J. Bio-Sci. 30(1): 69-78, 2022 (June) **ISSN 1023-8654**
http://www.banglajol.info/index.php/JBS/index
DOI: https://doi.org/10.3329/jbs.v30i1.63102

IN VITRO SHOOT REGENERATION FROM NODAL AND SHOOT TIP EXPLANTS OF A THREATENED MEDICINAL PLANT - *SMILAX ZEYLANICA* L.

Mustafa Abul Kalam Azad*, Mithun Adhikari, Md. Nur Alam Sarker, A N M Maruf Hasan and Muhammad Nurul Amin

Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract

An efficient protocol for *in vitro* clonal propagation of *Smilax zeylanica* was developed using nodal and shoot tip segments from field grown plants. Nodal explants performed better than shoot tip explants when two distinct explants were tested for shoot proliferation. The best results for shoot proliferation were achieved with MS medium supplemented with BAP and Kn alone or in combination with NAA and IBA at various concentrations. On MS medium containing 4.0 µM BAP, the greatest number of shoots were produced. *In vitro* proliferating shoots were transplanted to full strength MS medium fortified with IBA and NAA separately at varying concentrations (1.0 - 6.0 µM) in order to induce adventitious roots. The medium containing 2.0 µM IBA showed the best rooting reactions. Plantlets having well developed root system were transferred to soil and successfully acclimatized with 80% survival rate under *ex vitro* condition.

***Key words*:** *In vitro* propagation, Plant growth regulators, Axillary shoot proliferation, Basal medium

Introduction

The plant *Smilax* sp. is a dioecious climbing shrub with woody stem belongs to the monocotyledon family Smilacaceae. This plant is common throughout India and is also indigenous to various regions of the Indian Subcontinent and Asia, including Bangladesh, Myanmar, Malaysia, Java, and the Solomon Islands (Dhanya et al. 2018). More than 300 species of the genus *Smilax* exist around the world, 24 of which are found in India. In English they are called Wild Sarsaparilla, Indian Sarsaparilla or Black Creeper also known as Catbriers, Greenbriers and Smilax. In Bangladesh it is better known as Kumarilata or Kumarika but in some places this plant is also known as Bulkumia, Beral Achra. The *Smilax zeylanica* is a hard climbing cut ascending plant. The apex of the leaf is narrow, the stalk is round and the upper side of the leaf is smooth (Kamble and Lobo, 2022).

Sarsaparilla was manufactured from the bark of the *Smilax regelii* into soft beverages, tea, beer, and soda from its rhizomes, and was highly well-liked all across the world. In the nineteenth century, sarsaparilla drink was widely used as a patent medicine in America. It was thought to be a cure for several skin and blood issues at the time. Drinking sarsaparilla has a diuretic effect that helps prevent venereal disease and, as a result, aids in cleansing the urethra after sexual activity. The currently marketed sarsaparilla-flavored beverages do not include *Smilex* sp. It can be said that the original sarsaparilla is now rarely prepared. (Thirunganasampanand et al. 2008). The *Smilax* sp. was listed with the USP as a syphilis treatment from 1820 to 1910. Many chemical components, such as saponin, resin, diosgenin, tannin, cytosterol, coumarin,

*Author for correspondence: drmakazad@gmail.com

rutin, and smilasparic acid, are seperated from the root during virginity. These alkaloids are used to treat gonorrhea, arthritis, leg discomfort, bloating, anemia, diarrhea, and fever as well as syphilis and other diseases. When treating arthritis, its rhizome is dried and ground up (Rajesh and Perumal 2014). Some American researchers asserted in 2006 and 2007 that Smilax's rhizome had successful and antiviral outcomes against liver cancer (Hooda et al. 2011). Due to the difficulty in rooted, conventional vegetative cutting propagation is not used. Micropropagation is superior to traditional vegetative propagation in many ways, and it can be utilized to support conservation efforts and make use of genetic variety. In this article, we describe a dependable technique for *S. zeylanica in vitro* large-scale multiplication using nodal and shoot tip explants as part of the conservation effort.

Materials and Methods

The fast-expanding shoots' apical segments (7 to 10 cm) were taken from mature, field-grown plants and carefully cleaned under running water. Following a 10-minute treatment with 1% savlon and constant shaking, the shoot segments were rinsed three to four times with distilled water. The sample was moved to a sterilized conical flask under a running laminar airflow cabinet for surface sterilization. With 0.1% $HgCl_2$, the surface was disinfested for varying duration of time. The material was then rinsed at least four to five times with sterile distilled water to get rid of any remaining traces of the sterilant. From the surface-sterilized material, the segments with a shoot tip and a single node (1.0 to 1.5 cm) were prepared and used as explants for *in vitro* cultivation. First, field-grown sterilized nodal and shoot tip explants were cultured on MS media (Murashige and Skoog 1962) with various concentrations (2.0 - 8.0 µM) of BAP (6 - benzylaminopurine) and Kn (N^6-furfuryldenine) in order to choose the best explant for axillary shoot proliferation. Second, *in vitro* grown nodal explants were initially cultivated on four different basal medium, namely MS, MMS_1, MMS_2, and WPM (Woody plant medium) (Lloyd and McCown 1980), supplemented with 4.0 µM BAP, to examine the influence of basal medium on *in vitro* shoot multiplication. In addition, to assess the effectiveness of shoot induction methods from nodal explants, different concentrations (1.0 - 4.0 µM) of NAA (α-naphthaleneacetic acid) or IBA (indole-3-butyric acid) were mixed with 2.0 - 6.0 µM BAP or Kn. By cutting off the basal leaves of usable shoots, microshoots of 1-3 cm length were prepared, and they were then individually cultivated in 25 mm x 150 mm culture tubes with 15-20 ml of full-strength MS media supplemented with NAA, IBA, or IAA (1.0 - 6.0 µM).

The rooted plantlets were then placed in the tiny plastic pots with the sterile soil mixture (garden soil, sand and compost in 2:1:1 ratio). Plantlets that had been transferred were hardened in growth chamber conditions for 25 days before being moved to outdoor conditions. Both the total number of plants moved into pots and the number of plants that survived outdoors were counted. All media made with 0.8% (w/v) agar and 3% (w/v) sucrose (Sigma Chemical Co. USA). Before the medium was autoclaved at 121°C for 20 minutes at 1.2 kg/cm^2 pressure, the pH was adjusted to 5.7 ± 1.0 The cultures were grown at 25 ± 1°C for a 16 h photoperiod under the illumination of a cool-white fluorescence tube lamp with a light intensity of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After 8 weeks of culture incubation, all experimental results were recorded. Only the data were recorded after 4 weeks of culture in the case of the rooting experiment. A total of 15 to 20 explants were used throughout all studies, and each experiment was run three times. For all numerical data, mean and standard deviation were determined. The Duncan's Multiple Range Test (DMRT) was used to compare the mean data for each treatment at a p-value of 0.05%.

Result and Discussion

The sterile, living explants were grown on MS media that had been treated with BAP and Kn at various concentrations (2.0, 4.0, 6.0, and 8.0 µM) to promote axillary shoot proliferation. The optimum performance for the proliferation of axillary shoots was demonstrated by nodal explants of mature plant origin that were grown on 4.0 µM BAP. Ninety percent of cultivated explants grew shoots on this media. The total number of shoots per culture, the number of useable shoots per culture, and the average length of a shoot were all noted to be 6.5 ± 1.0, 5.9 ± 1.1, and 6.0 ± 0.6 cm, respectively (Fig. 1A, C). On the other hand, when shoot tip explants were cultivated on the same medium, 75% of the cultures developed shoots. The number of total shoots per culture, the number of useable shoots per culture, and the average length of the shoots in this example were, respectively, 4.3 ± 0.5, 4.1 ± 0.2, and 5.3 ± 0.4 cm (Fig. 1B, D). The types and concentrations of the cytokinins utilized had a significant impact on the proliferation of axillary shoots from the nodal and shoot tip segments of mature plants as well as *in vitro* grown shoot origin. At the majority of the concentrations, the cytokinin BAP was noticeably more effective than Kn in promoting axillary shoot proliferation. The percentage of explants exhibiting proliferation, the number of shoots per culture, and the average length of shoot steadily decreased with the increase in cytokinin concentrations from 6.0 to 8.0 µM. (Table 1). Results of this experiment indicated the high regenerative capacity of nodal explants of *S. zeylanica* than shoot tip explants. Similar results were noted in *Dalbergia congestiflora* (Hernández-García et al. 2021), *Aegle marmelos* (Mandal and Parsai 2020), and many other species. On the contrary of these findings, Zaib-Un-Nisa et al. (2019) in *Saussurea lappa*, Joshi and Mathur (2015) in *Anthocephalus cadamba,* and Naz et al. (2015) in *Althaea officinalis* observed increased shoot proliferation from the seedling explants rather than cotyledonary nodes. Debergh and Read (1990) hypothesized that the different responses of various explants from the same plant are more species-specific, whereas Lane (1978) proposed that the level of endogenous hormones in the buds of various stem regions is the cause of the various responses of various explants from the same plant. Based on the current findings, additional research should be done on *S. zeylanica* to determine the precise cause of the variable responses of the explants.

In comparison to the other three media (WPM, MMS_1 and MMS_2) studied the full-strength MS medium considerably increased shoot proliferation from nodal explants. On MS medium, maximum 90.0% explants resulted in the highest 6.5 ± 1.0 shoot production. The longest shoots (6.0 ± 0.6 cm) were formed by explants in MMS_1 medium, but this medium's percentage of response and multiplication rate were both lower than those of full-strength MS media (Table 2). This study revealed that full strength MS medium was preferred for axillary shoot proliferation from nodal of *S. zeylanica* while MMS_1 showed a little effect in terms of shoot proliferation. Full strength MS medium has been proved best for axillary shoot proliferation in many other species such as *Costus speciosus* (Jothi et al. 2022), *Cordia subcordata* (Xiong et al. 2021), and *Phellodendron amurense* (Azad et al. 2005). Similar results were also observed in some other plants, like *Salvia tomentosa* (Martini et al. 2022), *Helianthus verticillatus* (Nowakowska et al. 2020), and many other plant species.

Table 1: Effects of different concentrations of cytokinin in MS medium on direct regeneration of shoot from nodal and shoot tip segments of *Smilax zeylanica*.

Growth regulators (µM)	Type of explants	Explant showing proliferation (%)	No. of shoo/ culture	No. of usable shoot/ culture	Average length of shoots (cm)
Nil	NS	20.0	1.8 ± 0.6^{e}	1.2 ± 0.3^{f}	2.8 ± 0.1^{d}
	ST	15.0	1.2 ± 0.7^{e}	1.0 ± 0.4^{f}	1.8 ± 0.2^{e}
BAP					
2.0	NS	80.0	3.7 ± 0.1^{d}	3.1 ± 0.2^{d}	4.2 ± 0.4^{c}
	ST	65.0	3.2 ± 0.9^{d}	2.8 ± 0.7^{d}	3.3 ± 0.7^{d}
4.0	NS	90.0	6.5 ± 1.0^{a}	5.9 ± 1.1^{a}	6.0 ± 0.6^{a}
	ST	75.0	4.3 ± 0.5^{c}	4.1 ± 0.2^{c}	5.3 ± 0.4^{b}
6.0	NS	75.0	5.0 ± 0.6^{b}	4.8 ± 0.4^{b}	5.1 ± 0.7^{b}
	ST	45.0	3.5 ± 0.7^{d}	3.1 ± 0.3^{d}	4.5 ± 0.1^{c}
8.0	NS	55.0	2.2 ± 0.3^{e}	2.0 ± 0.3^{e}	3.2 ± 0.8^{d}
	ST	35.0	1.9 ± 0.4^{e}	1.3 ± 0.2^{f}	2.9 ± 0.4^{d}
Kn					
2.0	NS	40.0	2.3 ± 0.8^{e}	2.1 ± 0.3^{e}	2.3 ± 0.4^{d}
	ST	35.0	1.7 ± 0.1^{e}	1.2 ± 0.4^{f}	1.7 ± 0.8^{e}
4.0	NS	60.0	3.6 ± 0.4^{d}	3.1 ± 0.2^{d}	3.6 ± 0.3^{d}
	ST	55.0	2.5 ± 0.6^{e}	2.2 ± 0.5^{e}	2.5 ± 0.2^{d}
6.0	NS	50.0	3.5 ± 0.9^{d}	3.1 ± 0.1^{d}	3.5 ± 0.5^{d}
	ST	45.0	2.6 ± 0.7^{e}	2.2 ± 0.4^{e}	2.6 ± 0.1^{d}
8.0	NS	25.0	1.6 ± 0.7^{e}	1.2 ± 0.3^{f}	1.6 ± 0.3^{e}
	ST	20.0	1.3 ± 0.6^{e}	1.0 ± 0.2^{f}	1.3 ± 0.5^{e}

Values represent means ± standard error of 20 explants per treatment and data were recorded after eight weeks of culture. Means followed by the same letters are not significantly different by Duncan's multiple Range Test at 0.05% probability level.

Table 2: Effects of different basal media containing 4.0 µM BAP on *in vitro* shoot multiplication from nodal explants of *Smilax zeylanica.*

Basal medium	Explant responded (%)	No. of total shoots/ culture	No. of usable shoots/ culture	Average length of shoots (cm)
MS	90.0	6.5 ± 1.0^{a}	5.9 ± 1.1^{a}	5.1 ± 0.2^{b}
MMS_1	85.0	5.8 ± 0.5^{b}	4.2 ± 0.3^{b}	6.0 ± 0.6^{a}
MMS_2	70.0	3.4 ± 0.8^{c}	3.5 ± 0.5^{c}	4.3 ± 0.7^{c}
WPM	65.0	2.7 ± 0.6^{d}	2.1 ± 0.4^{d}	3.5 ± 0.4^{d}

Values represent means ± standard error of 20 explants per treatment. Means followed by the same letters are not significantly different by Duncan's multiple Range Test at 0.05% probability level. MS = full strength MS medium; MMS_1 = ½ strength of major salts and full strength of minor salts and vitamins; MMS_2 = ½ strength of both major and minor salts, and full strength of vitamins.

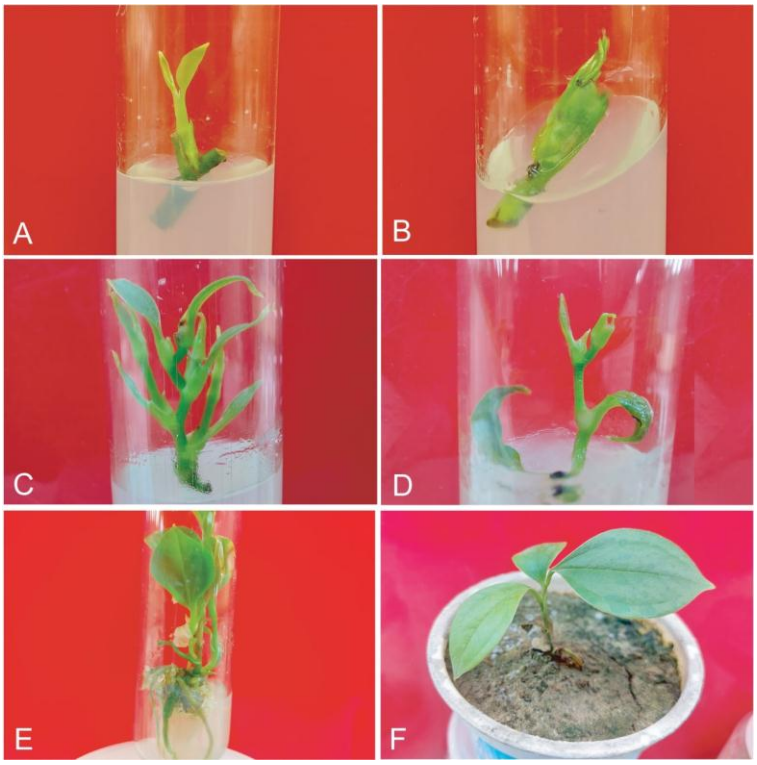

Fig. 1: *In vitro* regeneration of plantlets from the explants of filed grown mature plant of *Smilax zeylanica* L. A-B. Development of axillary shoots from nodal (Fig. A) and shoot tip (Fig. B) explants after four weeks of culture on MS medium containing 4.0 µM BAP, C-D. Multiple shoot formation and shoot elongation from the same explants after ten weeks of culture, E: Development of roots from the bases of micro-cuttings on MS medium containing 2.0 µM IBA after 8 weeks of culture, F. Acclimatized plantlets after 3 weeks of transplantation onto soil mix.

According to Rajeswari and Paliwal (2008) in *Pterocarpus santalinus*, cotyledonary node explants cultivated on MS medium devoid of a growth regulator produced 0.92±0.19 shoots with 0.64±0.14 cm lengths. This may be because cotyledonary nodes contain endogenous cytokinin. Exogenous cytokinin, however, significantly increased the rate of shoot multiplication when added to MS medium, demonstrating the need for exogenous cytokinin availability in the medium for enhanced axillary shoot proliferation. The multiplication and elongation of axillary shoots were significantly affected by the addition of NAA or IBA to BAP. In all auxin-cytokinin combinations, the average number of shoots and the average length of shoots both increased significantly. In the instance of *Centella asiatica,* Jaheduzzaman et al. (2012) supported this agreement.

Nodal explants produced the most shoots (7.6±0.2), which were 6.7±0.4 cm long, on a medium containing 4.0 µM BAP + 2.0 µM IBA (Table 3). The findings showed that the production of shoot buds required exogenous auxin, and they also showed that NAA or IBA with BAP had a favorable effect on *S. zeylanica's in vitro* shoot proliferation. According to Slupski et al. (2011), auxins (NAA, IBA, and IAA) combined with BA were beneficial in promoting the growth of shoots from *Codonopsis pilosula's* cotyledonary nodes.

Table 3: Effects of different concentrations and combinations of cytokinins and auxins on induction of axillary shoots from nodal segments of *Smilax zeylanica*.

Growth regulators (µM)	Shoot formation (%)	No. of total shoot / culture	No. of usable shoot / culture	Average length of shoot / culture (cm)	*Intensity of callus growth
BAP + IBA					
2.0 + 1.0	75.0	5.6 ± 0.4^{c}	4.1 ± 0.2^{c}	5.4 ± 1.1^{b}	-
4.0 + 2.0	80.0	7.6 ± 0.2^{a}	6.3 ± 0.4^{a}	6.7 ± 0.4^{a}	-
6.0 + 4.0	50.0	4.1 ± 0.1^{d}	3.3 ± 0.8^{c}	3.9 ± 0.2^{d}	+
BAP + NAA					
2.0 + 1.0	55.0	4.2 ± 0.3^{d}	3.9 ± 0.2^{c}	4.2 ± 1.0^{e}	-
4.0 + 2.0	70.0	6.5 ± 0.6^{b}	5.4 ± 0.5^{b}	5.7 ± 0.5^{b}	+
6.0 + 4.0	30.0	3.4 ± 0.1^{d}	2.8 ± 0.5^{d}	3.1 ± 0.3^{e}	++
Kn + IBA					
2.0 + 1.0	30.0	3.1 ± 0.2^{d}	2.2 ± 0.3^{e}	3.6 ± 0.5^{c}	-
4.0 + 2.0	50.0	4.1 ± 0.3^{d}	3.1 ± 0.1^{d}	4.3 ± 0.5^{b}	-
6.0 + 4.0	15.0	2.2 ± 0.1^{d}	1.9 ± 0.2^{e}	3.0 ± 0.1^{c}	+
Kn + NAA					
2.0 + 1.0	10.0	2.4 ± 0.3^{d}	2.0 ± 0.2^{e}	3.0± 0.1^{e}	-
4.0 + 2.0	30.0	3.5 ± 0.4^{d}	2.5 ± 0.5^{d}	3.5 ± 0.2^{d}	-
6.0 + 4.0	5.0	1.2 ± 0.8^{d}	1.0 ± 0.2^{d}	2.2 ± 0.4^{d}	+

*Intensity of callusing: (+) slight callusing, (++) considerable callusing and (-) no callusing. Values represent means ± standard error of 20 explants per treatment and data were recorded after eight weeks of culture. Means followed by the same letters are not significantly different by Duncan's multiple Range Test at 0.05% probability level.

The earliest axillary bud initiation in *Gerbera jamesonii* within 5 days of the inoculants was promoted by MS medium supplemented with a low amount of NAA (0.5 mg/l) and BAP (1.5 mg/l), according to Gantait et al. (2010). In addition, MS medium in combination with IBA or NAA was acceptable for axillary shoot proliferation from the nodal segments of *Centella asiatica*, according to Tiwari et al. (2013). Similar findings in *Alstroemeria* were reported by Seyyedyousefi et al. (2013). This result concurs with Khatri et al. (2019) in *Chlorophytum borivilianum* and Salekjalali (2012) in *Rosa damascena*. Dewir et al. (2018) demonstrated that in a number of cases, cytokinins alone are enough for optimal shoot multiplication as also indicated by the works of Nazira et al. (2021) and Meena et al. (2012). Many researchers suggested that incorporation of low-level auxin with BAP enhanced shoot induction in different medicinal plants, including *Sarcostemma acidum* (Choudhary and Kataria 2022), *Pimpinella anisum* (Amer and Omar 2019), and many other plants species.

Poor rooting was seen when microshoots with a length of 2-4 cm were cultivated on auxin-free media (data not shown). When IBA or NAA were introduced to MS medium in varying concentrations, rooting frequency increased significantly. IBA was significantly more effective than NAA at influencing the rate of root induction in the culture medium. The highest number of roots per shoot and longest roots with significant lateral roots were achieved with 2.0 µM IBA among the various IBA concentrations tested (Fig. 1E). When it comes to microshoot roots, NAA was found to be less effective than IBA. However, NAA generated thick, sensitive, and hairy roots. *S. zeylanica* responded poorly to all IAA concentrations. In this study, it was established that IBA was the best auxin when compared to NAA in terms of all rooting parameters (Table 4). Documented literature shows that IBA has been found suitable for rooting in a number of medicinal herbs like *P. heterophylla* (Wang et al. 2020), *V. arctostaphylos* (Bakhshipour et al. 2019), *H. enneaspermus* (Shekhawat and Manokari 2018), and many other plant species. The preceding evidence was confirmed by our findings. As a result, it was established that IBA is the best auxin for root induction of herbaceous medicinal plants.

Table 4: Effects of auxin on adventitious root formation on MS medium of *in vitro* developed micro-shoots. 20 explants were used in each treatment and data ($\bar{X}$±S.E) were recorded after 4 weeks of culture.

PGR (µM)		Explants rooted (%)	No. of roots / rooted cutting	Average length of root (cm)	*Intensity of callus growth
IBA	1.0	65.0	2.5 ± 0.5^{f}	4.5 ± 0.6^{b}	-
	2.0	90.0	8.0 ± 0.6^{a}	6.8 ± 0.8^{a}	-
	4.0	70.0	7.2 ± 0.7^{b}	5.2 ± 0.7^{b}	-
	6.0	60.0	5.5 ± 0.7^{c}	4.8 ± 0.6^{b}	+
NAA	1.0	60.0	6.8 ± 0.7^{b}	2.0 ± 0.6^{e}	-
	2.0	80.0	7.2 ± 0.6^{b}	5.5 ± 0.5^{b}	-
	4.0	65.0	6.4 ± 0.8^{b}	4.2 ± 0.2^{b}	+
	6.0	50.0	5.5 ± 0.7^{c}	3.7 ± 0.8^{c}	++
IAA	1.0	30.0	1.8 ± 0.5^{g}	1.8 ± 0.6^{e}	-
	2.0	55.0	3.5 ± 0.6^{e}	3.4 ± 0.2^{c}	-
	4.0	65.0	4.2 ± 0.7^{d}	3.1 ± 0.3^{c}	-
	6.0	45.0	2.8 ± 0.1^{f}	2.5 ± 0.8^{d}	-

*Intensity of callusing: (+) slight callusing, (++) considerable callusing and (-) no callusing. Values represent means ± standard error of 20 explants per treatment and data were recorded after eight weeks of culture. Means followed by the same letters are not significantly different by DMRT at 0.05% probability level.

After microshoots were successfully rooted, efforts were made to establish regeneration plantlets onto soil. Plantlets had been placed in little plastic pots with a soil mixture (garden soil: sand: compost, 2:1:1) and kept in a humid *ex vitro* environment in the growth room (Fig. 1F). When the *in vitro*-derived plantlets were kept in the growing room for 25 days before being moved to outdoor conditions, they adapted to *ex vitro* conditions more readily. After 25 days of transplantation, 80% of the plantlets had survived and adjusted well under *ex vitro* conditions.

Conflict of interest: the authors hereby declare no conflict of interest regarding the publication of this article.

Contribution: Authors contributed equally in the research and writing of this article.

References

Amer A and Omar H (2019). *In vitro* propagation of the multipurpose Egyptian medicinal plant *Pimpinella anisum*. Egyptian Pharmaceutical Journal. 18(3):254.

Azad MAK, Yokota S, Ohkubo T, Andoh Y, Yahara S and Yoshizawa N (2005). *In vitro* regeneration of the medicinal woody plant *Phellodendron amurense* Rupr. through excised leaves. Plant Cell, Tissue and Organ Culture, 80(1): 43-50.

Bakhshipour M, Mafakheri M, Kordrostami M, Zakir A, Rahimi N, Feizi F and Mohseni M (2019). *In vitro* multiplication, genetic fidelity and phytochemical potentials of *Vaccinium arctostaphylos* L.: An endangered medicinal plant. Industrial Crops and Products. 141: 111812.

Choudhary P and Kataria V (2022). *In vitro* culture in combination with aeroponics is an efficient means of mass propagation of *Sarcostemma acidum*: a rare medicinal plant of Indian arid zone. *In vitro* Cellular & Developmental Biol-Plant. 14: 1-10.

Deberg PC and Read RE (1990). Micropropagtaion, 1-12 p. *In*: Debergh PC, Zimmerman RH (Eds.). Micropropagation: Technology and Application, Kluwer Academic Publ., Dordrecht, The Netherlands.

Dewir YH, Aldubai AA, El-Hendawy S, Alsadon AA, Seliem MK and Naidoo Y (2018). Micropropagation of buttonwood tree (*Conocarpus erectus*) through axillary shoot proliferation. Hort. Sci. 53(5): 687-91.

Dhanya Shree VS, Ayesha A, Noorain SGK, Sahana BK, and Kekuda PTR (2018). Preliminary phytochemical analysis, antimicrobial and antioxidant activity of *Smilax zeylanica* L. (Smilacaceae). Journal of Drug Delivery and Therapeutics. 8(4):237-43.

Gantait S, Mandal N, Bhattacharyya S. and Das PK (2010). An elite protocol for accelerated quality-cloning in *Gerbera jamesonii* Bolus cv. Sciella. *In vitro* Cell. Dev. Biol. -Plant 46(6): 537-548.

Hernández-García A, Ambriz-Parra E, López-Albarrán P, Cruz-de León J and Salgado-Garciglia R (2021). *In vitro* propagation from axillary buds of the endangered tree *Dalbergia congestiflora* Pittier (Fabaceae). Plant Biotechnol (Tokyo). 38(4): 409-414.

Hooda V, Shama GN and Singla RK (2011). *Smilax zeylania* Linn. A Natural Therapeutic Hub. Pharmacologyonline 2:151-154.

Jaheduzzaman, Habib MA, Akter S, Banu NA, Rahman RB, Banu TA., Khan S and Islam S (2012). *In vitro* regeneration of an important medicinal plant *Centella asiatica* L. Urban. Bangladesh J. Sci. Ind. Res. 47(3): 269-272.

Joshi A and Mathur N (2015). *In vitro* propagation and conservation of *Anthocephalus cadamba* through apical bud and nodal explants a valuable medicinal plant. CIB Tech J Biotechnol. 4: 8-18.

Jothi KB, Ramaswamy A, Srinivasan R, Kannan M, Muthu A and Samy P (2022). Development of an efficient *in vitro* regeneration system in *Costus speciosus* - an important herbal insulin plant. South African J. Bot. 149: 468-475.

Kamble A and Lobo V (2022). *Smilax zeylanica* linn: Pharmacognostic, Taxonomical and Phytochemical Status. Research & Reviews: Journal of Herbal Science. 11(1): 1-14.

Khatri P, Rana JS, Sindhu A and Jamdagni P (2019). Effect of additives on enhanced in-vitro shoot multiplication and their functional group identification of *Chlorophytum borivilianum* Sant. Et Fernand. SN Applied Sciences.1(9):1-10. https://doi.org/10.1007/s42452-019-1118-8

Lane WD (1978). Regeneration of apple from shoot meristem tips. Plant Sci Let., 13: 281-285.

Lloyd GB and McCown BH (1980). Commercially feasible micropropagation of mountain laurel (*Kalmia latifolia*) by use of shoot tip culture. Comb Proc Int Plant Prop Soc., 30: 421-437.

Mandal S and Parsai A (2020). The effect of additional additives and amino acids on the axillary shoot micropropagation of medicinal plant *Aegle marmelos* (Corr.) Linn. Intl. J Plant Biotechnol. 6(2): 37-49.

Martini AN, Vlachou G and Papafotiou M (2022). Effect of explant origin and medium plant growth regulators on *in vitro* shoot proliferation and rooting of *Salvia tomentosa*, a native sage of the Northeastern Mediterranean Basin. Agronom. 12(8):1-17. https://doi.org/10.3390/agronomy12081889

Meena MK, Singh N and Patni V (2012). *In vitro* multiple shoot induction through axillary bud of *Cocculus hirsutus* (L.) Diels: A threatened medicinal plant. African J. Biotechnol. 11(12):2952-2956.

Murashige T and Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco cultures. Physiol Plant 15(3):473-497.

Naz R, Anis M and Aref IM (2015). Management of cytokinin–auxin interactions for *in vitro* shoot proliferation of *Althaea officinalis* L.: a valuable medicinal plant. Rendiconti lincei. 26(3):323-34.

Nazirah A, Nor-Hasnida H, Mohd-Saifuldullah AW, Muhammad-Fuad Y, Ahmad-Zuhaidi Y and Rozidah K (2021). Development of an efficient micropropagation protocol for eucalyptus hybrid (*E. urophylla* × *E. grandis*) through axillary shoot proliferation. J. Tropical Forest Sci. 33(4):391-397.

Nowakowska M, Pavlović Ž, Nowicki M, Boggess SL and Trigiano RN (2020). *In vitro* propagation of an endangered *Helianthus verticillatus* by axillary bud proliferation. Plants 9(6): 1-15.

Rajesh V and Perumal P (2014). Chemopreventive and antioxidant activity by *Smilax zeylanica* leaf extract against N-nitrosodiethylamine induced hepatocarcinogenesis in wistar albino rats. Orient Pharm Exp Med. 14(2): 111-126.

Rajeswari V and Paliwal K (2008). *In vitro* plant regeneration of red sanders (*Pterocarpus santalinus* L.f.) from cotyledonary nodes. Ind J Biotech 2:541-546.

Salekjalali M (2012). Phloroglucinol, BAP and NAA enhance axillary shoot proliferation and other growth indicators *in vitro* culture of Damask Rose (*Rosa damascena* Mill.). Advances in Environmental Biology. 6(7): 1944-1949.

Seyyedyousefi SR, Kaviani B and Dehkaei NP (2013). The effect of different concentrations of NAA and BAP on micropropagation of Alstroemeria. European J. Experimental Biol. 3(5):133-136

Shekhawat MS and Manokari M (2018). *In vitro* multiplication, micromorphological studies and *ex vitro* rooting of *Hybanthus enneaspermus* (L.) F. Muell.-a rare medicinal plant. Acta Botanica Croatica. 77(1): 80-87.

Slupski W, Tubek B and Matkowski A (2011). Micropropagation of *Codonopsis pilosula* (Franch.) Nannf by axillary shoot multiplication. Acta Biologica Cracoviensia. Series Botanica. 53(2): 87-93.

Thirungnanasampanand R, Mutharian RVN and Bai VN (2008). *In vitro* propagation and free radical studies of *Smilax zeylanica* Vent African J. Biotechnol. 8 (3): 395-400.

Tiwari C, Bakshi M and Vichitra A (2013). A rapid two step protocol of *in vitro* propagation of an important medicinal herb *Centella asiatica* Linn. African J. Biotechnol. 12(10): 1084-1090.

Wang F, Xin X, Wei H, Qiu X and Liu B (2020). *In vitro* regeneration, *ex vitro* rooting and foliar stoma studies of *Pseudostellaria heterophylla* (Miq.) Pax. Agronomy. 10(7):1-11. https://doi.org/10.3390/agronomy10070949

Xiong Y, Chen X, Wu K, Teixeira da Silva JA, Zeng S and Ma G (2021). Shoot organogenesis and plant regeneration in *Cordia subcordata* Lam. *In vitro* Cellular & Developmental Biology-Plant. 23: 1-7.

Zaib-Un-Nisa SJ, Anwar M, Sajad SH, Farooq G and Ali H (2019). Micropropagation through apical shoot explants and morphogenic potential of different explants of *Saussurea lappa*: An endangered medicinal plant. Pure and Applied Biology. 8(1): 585-592.

(Manuscript received on 17 April 2022; Revised on 06 May 2022)