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STANDARDIZATION AND IMPROVING OF *IN VITRO* MICROPROPAGATION OF NIGHT JASMINE (*CESTRUM NOCTURNUM* L.)

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Abstract

Night Jasmine is one of the most important ornamental plants in all over the world. But, inadequate multiplication rate and disease transmittance are hampers it cultivation to meet the demand of high quality planting material for commercial cultivation. Therefore, an efficient *in vitro* micropropagation protocol considers a best alternative to overcome this problem. The present study was carried out to assess in the micropropagation of *Cestrum nocturnum* by using single nodes and shoot tips excised from soft cuttings using MS salts, 30 g × l^{-1} Sucrose, 7 g × l^{-1} agar and different concentrations of plant growth regulators in culture medium. The results was showed that the use of mercuric chloride (0.05%, $HgCl_2$) for 10 minutes was very effective in preventing contamination and gave the highest survival percentage (99%). The highest response (100%) was gained at initiation stage from lateral bud explants on MS medium supplemented with 1.5 mg × l^{-1} of BA with most of NAA concentrations. The significant differences were observed at multiplication stage between the lateral buds and terminal buds, since the lateral buds produced a higher number of new shoots and leaves as well as longer new shoots. At the rooting stage, the treatment with 1 mg × l^{-1} IBA gave the highest percentage of rooting (100%), the highest number of roots (14.1 root/explants) and the longest roots (8.48 cm) respectively, on half strength MS medium. Plantlets obtained were transferred to pots and acclimatized with 95% success. The role of plant tissue culture in meeting the ever increasing demand and requirements of man in the field of agriculture, forest, horticulture and medicine is highly impressive.

Key words : *Cestrum nocturnum*, Standardization, Transplantable Plantlets, Explants, Micropropagation

Introduction

The genus *Cestrum* contains more than 300 species and most of them are native to warm subtropical and tropical areas. *Cestrum nocturnum* is a member of the family Solanaceae. It is commonly known under other names Lady of the Night, Queen of the Night and Night blooming Jasmine because of its strongly scented flowers at night (Stern *et al*., 2004). Jasmines generally grow in all types of soils. However, they are better adapted to rich loamy or dry sandy and irrigated soil. Water logging or excessive watering is detrimental to the plants. It is now possible to develop methods for virus free plant regeneration, salinity tolerance, herbicide resistance, disease resistance, frost resistance, incorporation of high protein content and genetically engineer plants for desirable traits. Hybridization, clonal selection, mutation and ploidy breeding have been attempted as plant improvement methods. Plant extracts have shown larvicidal activity against the mosquito *Aedes aegypti* while showing no toxicity to fish (Patil *et al*., 2011). Plant extracts because hematological changes in the freshwater fish when exposed to sub lethal concentration of this plant (Pérez-Saad and Buznego, 2008). Due to successful research carried out during this period plant tissue culture is an established field in plant cell biotechnology (Khairul Islam *et al*., 2015).

The leaves of the plant have shown significant analgesic and bactericidal activity (Huang *et al*., 2006 and Catterjee *et al*., 2007). Local anesthetic effect, inhibitory effect on central nerve system and cardiac arrhythmic effect of plant is also documented (Zeng *et al*., 2003). Mature leaf holds a calcinogenic glycoside that escorts to vitamin D toxicity and is accountable for

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elevated serum calcium level (Mello, 2003). Some of glycosides such as (25*R*)-spirost-5-ene-2R, 3,-diol pentaglycosides (nocturnoside A), (25*R*)-spirost-5-en-3,-ol tetraglycoside (nocturnoside B) and seven steroidal saponins including four new ones and eight new steroidal glycosides have been isolated from the leaves of *C. nocturnum* (Mimaki *et al*., 2006). Several phytochemical studies have demonstrated the presence of important bioactive compounds in different parts of the plant: alkaloids, flavonol glycosides, steroidal saponins, fatty acids, essential oils, phenols and others (Jawale and Dama, 2010). Practitioners use the plant externally for skin disorders, but several scientific reports demonstrate that it exhibits a wide spectrum of pharmacological activity when administered systemically or in isolated organ preparations. For example, it is used to treat arterial hypotension and as an analgesic, abortive, diuretic, antispasmodic, dyspeptic, antiviral and smooth muscle relaxant; it also has negative inotropic and chronotropic actions (Jawale *et al*., 2012). Most of the species of Cestrum have found several applications in folk medicine. *Cestrum parqui* is used in Chilean folk medicine as antifebrile and for the treatment of fever and inflammation (Backhouse *et al*., 1996). Therefore, we have undertaken to investigate the antifungal activity of the essential oil and organic extracts from flowers of *C. nocturnum* growing in Bangladesh and the results are reported in this communication.

In traditional medicine, leaves of *C. nocturnum* have been used for their pharmacological significance in burns and swellings. It is also used for treating epilepsy and as stupefying charm medicine in West Indian Islands. The volatile oil is known to be mosquito-repellent and hence *C. nocturnum* is used to prevent malaria in several African Nations (Mimaki *et al*., 2006). Pharmacological studies on the plant proved that the leaves have significant analgesic and bactericidal activity (Huang *et al*., 2006). Tissue culture has found application in a number of areas of plant science, including basic physiology, production of natural and pharmaceutical compounds, plant pathology, germplasm preservation, breeding, recovery of transgenic plants, and propagation (Hartmann *et al*., 2002).

Materials and Methods

This study was conducted in the laboratory of plant tissues and cells culture in the Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh. It aims to propagate *Cestrum nocturnum* plant using the tissues culture technique. Several experiments including the test of response of the different explants were done to propagation and studying the effect of some growth regulators at three stages of growth (initiation, multiplication and rooting) as well to determine the optimum conditions and ways of acclimatization of plant produce by tissue culture and transplant them to the soil.

The methodology for media preparation involves preparation of stock solutions of highly purified chemicals and demineralized water. MS medium formulated by Murashige and Skoog (MS) is most widely used for many types of culture systems. The stock solutions are stored in glass or plastic containers and frozen till further requirement. Now a day, plant tissue culture media are commercially prepared and are available in the market as dry powders. The culture media is usually sterilized in an autoclave at 121^0C and 15 psi for 20 minutes. Hormones and other heat sensitive organic compounds is filter sterilized and added to the autoclaved medium. Several experiments including the test of response of the different explants were done to propagation and studying the effect of some growth regulators at three stages of growth (initiation, multiplication and rooting), as well to determine the optimum conditions and ways of acclimatization of plant produce by tissue culture and transplant them to the soil.

Initiation stage

Sterilization process was done for all explants by soaking them into two types of sterilizing solutions. After sterilization actively growing shoots 15–20 cm long were cut from 1-year-old *Cestrum nocturnum* grown in the greenhouse. Shoots were defoliated and washed with water for 60 minutes to remove soil and other superficial contamination, followed by tap water and liquid soap for 20 minutes, followed by three-five minute rinses in sterile distilled water. Then, they were cut into shorter sections 1.5 cm long including the [terminal (apical) bud] and single nodes with an axillary bud. Shoot tips and nodes with axillary buds were removed and disinfected by immersion in the solutions of the Mercuric Chloride ($HgCl_2$), (0.05%) w/v for 10 minutes. The disinfested tissues [explants] were rinsed 3–4 times with sterilized distilled water and the ends of explants exposed to sterilant were trimmed. The experiments were conducted with ten replicates and the explants were placed aseptically in 25 × 150 mm test tubes containing 15 ml of MS medium supplemented with different concentrations of growth regulators. Benzyladenine (BA) with 0, 1.5, 3 and 4.5 mg × l^{-1} was added to the culture medium to observe the response of cultured explants at the initiation stage. Ten explants were cultured (an explant in each test tube for each concentration). They were incubated at 25 ± 2^0C under

light conditions of 16 light hours and 8 darkness hours. The results were recorded after 4–6 weeks from planting Different concentrations of BA and NAA were tested to find out their effect on culture initiation when combined together. BA was used at 0, 1.5, 3 and 4.5 mg × l^{-1} and NAA at 0, 0.2, 0.4 and 0.6 mg × l^{-1}. Ten test tubes were used for each treatment. On the basis of stage I results, the produced microshoots from the treatments were moved to MS medium (multiplication stage medium) from the best treatment. Number and length of shoots were recorded after 6 weeks from planting.

Multiplication stage

In multiplication stage experiments included the effect of BA was tested at 0, 1.5, 3 and 4.5 mg × l^{-1} to discover its effects on number and length of new shoots as well as the effect of the interaction between BA and NAA on multiplication stage. BA was added at 0, 1.5, 3 and 4.5 mg × l^{-1}, while NAA at 0, 0.1, 0.2 and 0.3 mg × l^{-1}. GA_3 was added to MS medium at 3 mg × l^{-1} to all treatments, including the control. The effects of IBA, NAA and IAA added to the culture medium on micro shoot rooting were studied by carrying out several separate experiments by adding IBA, NAA and IAA (0, 0.5, 1 and 2 mg × l^{-1}). All these treatments were examined in half strength MS medium. As far as the rooting stage is concerned, features such as number of micro shoots, number of roots/shoots and root length (cm) were recorded. These evaluations were performed on a weekly basis for 4–6 consecutive weeks. At the end of six weeks, the results were compiled, averaged and expressed as a percentage or number for each treatment.

Rooting stage and acclimatization

To stimulate the emergence of adventitious roots on shoots this produced from the tissue plantation. After 6–8 weeks from *Cestrum nocturnum* shoot rooting, several micro plants were selected from those that showed good vegetative growth. They were washed under tap water to remove agar from the roots, which might be a source of contamination. They were then put in Benlate fungicide solution (0.1%) and planted in plastic pots filled with a sterilized mixture of peatmoss and river soil (1:1). In order to maintain high humidity in the culture environment, the pots were covered with a light plastic cover which permits light penetration and contains many openings to permit air entrance. Micro plants were watered and given a solution containing MS salts with 0.25 of original strength. The plastic cover was removed from time to time after two weeks from planting. After four weeks, the microplants were transplanted after being sprayed with Benlate fungicide (0.1%) as required.

Results and Discussion

Initiation stage

The effect of different concentrations of BA, NAA and their interactions on the percentage of response of lateral and terminal buds excised from soft cuttings of *Cestrum* cultured on MS medium. For lateral buds, it can be noticed that the concentration of BA (1.5 mg × l^{-1}) was significantly superior over the other concentrations for both lateral and terminal buds and gave the highest response percentage (100%). In case of lateral buds the treatment of 1.5 mg × l^{-1} BA with most of NAA concentrations gave the highest response (100%). However, in case of terminal buds, higher percentages of responses resulted from the treatment of 1.5 mgl^{-1} BA with 0, 0.2 mg × l^{-1} NAA concentration. Figure 1 shows the effects of BA, NAA concentrations and their interactions as well as types of buds on the average number of shoots, leaves and the length of new shoots at the initiation stage. It is clear that lateral buds produced more new shoots as well as a higher number of leaves and length of new shoots as compared with those from terminal buds. This may be due to cytokinin deficiency in the lateral buds (Snir and Erez, 1980). Using BA at 1.5 mg × l^{-1} resulted in obtaining the highest number of shoots and leaves as well as the highest shoot length in lateral and terminal buds (5.2, 4 shoots/explant, 19, 13.2 leaves/explant and 6.06, 5.54 cm; respectively). This indicates the necessity of cytokinin (BA) presence in initiation medium. This fact has been discussed in many published studies on the tissue culture of many fruit trees like pear (Hirabayashi *et al*., 1987), plum (Catterjee *et al*., 1986) and walnut. It is clear that the highest values of number of shoots, number of leaves and length of new shoots were obtained from the interaction between the low concentrations of both growth regulators for both lateral and terminal buds.The treatment was resulted in a significant increase in the average number of new shoots, average number of leaves and average length of new shoots on lateral buds as compared with those from terminal buds. But the lowest number of new shoots, average number of leaves and average length of new shoots were produced from the treatment free of plant growth regulators (2.6, 2.2 shoots/explants, 7.2, 6.6 leaves/explants and 4.78, 3.34 cm). These results are in agreement with what has been found (Singh *et al*., 1994), they found that using of cytokinins and auxins in this category is very important and the role of cytokinins at this stage is essential to break apical dominance in buds and to induce the subsidiary meristem grow into a shoot.

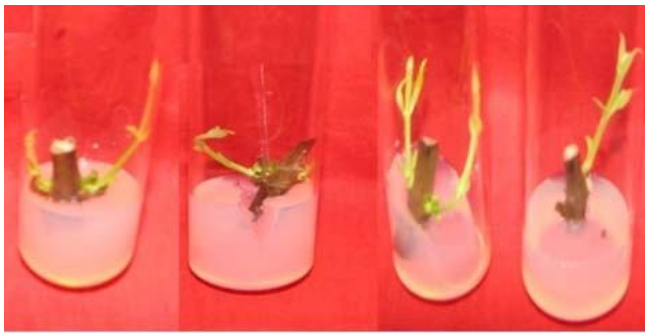

Fig. 1 : Shoot initiation of *Cestrum nocturnum* on the MS medium supplemented with BA + NAA at different concentrations after 4–6 weeks of culture.

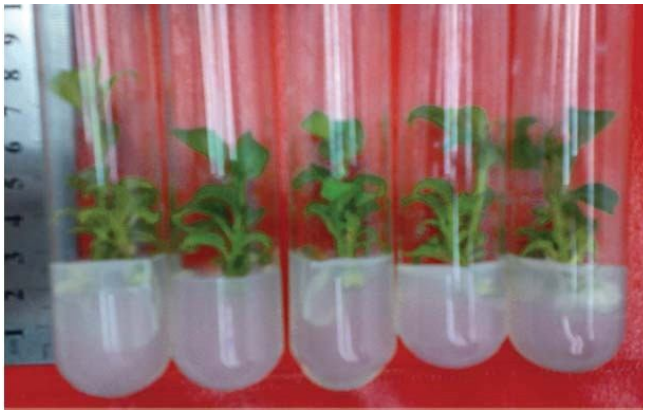

Fig. 2 : Shoot multiplication of *Cestrum nocturnum* on MS medium supplemented with BA + NAA at the different concentrations after 4–6 weeks of culture.

Fig. 3 : Root initiation of *Cestrum nocturnum* on MS medium supplemented with IBA, NAA and IAA at the different concentrations after 4–6 weeks of culture

Multiplication stage

During the multiplication stage (fig. 2) reveals the effects of different concentrations of BA, NAA and their interactions and types of buds on the average number of shoots, average number of leaves and lengths of new shoots at the multiplication stage. Significant differences were recorded between the lateral and terminals buds in

Fig. 4 : Microplants established in the pots after 6–10 weeks of transfer *ex vitro*.

which lateral buds produced higher numbers of new shoot, leaves and lengths of new shoots. It is thought that cytokinins promote the formation of woody tissues neighboring to the vascular tissues of the bud and stem, thus will make easy the translocation of water and nutrients, which cause bud initiation (Mohammed and Younis, 1991). It can be noticed that the using low concentrations of BA (1.5 mg × l^{-1}) led to get the highest responses in number of shoots (4.4 and 4.2 shoots/ explant), number of leaves (15.4 and 4.2 leaves/explant) and length of new shoots (5.36 and 4.86 cm) for lateral and terminal buds respectively. These results agree with those reported (Brookner, 1991) in their studies on the importance of cytokinins in shoot multiplication. The effect of interaction between cytokinins and auxins in vegetative multiplication and increasing growth lengths can be interpreted by the increase of cytokinins role in the presence of auxins as (Mohammed and Younis, 1991) reported that movement of cytokinins is generally activated in the presence of auxins, so a larger number of buds will have a chance to grow and start to produce shoots (Thanhran, 1921). These results are in agreement with those reported (Roy *et al*., 2004), who emphasized the importance of the interaction between auxins and cytokinins in the vegetative multiplication processes.

Rooting stage

The microshoots were transferred from multiplication medium and placed in half strength MS macro and micro elements supplemented with different concentrations of IBA, NAA and IAA (0–2 mg × l^{-1}). The micro shoots showed different responses to the rooting after 4–6 weeks

of culture (fig. 3). The highest percentage of rooting (100%) was obtained on half strength MS medium supplemented with 0.5, 1 mgl^{-1} NAA and IBA, respectively. On the other hand, in case of IAA, the highest rooting percentage of *Cestrum* shoots cultured in half strength MS (90%) were obtained at a concentration of 1 mg × l^{-1} IAA. Endogenous hormones might have a role in promoting plants to root (Peak *et al*., 1987), until the hormonal balance reached its optimal level to push the roots to grow and develop in the presence of exogenous hormones, since increasing auxin concentration promotes root formation on shoot bases (George and Shermington, 1984). The effects of IBA, NAA and IAA concentrations on the average number of roots per shoot and average root length. Concerning the effect of NAA, it is clear that the highest values for root numbers per shoot (7 roots/explant) and root length (5.46 cm), respectively were obtained at the concentration of 0.5 mgl^{-1} NAA in half strength MS medium.

These results prove that auxins have a role in the rooting process, since they promote adventitious root initiation in the bases of cultured shoots (Saleh, 1990). These results are in the agreement with those found (Abdullah *et al*., 2003), who observed that reducing the levels of MS salts in the medium to half increased rooting of the many tree species. Decreasing the level of salts in the medium means decreasing the level of nitrogen in the medium to half or quarter; this will result in decreasing the nitrogen level in the shoots, which may cause the percentage of carbohydrates to be increased to the nitrogen level and this in turn may result in increasing the percentage of root primordia and root numbers (Gawel *et al*., 1990).

Acclimatization stage

The rooting shoots were taken to pots containing loam soil or peat moss alone or as a mixture of both of them with a ratio 1:1 and were incubated in the growth room in the same conditions initiation stage by covering them with plastic lifted gradually at the beginning of the third week. The best percentage of survival plants (100%) in the medium consisting of the loam soil or mixture of loam soil and peat moss, which was significantly superior with the peat moss alone (70%) after 4 weeks of acclimatization had been studied. Figure 4 shows that the micro plants of *Cestrum* were carefully removed from rooting media and transferred to the greenhouse in small plastic pots with medium consisting of peat moss and river soil (1:1). The plants were finally hardened by gradually reducing the humidity. After four weeks from transplanting, the survival percentage reached 90% of plants. This protocol for vegetative micro propagation agrees with what has been found by many researches in the case of fruit plants that were moved to open air field like apples [28], peaches (Reeves *et al*., 1983) and chestnut (Preece and Sutter, 1991).

Conclusion

Plant tissue culture offers tremendous opportunities in plant propagation, plant improvement and production of plants with desirable agronomical features. The study was carried out to assess in the micro propagation of *Cestrum nocturnum* by using single nodes and shoot tips excised from soft cuttings using MS salts, sucrose, agar and different concentrations of plant growth regulators. The results revealed that the use of mercuric chloride for 7 minutes was very effective in preventing contamination. The highest response was gained at initiation stage from lateral bud explants on MS medium supplemented with BA with most of NAA concentrations. At rooting stage, the treatment with IBA gave the highest percentage of rooting (100%), the highest number of roots and the longest roots respectively on half strength MS medium. Plantlets obtained were transferred to pots and acclimatized with 90% success. Micro propagation is an important technology and tissue culture has found application in a number of areas of plant science, including basic physiology, production of natural and pharmaceutical compounds, plant pathology, germplasm preservation, breeding, recovery of transgenic plants and propagation. It is now possible to develop methods for virus free plant regeneration, salinity tolerance, herbicide resistance, disease resistance, frost resistance, incorporation of high protein content and genetically engineer plants for desirable traits. Advances in plant tissue culture will enable rapid multiplication and sustainable use of medicinal plants for future generations.

Conflicts of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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