IN VITRO PROPAGATION OF SMILAX MYOSOTIFLORA (UBI JAGA) FOR CONSERVATION AND COMMERCIAL PURPOSES

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ABSTRACT

In vitro propagation technique has been known to efficiently mass propagate valuable plant species. Threatened species, commercial valued and medicinal species are species that suitable to be propagated using tissue culture technology. Smilax sp., a genus belongs to Smilacaceae family has about 350 species and is reported distributed in tropical, subtropical and temperate region. Indigenous people from America and Asia have been using the Smilax rhizomes as tonic, diuretic and sudorific for century. The morphology of Smilax sp. consists of long, thin thorny stem, branches with tendrils which attach to other plants or objects and grow steadily upward. Smilax myosotiflora, locally known as Ubi jaga in Malaysia, has been used in traditional practice as an energy booster and for man health. The rhizome extract was proven to increase men sexual drive and the leaves were reported to have potential to cure syphilis. Currently, the source of the raw materials was collected from the wild where difficulty arises during harvesting the rhizome from the soil. Special technique is required to harvest the rhizome and identification of the S. myosotiflora is quite difficult since the morphology between Smilax sp. is very much similar to each other. Due to these issues, an alternative for sustainable production of S. myosotiflora raw material is required, hence production of this species in tissue culture has been taken and reported in this paper. S. myosotiflora has been introduced into tissue culture using nodal segment. The introduced clean cultures have been propagated in culture media consist of Murashige and Skoog (MS) basal medium supplemented with different range of 6-Benzylaminopurine, BAP for shoot multiplication and Indole-3-butyric acid, IBA for root induction. The shoot multiplication and root induction medium of S. myosotiflora have been determined where MS basal medium supplemented with 2.0 mg/L BAP induced the highest percentage of shoot formation whereas ½ MS basal medium added with 0.5 mg/L IBA induced the highest percentage of shoot rooted in vitro. The complete plantlets obtained were used for acclimatization experiment in the nursery.

Keywords: *Smilax myosotiflora* (Ubi jaga), Micropropagation, Murashige and Skoog (MS) basal media

INTRODUCTION

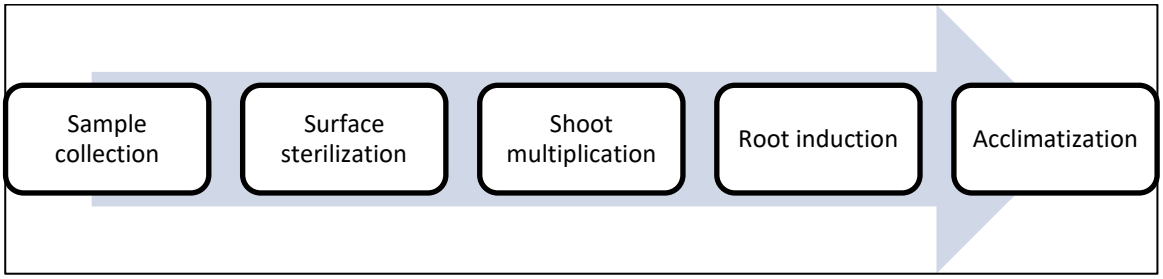
Plant species that are facing endanger risk due to illegal logging, in addition of low fruiting rate, occurrences of natural disasters or diseases, as well as trees that are commercially valuable but difficult to find in large quantities were situation that called for alternative propagation approach. Alternative propagation approach are including grafting, cutting and micropropagation or tissue culture. Micropropagation started from selection of explant sources from plant parts to initiate culture, shoot multiplication, root induction and acclimatization before replanting to the field or original habitat. The major aim is to obtain a mass plantlets production in sterile media culture and production of uniform seedlings. *Smilax myosotiflora* or locally known as Ubi jaga has been proven to have commercial value from previous research report on its medicinal properties (Asiah et al. 2007; Dasuki et al. 2012; Wan et al. 2016). Naturally, *S. myosotiflora* required huge effort to find in the forest and even special techniques are required to remove the rhizomes from the soil. These difficulties cause the availability of *S. myosotiflora* rhizomes to be lower than market demand. Therefore, there is a need to produce this species using tissue culture technique. So far, no report on tissue culture protocol development of *S. myosotiflora* has been published. However, tissue culture protocol from similar genus have been reported and were used as references for initiation of *S. myosotiflora* in this study (Jirakiattikul et al. 2013; Thirugnanasampandan et al. 2009). The establishment of *S. myosotiflora* in tissue culture will be used for advance tissue culture techniques such as cell or organ culture using temporary immersion bioreactor system to harness the medicinal potential of bioactive compound in the *S. myosotiflora* later.

For *S. myosotiflora* tissue culture protocol development, different parts of the plant are found suitable to be used as explants for initiation into the culture media. Nodal segments, rhizomes and seeds are the parts of *S. myosotiflora* that can be used as explants. Nodal segments, rhizomes and seeds have meristem in which cells actively divide and usually new shoot growth occurs. In this study, nodal segment was used as source material for tissue culture establishment of *S. myosotifolia*.

MATERIALS AND METHOD

General method of plant tissue culture protocol development was illustrated in the Figure 1. Tissue culture protocol development is started with sample collection, followed by surface sterilization, shoot multiplication, root induction and ended with acclimatization. Different species or plant part such as leave, nodal segment, root, rhizome or seed would require some modification during the protocol development process. In this paper, tissue culture protocol development of *S. myosotiflora* sample from nodal segment were explained.

Fig. 1 Tissue culture protocol development of *S. myosotiflora*



Sample collection and surface sterilization

S. myosotiflora rhizome and nodal segment were collected from FRIM, Kepong. The samples were washed under running tap water to remove macro contaminant. Commercial bleach, Clorox® and ethanol were used in the laminar flow to remove the micro contaminant. Fungicide, Thiram® at 1.0 g/L concentration was also used to reduce the contaminant from fungi. The clean samples were washed with sterilized distilled water before cultured into media.

Complete plantlet production

Shoots germinated from the nodal segment were subculture into new media, Murashige and Skoog, MS basal media supplemented with cytokinin, BAP at 2.0 mg/L. The new shoots were let elongated up to 2.5 – 3.0 cm before sub cultured into rooting media. All cultures were kept in growth room with parameters; 22 ±2°C, 2000 Lux and 16-hour light. The rooting process took about 8 weeks for the completed plantlets ready to be acclimatized in the nursery.

Acclimatization and hardening in the nursery

Acclimatization process required the complete plantlets to be removed from the media, washed under tap water to remove agar residue, dipped into fungicide and planted into Jiffy®, a commercial media consist of coconut fibre. These plantlets were kept in acclimatization chamber for 1 month where the humidity in the chamber were gradually decreased. After a while, the whole cover of the chamber was removed and the plantlets were transferred into polybag with new potting media (baked soil: peat moss, 1:1). The plantlets were kept under shades and continue hardening before ready to be planted in the field.

RESULTS AND DISCUSSION

Surface sterilization and culture initiation

Each type of explant used to be introduced in the culture media must first be surface sterilized to ensure that no microorganisms are attached to the plant samples. Microorganisms that typically have a faster growth rate than plant cells which they will compete with the plant cells to obtain the nutrients supplied in the culture media. This in turn will cause the growth of the desired plant to be stunted.

Surface sterilization of the *S. myosotiflora* nodal segment was carried out using sterilization solution such as ethanol and Clorox® under a laminar flow (Fig. 2A). Double surface sterilization using Clorox®, with concentration of 50% and 20% with exposure duration 15 min and 5 min respectively successfully produced clean culture of *S. myosotiflora*. Meristem cells in the nodal portion of the segment are more susceptible to destruct when exposed to Clorox® at higher concentrations. Beside nodal segment, rhizome and seed can also be used as explants for establishment into tissue culture media. Morphologically, the structure of the nodal segment and rhizome part are difference where the nodal segment is softer than rhizome. While embryo in the seed was protected by seed coat. Higher concentration of Clorox® can be applied during surface sterilization if the source from rhizomes and seeds, but the drawback is that the success rate of clean culture from rhizome is lower due to the high concentration of microflora reside in the soil where the rhizome grows and the source of seed is limited compared to nodal segment. In this study, only surface sterilization of the nodal segment part was discussed.

Shoots multiplication

New shoots emerged from nodal explants was transferred into new fresh media supplemented with the shoot multiplication hormone, cytokinin. Research to identify the suitable cytokinin concentrations, (in this study, Benzyl amino purine (BAP) hormone were used) were conducted where media with suitable BAP hormones concentration able to produce high number of new shoots with normal shoot and leaves morphology. If the concentration of BAP is too high for the species, the number of new shoots produced maybe high, however the new shoots are stunted (Arab et al. 2014). For *S. myosotiflora*, Murashige and Skoog media with the addition of cytokinin hormones (BAP, 2.0 mg/L) and sucrose (3%) were found suitable for the multiplication of *S. myosotiflora* shoots with more than 70% explants produce new shoots (Fig. 2B). Similar with other *Smilax sp*., *Smilax glabra*, the most suitable media for shoot multiplication was MS + 2.0 mg/L BAP with addition of 0.5 mg/L 1-Naphthaleneacetic acid, NAA (Dong et al., 2014). While *S. zeylanica*, require 5 mg/L Kinetin, another type of cytokinin to induce shoot development and 1 mg/L BAP for shoot multiplication (Jha et al., 1986).

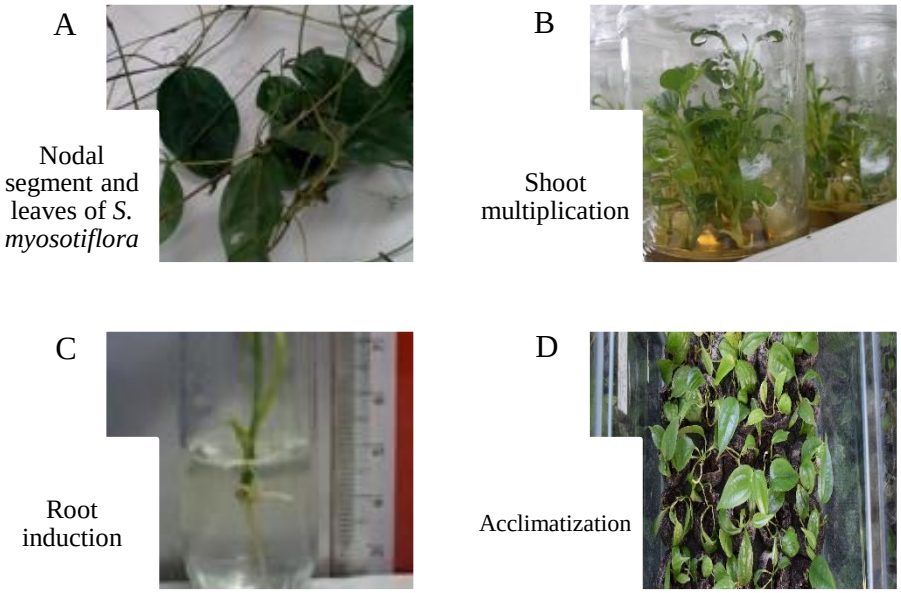
Rooting induction

The next stage is to induce rooting for complete plantlet production. *S. myosotiflora* shoots that had reached a suitable height of more than 2.5 – 3.0 cm were cultured into ½ MS basal media with the addition of auxin hormone, (IBA) which is a type of root-inducing hormone. An approximate of 8 weeks of culture is required for root production in the root inducing semi-solid media ½ MS basal media + 0.5 mg/L IBA (Fig. 2C). A mix of IBA 0.5 mg/L + NAA 0.5 mg/L was optimal for root induction of *S. glabra* (Dong et al., 2014). During the duration in media culture, no rhizome development have been observed so far. Further study need to be carried out to induce the production of *S. myosotiflora* rhizome in tissue culture.

Acclimatization in the nursery

The complete plantlets were transferred to acclimatize chamber to gradually adapting the plantlets into *ex-vitro* environment (Fig. 2D). The anatomy of the leaves of tissue-culture derived plantlets were different with seedling that established *ex-vitro* where the stomata were still not fully functioning to regulate water vaporization process. In addition to immature stomata function, epicuticle wax formation and photosynthetic tissue formation in leaves of tissue-culture derived plantlets are also low (Lamhamedi et al., 2003). Immature stomata function will cause the plantlets to wither quickly when exposed to low humidity and high temperatures resulting low survival rate. Therefore, the process of acclimatization plays an important role in ensuring that the plantlets are ready to survive in the *ex-vitro* environment.

Fig. 2 Tissue culture process of *S. myosotiflora* A) nodal segment of *S. myosotiflora* for surface sterilization (B), shoot multiplication (C), root induction and (D) acclimatization.



CONCLUSION

An alternative approach for propagation has been provided, which can promote the artificial cultivation of *S. myosotiflora* through tissue culture technique. The plantlets generated from tissue culture techniques will benefit the stakeholders such as traditional practitioner and product development for sustainable raw material while securing the *S. myosotiflora* in the wild from over exploitation.

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