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REVIEW ARTICLE

IN-VITRO REGENERATION OF ORTHOSIPHON STAMINEUS (MISAI KUCING) USING AXILLARY BUD

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ABSTRACT

Orthosiphon stamineus or known as Misai kucing is a popular herbal tea plant that helps in treating the ailments of kidney and bladder, diabetes mellitus and gout. Due to high demand of this medicinal plant, a large propagation of this plant is recommended. In this study, a protocol for regeneration of *Orthosiphon stamineus* using axillary bud as explant was established. Murashige and Skoog (MS) medium supplemented with various concentrations of 6-benzylaminopurine (BAP) were evaluated for their effects on shoot induction. Results obtained revealed that the best shooting ability was observed when explants were cultured on MS medium supplemented with 0.2 ppm of BAP. Subsequently, shoot elongation of established shoot was obtained in medium containing gibberelic acid (GA_3) with concentration of 0.5 ppm and 1.0 ppm. The best shoot elongation was achieved with medium supplemented with 0.5 ppm of GA_3. Subsequently, the elongated explants were transformed to root induction medium with the addition of either 0.2 ppm of 1-naphthaleneacetic acid (NAA) or 0.5 ppm NAA. The explants showed positive response in medium supplemented with 0.2 ppm of NAA. From this study it could be confirmed that suitable concentration of plant growth regulators could be used in the regeneration of *Orthosiphon stamineus* through tissue culture technique.

KEYWORDS

Orthosiphon stamineus, regeneration, axillary bud, plant growth regulator

1. INTRODUCTION

O. stamineus or Misai Kucing which comes from the Lamiaceae family is one of the medicinal herbs used in Southeast Asia [1]. It is locally known as Misai Kucing because the features of its bluish flowers look like a cat's whiskers. This herb was cultivated a long time ago and is now grown as garden plant and for wild type plant it can be seen growing along the forest edges, roadsides and wastelands. The plant grows to a height of 1.5 m and consists of leaves, petiole, stem, flowers and stamens [2].

O. stamineus has unique features and several benefits for human consumption which it has been used traditionally to treat eruptive fever, epilepsy, gallstone, hepatitis, rheumatism, hypertension and renal stones [3]. *O. stamineus* plant contains various bio-active compounds that make this plant usable as herbal medicine. Such properties are 5-hydroxy-6, 7, 3, 4-tetramethoxyflavone, 4, 5, 6, 7-tetramethoxyflavone, terpenoids, inositol, myo-inositol, carotenoids and saponins [4]. The chemical constituents of this medicinal plant were identified and show to have the properties of antiallergic, antihypertensive, anti-inflammatory, antioxidant and diuretic [5]. In Myanmar, leaves of *O. stamineus* have been used as anti-diabetic drugs and remedy to treat urinary tract and renal disease, while in Japan, it has been consumed as java tea that helps to facilitate the detoxification inside the body [6].

Because of the medicinal properties that the plant possessed there is a high demand of this plant nowadays. Hence, researchers need to find a way on how to propagate the plant in a large number. To develop a mass propagation of this herbal plant, tissue culture of *O. stamineus* using axillary bud has been proposed.

2. MATERIAL AND METHODS

2.1 Surface sterilization of explants

Surface sterilization of explants was performed in laminar flow chamber. Stems of plant were first collected from *O. stamineus* in the field and put into a sterile Duran bottle. Stems were initially washed with tap water to remove adhered particles and surface pathogens followed by washing with sterile distilled water for 4 times. Subsequently, they were sterilized using 70% ethanol with a drop of Teepol for 1 minute. The sterilized explants were then rinsed 5 times with sterile distilled water. Consequently, the explants were sterilized using 0.1% $HgCl_2$ for 5 minutes with one drop of Teepol and finally were rinsed thoroughly with sterile distilled water for 5 times. The sterilized stems were cuts using sterile scalpels and forceps and only the part of the axillary buds was left for culturing.

2.2 Shoot induction

For shoot induction, stems with the part of axillary bud were cultured on MS basal medium containing 3% sucrose and 0.8% agar and supplemented with a range of BAP concentrations (0, 0.2, 0.4, 0.6, 0.8, and

1.0 ppm). Each treatment consisted of 12 replicates and each jar contained 4 explants. The culture was incubated under continuous light at 25±2°C. Observation was carried out once a week to record for the presence of shoots. After shoots were induced, the explants were left for another couple of weeks in the same medium to let them branch.

2.3 Elongation of shoot

Healthy shoots induced from axillary buds were subcultured on MS medium supplemented with different concentrations of GA_3 (0.5 and 1.0 ppm) to elongate the shoots. Cultures were maintained at 25±2°C under continuous light. Observation was carried out once a week to observe the elongation of the shoots.

2.4 Root induction

The well-developed shoots were excised and transferred on MS medium supplemented with different concentrations of NAA (0.2 and 0.5 ppm) to induce root formation. The cultures were again maintained in light at 25±2°C. Observation was carried out once a week to record the number of roots per treatment.

3. RESULTS AND DISCUSSION

After surface sterilization, axillary buds were cultured on five concentrations of BAP (0 ppm, 0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm and 1.0 ppm) to induce the formation of shoots. As shown in Table 1, for control treatment (0 ppm BAP), there was no response because no plant growth regulator was added. Explants also did not give any response when cultured on MS medium supplemented with 0.6 ppm and 0.8 ppm BAP. Three concentrations of BAP (0.2, 0.4 and 1.0 ppm) gave response towards the explants with the highest percentage of response (33.3%) was obtained on MS medium with 0.2 ppm BAP. With 0.2 ppm BAP, little tiny shoots started to arise from the side edge of the explants one week after culturing. After two weeks the shoots became longer, and more shoots were induced (Figure 1). The shoots looked healthy and green. In upcoming weeks more, shoots were formed and became cluster of basal shoots. When explants were cultured on 0.4 ppm and 1.0 ppm of BAP, percentage of 8.33% was obtained for shoot induction. However, more shoots per explant were obtained on MS medium with 0.4 ppm BAP (4 shoots/explant) compared to 1 shoot/explant on 1.0 ppm BAP. BAP is one of the cytokinins that are commonly used for inducing rapid multiplication of shoots, buds and meristem. When BAP was used at 0.2 ppm concentration, it gave the best response towards leaf explants of *Ortosiphon aristatus* while for stem BAP at the same concentration also gave a higher average number of shoots per explant [7]. It was reported that 0.2 ppm of BAP increased the number of shoots of the mono-stem nodal segment explant of *Lamium album*, a plant which is the same family members of the *O. stamineus* [8].

Table 1: Effects of BAP on shoot induction using axillary bud explant of *O. stamineus*

Concentration of BAP (ppm)	Percentage of response (%)	Number of shoots per explant	Week of response
0	0	0	-
0.2	33.3	4	2-3 weeks
0.4	8.33	4	2-3 weeks
0.6	0	0	-
0.8	0	0	-
1.0	8.33	1	3-4 weeks

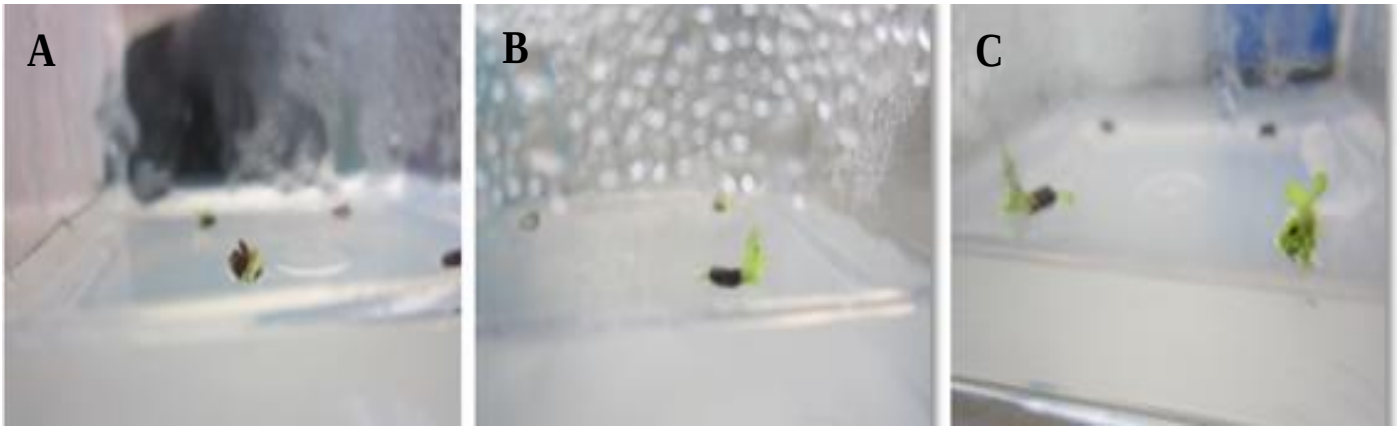

Figure 1: Shoot induction from axillary bud of *O. stamineus* medium with 0.2 ppm BAP after: A) 1 week of culture; B) 2 weeks of culture and C) 3 weeks of culture

For the elongation of shoots, induced shoots were either transferred into MS medium containing 0.5 ppm or 1.0 ppm GA_3. On medium with 0.5 ppm GA_3, the shoots started to elongate after one week of culturing. After two weeks more, shoots elongated vigorously. For this treatment the average length of shoot is approximately 4.5 cm. The explant also started to branch in this medium and two roots were also formed. When GA_3 was added at concentration of 1.0 ppm, shoots elongation can be observed after two weeks of culturing and the average length of shoot measured was 4 cm (Figure 2). From the results obtained it can be shown that 0.5 ppm is the best concentration of GA_3 used to elongate the shoots. Hence this concentration was used to produce more elongated shoots which to be used for root induction study. The use of GA_3 in tissue culture primarily is to stimulate cell elongation thus producing shoots. Also, the use of this PGR is a useful method to convert buds to functioning shoots. However, GA_3 is seldom used in tissue culture at early stage of bud culture because it reduces the number of buds. When this plant growth regulator is used with a reduced cytokinin concentration, which is BAP, it helps promote shoots elongation. With the reduction of cytokinin it helps the buds to elongate which is aided by the cell elongating effect of using GA_3 [9].

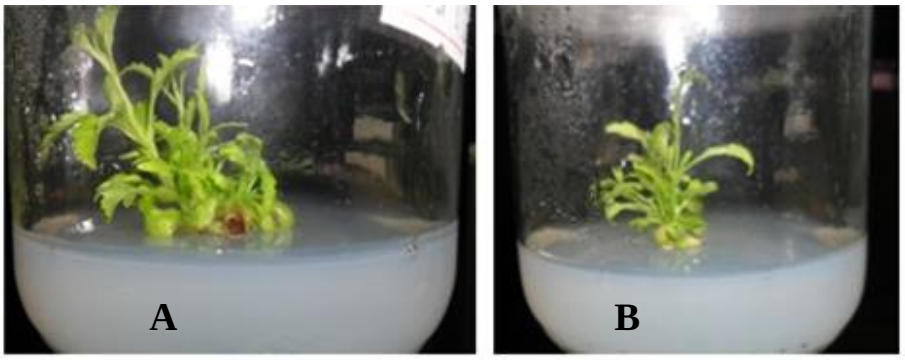

Figure 2: Elongation of established shoots of *O. stamineus* on MS medium with concentration of A) 0.5 ppm GA_3 and B) 1.0 ppm GA_3 after 2 weeks of culturing

After elongation stage, explants that produce abundant shoots were excised and were cultured on MS medium with two different concentrations of NAA which 0.2 ppm and 0.5 ppm were. After week 4 of culturing, it was observed that 0.2 ppm of NAA gave 100% response while 0.5 ppm NAA did not gave any response (Figure 3). The longest root induced on 0.2 ppm NAA was 3.5 cm. NAA is a synthetic analogue of IAA, another type of auxin which has been used for callus induction and growth of callus and suspension cultures. Besides that, it is also used for root induction [10]. Since NAA is a synthetic auxin, it possesses stability in plant tissue because the enzymes that process and degrade IAA do not recognize synthetic auxins. Synthetic auxins are also more effective plant growth regulators that can last for an extended length of time. NAA is not light sensitive and for this reason NAA was used to produce high amounts of roots in this experiment [11].

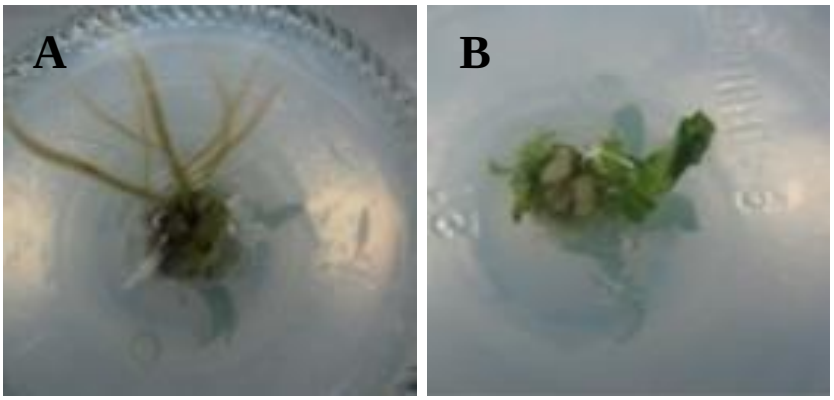

Figure 3: Root induction of axillary bud in MS medium containing different concentrations of NAA after 4 weeks. A) Root induced in 0.2 ppm NAA and B) no roots induced in 0.5 ppm NAA

4. CONCLUSION

In summary, this study showed that shoots were successfully induced on MS medium supplemented with BAP at concentration of 0.2 ppm. Shoot elongation was achieved using GA_3 at concentration of 0.5 ppm. Furthermore, the explants successfully branched, and root induction was attained by culturing the explants in 0.2 ppm of NAA. Therefore, this study proved that plantlet regeneration of *O. stamineus* is possible using axillary bud explants.

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