Mass Propagation of *Lagenaria siceraria* through *in Vitro* Culture

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***Abstract*—This aim of this research is to study and investigate mass propagation of *Lagenaria siceraria* through tissue culture system. This plant is commonly known as 'bottle guard' or 'calabash' and belongs to the family Cucurbitaceae. It is commonly cultivated in the tropical and subtropical regions of the world. The plant is grown for its fruit, which usually harvested and consumed as vegetable. Complete plant regeneration was successfully achieved when aseptic stem explant was cultured on MS medium supplemented with various combinations of plant growth regulators. MS medium supplemented with 2.0 mg/L Benyzlaminopurine (BAP) and 0.5 mg/L Napthalene Acetic Acid (NAA) was found to be the optimum medium and stem explant was the most responsive explant producing 4.633 ± 0.084 shoots per explant. Basal MS medium (without hormone) was optimum for root induction. Established plantlets were later acclimatized to the green house for further growth and development process. This research was successfully achieved and could serve as a good alternative to crop production in order to maintain the food security chain all over the world.**

***Index Terms*—*Lagenaria siceraria*, tissue culture, regeneration, MS medium, acclimatization**

I. Introduction

Tissue culture technique has long been used since the cell theory was established. Many scientists have tried to prove the totipotency concept, which is the ability of a single cell to form complete individual. Today, tissue culture technique is being used widely realizing its potentials in mass propagation and preservation of elite plants. The most important aspect in plant tissue culture is the capability of cultured cells and tissues to regenerate into complete plants. This technology is being utilized commercially in the ornamentals industry and in other plant production organizations worldwide [1]-[4]. Many temperate and tropical plants have been successfully propagated via tissue culture. An efficiently reproducible and rapid *in vitro* regeneration system is an important aspect in improving the conventional plant breeding programmes. One of the methods where specific traits can be added with minimal alteration of the target plant genome is through genetic transformation. Therefore, direct shoot formation from the explants is more desirable compared to intermediate callus phase. In the present work, propagation of *Lagenaria siceraria* through tissue culture techniques was done and the factors influencing the growth of this plant were studied. *Lagenaria siceraria* is also commonly known as calabash or bottle gourd and as all other gourds, belongs to Cucurbitaceae. It probably originated in tropical Africa and now has a pantropical distribution. It is a monoecious annual vine with a long, ribbed stem and strong tendrils. The mature fruit of bottle gourd is not a highly desirable food as it has little flesh and an offensive smell with a bitter taste. Its shell, however, becomes tough when it matures and makes it attractive for handicrafts. It is interesting that this species is exclusively used as a rootstock for watermelon and squash, especially for production in the winter greenhouse, because it possesses excellent tolerance to low soil temperature and to soil-borne pathogens such as *Fusarium oxysporum* f. sp. *niveum*. The cultivation of grafted vegetables has been successfully practised in many Asian countries for several decades and it is increasing in Europe [5].

Lagenaria siceraria fruit consists all the essential constituents that are necessary for the good health of human being. Traditionally, the fruits have been used as cardiotonic and cardioprotective drug. In addition, it has also been used as aphrodisiac, diuretic, antidote to certain poisons, scorpion sting s and alternative purgative. This plant is also used to relieve pain, ulcers and fever. Scientific researchers have also shown that *Lagenaria siceraria* have antifungal, antibacterial, anti-allergic, anti-inflammatory, antioxidant, analgesic, free radical scavenging, cytotoxic, antidiabetic as well as memory enhancing properties. Plant propagation by tissue culture technique is mainly aimed to produce plants with very high multiplication rates. Through indirect organogenesis, multiple shoots can be produced *in vitro* from callus. In the present study, experiments were conducted to investigate organogenesis from various sources of *Lagenaria* explants. The effects of various concentrations of plant growth regulators on the multiplication of shoots were examined.

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II. Materials and Methods

Initially seed coat of *Lagenaria siceraria* was removed manually. Then, the seed were rinsed under running tap water for approximately three hours. After that, the seeds were sterilized in 70% ethanol for 3 minutes, followed by 50%, 20% and 10% solution sodium hypochlorite for 10 minutes respectively. The seeds were rinsed with distilled water in 1 minute subsequently after sterilizing with 70% ethanol and series of sodium hypochlorite. Next, the seeds were transferred into the laminar flow. The seeds were sterilized again using 70% ethanol followed by 3 times rinsing with sterile distilled water. Then, the seeds were blot dried on sterile filter paper before cultured on MS basal medium [6]. The MS basal medium was prepared using distilled water, 30g/L sucrose, 8g/L agar and MS medium. All the substances were mixed and autoclaved at 121℃/18 psi for 20 minutes. The medium pH was set to 5.8 prior to autoclaving. The seeds were incubated at 27℃ under 16 hours of photoperiod. Plantlets were transferred to soil and maintained in culture room for 2-3 weeks for adaptation process before being transferred to field environment. *In vitro* propagated *Lagenaria* plants were compared morphologically with the intact plant. Survival rate of micropropagated plants that were transferred to soil were investigated.

III. Results and Discussion

Plant hormones and type of explants play very important roles in determining regeneration of *Lagenaria siceraria in vitro*. Many commercial plants are being propagated by *in vitro* culture on the culture medium containing auxins and cytokinins [7], [8]. Edible, ornamental plants and woody plant species are also propagated commercially by axillary bud proliferation [2], [3], [4]. Leaf explant promoted callus development. However, in this study, at a very high concentration of BAP and other growth regulators, development and growth of shoots were inhibited. The first step towards de novo regeneration is to establish callus or cell suspension cultures. Tissues of explants generally show distinct planes of cell division, various specializations of cells and organization into specialized structures such as the vascular system. Formation of callus from explants tissues involves the development of progressively more random planes of cell division, less frequent specialization of cells and loss of organized structures [9]. In *Lagenaria siceraria*, BAP was required for the formation of callus. Based on the present study, all combinations of hormones used were able to induce callus. Callus with good and compact structure was obtained when explant was cultured on MS medium supplemented with the combination of BAP and NAA. Relatively, in this experiment, callus formation was optimum when leaf explant was cultured on MS medium supplemented with 1.0 mg/l BAP + 1.0 mg/l NAA (Table I, Fig. 1a). This callus gave the highest dry weight. Meanwhile, stem explants cultured on MS medium supplemented with 1.0 mg/l BAP + 2.0 mg/l NAA also produced good, green compact callus (Fig. 1b). Callus formed in these experiments were mainly green. However, white, creamy friable callus was also obtained when explants were cultured on MS medium supplemented with 2, 4-D alone. The lowest callus formation was obtained when root explant was cultured on MS medium supplemented with 1.5 mg/l BAP + 1.0 mg/l NAA. Callus is capable of forming adventitious roots. Root formation occurred when explants were cultured on medium with higher auxin concentration and lower cytokinin concentration.

In the meantime, various explants have been used for direct shoot formation. Different types of auxin and cytokinin combinations were used in order to obtain complete regeneration of *Lagenaria in vitro*. The right combination of auxin and cytokinin in the culture medium determined the effectiveness of micropropagation of *Lagenaria* shoots. In the present study, regeneration of shoots increased when BAP was added with NAA. Highest numbers of shoots from stem explants (4.633 ± 0.084 per explant) were obtained when explants were cultured on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA (Table I, Fig. 1c). Shoots were obtained after 28 days of culture and shoot growth was normal. The lowest shoot formation was observed when explants were cultured on MS medium supplemented with 0.5 mg/l BAP and 2.0 m/l NAA with 0.550 ± 0.300 shoots per explant. Addition of strong auxin (NAA) with BAP promoted better shoot formation compared to weak auxin (IAA) [10]. Maximum shoot regeneration of *Lagenaria siceraria* was observed when cotyledons were cultured on MS medium added with 3.0 mg/l BA and 0.5 mg/l $AgNO_3$ [11]. Meanwhile, according to Mendi (2009) MS medium supplemented with 1.0 mg/l BA was optimal for shoot formation capacity of *Lagenaria siceraria* [12]. Higher addition of auxin compared to cytokinin in the culture medium resulted in the inhibition of shoot formation.

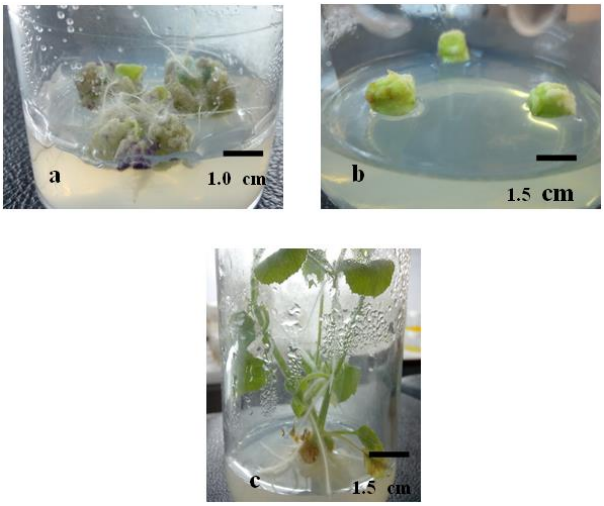

Figure 1. (a) Callus with hairy roots formed from leaf explants on MS medium supplemented with 1.0 mg/l BAP and 1.0 mg/l NAA. (b) Callus formed from stem explants on MS medium added with 1.0 mg/l BAP and 2.0 mg/l NAA. (c) Regeneration of shoots from petiole explants cultured on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA.

TABLE I. SHOOT FORMATION AND CALLUS INDUCTION OF *LAGENARIA SICERARIA* WHEN CULTURED ON MS MEDIUM SUPPLEMENTED WITH VARIOUS CONCENTRATION OF BAP AND NAA USING LEAF STEM AND ROOT EXPLANTS. MEANS FOLLOWED BY THE SAME LETTER IN THE SAME COLUMN DID NOT DIFFER ACCORDING TO DUNCAN'S MULTIPLE RANGE (DMRT) TEST AT 5% SIGNIFICANCE LEVEL

concentration	explants	shoot/ explant	weight of callus/explant (g)
MS media + 0.5 mg/l BAP + 0.5 mg/l NAA	Leaf Stem Root	- 1.566 ±0.341$_{g}$ -	0.247 ±0.024$_{f}$ 0.176 ±0.039$_{fg}$ 0.124 ±0.005$_{g}$
MS media + 1.0 mg/l BAP + 0.5 mg/l NAA	Leaf Stem Root	- 3.96 ±0.023$_{c}$ -	0.888±0.039$_{bc}$ 0.247 ±0.044$_{f}$ 0.388 ±0.151$_{ef}$
MS media + 1.5 mg/l BAP + 0.5 mg/l NAA	Leaf Stem Root	- 4.030 ±0.120$_{bc}$ -	0.932±0.124$_{bc}$ 0.667 ±0.047$_{cd}$ 0.625±0.257$_{d}$
MS media + 2.0 mg/l BAP + 0.5 mg/l NAA	Leaf Stem Root	- 4.633 ±0.084$_{a}$ -	0.627 ±0.055$_{d}$ 0.623 ±0.150$_{d}$ 0.621±0.111$_{d}$
MS media + 0.5 mg/l BAP + 1.0 mg/l NAA	Leaf Stem Root	- 2.838 ±0.233$_{ef}$ -	1.123 ±0.131$_{b}$ 1.128 ±0.118$_{b}$ 1.238 ±0.121$_{b}$
MS media + 1.0 mg/l BAP + 1.0 mg/l NAA	Leaf Stem Root	- 3.774 ±0.139$_{d}$ -	1.446 ±0.643$_{a}$ 0.925 ±0.119$_{bc}$ 0.629 ±0.136$_{d}$
MS media + 1.5 mg/l BAP + 1.0 mg/l NAA	Leaf Stem Root	- 3.867 ±0.143$_{cd}$ -	0.221±0.026$_{f}$ 0.219 ±0.216$_{f}$ 0.115 ±0.118$_{g}$
MS media + 2.0 mg/l BAP + 1.0 mg/l NAA	Leaf Stem Root	- 4.060 ±0.500$_{b}$ -	0.193 ±0.041$_{fg}$ 0.713 ±0.179$_{c}$ 0.743 ±0.031$_{c}$
MS media + 0.5 mg/l BAP + 1.5 mg/l NAA	Leaf Stem Root	- - -	0.127 ±0.118$_{g}$ 0.166 ±0.156$_{fg}$ 0.916 ±0.097$_{bc}$
MS media + 1.0 mg/l BAP + 1.5 mg/l NAA	Leaf Stem Root	- 0.665 ±0.520$_{hij}$ -	0.539 ±0.155$_{de}$ 0.129 ±0.114$_{g}$ 0.727 ±0.086$_{c}$
MS media + 1.5 mg/l BAP + 1.5 mg/l NAA	Leaf Stem Root	- 2.445 ±0.200$_{ef}$ -	0.882 ±0.180$_{bc}$ 0.621 ±0.094$_{d}$ 0.355 ±0.121$_{ef}$
MS media + 2.0 mg/l BAP + 1.5 mg/l NAA	Leaf Stem Root	- 2.877 ±0.330$_{ef}$ -	0.741 ±0.063$_{cd}$ 0.627 ±0.036$_{e}$ 0.397 ±0.149$_{f}$
MS media + 0.5 mg/l BAP + 2.0 mg/l NAA	Leaf Stem Root	- 0.550 ±0.300$_{hij}$ -	0.629 ±0.121$_{d}$ 0.515 ±0.282$_{de}$ 0.711±0.2123$_{c}$
MS media + 1.0 mg/l BAP + 2.0 mg/l NAA	Leaf Stem Root	- 0.680 ±0.078$_{hi}$ -	0.825 ±0.231$_{bc}$ 1.232 ±0.038$_{b}$ 0.147 ±0.164$_{fg}$
MS media + 1.5 mg/l BAP + 2.0 mg/l NAA	Leaf Stem Root	- 0.750 ±0.560$_{h}$ -	0.145 ±0.082$_{fg}$ 0.672 ±0.095$_{cd}$ 0.707 ±0.110$_{cd}$
MS media + 2.0 mg/l BAP + 2.0 mg/l NAA	Leaf Stem Root	- 2.908 ±0.103$_{e}$ -	0.442 ±0.224$_{e}$ 0.354±0.268$_{ef}$ 0.365±0.116$_{ef}$

BAP strongly enhanced regeneration of shoots in petiole explants of *Lagenaria siceraria*. Cytokinin especially BAP, are reported to overcome apical dominance, release lateral buds from dormancy and promote shoot formation [13]. Superiority of BAP in inducing multiple shoot formation has also been reported for a number of plants such as *Tridax procumbens* [14], *Cypripedium flavum* [15] and *Medicago truncatula* [16]. As for BAP alone, the best effect was obtained at 10.0 µM and a similar optimal concentration was also reported for *Ceropegia sahyadrica* [17] and *Andrographis paniculata* [18]. Number of shoots and number of leaves per explant of *Portulaca grandiflora* increased when low kinetin concentration was supplemented with BAP [18]. Optimum response was obtained when 8.0 µM kinetin was added with 2.0 µM BAP. Similar results have also been reported in *Lagenaria siceraria* [19].

The present study showed that only stem explant produced optimum results for shoot formation. Rooted plantlets of *Lagenaria* were successfully established and all plantlets were transferred to soil and maintained in the green house. Plantlets survival rate achieved was 85.00%.

IV. CONCLUSION

The main purpose for mass propagation of plants is for its edible, medicinal and aesthetic values. Thus, improvements of plant quality need to be studied and more research need to be done. One of the most important techniques in plant improvements is via micropropagation. Successful *in vitro* propagation of ornamental plants is now being widely used in commercialization purposes. In conclusion, the research done has proven that micropropagation of *Lagenaria siceraria in vitro* could be successfully obtained. Stem explants have been identified as the most regenerative explant for multiple shoot formation. Studies of *Lagenaria* clonal propagation could also be efficiently adapted for other ornamental plants.

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