Micropropagation of *Boesenbergia pulchella* (Ridl.) Merr., a Potential Ornamental Plant

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Abstract

Shoot tips of *Boesenbergia pulchella* (Ridl.) Merr. were cultured on Gamborg B5 medium containing 30% (w/v) sucrose and 2.8% (w/v) Gelrite. Various concentration of plant growth regulators (PGR) were supplemented to B5 media, i.e., 6-benzylaminopurine (BAP) at 1-4 mg/l alone or in combination with α-naphtalene acetic acid (NAA) at 0.1 mg/l or thidiazuron (TDZ) at 0.1-0.7 mg/l. Multiple shoot formation were found on both media supplemented with TDZ and BAP. A maximum of 11 shoots were produced after treatment with TDZ at 0.3 mg/l, which were the highest among other treatments. Acclimatization were conducted on (1:1 v:v) soil and vermiculite.

Introduction

Boesenbergia pulchella (Ridl.) Merr. is a small herb known as 'jerangau' locally. This plant is a member of one of the most advanced monocotyledonous plant family, Zingiberaceae. Commonly, this attractive plant can be found on the forest floor. They have beautiful glossy green leaves and the inflorescences are lanceolate on separate leafless shoot. The flower is small, open from apex to bottom with white and red labellum. Since they possess beautiful flower and foliage, they can be introduced as ornamental plants and have the potential to be commercialized, either as indoor potted plant or garden plant, even for landscaping purposes.

To achieve the goal of commercializing this plant, a large number of planting materials are needed. Propagation through conventional technique via rhizome cutting is very slow. This plant will produce shoot once they flower, which takes about three to four months after the shoot emerges. The highest number of shoot produced is two per mother plant, usually it is just one, and this restricts the use of conventional means. Micropropagation

enables mass production of clone and thus could satisfy the demand for planting material. The other benefit of using this technique is that the plantlet is disease-free and true-to-type.

Successful micropropagation of a number of species in Zingiberaceae such as *Zingiber officinale* Roscoe, *Curcuma longa* L., *Kaempferia galangal* L. and *Alpinia galanga* (L.) Willd. were reported (Bhagyalakshmi and Singh, 1988; Shirgukar *et al*., 2001; Shirin *et al*., 2000; Borthakur *et al*., 1999). However, to date, no successful micropropagation protocol has been reported for this species.

The present investigation is an attempt towards establishing a reliable *in vitro* regeneration protocol for *Boesenbergia pulchella* Ridley for use in large-scale propagation. Different concentrations of BAP with or without NAA and TDZ were tested to find the best PGR that can induce the optimum multiplication rate for this species.

Materials and Methods

Explants sources and sterilization

The stock plants for this study were collected from Gunung Ampungan in Kota Samarahan district. Rhizome buds between 1-1.5 cm were selected as initial explants. The fresh buds collected were cleaned of soil dirt and left under running tap water for one to one and a half hour. Then the buds were immersed in 75% (w/v) ethanol for one minute. Without rinsing, they were agitated in 30% (w/v) Clorox (5.25% w/v sodium hypoclorite) added with 0.1ml/l Tween 20 for 20 minutes with constant agitation. After that they were rinsed with sterile-distilled water four times. Under aseptic condition the buds scale were peeled-off and they were trimmed to about 0.5 cm long.

Establishment of axenic culture

The trimmed buds were cultured on Gamborg B5 medium, gelled with 2.8g/l Gelrite, 30% sucrose. The pH was adjusted to 5.7-5.8 with 1N KOH or 0.1 N HCL prior to autoclaving. Tetracycline at 10 mg/l and 1 ml/l Plant Preservative Mixture (PPM) were added to the medium to reduce contamination. After 15 days, the axenic culture were cut into half and subcultured onto B5 medium supplemented with BAP at 1 mg/l for 4 weeks to induce more shoots. The shoots were subcultured on B5 media for 2 weeks before they were used in subsequent experiment.

To study the effects of different types and concentrations of PGR on shoot multiplication, different treatments were used, i.e., BAP at 1, 2, 3 and 4 mg/l alone or each added with 0.1 mg/l NAA and TDZ at 0.1, 0.3, 0.5 and 0.7 mg/l.

Rooting and acclimatization
Rooting was induced on B5 medium without plant growth regulator. Plantlet at about 6-8 cm height were taken out from the vessel and washed thoroughly with tap water before they were transferred to plastic pot containing 1:1 soil and vermiculite. The plants were covered with plastic to retain moisture.

Results and Discussion

After one week, the explants turned greenish from white. For establishment of axenic culture, B5 medium with and without tetracycline and PPM were used. However, addition of tetracycline and PPM did not reduce contamination satisfactorily if compared with explants cultured on B5 medium only. About 50% of the explants were discarded for bacterial contamination. The result showed that tetracycline was not beneficial to control bacterial contamination for this species.

Induction of multiple shoots
In this study, excised shoot obtained from *in vitro* raised plants were used as explants. Shoots were divided into half prior to culture on different treatment of PGR. Treatment with BAP alone or in combination with NAA showed variability in terms of number of shoots produced per explant (Table 1). Optimum concentration for shoot multiplication was found on medium supplemented with BAP at 3 mg/l, which produced 6.6 shoots per explants. However, in terms of number of days the first bud sprouted, the duration was not really different between one treatment to another. After two weeks, at least one bud sprouted for each treatment. The basal part of the explant was enlarging before the new buds sprouted from the lateral side.

For treatment with BAP added with NAA 0.1 mg/l, the number of shoots produced was not much different with the treatment using BAP alone. The highest number of shoots was obtained on B5 medium supplemented with BAP at 3 mg/l added with NAA at 0.1 mg/l with a mean of 6.8 shoots per explant. Hence, this proved that addition of NAA was not beneficial in increasing the number of shoots. Miachir *et al*. (2004) also reported a similar finding on *Curcuma zedoaria* (Christm.) Roscoe where addition of NAA with BAP was not effective for shoot multiplication. However, results from this study showed that explants that were subjected to this treatment produced roots faster than treatment with BAP alone or TDZ. This was probably due to the fact that NAA is an auxin that helps in promoting root development.

In TDZ supplemented media, highest number of shoots obtained was on medium incorporated with TDZ at 0.3 mg/l. Eleven shoots per

explants were developed in the above medium and this was the highest among other treatments. However, more shoot clusters were formed on media supplemented with TDZ. These clusters were later subcultured on fresh medium and were able to regenerate into multiple shoots. The clusters were first separated into smaller clump, since the regeneration was faster if compared to larger clump based on the observation.

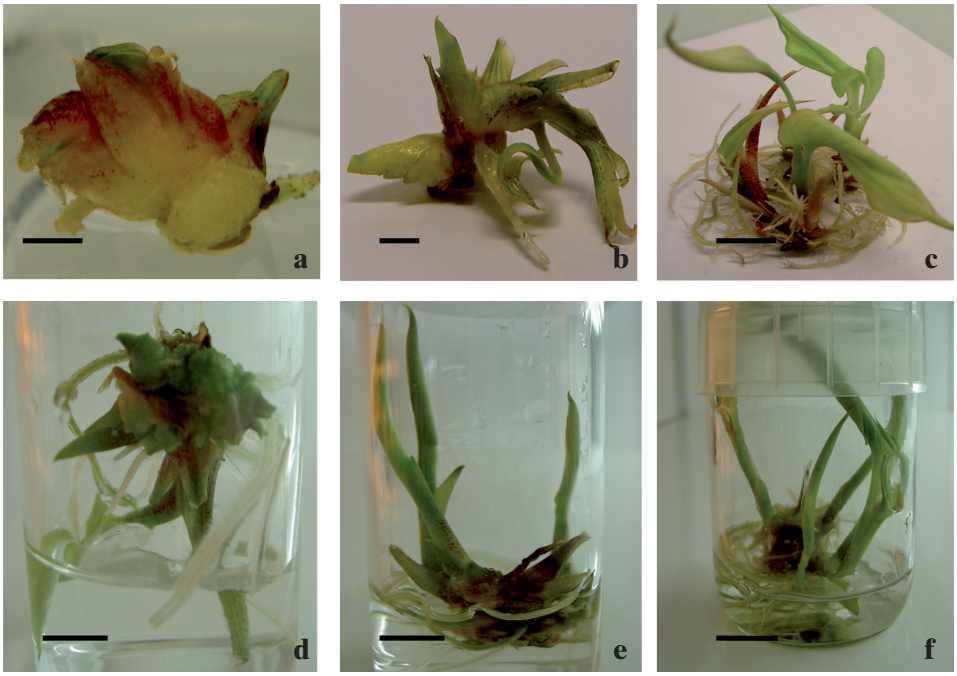
Table 1. Shoots multiplication under different growth regulators after 12 weeks of culture.

Growth regulator (mg/l)	*No of shoots/explant	Days to induction of new shoots
BAP (1)	3.60 ± 0.68	13
BAP (2)	4.40 ± 0.86	15
BAP (3)	6.60 ± 1.03	13
BAP (4)	4.20 ± 1.20	15
BAP (1) + NAA (0.1)	4.60 ± 0.75	13
BAP (2) + NAA (0.1)	4.00 ± 0.84	15
BAP (3) + NAA (0.1)	6.80 ± 1.24	15
BAP (4) + NAA (0.1)	3.96 ± 1.77	12
TDZ (0.1)	9.20 ± 3.44	12
TDZ (0.3)	11.00 ± 1.52	15
TDZ (0.5)	9.80 ± 1.74	11
TDZ (0.7)	6.00 ± 1.14	20

* Data expressed as mean ± SE from 5 replicates

Among the shoot multiplication studies conducted, it is shown that TDZ was able to regenerate a high number of shoots even at lower concentration. This accords with Tefera and Wannakrairoj (2000) where they managed to obtain 15.52 shoots on treatment with TDZ at 0.5 mg/l for multiplication of *Curcuma longa*. However for BAP, a higher concentration is needed to obtain more shoots. In fact, previous studies on other Zingiberaceae species did use a high BAP concentration. Nayak (2000) obtained the highest shoot multiplication of *Curcuma aromatica* Salisb. on medium supplemented with BAP at 5 mg/l and Samsudeen *et al*. (2000) used 10 mg/l BAP to induce organogenesis in *Zingiber officinale*.

Rooting was relatively easy for this species. Vigorous roots were formed on B5 medium without growth regulator. Plants were successfully acclimatized with survival rate of 80% after two months.



Figures a-c. Shoots multiplication on B5 medium supplemented with BAP at 3 mg/l after 4, 8 and 12 weeks of culture (bar = 0.5, 1 and 2 cm); **Figures d-f.** Shoots multiplication on B5 medium supplemented with TDZ at 3 mg/l after 4, 8 and 10 weeks of culture (bar = 1, 1.5 and 2.5 cm).

Conclusions

In vitro technique is a useful approach for propagating plants on large scale. For ginger species, propagation through conventional technique is time consuming and prone to spread disease by rhizome cuttings.

Shoots multiplication of *Boesenbergia pulchella* can be obtained using TDZ and BAP. While TDZ can produce shoots at a lower concentration, BAP is needed at higher concentration to produce similar results. Addition of NAA is not beneficial for shoot multiplication of this species.

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