IN VITRO INDUCTION OF ADVENTITIOUS ROOT FROM SHOOT BUD OF *BOESENBERGIA ROTUNDA* (ZINGIBERACEAE): EFFECT OF PLANT GROWTH REGULATORS

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ABSTRACT: *Boesenbergia rotunda is an important medicinal plant. It requires a long period of time to produce in large scale. Secondary metabolites in B. rotunda show that it has many biological and pharmacological activities such as antioxidant, antibacterial, antitumor, anti-inflammatory and anti-cancer activities. However, this plant has low content of secondary metabolites in cell suspension cultures and callus culture. This study was conducted to establish the protocol for adventitious root induction from shoot bud of B. rotunda. Surface sterilization with T3 (60% Clorox for 30 minutes; 20% Clorox for 15 minutes) was the best treatment to establish the aseptic explants with mean value of 4.667 ± 0.724. For the adventitious root induction, half strength of Murashige and Skoog (MS) was supplemented with various concentrations of auxins; 1-Naphthaleneacetic acid (NAA), Indole-3-butyric acid (IBA), 2,4-Dichlorophenoxyacetic acid (2,4-D) and Indole-3-acetic acid (*IAA*) with a range (0.5 mg/L-3.0 mg/L) either alone or with combination of cytokinins; kinetin (Kn) and 6-Benzylaminopurine (BAP) with a range of 0.1 mg/L to 0.5 mg/L. The most effective treatment for adventitious root induction was supplemented with 0.5 mg/L NAA which produced the highest number and length of roots with a mean value of 7.667 ± 2.082 and 1.733 ± 0.416 respectively. The most effective adventitious root induction for combination of auxin and cytokinin was supplemented with 0.5 mg/L NAA+0.1 mg/L Kn that produced the highest number and length of roots with a mean value of 3.400 ± 2.408 and 1.680 ± 1.211 respectively. The result shows that the culture condition is a good approach for enhancing the secondary metabolite content in adventitious root cultures.*

Keywords: Adventitious root, medicinal plant, *Boesenbergia rotunda*, Zingiberaceae, culture condition

1. INTRODUCTION

Boesenbergia rotunda L. (*B. rotunda*) is formerly known as *Boesenbergia* or *Kaempferia pandurata* (Roxb. Schltr.). As regards its natural activities, the rhizomes of this plant have been used for the treatment of peptic ulcer, as well as colic, oral diseases, urinary disorders, dysentery, inflammation [1], gastrointestinal disorder, and aphrodisiac [2]. Conventionally, *B. rotunda* requires a long period of time to produce in large scale as it is usually propagated through rhizome cuttings. [3] reported the conventional way of propagation is by splitting the rhizome of *Kaempferia galangal*, which also belongs to family Zingiberaceae is not suitable for rapid planting to meet the need of propagules and it takes years to build up for commercial quantities. *Boesenbergia* species is extremely uncommon compared to other genera because primarily, they were found in very damp, in the shady areas and usually near to streams or in swampy conditions. The plants' life cycle was about five months and breeding is seriously handicapped by poor fruit setting [4] since *B. rotunda* is a monocotyledonous plant. Furthermore, pathogenic fungi were readily transmitted through traditional practices and *B. rotunda* is high susceptibility to rot diseases. Application of tissue culture techniques is a solution to this problem [5]. Hence, for the biotechnological approach, *in vitro* culture is the best method as a continuous source supplies of disease-free planting material for commercial utilization because it offers an opportunity to exploit the cell, tissue, organ or the entire organism by growing them *in vitro*. *In vitro* techniques is known to be more consistent, can be controlled and relatively more stable. Shoot cultures and root cultures for the production of medicinally important compounds have been attempted, but no research on induction of adventitious root in *B. rotunda*. Therefore, this study was attempted to establish the protocol for *in vitro* adventitious root induction from shoot bud of *B. rotunda*.

2. MATERIALS AND METHODS

2.1 Plant material

The tissue culture experiment was conducted in the laboratory at the Faculty of Plantation and Agrotechnology, UiTM Puncak Alam. Rhizomes of *B. rotunda* were collected from Kuala Krau, Pahang. Healthy rhizomes with active shoot bud were excised to be used as explants and were washed thoroughly under running tap water to remove adhering soil particles.

2.2 Culture Media and Growth Condition

Half strength of Murashige and Skoog (MS) medium [6] containing 30.0 g/L sucrose, solidified with 2.0 g/L gelrite were used. The pH of all media was adjusted to 5.8 with 1.0 M sodium hydroxide (NaOH) and/or 1.0 M hydrochloric acid (HCl) prior to autoclaving. The cultures were maintained in a growth room at 25±1ºC under darkness.

2.3 Establishment of aseptic explants

To control the microbial contamination, explants were placed under running tap water for 20 min, then were soaked with 60% concentration of Clorox™ (5.25% sodium hypochlorite, NaOCl is the active ingredient) with 4 drops of Tween 20™ for 30 min, followed by rinsing once with sterilized distilled water. The outer layer was trimmed and the explants were further disinfected with 20% concentration of Clorox™ with 4 drops of Tween 20™ with three different treatments; T1, T2, T3 with time exposures of (5, 10, 15) min respectively and were rinsed with sterile distilled water for eight times. The explants were dipped in 95% ethanol (EtOH) for 1 min and were rinsed twice with sterile distilled water. The explants were air dried in the sterile plate and cultured onto initiation media. All steps were carried out in laminar air flows. This experiment was done in fifteen replicates with five explants per replicate.

Table 1. The number of survival, contaminated and dead aseptic cultures of *B. rotunda* obtained from surface sterilization treatments.

Treatments	Number of survival (Mean±SD)	Number of contaminated (Mean±SD)	Number of dead (Mean±SD)
T1	2.067 ±1.710ab	2.867±1.807bc	0.067±0.258abc
T2	3.267±2.187abc	1.733±2.187bc	0.00±0.00abc
T3	4.667±0.724bc	0.133±0.352a	0.200±0.561abc

*Data represent mean and standard deviation. Values are the mean of fifteen replicates. Mean values followed by the same alphabet are not significantly differencing at p=0.05 (by Tukey's Test)

2.4 Adventitious root induction and culture condition

Half strength of MS medium containing 30 g/L sucrose, solidified with 2 g/L gelrite were supplemented with various concentrations of plant growth regulators (PGRs); NAA (0.5 to 3.0 mg/L), IBA (0.5 to 3.0 mg/L), 2,4-D (0.5 to 3.0 mg/L) and IAA (0.5 to 3.0 mg/L) either alone or with combination of cytokinin; Kn (0.1 to 0.5 mg/L) and BAP (0.1 to 0.5 mg/L). MS medium without PGRs was used as a control treatment. Rooted cultures were maintained on the same media by regular subculturing at 4-week interval over a period of 8 weeks. Each treatment using PGRs alone and treatment using a combination of cytokinin consisted of five replications. Each treatment was replicated with three aseptic explants per replicate.

2.5 Experimental design and statistical analysis

The data of each parameter were analyzed using One-Way ANOVA followed by mean comparison with Tukey test (HSD) at $p \leq 0.05$ using SPSS version 16.0. For adventitious root induction, the growth parameters such as number of adventitious roots formed and length of adventitious roots formed from each explant were observed, recorded and analyzed using One-Way ANOVA followed by mean comparison with Tukey test (HSD) at $p \leq 0.05$ using SPSS version 16.0.

3. RESULTS AND DISCUSSIONS

3.1 Effects of different time exposures of Clorox™ on aseptic explants

The success of *in vitro* techniques largely depends on the availability of efficient tissue culture protocols. Being a monocotyledonous crop, *B. rotunda* was one of Zingiberaceae species that was difficult to establish the initial of contamination-free cultures due to the fact that rhizomes originate from the field [7]. In this study, the best treatment for establishing an aseptic culture from shoot bud of *B. rotunda* with the highest survival rate was T3 with mean value 4.667 ± 0.724, followed by T2 (3.267 ± 2.187) and T1 (2.067 ± 1.710) (Table 1). Among three treatments tested, only T3 shown the significantly different (p=0.05) for the number of survival rate explants. [8] reported that double sterilization method yielded 80% of contamination free nodal explants in *P. santalinus* by using 70% EtOH for 2 min followed by 0.1% (w/v) aqueous mercuric chloride solution for 7 min. However, mercuric chloride is a hazardous chemical with disposal problems. Therefore, it is more eco-friendly and secure to propose other surface sterilization procedure that does not use mercuric chloride. For this study, sodium hypochlorite was used to replace the mercuric chloride. The present studies showed the applicability using 60% and 20% Clorox™ for 30 min and 15 min respectively followed by EtOH for 1 min could establish more than 80% aseptic and surviving explants. The explants free with contamination after four weeks cultured in half strength of MS medium (Figure 1B). This is concurrent to previous research by [9] that demonstrated the applicability of 15 % Clorox™ for 10 min, followed by 70 % EtOH for 2 min as surface sterilization agents for immature stem cuttings of *P. santalinus*.

3.2 Effects of different PGRs for adventitious root induction

As the protocol for adventitious root induction of *B. rotunda* has not been published, a broad range of treatments was designed to determine the most effective PGRs condition for the adventitious root induction. The half strength of MS medium was supplemented with various concentration of PGRs (NAA, IBA, 2,4-D, IAA) with a range of 0.5 to 3.0 mg/L either alone or with a combination of Kn and BAP with a range of 0.1 to 0.5 mg/L has been used. The culture medium is significant aspect on micropropagation of plant species, the culture environments such as light and temperature are also one of the important factors. The cultures were generally incubated under the photoperiod regime of darkness with 60-70% relative humidity and temperature of 22 ± 2ºC in the culture room [10]. In this study, the best treatment for adventitious root induction was T1, which was supplemented with 0.5 mg/L NAA after had been incubated for eight weeks onto half strength of MS media (Figure 2). Among the treatments tested, only T1 had shown significant different (p=0.05) for the rooting induction and also produced the highest number and length of roots with a mean value of 7.667 ± 2.082 and 1.733 ± 0.416 respectively (Table 2). The number of roots induced differed as the concentration of PGRs varied. According to Table 2, the second highest number of roots produced was T5 with a mean value of 4.333 ± 7.506, followed by T7 (2.000 ± 3.464), T8 (1.000 ± 1.732) and T2 (0.333 ± 0.577) respectively. These results are in accordance with [11] which reported that high concentrations of plant growth regulator reduce shoot elongation and rooting on micropropagation. Subsequently, the second highest length of roots produced was T5 with a mean value of 1.000 ± 1.732, followed by T7 (0.723 ± 1.253), T8 (0.667 ± 1.155) and T2 (0.500 ± 0.866) respectively.

Figure 1. A. Shoot buds of *B. rotunda*. Bar at 2.0 cm. B. Aseptic culture after 4 weeks. Bar at 1.0 cm.

The result was concurrent with research done by [12] which mentioned that NAA was more effective than IBA in root induction. These results were contrary to study done by [13], which stated that IBA was more effective when compared to NAA for root formation in meristem culture. According to [14], the maximum rooting to multiple shoots was noted on the half strength of MS medium with 0.5 mg/L NAA in turmeric, which supported this research as low concentrations of NAA and IBA produced multiple shoots and root induction.

Treatments that supplemented with 2,4-D and IAA shows no response for adventitious root induction. However, within four weeks of culture, swelling of explants was observed and after another two weeks, callus emerged from the explants. The callus was friable, compact and cream coloured. This result was supported by a previous study done by [15] stated that the addition of 2,4-D and IAA produced callus with 53.4% after four weeks of culture. Contrary, research done by [16] reported that NAA alone or in combination with BAP multiples callus faster than 2,4-D supplements. Auxin was an essential factor for induction rather than initiation of roots in plants [17], which verified the hypothesis that the adventitious root formation initially occurred in two phases: an auxin-sensitive phase and an auxin-insensitive phase [18].

As a synthetic auxin, NAA was generally used at a relatively low dose to elicit auxin-type responses in cell growth, cell division, fruit setting, rooting, etc [19, 20].The addition of cytokinin (Kn, BAP) to auxin (NAA) with low concentration into half strength of MS medium facilitated the induction of roots, more than when NAA was used singly. The best root induction medium i.e. ½ MS + 0.5 mg/L NAA (Table 2) with the addition of cytokinin was T1, which was supplemented with 0.1 mg/L Kn (Table 3), with a mean value of 3.400 ± 2.408 for number of roots and an average length of root per culture with a mean value of 1.680 ± 1.211. The second best treatment was T6 which was supplemented with 0.1 mg/L BAP with a mean value of 0.800 ± 1.789 for a number of roots, with an average length of root per culture of 0.720 ± 1.610 respectively (Table 3). The roots were with tufts of root hairs (Figure 3). As the culture period went on along for eight weeks, the narrow and white roots became very long and diverged. As reported by [20, 21], the adventitious root production was increased rapidly at lower concentrations of NAA, while the number of roots was decreased at higher concentration, which was similar to this study. According to [22], the combination of BAP & NAA was effective for the multiplication of *Kaempferia galanga* L. The result of this study was differ with [23] which reported that medium containing BAP decreased root induction for *Kaempferia galanga* L. and in ginger [13].

4. CONCLUSION

B. rotunda is an important aromatic and medicinal plant. It is traditionally propagated through rhizome segments. For conventional propagation, it is considered as low multiplication rate as the plant easily attacked by fungus. An optimum size of sterilized explants and proper culture medium essentially required *in vitro* cultures. The best treatment to establish aseptic explants wasdouble sterilization method using 60% and 20% Clorox® with 30 min and 15 min exposure respectively followed by 95% EtOH for 1 min.

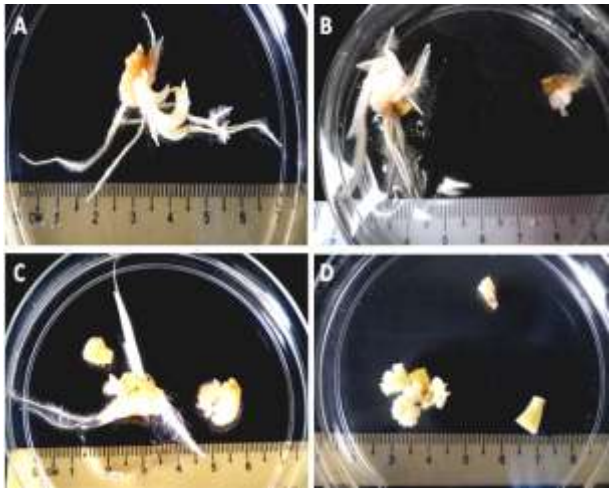

Figure 2: Adventitious roots induction when supplemented with different concentration of PGRs; (A) 0.5 mg/L NAA; (B) 2.5 mg/L NAA; (C) 0.5 mg/L IBA; (D) 0.5 mg/L 2,4-D

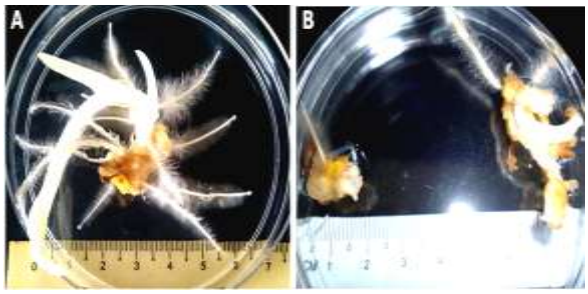

Figure 3: Adventitious roots induction when supplemented with combination of PGRs;
(A) 0.5 mg/L NAA+0.1 mg/L Kn; (B) 0.5 mg/L NAA+0.1 mg/L

The half strength of MS medium supplemented with a low concentration of PGRs (NAA, IBA, 2,4-D, IAA) promotes rooting in *B. rotunda*. ½ MS+0.5mg/L NAA+0.1 mg/L Kn was the ideal plant growth regulator for the adventitious root formation. This study is useful as a baseline for chemical properties, molecular biology and pharmacology research of *B. rotunda*.

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The response of different treatment concentrations on the number of roots and length of roots of *B. rotunda* cultured onto half strength of MS medium after 8 weeks of culture.

Table 2. The number of roots, average length of roots, average number of shoots and number of calli of the adventitious root of *B. rotunda* produced for each treatment with different PGRs after eight weeks in cultures.

Treatments	PGRs (mg/L)	Number of roots (Mean±SD)	Average length of roots (Mean±SD)	Average number of shoots (Mean±SD)	Number of calli (Mean±SD)
T1	0.5 NAA	7.667 ± 2.082d	1.733 ± 0.416cd	0.000 ± 0.000ab	0.000 ± 0.000ab
T2	1.0 NAA	0.333 ± 0.577ab	0.500 ± 0.866bc	2.000 ± 3.464c	0.000 ± 0.000ab
T3	1.5 NAA	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab
T4	2.0 NAA	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab
T5	2.5 NAA	4.333 ± 7.506c	1.000 ± 1.732bc	0.000 ± 0.000ab	0.000 ± 0.000ab
T6	3.0 NAA	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab
T7	0.5 IBA	2.000 ± 3.464bc	0.723 ± 1.253bc	0.333 ± 0.577ab	0.000 ± 0.000ab
T8	1.0 IBA	1.000 ± 1.732ab	0.667 ± 1.155bc	0.000 ± 0.000ab	0.000 ± 0.000ab
T9	1.5 IBA	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab
T10	2.0 IBA	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab
T11	2.5 IBA	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab
T12	3.0 IBA	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab
T13	0.5 2,4-D	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	1.000 ± 1.000bc
T14	1.0 2,4-D	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab
T15	1.5 2,4-D	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab
T16	2.0 2,4-D	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab
T17	2.5 2,4-D	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab
T18	3.0 2,4-D	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab
T19	0.5 IAA	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.333 ± 0.577b
T20	1.0 IAA	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.333 ± 0.577b
T21	1.5 IAA	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	1.000 ± 1.000bc
T22	2.0 IAA	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.333 ± 0.577b
T23	2.5 IAA	0.000 ± 0.000ab	0.000 ± 0.000ab	0.000 ± 0.000ab	0.333 ± 0.577b
T24	3.0 IAA	0.000 ± 0.000ab	0.000 ± 0.000ab	4.333 ± 1.528d	2.333 ± 0.577d

*Data represent mean and standard deviation. Values are the mean of five replicates. Mean values followed by the same alphabet are not significantly differencing at p=0.05 (by Tukey's Test)

Table 3. The number of roots and the average length of roots of the adventitious root of *B. rotunda* produced for each treatment with different concentration of PGRs after eight weeks in cultures.

Treatments	PGRs (mg/L)	Number of roots (Mean±SD)	Average length of roots (Mean±SD)
T1	0.5 NAA + 0.1 KIN	3.400 ± 2.408cd	1.680 ± 1.211cd
T2	0.5 NAA + 0.2 KIN	0.000 ± 0.000ab	0.000 ± 0.000ab
T3	0.5 NAA + 0.3 KIN	0.000 ± 0.000ab	0.000 ± 0.000ab
T4	0.5 NAA + 0.4 KIN	0.000 ± 0.000ab	0.000 ± 0.000ab
T5	0.5 NAA + 0.5 KIN	0.000 ± 0.000ab	0.000 ± 0.000ab
T6	0.5 NAA + 0.1 BAP	0.800 ± 1.789b	0.720 ± 1.610bc
T7	0.5 NAA + 0.2 BAP	0.000 ± 0.000ab	0.000 ± 0.000ab
T8	0.5 NAA + 0.3 BAP	0.000 ± 0.000ab	0.000 ± 0.000ab
T9	0.5 NAA + 0.4 BAP	0.000 ± 0.000ab	0.000 ± 0.000ab
T10	0.5 NAA + 0.5 BAP	0.000 ± 0.000ab	0.000 ± 0.000ab

*Data represent mean and standard deviation. Values are the mean of five replicates. Mean values followed by the same alphabet are not significantly differencing at p=0.05 (by Tukey's Test)

REFERENCES

[1]Trakoontivakorn, G., Nakahara, K., Shinmoto, H., Takenaka, M., Onishi-Kameyama, M., Ono, H., Yoshida, M., Nagata, T. and Tsushida, T. Structural analysis of a novel antimutagenic compound,4- hydroxypanduratin A, and the antimutagenic activity of flavonoids in a Thai spice, fingerroot (*Boesenbergia pandurata* Schult.) against mutagenic heterocyclic amines. *Journal Agriculture Food Chemistry*,**49**: 3046-3050 (2001).

[2]Saralamp, P., Chuakul, W., Temsiririrkkul, R. and Clayton, T. *Medicinal Plants in Thailand*, Vol I, Amarin Printing and Publishing Public Co., Ltd., Bangkok, p. 49(1996).

[3]Kalpana, M. and Anbazhagan, M. *In vitro* production of *Kaempferia galanga* (L.) - An endangered medicinal plant. *Journal of Phytology*, **1(1)**: 56-61(2009).

[4]Larsen, K., Ibrahim, H., Khaw, S.H. and Saw, L.G., *Gingers of Peninsular Malaysia and Singapore.* Nat. Hist. Publ., Borneo (1999).

[5]Nayak, S. and Naik, P.K. Factors Effecting *In vitro* Microrhizome Formation and Growth in *Curcuma longa* L. and Improved Field Performance of Micropropagated Plants. *Science Asia*, **32**: 31-37(2006).

[6]Dani, F., Spiridon, K., Athanasios, S.E., Georgia, M., and Helen-Isis, A.C. Effect of Different Strength of Medium on Organogenesis, Phenolic Accumulation and Antioxidant Activity of

Spearmint (*Menta spicata* L.). *The Open Horticulture Journal*, **3**:31-35(2010).

[7]Hosoki, T. and Sagawa, Y. Clonal propagation of ginger (*Zingiber officinale* Rosc.) through tissue culture. *Hort. Sci.* **12**:451- 452(1977).

[8]Prakash, E., Khan, P.S.S.V., Rao, T.J.V.S. and Meru, E. S. Micropropagation of red sanders (*Pterocarpus santalinus* L.) using mature nodal explants. *Journal of Forest Research*, **11**:329- 335(2006).

[9]Warakagoda, P.S. and Subasinghe, S. *In vitro P. santalinus* L. (Red Sandalwood) through tissue culture. *J. Natn. Sci. Foundation Sri Lanka*, **41(1)**:53- 63 (2013).

[10]Lincy, A. and Sasikumar, B. Enhanced adventitious shoot regeneration from aerial stem explants of ginger using TDZ and its histological studies. *Turk. J. Bot.*, **34**: 21-29(2010).

[11]Thayamini, H. S. *In vitro* Propagation of Ginger (*Zingiber officinale* Rosc.) through Direct Organogenesis: A Review. *Pakistan Journal of Biological Sciences*, 1028-8880(2014).

[12]Kambaska, K.B., and Santilata, S. Effect of plant growth regulator on micropropagation of ginger (*Zingiber officinale* Rosc.) cv- Suruchi. *J. Agric. Technol.*, **5**: 271-280(2009).

[13]Bhagyalakshmi, N. and Singh, N.S. Meristem culture and micropropagation of a variety of ginger (*Zingiber officinale* Rosc.) with a high yield of oleoresin. *J. Hort. Sci.* **63**:321– 327(1988).

[14]Dipti, T., Ghorade, R.B., Swati, M., Pawar, B.V. and Ekta, S. Rapid multiplication of turmeric by micropropagation. *Ann. Plant Physiol.*,**19**:35-37(2005).

[15]Yusuf, N. A., Annuar, M. S. M., and Khalid, N. Existence of bioactive flavonoids in rhizomes and plant cell cultures of *Boesenbergia rotunda* (L.) Mansf. Kulturpfl. *Australian Journal of Crop Science*, **7(6)**:730-734(2013).

[16]Chirangini, P., Sinha, S. K. and Sharma G. J. *In vitro* propagation and microrhizome induction in *Kaempferia galanga* Linn. and *K. rotunda Linn. Indian Journal of Biotechnology*, **4**: 404-408(2005).

[17]Nag, S., Saha, K. and Choudhuri, M.A. Role of auxin and polyamines in adventitious root formation in relation to changes in compounds involved in rooting. *J. Plant Growth Regul.* **20**: 182–194(2001).

[18]Hartmann, H.T., Kester, D.E. and Davies, F.T. Plant propagation: principles and practices. India: Prentice Hall. (1968).

[19]Srivastava, L. M. Plant growth and development: hormones and environment. San Diego (CA): Academic(2002).

[20]Sun, Y. L. and Hong, S. K. Effects of plant growth regulators and L-glutamic acid on shoot organogenesis in the halophyte *Leymus chinensis* (Trin.). *Plant Cell Tiss Organ Cult* **100**: 317–328(2010).

[21]Khan, S. and Bi, T. B. Direct shoot regeneration system for date palm (*Phoenix dactylifera* L.) cv. Dhakki as a means of micropropagation. *Pak. J. Bot.* **4419** 65– 71(2012).

[22]Shirin, F., Kumar, S. and Mishra Y. *In vitro* plantlet production system of *Kaempferia galanga*, a rare Indian medicinal herb. *Plant Cell, Tissue and Organ Culture* **63**: 193-197(2000).

[23]Chithra, M., Martin, K.P., Sunandakumaria, C. and Madhusoodanana, P.V. Protocol for rapid propagation, and to overcome delayed rhizome formation in. *Horticulturae*,**104**:13-120(2005)