Multiple Shoot Regeneration from Nodal Explants of Gynura procumbens (Lour.) Merr

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Authors’ contributions

This work was carried out in collaboration between all authors. Author ZA designed the study, wrote the protocol and interpreted the data. Author YN anchored the field study, gathered the initial data and performed preliminary data analysis. All authors read and approved the final manuscript.

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ABSTRACT

Gynura procumbens (Lour.) Merr. is an important medicinal plants and used to treat various ailments such as diabetes, hypertension and urinary tract infection. Multiple shoot formation was induced from nodal segment of G. procumbens cultured on Murashige and Skoog (MS) medium containing various concentrations 6-benzylaminopurine (BAP). The MS medium with 3 mg/l BAP gave the highest number of shoots per explant. All the micro-shoots produced normal roots within two weeks of culture on basic MS medium without any plant growth regulators. Regenerated plants were aclimatised before transferred to field condition and showed similar morphology to field grown plants.

Keywords: Gynura procumbens; tissue culture; nodal segment; shoot regeneration.

1. INTRODUCTION

Gynura procumbens is widely distributed in South East Asian countries such as Malaysia, Indonesia and Thailand. This plant is commonly known as ‘sambung nyawa’, ‘kecam akar’ or ‘daun dewa’ by the Malays and belongs to a family of Asteraceae. This plant traditionally been used for the treatment of fevers, rashes, kidney disease, migraine, constipation, hypertension,
diabetes mellitus and cancer. Recent pharmacological studies of *G. procumbens* revealed the anti-Herpes virus activity [1], anti-hyperglycemic [2], anti-inflammatory [3], anti-ulcer [4], anti-hyperlipidemic [5] and blood pressure reduction capabilities [6]. Due to medicinal values, there is a great potential to develop various products from this species. In order to maintain a sustainable raw material supply in manufacturing of *G. procumbens* products, propagation of this plant on a large scale will be a key step. The *in vitro* culture techniques can be used as the alternative for the superior planting material and continuous provision of plantlet stocks for large scale field cultivation.

In the present study, we investigated the suitable regeneration protocol of *G. procumbens* using nodal segments derived from adult plants. The protocol can be used at a large scale for clonal propagation of the species.

2. MATERIALS AND METHODS

Plants of *G. procumbens* were raised in the glasshouse. Nodal segments were collected from the grown plants and washed with detergent and rinsed under running tap water. They were surface sterilized with 95% (v/v) ethanol for 30 s and further surfaced sterilized with 20% Clorox® for 15 min, and rinsed three times with sterile distilled water. They were again surface sterilized second time with 5% Clorox® and rinsed again with sterile distilled water. Nodal segments were cut into 0.5-1.0 cm and used as explants for the induction of multiple shoots. The nodal segments were inoculated onto Murashige and Skoog medium [7] containing 30 g/L sucrose and 2.8 g/L gelrite for 5 days to determine the percentage of aseptic nodal segments. Healthy and aseptic explants were then transferred onto MS medium supplemented with 0 – 10 mg/L BAP.

The pH of the medium was adjusted to 5.7 using 0.1 N NaOH or 0.1 N HCl before autoclaving at 121°C for 15 min and adding agar. All cultures were maintained at 16 hr photoperiod with 3000 lux light intensity at 25±2°C. Observation were made at regular intervals and tabulated. For each treatment 25 replicates were used and all experiments were conducted thrice.

3. RESULTS AND DISCUSSION

The nodal segments were used as initial explants for establishment of *in vitro* culture system of *G. procumbens*. High aseptic nodal segments (90%) were successfully established using double sterilized technique (20% and 5% Clorox®) The nodal segments were then cultured on MS medium supplemented with 1-10 mg/L BAP to determine the best concentration for induction of multiple shoots.

Initially one shoot bud per explant emerged after 5-8 days of inoculation and gradually the number of shoot buds per explant increased, depending on the BAP concentration supplemented in the medium. The shoots proliferate from the node via axillary branching of buds from the explants. After 6 weeks, 100% of the explants produced shoots on induction medium. An average of 3 to 8 shoots formed from each nodal segment (Fig. 1b). Nodal segments cultured on PGR-free MS medium induced an average single shoot per explant (Fig. 1a). MS supplemented with 3 mg/L BAP induced highest number of shoots with 8.2 shoots per explant (Fig. 2).

![In vitro micropropagation of *G. procumbens*. (a) Sterilized nodal segment after 5 days on MSO. (b) Axillary branching of buds from the nodal segments of *G. procumbens* after 4 weeks of culture on MS medium supplemented MS BAP (3 mg/L)](attachment:fig1.png)

Our results showed that BAP is very effective in induction of multiple shoot from nodal segment. BAP is regarded the most effective cytokinin for shoot induction and is widely used in *in vitro* propagation of plants [8-9]. The enhanced rate of multiple shoot induction in cultures supplemented with BAP may be largely ascribed due to increased rates of cell division induced by cytokinin (BAP) in the terminal and axillary meristematic zone of explant tissues. Cells in this zone divide with the faster pace and thus, produced large number of shoots [10].
However, on lowering the concentration of BAP from the optimum concentration (3 mg/l), the number of shoots per explant declined. In general, the number of adventitious shoot buds per explant increased up to 3 mg/L concentration and declined with the increase or decrease in concentration of each cytokinin beyond their optimal level. Reduction in shoot number at concentrations higher/lower than optimal level has also been reported for several medicinal plants [11-13]. Higher concentrations of BAP not only reduced the number of shoots formed but also resulted in stunted growth of the shoots. Similar results were reported in G. procumbens by [14,15].

3.1 In Vitro Rooting and Acclimatization

The regenerated microshoots were excised and placed on MS medium without any plant growth regulator. Shoot elongation was simultaneously observed along with root induction (Fig. 3a). 100% of root initiation occurred directly from the cut ends of microshoots after 2 weeks of culture. This result showed that MS medium without the addition of any auxin was sufficient for the establishment of in vitro rooting of the microshoots of G. procumbens. Rooted plantlets (Fig. 3a) were removed from medium, washed thoroughly and placed in a mixture of sterilized vermiculite and sterilized soil (1:1), before acclimatized in greenhouse (Fig. 3b). The plantlets was transplanted into bigger earthen pot with 85% survival.

4. CONCLUSION

Here, we report an efficient protocol for clonal multiplication of this G. procumbens using nodal segments explants. The protocol can be suitably exploited for mass multiplication on a large scale for commercial production The system could also be explored to produce important compounds of medicinal value from cultured cells/tissues/organs or plantlets. It would also ensure a continuous supply of plants in limited time and space for this valuable medicinal herb, thereby ruling out the dependency on natural source to fulfill the growing demands for the pharmaceutical industry.
COMPETING INTERESTS

Authors have declared that no competing interests exist.

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