An Efficient And Quick Protocol For *In Vitro* Multiplication Of Snake Plant, *Sansevieria Trifasciata* Var. Laurentii [Prain]

Jaspreet Kaur
Chandigarh University
GAURAV MUDGAL (gauravmdgl@gmail.com)
Chandigarh University https://orcid.org/0000-0003-4303-4983

Research Article

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Abstract

An efficient protocol was developed for quick propagation of snake plant under *in vitro* conditions. Leaf segments were surface sterilized and inoculated on Murashige and Skoog media supplemented with 3% sucrose, 0.8% agar, and various concentrations of indole-3-butyric acid. Cultures were maintained for 4-5 weeks at standard conditions to allow root induction and elongation. Shoot induction is triggered upon altering the culture room temperature to 37±2°C. Multiple shoots were witnessed at higher IBA concentrations. Another 5 weeks later, individual plantlets were excised and hardened for 2 weeks in soil preparation contained in small cups before transferring to big sized pots.

Highlights

- Two step quick *in vitro* propagation without subculturing or use of any cytokinins.
- Greater reproducibility with 90-95% regeneration and viable propagules.

Introduction

Snake plants represent about 70 species of monocot angiosperms in the genus *Sansevieria* belonging to the *Asparagaceae* family of the order *Nolinoideae*. Besides their ornamental use *Sansevieria* species have been exploited for their multiple attributes such as a fibre crop, bio-composites (Sreenivasan et al. 2011), ethnomedicinal prospects (Anbu et al. 2009; Andhare et al. 2012), and as valuable resource of phytochemicals (Mimaki et al. 1996; Tchegnitegni et al. 2017; Teponno et al. 2016). The species Sanse*vieria trifasciata* (ST) is a succulent, native to Tropical Canary Isles, subtropical Japan, India, Nigeria, Zaire, Thailand and Brazil and is known by several names such as the Mother in Law's tongue, bow-string hemp, zebra lily, cow tongue, leopard lily, good luck plant and devil's tongue (Takawira and Nordal 2001). It is included in the NASA's global list of top 12 pollution-absorbing plants (Wolverton et al. 1989) which is attributable to its Crassulacean Acid Metabolism (CAM) (Boraphech and Thiravetyan 2015; Kim and Lee 2008). With these as well as features that of a house plant in being shade tolerant (Hessayon 1996), the economic importance of ST has seen an up-surging growth. It is in popular demand particularly due to the growing concerns of skyscraper syndrome and air pollution especially in residential areas close to industrial setting (Sriprapat et al. 2014; Yuningsih et al. 2014). For fibre and above moonlighting bioprospects, production of ST foresees a highly mechanized industry (Irga et al. 2018). However, natural breeding (through seeds) and/or conventional vegetative propagation (grafting with rhizomes and leaf cuttings) techniques are variously constrained viz., one propagule per graft and/or per seed, higher chances of cross contamination (Takawira and Nordal 2001), rare flowering frequency, unviable seed counts, unfavourable phenotypes (Joyner et al. 1951) and climatic factors that limit fibre yield (Ramanaiah et al. 2013). These approaches thus culminate into slow and unproductive regeneration. For these reasons, *in vitro* clonal propagation using plant tissue culture methods seem the only resort at catering to a growing demand for ST plants.

We studied the effects of a plant growth regulator IBA (Indole 3 btyric acid) and a shift in culture room temperature on productive regeneration efficiency in ST which catered successfully devising a rapid, easy and proficient *in vitro* clonal propagation protocol for this valuable plant.

Material And Methods

ST plants (Fig. 1, centre) were obtained from the botanical garden of Chandigarh University, Punjab and micropropagated following the steps (as in Fig. 1) outlined below:

1. One fully expanded healthy green leaf was selected and excised from a mother plant by cutting it at 3/4th length towards the base (to avoid the bottom white segment).
2. Leaf was divided into 4–5 segments and washed thoroughly in a suitably sized jam bottle under running tap water for half an hour, followed by further rinsing in froth with 2 drops of Tween-20 and clearing thrice with ample distilled water for another half hour.
3. Under the laminar flow hood (for all that follow from here), surface sterilization followed rinsing the leaf segments in 1% v/v aqueous solution of Dettol for 1 minute and clearing with 2x5 min washes of sterile distilled water.
4. Next, plant material was serially treated with 0.1% v/v solution of mercuric chloride and 70% ethanol, each for 45 seconds followed by 3x5 min washes with sterile distilled water. Leaf segments were continuously shaken during these sterilization steps.
5. *For root induction and maintenance*, leaf segments were further excised to $1cm^2$ explants, each of which were aseptically transferred to glass jam jars (400 ml volumetric capacity), each containing 100 ml of autoclaved (121 °C, 20 min) MS (Murashige and Skoog, 1962) basal media (Himedia, cat# PT099) supplemented with various concentrations (1.00–10.00 mgL^{-1}) of Indole-3-butyric acid (IBA) (Himedia, cat# PCT0804), 0.8% agar (Himedia, cat# RM7695) and 3% sucrose (Himedia, cat# PCT0607). The pH was adjusted to 5.88 (± 0.01, prior to mixing agar and autoclaving). These explants in jars were incubated continuously in a controlled culture room with temperature 22 ± 2°C, relative humidity approximately 60–65%, and with photoperiods of 16:8 hours light/dark for at least 4–5 weeks. Within this time appreciable root growth in length and numbers is attained for the next steps. No subcultures were attempted.
6. *For shoot induction*, the culture room temperature was abruptly increased 37°C (± 2°C) and maintained until further 4–5 weeks until shoot-buds appear on the rooted explants in the jars and attain suitable number and length.
7. Plantlet acclimatization involved removal of plantlets from jar vessels (after 10–12 weeks of inoculation and incubation in above steps), cleaning gently with sterile distilled water (to remove trace gelling agent) and transfer to plastic cups consisting 100 gms of 1:5 proportioned autoclaved cocopeat: garden soil mixture. Cups were properly covered with caps (with a small vent) in order to control from drastic shifts in relative humidity. These plantlets were incubated in a plant growth chamber under similar conditioning as in culture room but at temperatures not fluctuating more or less than between 28°C, and relative humidity of 45–55%. The plantlets were hardened for 2 weeks

with spray watering (1.0 ml sterile tap water) the cup soil once a week. These ensured healthy growth of shoots.

8. For further acclimatization, plantlets were transferred to big styrofoam pot(s) containing 10 Kgs of autoclaved cocopeat:sand:garden soil (1:2:5 proportioned) mixture and maintained with 50 ml spray watering the upper soil at least every 3 or 4 weeks. Pots were exposed to mild sunlight (one full day per week) but otherwise kept in shade henceforth. Use of any microbicidal agent in soil mixture is not recommended.

Compilation Of Data And Statistical Analysis

Absolute growth parameters viz., number and length of root(s), shoot(s) per explant were recorded. All treatments were done thrice (n = 20 for each). Statistical analyses involved the use of ANOVA. Significance levels in each treatment were agreed at $P < 0.05$. Experimental outcomes were expressed in mean ± standard error (SE) (see Fig. 2 and Online resource 1).

Results And Discussion

The quick *in vitro* micropropagation protocol for ST presented above (Fig. 1) founds on the results from experiments studying the effect of IBA and culture incubation temperature. We witnessed IBA could trigger root induction in ST leaf explants and offers concentration dependent increments in both root number and length (1–10 mg^{-L}) in MS media (Fig. 2a).

In our hands, root emergence from ST explants occurs normally within two weeks post inoculation and is not significantly influenced by subculturing on the same media (data not shown). This minimises the labour, time and resources for mass propagating. IBA concentrations below 1 mg^{-L} resulted only few roots per explant which later failed to offer any shoot primordia (data not shown). Within a month of culturing, root growth was found prominent within the range of 5–10 mg^{-L} IBA, wherein best root length (approx. 8 cm) and numbers (approx. 17) were recorded at 10 mg^{-L} (Fig. 2a and online resource 1). Of the 20 replicates of explants in each of the IBA concentrations, highest rooting percentage was shown with 7.5mg^{-L} (95%). Increasing the concentration of IBA, number of roots per explant also increases. Rooting response did not change significantly while maintaining the explants in the same media or subculturing them into fresh media supplemented with or without IBA concentrations (data not shown).

Explants (with or without rhizogenesis) exhibited no shoot regeneration when culture vessels were incubated at 22 ± 2°C for any length of time, both with and without subcultring. However, upon a drastic shift of culture room temperatures to 37°C ± 2°C, on the 5th week post inoculation, only the root bearing explant(s) exhibited minute caulogenetic protrusions (Step 6 in Fig. 1). These were apparently visible as shoot primordia directly emerging from explants following 2 weeks. Callusing was never observed in any of the explants. As seen with root growth, caulogenesis also depicted increments in shoot number and length at increasing concentrations of IBA in media (Fig. 2b and online resource 1). Again, subcultring

shoot regenerated explants over fresh media with or without supplementation of IBA did not significantly influence the shoot growth parameters observed at respective IBA concentration. None of the explants without roots in culture showed caulogenesis. After 5 weeks at 37 ± 2°C, highest shoot regeneration (90%) was recorded at $10mgl^{-L}$ IBA which accounted for more explants (14.9 ± 0.99) with maximum number (8.5 ± 0.34) and length (7.95 ± 0.23) of shoots. IBA at $1mgl^{-1}$ was least responsive to shoot growth parameters. Hence, 10 mg^{-L} IBA proved the best concentration for rhizogenesis as well as culture temperature shift-assisted shoot formation (Fig. 2 and online resource 1). Following the observations at 5th week post incubation temperature shift, *in vitro* plantlets were found amenable to hardening treatment on soil preparation in plastic cups, followed by acclimatization on big styrofoam pots.

Only a few tissue culture attempts have been reported in literature for ST (Sarmast et al. 2009; Sarmast et al. 2014; Torres 1989; Yusnita et al. 2011), however they suffer with longer culturing times, few productive shoots per explant and/or callusing limitations. A quick and efficient protocol offered by us may suffice the growing need at bioprospecting ST into various applications talked earlier. Nonetheless, it can favour future conservation efforts for specific genotypes and biotechnology in this underexplored *Asparagaceae* family member.

Declarations

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Figures

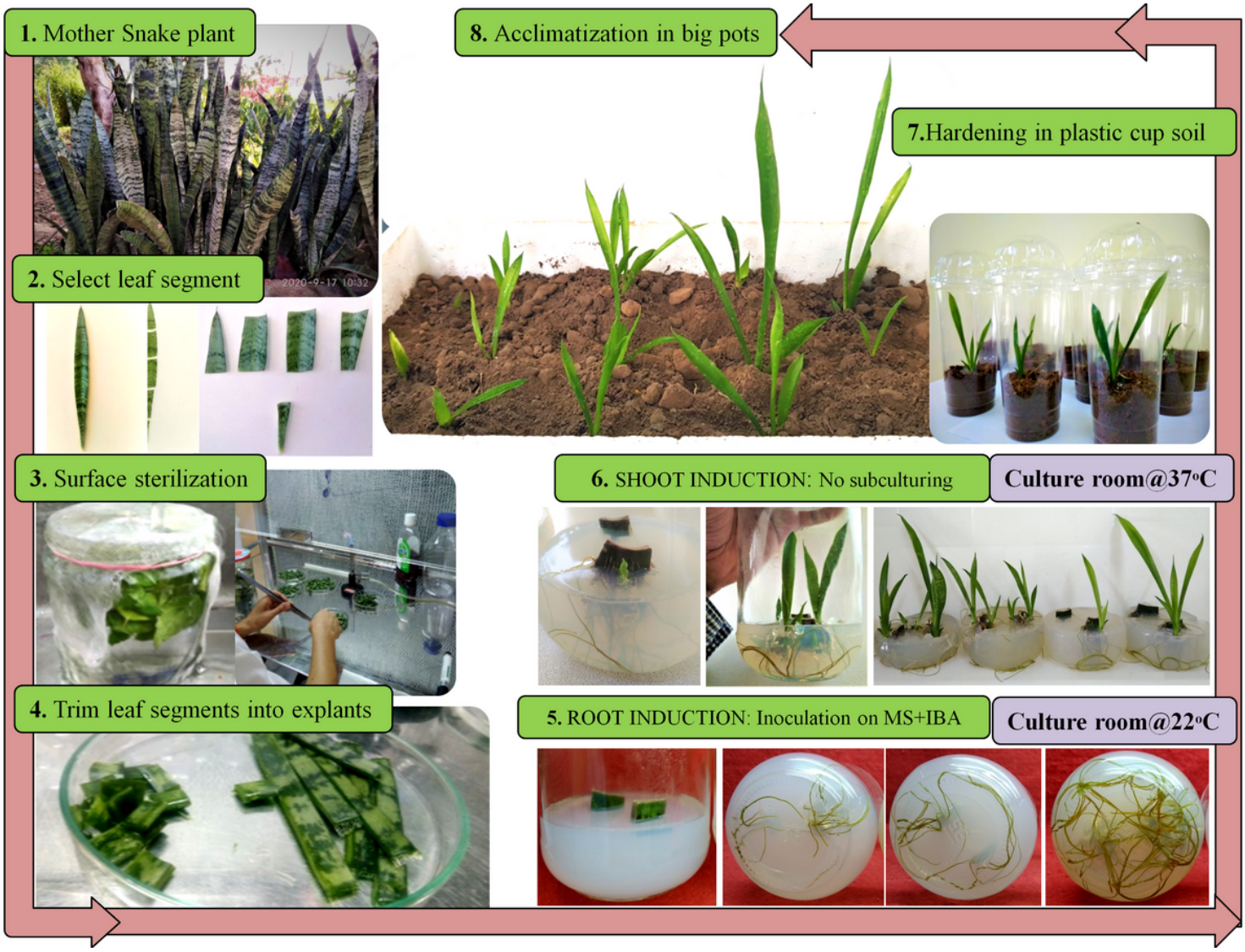

Figure 1

Sequential steps in quick in vitro mass propagation Snake plant (ST).

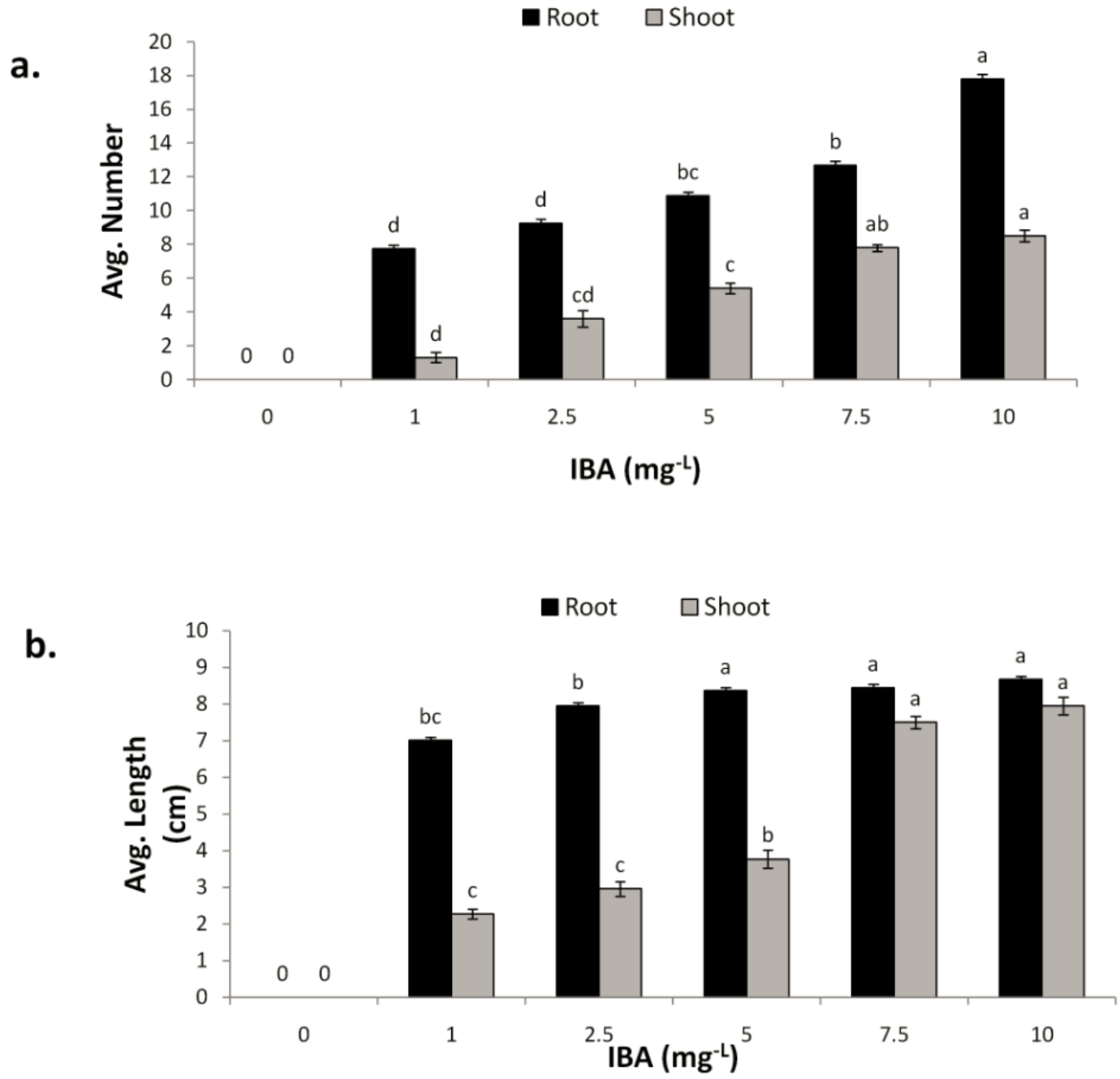

Figure 2

Effect of IBA (a) and culture room temperature shift (b) on micropropagation of ST. Statistical analysis was performed using ANOVA. Results are mean of 20 replicates per trial. All experiments were repeated thrice. Mean separations were validated using Duncan's multiple range test at 5% level of significance. All values related in a group in are depicted as lowercase letters and the data was significant at $p<0.05$. Refer online resource 1 for comprehensive data.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Onlineresource1.pdf