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Effect of Cytokinins on *In vitro* Propagation of *Gynura procumbens* (Lour) Merr

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Original Research Article

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Abstract: Two cytokinins, benzylaminopurine (BA) and kinetin were investigated for multiple shoot induction of *Gynura procumbens* (Lour.) Merr. Nodal segments of *G. procumbens* were cultured on Murashige and Skoog (MS) agar medium supplemented with BA or Kinetin at the concentration of 1- 10 mg/L. BA proved superior to kinetin in producing multiple shoots. Optimum adventitious shoot buds induction occurred at 4 mg/L BA where an average of 21 shoot buds were produced after 8 weeks of culture. Meanwhile, kinetin produced the highest shoots buds induction at 6 mg/L (19 shoots per explants). *In vitro* derived shoots were elongated on growth regulator-free MS medium and produced normal roots within two weeks of culture. The plantlets were grew well without any phenotypic aberrations. This protocol could be useful for germplasm conservation, cultivation, and genetic improvement of *G. procumbens*.
Keywords: Medicinal plant, nodal segment, benzylaminopurine, kinetin, micropropagation.

INTRODUCTION

Herbs have been used as traditional medicine, especially in the rural parts of developing countries [1]. In the last decade the use of herbal medicine has seen a significant increase [2,3,4]. *G. procumbens* is one of the popular tropical medicinal plants in Malaysia. This plant is commonly known as 'sambung nyawa', 'kecam akar' or 'daun dewa' by the Malays and belongs to a member of Asteraceae (Compositae) family. It is also grown widely in South East Asian countries such as Indonesia and Thailand. This plant has hairy green leaves arranged alternately on purple stem and shows extensive branching.

This annual evergreen shrub with a fleshy stem can grow approximately 10-25 cm in height. The leaves are edible as 'ulam' and can be used as a flavouring agent in food preparation.

This plant has long been used as ethnoherbal products to treat various ailments such of fever, rashes, kidney disease, migraine, constipation, hypertension, diabetes mellitus and cancer [5]. Recently, pharmacological studies reported that *G. procumbens* also has anti-Herpes virus [6], anti-hyperlipidemic [7], anti-inflammatory [8], anti-hyperglycemic [9], anti-ulcer [10] and blood pressure reduction capabilities [11].

Due to medicinal values, there is a great potential to develop various products from this plant. The *In vitro* culture techniques can be used as an alternative to produce superior planting material and continuous provision of plantlet stocks for large scale field cultivation. Through this technique, it will enable rapid multiplication and sustainable use of medicinal plants for future generations. Tissue culturing of medicinal plants is widely used to produce active compounds for herbal and pharmaceutical industries. We therefore identified the suitable plant growth hormone to regenerate *G. procumbens*, using nodal segments derived from adult plants. This protocol can be used for clonal propagation of this species.

MATERIALS AND METHODS

Preparation of explants

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 dipped in ethanol for 1 min and further surfaced sterilized with 20% Clorox® (Sodium hypoclorite, 5.2%) for 20 min, and rinsed with sterile distilled water for three times. 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 onto MS [12] medium containing 30 g/L sucrose and 2.8 g/L gelrite for 5 days before transferred onto MS medium supplemented with Kinetin or BA

Shoot multiplication and elongation

Aseptic nodal segments were used for the establishment of best shoot proliferation medium by transferring them onto MS medium supplemented with BA or kinetin. MS medium containing 30 g/L sucrose, 2.8 g/L gelrite and supplemented with 0 – 10 mg/L BA or kinetin. The pH of the medium was adjusted to 5.7, using 0.1 N NaOH or 0.1 N HCl before autoclaving at 15 psi (1.04 kg/cm2) for 15 min at 121°C. All cultures were maintained at 16 hr photoperiod with 3000 lux light intensity at 25 ± 20C.

RESULTS AND DISCUSSION

Shoot multiplication

The sterilized nodal segments were cultured on MS medium without any PGR for 5 days. The use of basal MS medium without any PGR is adopted as it is the usual method to reveal and eliminate the contamination in initiation culture stage in many laboratories [13].

Cleaned and healthy nodal segments were cultured on MS medium supplemented with 1-10 mg/L of either BA or kinetin to determine the best concentration for production 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 cytokinin concentration in all treatment. Multiple shoots appeared to proliferate directly from the node via axillary branching of buds from the explants without the intervention of callus.

After 8 weeks, results showed 100% of the explants produced shoots on induction medium. An average of 2 to 21 shoots was formed from each nodal segment depending on concentration and types of cytokinin used. The growth regulators applied externally during *In vitro* studies might disturb the internal polarity and change the genetically programmed physiology of explants resulting in organogenesis.

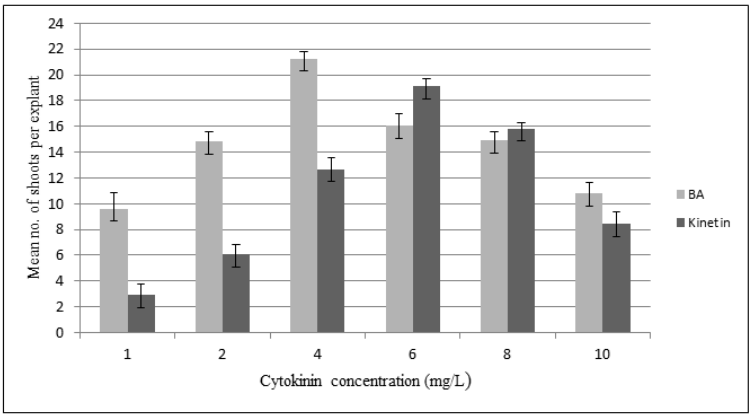

Fig-1: Effect of different concentration of BA and kinetin on shoot induction of *G. procumbens*

BA was more effective than kinetin for mean number of shoot buds produced per explant. MS supplemented with 4 mg/L BA induced the number of shoots, (21.27±.51 shoots per explant) (Figure 1). Meanwhile, kinetin also induced multiple shoots, but not as effective as BAP. Kinetin produced the highest shoots buds induction at 6 mg/L (19.14 ± 0.52 shoots per explant). A similar result about the effectiveness of BAP was also reported in *Aloe vera*[14], *Peganum harmala* [15], *Prosopis cinerari* [16], *Spilanthes acmella* [17]. Nodal segments cultured on MS medium without any growth regulator were differentiated into single shoot per explants. The enhanced rate of multiple shoot induction in cultures supplemented with BA/kinetin may be largely ascribed due to increased rates of cell division induced by cytokinin in the terminal and axillary meristematic zone of explant tissues. Cells in this zone divided with faster pace and thus, produced large number of shoots [18].

The stimulating effect of BA on multiple shoot formation has been reported earlier for several medicinal plant species including *Bacopa monniera* [19], *Vitex trifolia* [20] and *Mucuna pruriens*]21]. This PGR is considered as the most useful cytokinin in bud breaking in plants, and has been widely used in plant micropropagation [22,23].

However, on lowering the concentration of BA or kinetin from their optimum concentration (4 mg/l and 6 mg/l respectively), the number of shoots per explant was reduced. In general, the number of adventitious shoot buds per explant increased up to a certain 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 [24, 20, 25]. Higher concentrations of BA or kinetin not only reduced the number of shoots formed but also resulted in stunted growth of the shoots. Shoot multiplication and subsequent elongation were achieved on the same medium.

***In vitro* rooting and acclimatization**

The regenerated shoots were excised and placed on the MS medium without any growth regulator. Shoot elongation was simultaneously observed along with root induction (Fig. 2c). 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 auxsin was sufficient for the establishment of *In vitro* rooting of the micro-shoots of *G. procumbens*. Rooted plantlets were removed from agar, washed thoroughly and placed in a mixture of sterilized vermiculite soil (1:1), before being acclimatized in greenhouse (Fig 2e).

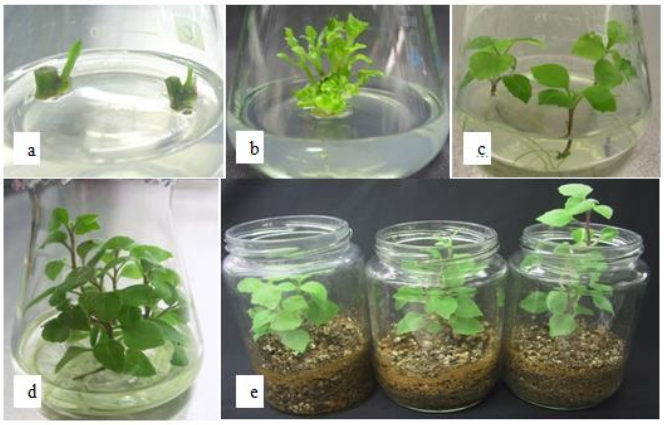

Fig-*2*: *In vitro* micropropagation of *G. procumbens*. (a) Sterilized nodal segment after 5 days on MS media without PGR. (b) Axillary branching of buds from the nodal segments of *G. procumbens* after 4 weeks of culture on MS medium supplemented with BA (4mg/L). (c &d) Elongation and rooting of *G. procumbens* on MS without PGR and (e). Acclimatization of *In vitro* plantlets of *G. procumbens* in a mixture of sterilized vermiculite soil (1:1)

CONCLUSION

The micropropagation system described in this study could form the basis to develop protocols for a continuous supply of *G. procumbens* plants, and to fulfill the growing demands for this valuable medicinal plant. 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.

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