concentration of BAP and NAA showed highest leaves proliferation/shoots. The highest shoot length was achieved in the treatment AS 4, followed by AS 3 and AS 14. In other treatment the shoot length was less than 4 cm. The response of different media on shoot length was recorded highest in BAP and in combination of BAP and NAA. This showed that the combination of cytokinins and auxin in the culture medium enhanced the response of shoot development. Similar results were also obtained for *Spathiphyllum floribundum* when cultured on media with BAP alone, however adding of NAA in media enhance the growth of shoot¹⁴. Abbas *et al.*⁶, found that highest growth in shootlets was achieved in MS media having 4.5 mg L⁻¹ BAP.

The data on effect of different concentration of IBA and IAA in root proliferation and root length is presented in Table 2. The results showed that MS media AsR5 has highest root proliferation. Media AsR4 has 6.06±0.15 root/shoot followed by media AsR3 as shown in Fig. 2. The root proliferation of less than 4 roots/shoots was achieved in the other media examined. Thus it is concluded that the MS media of 1 mg L⁻¹ IBA+0.2 mg L⁻¹ IAA should be used for the purpose of root proliferation in *A. subulatum*. Rout *et al.*¹⁵, indicated that the use of either IAA or IBA in the culture medium positively affect the root induction in *Acacia chundra*.



Fig. 1(a-h): (a, b) Bud initiation, (c, d) Shootlets proliferation (e, f) Shootlets multiplication, (g) Bud proliferation, (h) Acclimatization of micro-propagated plants of *A. subulatum* under shad net house conditions

Table 2: Effect of MS medium supplement with different growth regulators concentrations IBA and IAA on rooting and root length after 4 weeks of observation under incubation (Illumination/dark period: 16/8 h day⁻¹)

| Codes | MS medium supplemented with | Rooting/shoots | Root length (cm) |
|-------|---|----------------|------------------|
| AsRC | Free of growth regulators | 1.36±0.02* | 2.73±0.03 |
| AsR1 | 0.2 mg L ⁻¹ IBA+0.2 mg L ⁻¹ IAA | 2.18±0.07 | 3.14±0.04 |
| AsR2 | 0.4 mg L ⁻¹ IBA+0.2 mg L ⁻¹ IAA | 3.52±0.03 | 4.92±0.03 |
| AsR3 | 0.6 mg L ⁻¹ IBA+0.2 mg L ⁻¹ IAA | 4.14±0.04 | 5.12±0.03 |
| AsR4 | 0.8 mg L ⁻¹ IBA+0.2 mg L ⁻¹ IAA | 6.06±0.15 | 5.32±0.04 |
| AsR5 | 1 mg L ⁻¹ IBA+0.2 mg L ⁻¹ IAA | 7.79±0.11 | 6.24±0.04 |
| AsR6 | 0.2 mg L ⁻¹ IBA | 1.95±0.03 | 2.21±0.03 |
| AsR7 | 0.4 mg L ⁻¹ IBA | 2.32±0.02 | 2.53±0.03 |
| AsR8 | 0.6 mg L ⁻¹ IBA | 2.62±0.02 | 3.81±0.03 |
| AsR9 | 0.8 mg L ⁻¹ IBA | 3.14±0.03 | 4.92±0.04 |
| AsR10 | 1 mg L ⁻¹ IBA | 3.38±0.02 | 5.20±0.05 |

*±Standard deviation (SD)

Datta *et al.*¹⁶, used MS supplemented with 0.2 mg L⁻¹ IBA for rooting of *in vitro* propagated shootlets of *J. curcas* and

reported 75% root induction. Thepsamran *et al.*¹⁷, cultured *in vitro* produced shootlets of *J. curcas* on MS media



Fig. 2(a-k): (a-e) Root proliferation and elongation, (f-h) Mature root lets, (i) Plants developed through *in vitro* multiplication, (j,k) Hardening and acclimatization of the plant lets in shade net house conditions

supplemented with 1.0 mg L^{-1} IBA for rooting. Kaewpoo and Te-chato¹⁸, reported that, MS media supplemented with 0.5 mg L^{-1} IBA was best for rooting of regenerated shootlets of *J. curcas*. Also, Akanksha *et al.*¹⁹, rooted *in vitro* propagated shootlets of *J. curcas* on MS media supplemented with 0.3 mg L^{-1} IBA and reported that IBA was more effective than IAA for rooting with rooting frequency of 86.67%. Moreover, the influence of different type of nutrient media at full and half strength on root proliferation and root length was also examined. In present study, it was observed that half strength media of MS, NN and Hiller's supplemented with 1 mg L^{-1} IBA improved root proliferation and root development except half strength media of B5 supplemented with 1 mg L^{-1} IBA. Highest root proliferation was achieved in half strength MS media as compared to full strength MS media. Likewise, in NN media, the root proliferation was higher in half strength media as compared to full strength media (Table 3). Similar trend was observed for Hiller's media, where root proliferation and

length of root was higher in half strength media as compared to full strength media (Table 3). The other studies also confirmed that half strength of media were more effective than full strength of media⁸. Also, it was observed that the root system formed with half strength media were well developed and widely branched. Further, result shows clear cut relationship between strength of media and root length, as the strength of media lowers, the development of root increase (Table 3). The finding of present study on effect of different strength of media on root proliferation and root length was in accordance with the findings of other studies^{7,20}. The *in vitro* derived plants were acclimatized in different substrate under shade net house conditions. Highest survivability was achieved in substrate Peatmoss, followed by substrate Soil: Sand: Cow dung (1:1:1), Peatmoss: Vermiculite (1:1). Substrate Peatmoss: Sand (1:1) showed the lowest survivability. The higher survivability of *in vitro* plantlets may be attributed to good climatic conditions with high

Table 3: Effect of different type of nutrient media at full and half strength supplemented with 1 mg L⁻¹ IBA on root development and root length

| Code | Type of nutrient media | Number of root/shoot | Root length (cm) |
|------|---|----------------------|------------------|
| AC | Sucrose and agar | 2.12±0.10* | 0.69±0.04 |
| A1 | Full strength MS+1 mg L ⁻¹ IBA | 6.13±0.11 | 3.35±0.06 |
| A2 | Half strength MS+1 mg L ⁻¹ IBA | 8.10±0.01 | 4.87±0.05 |
| A3 | Full strength NN+1 mg L ⁻¹ IBA | 4.08±0.07 | 2.94±0.03 |
| A4 | Half strength NN+1 mg L ⁻¹ IBA | 5.23±0.13 | 3.21±0.09 |
| A5 | Full strength B5+1 mg L ⁻¹ IBA | 4.90±0.05 | 3.39±0.03 |
| A6 | Half strength B5+1 mg L ⁻¹ IBA | 3.84±0.09 | 2.78±0.04 |
| A7 | Full strength Hiller's+1 mg L ⁻¹ IBA | 3.87±0.02 | 2.33±0.09 |
| A8 | Half strength Hiller's+1 mg L ⁻¹ IBA | 3.90±0.15 | 2.45±0.05 |

*±: Standard deviation (SD)

humidity. Moreover, high humidity and good climatic conditions under shade net house provides good environment to plantlets to rapidly transit from the heterotrophic to the photoautotrophic stage. Cha-um *et al.*²¹, also reported that *in vitro* acclimatization of ginger plantlets under high relative humidity with CO₂ enrichment produced plantlets with vigorous shoot and root systems. Highest plant height was achieved in substrate Peatmoss, followed by substrate Soil: Sand: Cow dung (1:1:1) and Peatmoss: Sand (1:1). Lowest plant height was recorded for substrate Peatmoss: Vermiculite. Likewise, highest number of leaves/plant was also achieved in substrate Peatmoss, while lowest number of leaves/plant was recorded for substrate Peatmoss: Vermiculite.

CONCLUSION

The *in vitro* propagation of *Amomum subulatum* indicated that Media AS 4 is good for micro-propagation as highest number of shoot proliferation, highest number of leaves/shoots and highest shoot length is achieved in this media. Likewise, for root proliferation and root length the best media was AS5. MS media with half strength and with 1 mg L⁻¹ of IBA is found good for root development. Survivability during hardening of plantlets was found higher in substrate Peatmoss. However, other substrates are also comparatively good in terms of survivability of plantlets during hardening.

SIGNIFICANCE STATEMENTS

This study evaluates the effects of the different concentration of phytohormones in the shoot and root proliferation of *Amomum subulatum*. The present study revealed that BAP at a comparatively higher concentration exhibit maximum shoot proliferation while higher concentration of IBA with lower concentration of IAA showed higher root proliferation. The findings of this study are useful as a platform for further development to improve the protocol and to produce superior quality plants.

REFERENCES

1. Bisht, V.K., J.S. Negi, A.K. Bhandari and R.C. Sundriyal, 2011. *Amomum subulatum* Roxb: Traditional, phytochemical and biological activities-An overview. Afr. J. Agric. Res., 6: 5386-5390.
2. Bhandari, A.K., V.K. Bisht, J.S. Negi and M. Baunthiyal, 2013. 1, 8-Cineole: A predominant component in the essential oil of large cardamom (*Amomum subulatum* Roxb.). J. Med. Plants Res., 7: 1957-1960.
3. Baunthiyal, M., A.K. Bhandari, V.K. Bisht and N. Singh, 2016. A Socio-Pecuniary Citation for Geographical Indications, Awareness in India. In: Agro-Biodiversity Conservation and Sustainable Development, Sharma, P., N. Singh and P.K. Bharti (Eds.). Discovery Publishing House, Delhi, India, ISBN: 9789350567821, pp: 89-100.
4. Anonymous, 1998. Spices Board's annual report (1997-98). Spices Board, Sugandha Bhawan, Cochin, India.
5. Rajore, S. and A. Batra, 2007. An alternative source for regenerable organogenic callus induction in *Jatropha curcas* L. Indian J. Biotechnol., 6: 545-548.
6. Abbas, M.S., H.S. Taha, U.I. Aly, H.M. El-Shabrawi and E.S.I. Gaber, 2011. *In vitro* propagation of ginger (*Zingiber officinale* Rosco). J. Genet. Eng. Biotechnol., 9: 165-172.
7. Dhabhai, K., M.M. Sharma and A. Batra, 2010. *In vitro* clonal propagation of *Acacia nilotica* (L.)-A nitrogen fixing tree. Researcher, 2: 7-11.
8. Bhatia, P. and N. Ashwath, 2005. Effect of medium pH on shoot regeneration from the cotyledonary explants of tomato. Biotechnology, 4: 7-10.
9. Sajina, A., P.M. Mini, C.Z. John, K. Nirmal Babu, P.N. Ravindran and K.V. Peter, 1997. Micropropagation of large cardamom (*Amomum subulatum* Roxb.). J. Spices Aromatic Crops, 6: 145-148.
10. Shukla, S.K., S. Shukla, V. Koche and S.K. Mishra, 2007. *In vitro* propagation of tikur (*Curcuma angustifolia* Roxb.): A starch yielding plant. Indian J. Biotechnol., 6: 274-276.
11. Hedayat, M., G. Abdi and M. Khosh-Khui, 2009. Regeneration via direct organogenesis from leaf and petiole segments of pyrethrum [*Tanacetum cinerariifolium* (Trevir.) Schultz-Bip.]. Am.-Eurasian J. Agric. Environ. Sci., 6: 81-87.

12. Saritha, K.V. and C.V. Naidu, 2008. Direct shoot regeneration from leaf explants of *Spilanthes acmella*. *Biologia Plantarum*, 52: 334-338.
13. Pradhan, S., S. Pradhan, B.C. Basistha and K.B. Subba, 2014. *In vitro* micropropagation of *Amomum subulatum* (Zingiberaceae), a major traditional cash crop of Sikkim Himalaya. *Int. J. Life Sci. Biotechnol. Pharma Res.*, 3: 169-180.
14. Malamug, J.J.F., H. Inden and T. Asahira, 1991. Plantlet regeneration and propagation from ginger callus. *Scient. Hortic.*, 48: 89-97.
15. Rout, G.R., S.K. Senapati and S. Aparajita, 2008. Micropropagation of *Acacia chundra* (Roxb.) DC. *Hortic. Sci.*, 35: 22-26.
16. Datta, M.M., P. Mukherjee, B. Ghosh and T.B. Jha, 2007. *In vitro* clonal propagation of biodiesel plant (*Jatropha curcas* L.). *Curr. Sci.*, 93: 1438-1442.
17. Thepsamran, N., C. Thepsithar and A. Thongpukdee, 2008. *In vitro* induction of shoots and roots from *Jatropha curcas* L. explants. *J. Hortic. Sci. Biotechnol.*, 83: 106-112.
18. Kaewpoo, M. and S. Te-chato, 2009. Influence of explant types and plant growth regulators on multiple shoot formation from *Jatropha curcas*. *Sci. Asia*, 35: 353-357.
19. Akanksha, D., O. Shubham, G. Neelam, K.L. Pandey, S. Pramila and A. Mohommad, 2010. *In vitro* regeneration of *Jatropha curcas* L. using petiole explants. *Biochem. Cell. Arch.*, 10: 7-12.
20. Sivanesan, I. and B.R. Jeong, 2007. Direct shoot regeneration from nodal explants of *Sida cordifolia* Linn. *In vitro Cell Dev. Biol.-Plant*, 43: 436-441.
21. Cha-um, S., N.M. Tuan, K. Phimmakong and C. Kirdmanee, 2005. The *ex vitro* survival and growth of ginger (*Zingiber officinale* Rocs.) influence by *in vitro* acclimatization under high relative humidity and CO₂ enrichment conditions. *Asian J. Plant Sci.*, 4: 109-116.