Exploration of sterilization method and type of media for in vitro propagation of Bauhinia scandens

Abstract

This study aims to obtain an appropriate sterilization method and media for propagation of Bauhinia scandens in vitro. The study was conducted at the Tissue Culture Laboratory and Biology Greenhouse. The method used in this study was induction using cytokinin BAP (benzyl amino purine) in MS media. Explain obtained from seed seeds that have been germinated in polybags with soil media and compost. Sterilization for planting with tissue culture techniques using detergents, 70% alcohol, 20% and 10% Clorox, and sterile aqua dest. The media used were MS (Murashige and Skoog) media plus BAP (concentrations of 2 and 4 ppm), as well as 2,4-D (0.4, 1.0, and 1.5 ppm) and the tones, were added with activated charcoal. The results showed that the addition of BAP in MS media was able to trigger the growth of shoots in exploder node bauhinia at concentrations of 2 and 4 ppm. The addition of 2,4-D was able to induce callus in Bauhinia scandens leaves. At a concentration of 1 ppm the callus produced was white, runny with leaves that were not too curled while concentrations of 0.4 and 1.5 ppm produced green callus with curled leaves. More varied media treatments need to be performed on both nodes and leaf explants to produce plantlets through callus or shoot formation directly.

Keywords: exploration, sterilization, media, in vitro, propagation

Background

Bauhinia scandens is a unique plant that is already rare, especially in Semarang, Central Java. It is found to be the largest in Sam Poo Kong, around 600 years old with a diameter of 65 cm. As for the others found in the Pagerwungung Darupono Kaliwungu Kendal Nature Reserve. However, with the widespread observation or exploration that chain trees were found to be in the public cemetery/grave of Tugurejo Semarang with a diameter of 20-35 cm and in Silayur Mijen Diameter 4-10 cm. Also in the village of Pidi Kec Pengonon 5-10cm also in Goa Kreo diameter 20-25 cm, near the Sewu Sukorjo waterfall and in the Sukorjo Protection Forest, there are also, but all are still small. The uniqueness of this liana plant is always clustering suspected of breeding in addition to the seeds as well as their root buds so that clustered between 1-17 trees in CA Pagerwungung Darupono, 1-10 trees in Silayur Ngaliyan Semarang that can be counted significantly.

Breeding through seeds is quite difficult. It has been proven in a study conducted by Karsinah, explants of B. scandens cotyledons grown on media with 0.1-0.5 ppm 2,4-D and 0.5-2.0 ppm BAP produced callus growth, whereas used 1 mg/liter BAP to induce lateral durian buds in vitro.

Research methods

Research has been conducted in the experimental garden and tissue culture laboratory, Faculty of Mathematics and Natural Sciences, Semarang State University. The material used is chain tree seeds from Sam Poo Kong Semarang. Seed germination is carried out in the experimental garden by planting seeds in polybags containing soil media and compost. Sterilization for planting with tissue culture so that they can be used to obtain uniform seeds in large quantities.
After the buds begin to appear, nodes and leaves are transferred to MS media containing BAP cytokinins. For sterilization, ingredients such as detergents, bactericides, fungicides, distilled water, 70% alcohol, 10% Clorox (bayclin) solution, and tissue are used. Aluminum foil and paper umbrella are also used in the sterilization of tools. The media used were MS+2 ppm BAP and MS+4 ppm BAP for shoot induction from nodia, while for callus induction in leaves the MS media was used with an additional 2.4-D (0.4 ppm, 1ppm, and 1.5ppm) In vitro treatment, tools such as media bottles and heat-resistant caps or aluminum foil are needed. Tweezers, Bunsen lamps, scalpels, Petri dish, beakers, magnetic stirrers, autoclaves, digital scales, labels, and Laminar airflow (LAF) cabinets will also be used.6,7

Results and discussion

Chain tree germination is carried out in soil media because in vitro germination is difficult.1 With several methods of seed sterilization, contamination still occurs when planted in vitro media. Therefore, attempts to obtain a sterile explant source cannot be done. Of the several sterilization methods tried, the best results were obtained with 20% and 10% Clorox sterilization methods and 70% alcohol for explant nodia, with a soaking time of 5 minutes. For leaves, sterilize with Clorox 10% and 5% and 70% alcohol with soaking time in each solution for 3 minutes. The addition of BAP in MS media was able to induce shoots in nodia

Bauhinia scandens with BAP concentrations of 2 and 4 ppm. At a concentration of 2 ppm, BAP induces the formation of shoots only on nodes while a concentration of 4 ppm induces the formation of nodes and callus. This can be caused by cytokines that have been in explants since they have been taken from germination seeds. The origin of the explant, specifically the genotype and position of the explant in the parent plant will determine the level of endogenous hormones in the explant.8 Shoots that form on media with 2 ppm BAP require a longer time to grow, whereas with 4 ppm BAP shoots appear faster but with callus growth on the node part. The callus that grows is a dense callus with a smooth yellow outer surface (Figure 1).
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Conclusion
Research shows that BAP with concentrations of 2 and 4 ppm on MS media is able to induce shoots of Bauhinia scandens nodia in vitro. A concentration of 4 ppm also induces callus in addition to shoots. The addition of 2,4-D auxin was able to induce callus in explants of Bauhinia scandens leaves originating from seedlings aged 1 month, at concentrations of 1 and 1.5 ppm. A concentration of 0.4 ppm 2,4-D causes leaf explants to curl but no callus is formed. Exploration needs to be done with concentrations of growth regulators that are more varied and with certain combinations (for example auxin and cytokinin in one media).

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Conflicts of interests
Authors declare no conflict of interest exists.

Figure 2 Growth of Bauhinia Scandens nodia explants in vitro with use of MS +4 ppm BAP at 4 weeks after planting.

References


