An efficient and quick protocol for in vitro multiplication of snake plant, *Sansevieria trifasciata* var. Laurentii [Prain]

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Abstract

An efficient protocol was developed for quick propagation of snake plant under in vitro conditions. Leaf segments sized 1 cm 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 (1 to 10 mg/L). Cultures were maintained for 4 to 5 weeks at standard conditions to allow root induction and elongation. Shoot induction was triggered upon altering the daytime culture room temperature to 37 ± 2 °C. Multiple shoots were produced 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 30 × 20 × 20 cm sized pots. We discuss the probable events effectuating unusual shoot regeneration at relatively higher temperatures in media without supplementing any cytokinins.

Key message

This manuscript offers a unique protocol for rapid in vitro regeneration in snake plants using leaf explants cultured in airtight-sealed vessels. The success of the protocol relies on IBA led root induction at standard tissue culture temperatures, while shoot induction uses only an abrupt daytime shift to relatively high temperatures.

Keywords Mass propagation · Shoot formation · IBA · High temperature · Ethylene · Cytokinin

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; Teponno et al. 2016; Tchegnitegni et al. 2017). The species *Sansevieria 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 Nor-dal 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) (Kim and Lee 2008; Boraphech and Thiravetyan 2015). With these as well as features that of a house plant in being shade tolerant (Hessayon 1996), the economic importance of ST has seen reasonable 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...
For fibre and above moonlighting bioprospects, production of ST necessitates a highly mechanized industry (Irga et al. 2018). However, natural breeding (through seeds) and/or conventional vegetative propagation (rhizomes divisions and leaf cuttings) techniques are variously constrained viz., one propagule per division and/or per seed, higher chances of cross contamination (Takawira and Nordal 2001), rare flowering frequency, nonviable 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 poor 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-butyric acid) and a shift in culture room temperature on productive regeneration efficiency in ST which helped in successfully devising a rapid, easy and proficient in vitro clonal propagation protocol for this valuable plant.

### Material and methods

Snake plants (Fig. 1) 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 (avoid the bottom white segment to reduce contamination).
2. Leaf was divided into 4–5 segments (accommodable in a suitably sized jam bottle) and washed thoroughly under running tap water for half an hour, followed by further rinsing in froth with two drops of Tween-20 and clearing thrice with ample distilled water for another half hour.
3. Under the laminar flow hood (for all procedures that follow from here), surface sterilization followed rinsing the leaf segments in 1% v/v aqueous solution of Dettol.

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**Fig. 1** Sequential steps for quick in vitro mass propagation of Snake plant (ST)
for 1 min and clearing with 2 × 5 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 s followed by 3 × 5 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 1cm² explants, each of which were aseptically transferred to glass jam jars (400 mL volume 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 to 10.00 mg/L) 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 to 65%, and with photoperiods of 16:8 h light/dark for at least 4 to 5 weeks. Within this time appreciable root growth in length and numbers was attained for the next steps. No subcultures were attempted.

6. For shoot induction, culture room temperature, exclusively for the daytime (i.e. 16 h light period), was increased to 37 °C (± 2 °C) and maintained until further 4 to 5 weeks until shoot-buds appear on the rooted explants in the jars and attained suitable number and length.

7. Plantlet acclimatization involved removal of plantlets from jar vessels (10 to 12 weeks after inoculation and incubation in above steps), cleaning gently with sterile distilled water (to remove trace gelling agent) and transferred to plastic cups consisting 100 g 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 to 55%. The plantlets were hardened for 2 weeks with spray watering (1.0 mL sterile tap water) the cup soil once a week. These steps ensured healthy growth of shoots.

8. For further acclimatization, plantlets were transferred to big styrofoam pot(s) containing 10 K 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 70–75% shade henceforth. Use of any microbicidal agent in soil mixture was avoided (which may negatively influence the plants).

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 completed three times (n = 20 for each). Statistical analyses involved the use of ANOVA. Significance levels in each treatment were agreed at P < 0.05 using Duncan’s multiple range test. Experimental outcomes were expressed in mean ± standard error (SE) (see Fig. 2 and Online resource 1).

Results and discussions

The quick in vitro micropropagation protocol for ST presented above (Fig. 1) was based on the tested effect of various IBA concentrations over root induction and of subsequent shift in daytime culture incubation temperature to 37 °C on shoot induction (Fig. 2a, b). We witnessed IBA could trigger root induction in ST leaf explants and offers concentration dependent increments in both root number and length (1 to 10 mg/L) in MS media (Fig. 2a).

In our hands, root emerged from ST explants normally within two weeks post inoculation and was not significantly influenced by subculturing on the same media (data not shown). This minimizes the labour, time and resources for mass propagating. IBA concentrations below 1 mg/L resulted in only few roots per explant which later failed to form any shoot primordia (data not shown). Within a month of culturing, root growth was found prominent within the range of 5 to 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 7.5 mg/L (95%). While increasing the concentration of IBA, number of roots per explant also increased. Rooting response was similar 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 subculturing. However, upon a drastic shift of specifically the daytime culture room temperatures to 37 ± 2 °C, during the 5th week post inoculation, only the root bearing explant(s) exhibited minute caulogenetic protrusions (Step 6 in Fig. 1). These protrusions were apparently visible as shoot primordia directly emerging from explants following 2 weeks. More precisely, shoots emerged either laterally and/or from the
Fig. 2 Effect of IBA and culture temperature shift on micropropagation of ST. In a and b, effect of IBA and shift in daytime culture temperature (to 37 °C at the 5th week post inoculation) respectively on root and shoot growth in ST explants. Observations (in a and b) following 10 weeks post inoculation and values on the vertical axes correspond to effects per explant. Data in c, d, e and f from previous founding experiments, where in c, all cultures maintained for 20 weeks at 22 °C (constant day and night temperature i.e. without shift) supplemented with or without IBA; in d, e, and f daytime culture incubation temperature respectively altered to 30, 37 and 45 °C (i.e. Shift, as also indicated) at various weekly intervals to record the percentage response corresponding to root and shoot growth at the 20th week post inoculation (in d, e and f media supplemented with 10 mg/L IBA; Nil growth response at 0 mg/L, hence data not shown). No subculturing was attempted in any of the trials. Statistical analysis was performed using ANOVA. Results are mean of 20 replicates per trial (1 explant per replicate). 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 are depicted with lowercase letters and the data was significant at $p < 0.05$. 

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interior underneath the trimmed leaf segments (explants). No shoot emergence, however, could be recorded from explant tip and/or root. Also, we could not recover any significant differences in rhizogenesis and/or caulogenesis with changes in polarity and/or orientation of the ST explants. Profuse 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, subculturing 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 with 37 ± 2 °C, highest shoot regeneration (90%) was recorded at 10 mg/L IBA which accounted for more explants (14.9 ± 0.99) with maximum number (8.5 ± 0.34) and length (7.95 ± 0.23 cm) of shoots. IBA at 1 mg/L 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 amenable to hardening treatment on soil preparation in plastic cups, followed by acclimatization in big styrofoam pots.

The above protocol precisely founds over the results from previous expanded (20 weeks) trials with ST leaf segments (Fig. 2c-f). We figured that at constant 22 °C culture temperature, higher IBA concentrations (7.5 and 10 mg/L) could favour optimal root induction (Fig. 2c), of which 10 mg/L was later used to evaluate the effects from various daytime temperature shifts administered at weekly intervals in separate tests (Fig. 2d–f). These trials suggested an optimally quicker and productive shoot induction from the rooted ST explants at the 37 °C shift when administered by the 5th week post inoculation (Fig. 2e).

Overall, the protocol proposed herein is unique for the intermittent administration of higher (daytime) culturing temperature(s) that induce shoot formation (in rooted explants) without the exogenous supplementation of any cytokinin, but shoot induction otherwise, exclusively corresponded to proportionate initial treatment with an auxin (increasing concentrations of IBA). Though, with only these results in hand we do not vouch a hypothesis that solely the presence of an auxin could directly effectuate shoot formation. However, it infers an indirect effect that could interpret as if a caulogenetic threshold was achieved easily by the rooted explants which perhaps got thermally induced (effect exclusively at higher temperature shifts) to produce shoots, probably following an endogenous cytokinin biosynthesis (in the root). We reiterate that regardless of the temperature shifts none of the explants, cultured over MS basal (growth regulator free media) in any of the trials, could generate any shoot primordia.

Nonetheless, rapidly growing plant tissue cultures are known to release enormous ethylene (Thomas and Murashige 1979) and CO₂ which may accumulate in the headspace of air tight-sealed culture vessels during subsequent growth and may variously impact tissue culture systems (Biddington 1992). We routinely use sterile air-tight-sealed jam jars in tissue culture to minimize chances of contamination and humidity loss with time. Auxins, like IBA, which principally stimulate adventitious rooting, are also known to induce endogenous ethylene biosynthesis as shown in mung bean (Riov and Yang 1989) and Prunus avium shoot cultures (Biondi et al. 1990). IBA would work differently from other auxins like 2,4-D. At low concentrations, 2,4-D (below 50 µM) may stimulate ethylene biosynthesis, while at higher (more than 100 µM) it contrarily inhibits ethylene production (Yu et al. 1980). Ethylene may both initiate and inhibit callusing in different plants (Biddington 1992). Headspace ethylene accumulation can otherwise also result in shoot formation, an effect, which may substantiate in synergy with the accumulated CO₂, and these, in turn, may substitute the shoot forming effect of the cytokinins. This has been reported in rice, white cedar, Pinus radiata, Chychorium intybus and Paulownia kawakamii (Cornejo-Martin et al. 1979; Kumar et al. 1996, 1987; Lefebvre 1972; Nour and Thorpe 1994). Time could also play an added factor for ethylene’s early inhibitory and late stimulatory effects for in vitro shoot formation (Huxter et al. 1981).

How high temperature would top-up the above effects to induce shoot induction in ST, however, remains to be investigated. Recent literature could not reveal extensive in vitro attempts in many plants at relatively higher temperatures that would result in shoot formation, both inclusive and/or exclusive in relation to headspace accumulated ethylene and/or CO₂. In Tobacco, high ethylene production at 25, 30 and 35 °C reportedly triggered rhizogenesis while not at low ethylene production at 15 and 45 °C (Le Guyader 1987). Interestingly, ethylene production could be temperature sensitive, as reported in apple and mungbean with its optimum at about 30 °C, while inhibition from 35 to 40 °C (Yu et al. 1980). This may otherwise interpret that somehow at high temperatures ethylene accumulation diminishes for its caulogenesis inhibition, and/or cytokinin biosynthesis would be thermally induced.

Generally, cytokinins biosynthetically hail from roots from where they transport upward via xylem. Besides growth promotion, cytokinins have been implicated in long-distance signalling in plants. External influences may indirectly cause fluctuations in cytokinin synthesis and flow, thereby marking these fluctuations vital at regulating adaptive responses in propagules (Kudoyarova et al. 2014). A
high cytokinin flow in xylem from its root-retained reserves can result from influences that inhibit its accumulation in root cells (Kudoyarova et al. 2014). Moreover, various studies demystify cytokinins’ role at countering stresses like heat and drought. Alternatively, as various cytokinins are breakdown products of nucleic acids, higher temperatures might accumulate them from heat-labile precursor molecules (Nogué et al. 2003). Both ethylene and cytokinins are also known to share similar cell surface receptors (the histidine kinase sensors) in plants (Nogué et al. 2003).

In shoot microcultures of a tropical fruit tree, Hancornia speciosa, temperature shifts from 25 to 35 °C and later to 31 °C reportedly resulted in the release of auxillary buds followed with shoot branching (De Pereira-Netto and McCown 1999). Some tropical and subtropical plants stimulate in vitro bud breaks at 30 °C (Shekhawat et al. 1993). Phaseolus and Chrysanthemum vulgaris exhibit lateral branch elongation at higher ex vitro air temperatures (Kigel et al. 1991; Schoellhorn et al. 1996). In the anther cultures of Brussels sprouts (Brassica oleracea var. gemmifera) high ethylene production rates reportedly correspond to poor embryogenic response (Biddington and Robinson 1990, 1991). Appreciable embryogenesis, in these anther cultures, mandatorily requires 35 °C incubation, a temperature elevation known to inhibit ethylene biosynthesis (Dalton and Street 1976; Cheng et al. 1988). The temperature effect is so pronounced that even administration of aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis would also fail to promote embryogenesis in the absence of the high temperature (Biddington 1992). This could be nearly convincing in light to the effects witnessed in our previous attempts with ST explants. Precisely, predating the temperature shifts to the 2nd week post inoculation (when root emergence had just effected from IBA), we did witness some caulogenesis, which however was either lengthily delayed (9–10 week post temperature shift) and/or could not be productive enough to develop to healthy shoots (Fig. 2e). This might see its justification in that if caulogenesis had to effectuate from endogenous cytokinin biosynthesis (following thermal induction) in roots, the latter were immature enough to comply fully (Fig. 2e). The temperature shifts (preferably to 37 °C) at exactly the 5th week post inoculation (as stipulated also in the protocol) show optimally productive shoot induction, probably sufficed by roots matured for cytokinin anabolism.

Achieving optimal in vitro growth at higher thermoregime could be unusual, against a norm of propagating most plants at a constant temperature of 25 °C. However, many tropical and subtropical species have also been surveyed for their optimal growth at 27 °C (George 1996). Probably, tissue culture at relatively higher temperatures has not been tried in many plants, but it may offer potential prospects in tropical and desert species, particularly those tolerant to higher temperatures and less responsive to exogenous cytokinin treatments (De Pereira-Netto and McCown 1999). It thus concludes that in certain plant species (talked earlier) and possibly also in ST, relatively high temperatures favour embryogenesis. However, in case of ST, how it may occur in the absence of an exogenous cytokinin is intriguing and hints a crosstalk of mechanisms triggering a reduction in ethylene production/accumulation or an endogenous cytokinin biosynthesis or, otherwise in synergy with factors yet unknown (Wolverton et al. 1989). The mechanisms favouring such plasticity should be intact within plants’ genetic and evolutionary blueprints, besides the epigenetically governed stress signalling reflexes. We intend to answer these and other related queries in future.

Only a few tissue culture attempts have been reported in literature for ST (Torres 1989; Sarmast et al. 2009, 2014; Yusnita et al. 2011), however, they require 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, this protocol can favour future conservation efforts for specific genotypes and biotechnology in this underexplored Asparagaceae family member.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11240-021-02132-0.

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