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# Meta-topolin influenced shoot morphogenesis in *Sansevieria cylindrica* Bojer ex Hook. through leaf disc culture

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**Abstract**
Meta-toplolin (mT) is now considered as a potential phytohormone in the regulation of morphogenesis and other physiological activities in the plant cells. Meta-topolin (mT) is increasingly been recommended in plant tissue culture studies because of its ability to support spontaneous root formation in the regenerating shoots, thereby reducing the time span for complete plantlet development. The present study describes a protocol for enhanced shoot regeneration and plantlet development of *Sansevieria cylindrica* with an application of mT as compared to other cytokinins viz., benzyladenine (BA), kinetin (Kn) and adenine sulphate (AdS). Higher shoot regeneration (mean number of 32.6 ± 0.40shoot per explant) with simultaneous root formation was achieved on Murashige and Skoog (MS) medium + mT (12.5 µM) after 6 weeks of culture. The media containing routine cytokinins (CKs) also responded well with a maximum of 12.6 ± 0.67shoots per explant on MS medium + BA (10.0 µM) but without spontaneous rooting and comparatively exhibited lesser growth and leaf development than mT containing medium after 6 weeks of culture. The micro shoots regenerated on BA containing medium were excised and rooted best on half-strength MS medium containing IBA (5.0 µM). The plantlets produced on mT containing medium survived better (90%) than those obtained on BA containing medium (67%) when acclimatized in garden soil + coco peat (1:1) after 4 weeks of acclimatization.

**Keywords:** acclimatization, cytokinin, Meta-topolin, micropropagation, morphogenesis, *in vitro* rooting

## Introduction

Appropriate application of exogenous cytokinins (CKs) plays a central role in regulating shoot proliferation (Werner *et al.* 2001, Van Staden *et al.* 2008) [29, 26]. However, in some cases cytokinin negatively influence growth, rooting and percent survival during acclimatization and may cause hyperhydricity (Werbrouck *et al.* 1995, Ivanov and Van Staden *et al.* 2011) [28, 10]. Thus, the limitations of existing CKs have spurred the active search for new CKs. Consequently, a new class of aromatic CKs, the topolins were discovered and identified by Strnad (1997) [23] and Tarkowská *et al.* (2003) [24] in *Populus* species.

In the past few decades, use of topolins, especially the *meta*-topolin (mT) has shown promising results in terms of growth as well as minimizing various physiological disorders (Aremu *et al.* 2012, Amoo *et al.* 2014) [3, 1]. In many cases mT application showed a great relief in controlling hyperhydricity and shoot tip necrosis (Malá *et al.* 2013).

In the topolins' basic structure, presence of a hydroxyl group on the benzyl ring differentiates it from BA. Because small differences in a molecule's structure can have a large effect on a plants' response. Thus, the present study was aimed to compare the morphogenetic effect of routine cytokinins with the aromatic cytokinins (mT) in *Sansevieria cylindrical* Bojer ex Hook, an important agave species.

*S. cylindrica* is an important fiber yielding plant of dry land. It is mainly found in Tropical Africa to southern Asian countries. The plant in the wild, form diffused colonies but in pots they form thickets. Small greenish tubular flowers, many in numbers are borne on spikes. They are nicely fragrant although not showy. Propagation generally done through rhizome cuttings as well as leaves, which is a slower and cumbersome process coupled with limited number of propagules production. Because an excellent ability to thrive well in water scares regions and due to good fiber yielding quality, *S. cylindrica* becomes the plant of choice for cultivation among prominent fiber yielding plant in African and Arabian countries, leading to the surge in demand for propagules supply, which could not be met through conventional propagation methods. Thus, plant tissue culture is the only method to be adopted at industrial level for the supply, prompting to develop an efficient regeneration system. There are only two reports on *in vitro* micropropagation of *S. cylindrica* through leaf disc culture. Anis and Shahzad (2005) [2] reported direct organogenesis while Shahzad *et al.* (2009) [22] reported indirect organogenesis through leaf derived calli by using routine cytokinins and auxins.

## Materials and Methods

### 1. Preparation of explants and surface sterilization

Immature leaves, measuring 8-10 cm, were collected from a potted plant and 4-5 cm basal half were cut and kept under running tap water for 1 hour followed by cleaning in 5% laboline (Sigma-Aldrich, Germany) - a mild detergent, for 15 min. To disinfect the explants, 5% Bavistin (Carbendazim Powder, BASF India Ltd) exposure was given for 20 min and finally explants were transferred to laminar flow hood for surface sterilization with 0.1% $HgCl_2$ (Qualigens, India) for 5 min. Few drops of Tween-20 (Qualigens, India) were added in the sterilization solution as a wetting agent. The explants were then thoroughly washed with sterile double distill water (DDW), at least 5-6 times to remove the traces of sterilant. After discarding the 2-3 mm thick exposed surface, the cylindrical leaves were cut into disc of 3-4 mm thickness and used as explants for regeneration studies.

### 2. Media preparation and culture conditions

The MS medium (Murashige and Skoog 1962) with 3% (w/v) sucrose (Qualigens, India) and 0.8% agar (Qualigens, India) was used throughout the experiment. The pH of the medium was adjusted to 5.8 using 1 N NaOH or 1 N HCl before autoclaving at 121ºC and $1.03 \times 10^5$ $Nm^{-2}$ (Pascal) for 15 min. After inoculation the cultures were kept in the controlled environment of culture room at 25 ± 2ºC temperature under 16 h photoperiod with 50 µmol $m^{-2}s^{-1}$ photosynthetic photon flux density (PPFD) provided by cool white fluorescent tubes (40 W; Philips, China) and 55 ± 5% relative humidity.

### 3. Shoot regeneration medium

Explants were inoculated aseptically in culture tubes (25×150 mm) containing 15 ml MS medium supplemented with different cytokinins i.e., 6-benzyladenine (BA) and kinetin (Kn), growth additive i.e., adenine sulphate (AdS) and meta-topolin (mT)at different concentrations (1.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20, 40, 50, 55, 60 and 65 µM). The MS medium devoid of any plant growth regulator (PGR) was treated as control. After induction, explants were transferred to Erlenmeyer culture flasks (100 ml) containing 20 ml of fresh medium for proper shoot elongation and leaf expansion.

### 4. Effect of repeated subculture for short term maintenance and hyperhydricity of shoots

To compare the effect of subculturing on hyperhydricity, the proliferating tissue induced either on BA as well as mT supplemented nutrient media were multiplied by repeated subculturing on three different nutrient media viz., PGR-free MS basal medium (MS), MS medium supplemented with reduced concentration of BA and mT i.e., 5.0 µM and MS medium supplemented with optimal concentration of BA and mT i.e., 12.5 µM. The organogenic tissue was divided into 4 pieces and each piece was subcultured on fresh nutrient media after every 4 weeks. The effect of sub culturing was noticed up to 4 subculture passage.

### 5. Root induction in microshoots

For *in vitro* root induction healthy microshoots (3.0-4.0 cm) were excised and transferred to semi-solid half-strength MS basal medium supplemented with different concentrations of auxin i.e., indole-3-acetic acid (IAA), IBA, α-naphthalene acetic acid (NAA) (0.5, 1.0, 2.5 and 5.0 µM). Half-strength MS medium devoid of any auxin was treated as control.

### 6. Acclimatization

Rooted plantlets (developed from BA and mT containing nutrient media) were removed from the medium, washed gently under running tap water to remove agar from the roots, transplanted into thermocol cups (expanded polystyrene) containing sterile Soilrite™ (75 % Irish peat moss and 25% horticulture grade expanded perlite), vermicompost and garden soil and coco peat (1:1). Potted plantlets were covered with a transparent polythene membrane and kept in a culture room at 25 ± 2ºC temperature and 16 h photoperiod with 50 µmol $m^{-2}s^{-1}$ photosynthetic photon flux density (PPFD) provided by cool white fluorescent tubes (40 W; Philips, China) and 55 ± 5% relative humidity for 4 weeks and watered every three days. Polythene membranes were gradually opened after 2 weeks in order to acclimatize the plantlets to field conditions. After 4 weeks, the plantlets were transferred to pots containing normal garden soil and green manure (2:1) and maintained in a greenhouse under normal day length conditions.

### 7. Statistical Analysis

The data for each experiment viz. shoot induction and multiplication and *in vitro* root induction were collected after 6 weeks. All the experiments were conducted with a minimum of 20 replicates per treatment and each experiment was repeated thrice. The data were analyzed statistically by one-way ANOVA using SPSS version 12 (SPSS Inc., Chicago, IL, USA). The significance of differences among means was analyzed using Tukey's test at P=5% and data represented as mean ± standard error (SE).

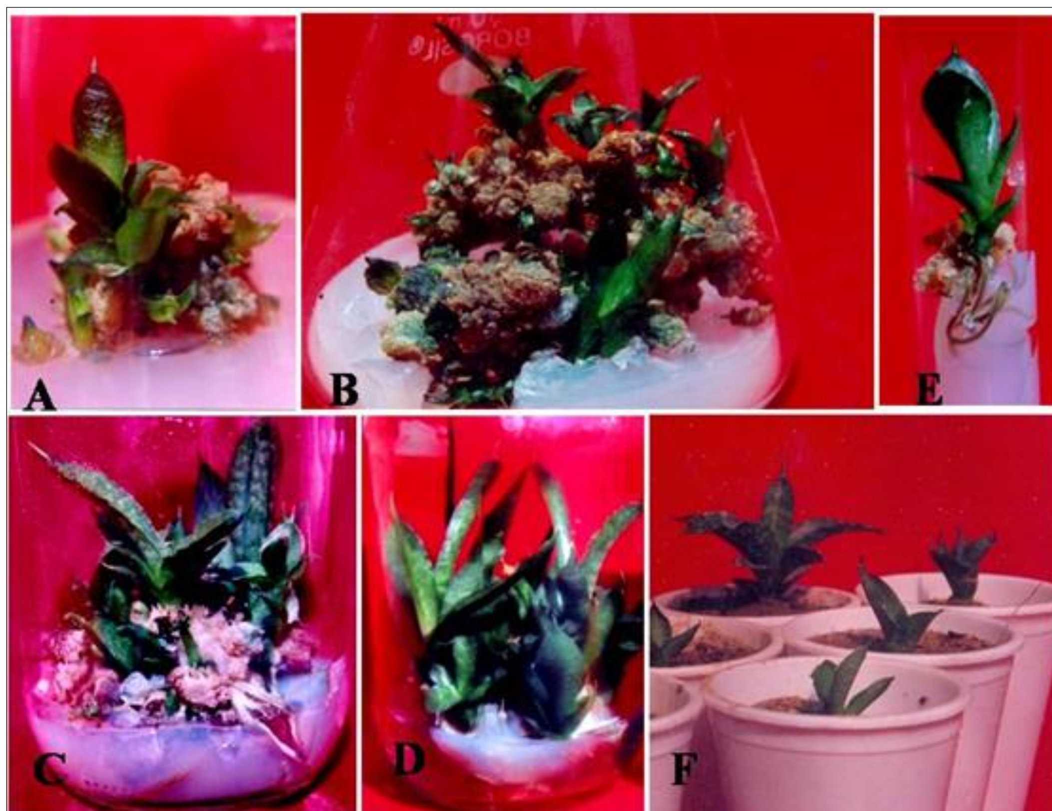


**Fig 1:** A. Shoot regeneration through leaf explants on MS medium + BA (10.0 µM)-2 weeks old culture; B. High frequency shoot regeneration through leaf explants on MS medium + mT (12.5 µM)-2 weeks old culture; C. Microshoots showing simultaneous rooting (arrow) on MS medium + mT (12.5 µM)- 4 weeks old culture; D. Shoot elongation on MS medium + mT (12.5 µM)-after first subculture passage; E. *In vitro* rooting of BA derived plantlet in half-strength MS medium Supplemented with IBA (5.0 µM) -4 week old culture; F. Acclimatized plantlets of *S. cylindrica* in garden soil and coco peat (1:1) – after 4 weeks of acclimatization.

**Table 1:** Effect of BA, Kn and AdS on direct shoot organogenesis through leaf disc culture after 6 weeks of culture

| **Treatments (µM)** | | | **% Response** | **Shoot number per explant** | **Shoot length (cm)** |
|---|---|---|---|---|---|
| BA | Kn | AdS | | | |
| - | - | - | 0 | $0.0 \pm 0.00^{k}$ | $0.0 \pm 0.00^{i}$ |
| 1.0 | | | 70 | $3.4 \pm 0.24^{ghi}$ | $1.9 \pm 0.12^{h}$ |
| 2.5 | | | 75 | $6.4 \pm 0.50^{de}$ | $2.5 \pm 0.14^{f}$ |
| 5.0 | | | 90 | $7.2 \pm 0.37^{d}$ | $2.9 \pm 0.06^{de}$ |
| 7.5 | | | 100 | $9.6 \pm 0.24^{c}$ | $3.3 \pm 0.08^{bc}$ |
| 10.0 | | | 100 | $12.6 \pm 0.67^{a}$ | $3.9 \pm 0.04^{a}$ |
| 12.5 | | | 100 | $8.6 \pm 0.24^{c}$ | $4.0 \pm 0.10^{a}$ |
| 15.0 | | | 80 | $5.8 \pm 0.37^{ef}$ | $2.5 \pm 0.09^{f}$ |
| | 1.0 | | 50 | $2.0 \pm 0.31^{j}$ | $2.5 \pm 0.06^{f}$ |
| | 2.5 | | 55 | $4.0 \pm 0.31^{gh}$ | $2.9 \pm 0.07^{de}$ |
| | 5.0 | | 70 | $5.6 \pm 0.40^{ef}$ | $3.0 \pm 0.10^{cd}$ |
| | 7.5 | | 80 | $8.6 \pm 0.40^{c}$ | $3.1 \pm 0.06^{cd}$ |
| | 10.0 | | 100 | $11.0 \pm 0.63^{b}$ | $3.2 \pm 0.09^{c}$ |
| | 12.5 | | 100 | $7.2 \pm 0.37^{d}$ | $3.5 \pm 0.04^{b}$ |
| | 15.0 | | 70 | $4.6 \pm 0.50^{fg}$ | $2.7 \pm 0.05^{ef}$ |
| | | 20 | 20 | $2.6 \pm 0.40^{ij}$ | $2.2 \pm 0.20^{g}$ |
| | | 40 | 40 | $2.8 \pm 0.37^{hij}$ | $2.6 \pm 0.04^{f}$ |
| | | 50 | 55 | $2.4 \pm 0.40^{ij}$ | $2.6 \pm 0.05^{f}$ |
| | | 55 | 55 | $3.6 \pm 0.40^{ghi}$ | $2.9 \pm 0.04^{de}$ |
| | | 60 | 60 | $4.6 \pm 0.40^{fg}$ | $2.9 \pm 0.03^{de}$ |
| | | 65 | 50 | $3.2 \pm 0.37^{hij}$ | $2.5 \pm 0.04^{f}$ |

Data represents Mean ± SE of 20 replicates per treatment in three repeated experiments.
Mean value followed by the same letter are not significantly different according to Tukey's Test at 5% probability.

**Table 2:** Effect of mT on direct shoot organogenesis through leaf disc culture after 6 weeks of culture

| mT (µM) | % Response | Shoot number per explant | Shoot length (cm) | Root number per explant | Root length (cm) |
|---|---|---|---|---|---|
| 1.0 | 40 | 7.8 ± 0.37[g] | 2.5 ± 0.02[g] | 0.0 ± 0.00[d] | 0.0 ± 0.00[f] |
| 2.5 | 50 | 11.6 ± 0.40[f] | 2.7 ± 0.04[f] | 0.0 ± 0.00[d] | 0.0 ± 0.00[f] |
| 5.0 | 70 | 15.8 ± 0.58[e] | 3.1 ± 0.07[e] | 2.6 ± 0.40[bc] | 3.1 ± 0.07[e] |
| 7.5 | 80 | 18.8 ± 0.31[d] | 3.5 ± 0.05[d] | 2.8 ± 0.37[bc] | 3.3 ± 0.10[d] |
| 10.0 | 100 | 25.6 ± 0.40[b] | 4.2 ± 0.06[b] | 3.4 ± 0.24[b] | 3.8 ± 0.05[c] |
| 12.5 | 100 | 32.6 ± 0.40[a] | 4.5 ± 0.05[a] | 4.6 ± 0.40[a] | 4.6 ± 0.05[a] |
| 15.0 | 80 | 24.0 ± 0.44[c] | 3.7 ±0.04[c] | 2.0 ± 0.31[c] | 4.1 ± 0.05[b] |

Data represents Mean ± SE of 20 replicates per treatment in three repeated experiments.
Mean value followed by the same letter are not significantly different according to Tukey's Test at 5% probability.

**Table 3:** Effect of subculture passages on proliferation efficiency and hyperhydricity

| Subculture passages | BA (12.5 µM) derived shoots | | | | mT (12.5 µM) derived shoots | | | |
|---|---|---|---|---|---|---|---|---|
| | Sub culturing on BA (5.0 µM) | | Sub culturing on BA (12.5 µM) | | Sub culturing on mT (5.0 µM) | | Sub culturing on mT (12.5 µM) | |
| | Shoot per explant | Hyper-hydricity | Shoot per explant | Hyper-hydricity | Shoot per explant | Hyper-hydricity | Shoot per explant | Hyper-hydricity |
| 1 | 10.6 ± 0.67[b] | - | 12.4 ± 1.14[b] | - | 26.0 ± 1.41[a] | - | 36.2 ± 0.83[a] | - |
| 2 | 11.2 ± 0.58[b] | - | 12.8 ± 0.83[b] | + | 23.4 ± 1.14[b] | - | 36.2 ± 0.83[a] | - |
| 3 | 12.6 ± 0.40[ab] | + | 13.2 ± 0.83[b] | ++ | 22.6 ± 1.34[b] | - | 33.4 ± 0.89[b] | - |
| 4 | 13.6 ± 0.40[a] | + | 15.6 ± 0.89[a] | +++ | 19.2 ± 0.83[c] | - | 32.8 ± 0.83[b] | - |

Data represents Mean ± SE of 20 replicates per treatment in three repeated experiments.
Mean value followed by the same letter are not significantly different according to Tukey's Test at 5% probability.
-: No hyperhydricity, +: Small hyperhydricity; + +: Moderate hyperhydricity; + + +: Intense hyperhydricity.

**Table 4:** Effect of different auxins on *in vitro* root induction of BA derived shoots after 4 weeks of culture

| Auxin (µM) | | | % Response | Root number per shoot | Root length (cm) |
|---|---|---|---|---|---|
| IAA | IBA | NAA | | | |
| - | - | - | 0 | 0.0 ± 0.00[h] | 0.00 ± 0.00[i] |
| 0.5 | | | 30 | 1.2 ± 0.20[efg] | 3.26 ± 0.18[f] |
| 1.0 | | | 40 | 2.0 ± 0.31[cde] | 4.48 ± 0.19[d] |
| 2.5 | | | 55 | 2.2 ± 0.37[bcd] | 5.68 ± 0.14[c] |
| 5.0 | | | 60 | 1.8 ± 0.20[cdef] | 4.20 ± 0.12[d] |
| | 0.5 | | 50 | 1.6 ± 0.24[cdef] | 3.54 ± 0.17[ef] |
| | 1.0 | | 55 | 2.4 ± 0.40[bc] | 4.20 ± 0.09[d] |
| | 2.5 | | 90 | 3.6 ± 0.50[a] | 7.70 ± 0.09[a] |
| | 5.0 | | 80 | 3.0 ± 0.31[ab] | 6.24 ± 0.08[b] |
| | | 0.5 | 40 | 0.6 ± 0.24[gh] | 2.24 ± 0.08[h] |
| | | 1.0 | 50 | 1.0 ± 0.00[fg] | 2.68 ± 0.09[g] |
| | | 2.5 | 70 | 1.8 ± 0.37[cdef] | 3.74 ± 0.06[e] |
| | | 5.0 | 80 | 1.4 ± 0.24[defg] | 3.26 ± 0.07[f] |

Values represent the mean of 20 replicates ± standard error (SE).
Mean values within the column with same superscript are not significantly different (P=5%; Tukey's Test).

**Table 5:** Effect of different planting substrate on *ex vitro* survival of BA and mT derived plantlets after 4 weeks of acclimatization

| Planting substrate | % Survival | |
|---|---|---|
| | BA derived plantlets | mT derived plantlets |
| Soilrite™ | 40 | 80 |
| Vermicompost | 60 | 85 |
| Garden soil + coco peat (1:1) | 67 | 90 |

**Results and discussion**

**1. Effect of routine cytokinins (BA, Kn, AdS) on shoot organogenesis**

The choice of cytokinin to use in tissue culture is determined by its cumulative efficiency in inducing an acceptable rate of shoot multiplication, normal shoots and roots development and eventual ability of plants to

acclimatize easily. The shoot and root growth responses and variations observed and analyzed in the present study are due to treatment effect as indicated in Table 1, 2 and 3. Mean number of shoots showed significant difference among the different cytokinins and growth additive tested.

The young leaf discs of *S. cylindrical* were used as initial explants. Leaf disc failed to response morphogenetically on PGR-free MS basal medium that necessitated the exogenous supplementation of PGRs in the nutrient medium. Between two types of routine cytokinins (BA and Kn) and one growth additive (AdS), BA (10.0 µM) was found more suitable for the onset of direct shoot organogenesis on all the concentration applied comparatively with others (Fig. 1A). On this treatment mean number of 12.6 ± 0.67 shoots per explant and 3.9 ± 0.04 cm shoot length were recorded in 100% of cultures after 6 weeks of incubation. Shoot bud initiation was noticed from the central position of the disc as a tough green bulges after 2 weeks of culture. Differentiation of leaves become prominent within a week of buds organization. On the treatment of 7.5 and 12.5µM BA mean number of shoots per explants remain 9.6 ± 0.24 and 8.6 ± 0.24 after 6 weeks of culture, however growth in shoots was slightly stimulated at 12.5 µM with mean shoot length of 4.08 ± 0.10 cm (Table 1). During the study it was also noticed that along with the direct shoot buds organization, callusing was accompanied on all the treatments tested. Callusing was heavily occurred when the explants were planted on 15.0 µM BA containing medium, thereby causing a drastic reduction in shoot formation as well as leads to brittle (hyperhydric) shoot development if kept for longer incubation.

Supplementation of Kn at the same concentration as BA, did not improve the regenerability of shoot buds but exhibited a similar pattern of regeneration response. Among various concentrations of Kn tested, 10.0 µM Kn was found optimal with the production of 11.0 ± 0.63 shoots with mean shoot length of 3.28± 0.09 cm in 100% cultures after same incubation period. On this treatment delayed buds organization was recorded i.e. 3 weeks after inoculation. The response was affected accordingly but the number of shoot regeneration was much lesser than BA containing media of corresponding concentrations. Lower treatment yielded poor harvest of regenerated shoots while the higher concentrations, beyond optimal, was contributed deform buds organization thereby hampering healthy propagule production. The stimulating effect of BA on direct organogenesis through leaf disc culture of *S. cylindrica* has also been reported by Anis and Shahzad (2005) [2]. They have reported a linear correlation with increased levels of BA concentration up to an optimum level (12.5 µM) and thereafter a gradual reduction with intense callogenesis was found. In another study conducted by Shahzad *et al.* (2009) [22] indirect shoot regeneration was reported to be more efficient for enhanced shoot production. They were able to produced shoots after subculturing the calli raised onto 2, 4-D (10 µM) on medium comprised of MS + BA + NAA.

In contrast to BA and Kn, AdS was found to be the least effective for the onset of organogenesis in *S. cylindrical* even after using higher concentration range. Among different concentrations of AdS tested, 60.0 µM AdS induced mean of 4.6 ± 0.40 shoots per explant with mean shoot length of 2.92 ± 0.03 cm in maximum 60% cultures after same incubation period of 6 weeks. These findings revealed that for the propagation of *Sansevieria*, use of AdS is not encouraging.

**2. Effect of mT on shoot organogenesis**

To improve the regeneration potential of leaf disc tissue, another experiment was designed with the application of mT in equimolar concentration as of other cytokinins tested. It was observed that application mT exhibited significantly improved response than the other cytokinins, with an added effect of subsequent rooting in the regenerants. Among various concentrations tested 12.5 µM mT responded best with the production of mean number of 32.6 ± 0.40 shoots per explant. On this medium initiation of shoot buds was recorded after2 weeks of inoculation while the root primordial were witnessed regenerating from the base of microshoot after 4 weeks (Fig. 1B & C). On this composition a very healthy shoot and root system was achieved. At the end of 6 weeks of culture, shoot attained mean shoot length of 4.58 ± 0.05 cm bearing mean number of 4.6 ± 0.40 roots with mean root length of 4.62 ± 0.05 cm. On increasing the concentration of mT to 15.0 µM, an intense callusing wasnoticed with a drastic reduction in shoot regeneration efficiency i.e., 19.0 ± 0.44shoot per explant, attaining shoot length of 3.70 ± 0.40 cm while only 2.0 ± 0.31 roots per microshoot were appeared, however with consistent root length of 4.14 ± 0.5 cm. Similarly on decreasing the concentration, all the parameters affected severely, and witnessed vanishing effect on subsequent rooting in the microshoots (Table 2). In the present study, it was found that equimolar concentration of mT to BA responded significantly better for shoot regeneration as in the same incubation period the number of regenerants increased to double with further advantage of subsequent rooting in microshoots. Complete plantlet production on single PGR treatment has its great applicability in micropropagation system as it reduced the rooting phase of generally 4-6 weeks.

Similar to the present study, Bairu *et al.* (2007) [4] described that in *Aloe polyphylla* mT was proved better than the other routine cytokinins, however, in their study 5.0 µM mT was found better both in terms of multiplication rate as well as subsequent rooting. Kaminek *et al.* (1987) [11] presented a report on the comparative analysis of BA and mT on growth and development of lateral buds in *D. poinsettia* and *Gerbera*, wherein mT was regarded as more active than the BA. Similarly Werbrouck *et al.* (1996) [29], while studying in *Spathiphyllum flouribumdum* tissue culture, revealed that BA derived shoots exhibited a severe reduction in rooting response as well as their survival while those obtained from the mT containing medium were more responsive for rooting and successful acclimatization. Hence a considerable number of studies are available wherein mT was referred as a potential alternative to BA for the development of improved micropropagation system in various important plant species (Pérez-Tornero and Burgos 2000, Gentile *et al.* 2014, Petri and Scorza 2010, Moyo *et al.* 2011,

Malá *et al.* 2013, Amoo *et al.* 2014) [20, 9, 17, 1]. However, in *prunus* root stock tissue culture studies made by Gentile *et al.* (2014) [9], it was found that although the application of mT did not improve the adventitious shoot regeneration with respect to BA but did a positive impact on quality plantlet production with reference to healthy growth and development. In some cases it was also observed that the use of topolins exhibited unfavorable response for multiple shoot regeneration and proliferation for example in *Rosa* hybrid (Bogaert *et al.* 2006) [5], in a citrus hybrid (Niedz and Evens 2010) [19] and in *Vaccinum corymbosum* (Meiners *et al.* 2007) [16].

**3. Effect of repeated subculture for short-term maintenance and hyperhydricity of shoots**

Generally, the incidence of hyperhydricity increased with an increase in the concentration of cytokinin. In the present study, during the subculturing of regenerating tissue on the optimized concentration of mT (12.5 µM) no hyperhydric shoots were recorded for mT derived shoots. Hyperhydricity was most severe on BA (12.5 µM) for BA derived shoots.However, when BA derived shoots were subcultured on to the reduced concentration i.e.5.0 µM BA,all the inducedshoots were healthier as compared to the shoots multiplied on 12.5 µM BA and mean number of 13.6 ± 0.40shoots per culture were found at fourth subculture passage. While, on 12.5 µM BA maximum of 15.6 ± 0.89 shoots per culture were noticed after fourth subculture passage but most of the shoots were hyperhydric. On this treatment hyperhydricity in the regenerating shoots was noticed just after second subculture passage. The shoots turned glassy, yellowish and started to die after fourth subculture passage (data not shown). Hyperhydricity is a common problem that has been observed in micropropagated plants which hinders the potential of *in vitro* technique for mass propagation (Kevers *et al.* 1984) [12]. Gentile *et al.* (2014) [9] have also found significant hyperhydricity on BA containing media.

Proliferation efficiency was the most effective on mT (12.0 µM) supplemented nutrient media as compared to proliferation noticed on other treatments of BA and mT. When mT (12.5 µM) derived shoots were subcultured on to the fresh nutrient medium having similar concentration of mT i.e., 12.5 µM, the highest mean number of shoots per culture (36.2 ± 0.83) were noticed after first subculture passage (Fig. 1D) that remain consistent up to second subculture passage thereafter a slight reduction (32.8 ± 0.83) in shoot proliferation efficiency was noticed. However all the shoots were healthy; none of them exhibited hyperhydricity as noticed with BA derived shoots. However, the shoots proliferated on reduced concentration of mT (5.0 µM) were also healthy and did not exhibit hyperhydricity but the proliferation efficiency was significantly lower than mT (12.5 µM) and a maximum of 26.0 ± 1.41 shoots were induced after first subculture passage (Table 3).

Apart from hyperhydricity, BA-derived microshoots failed to root during subculturing while on mT containing nutrient media simultaneous rooting was noticed after every subculture passage. Similar effect of mT and BA on hyperhydricity and rooting was noticed in *Aloe polyphylla* (Bairu *et al.* 2007) [4]. They have reported that on optimal concentration of 5.0 µM, there were no hyperhydric shoots occurrence in topolins [mT and 6-(-3-methoxybenzylamino)-9-b-D-ribofuranosylpurine (memTR)]-treated cultures. However, in some other reports, on *Malus x domestica* cultivars, use of topolins did not prevent hyperhydricity problem (Dobranszki *et al.* 2002, 2004, 2005) [8, 2].

**4. Effect of auxins on *in vitro* rooting**

As on mT supplemented nutrient medium simultaneous rooting was recorded in a single step therefore no separate experiment was needed for the development of complete plantlets. On the other hand, on BA supplemented nutrient medium, no rooting was found even after different subculture passages, therefore a separate experiment was set for *in vitro* root induction of BA derived shoots. Among different concentrations of auxins tested, half-strength MS medium supplemented with IBA (2.5 µM) was the most effective where the highest of 3.6 ± 0.50 roots per shoot and 7.70 ± 0.09cm root length were noticed after 6 weeks of culture (Table 4, Fig. 1E). Optimum rooting with half-strength MS medium supplemented with IBA has previously been reported by Anis and Shahzad (2005) [2] for *S. cylindrica*. However, in the present study, complete plantlets production with well developed shoot and root system on mT supplemented media is of great significance, as in case of *Aloe polypholia* (Bairu *et al.* 2007) [4].

**5. Effect of planting substrateson *ex vitro* acclimatization**

The experiment conducted for comparative *ex vitro* acclimatization success of mT (12.5 µM) induced plantlets and IBA rooted microshoots obtained from BA (10.0 µM) supplemented nutrient medium, clearly revealed that the plantlets obtained from mT supplemented medium survived better (90%) in garden soil + coco peat that that of BA-IBA derived plantlets (65%) (Table 5, Fig. 1F). The detrimental effect of BA during acclimatization of various plant species have been well documented (Valero-Aracama *et al.* 2010, Aremu *et al.* 2012) [3]. Aremu *et al.* (2012) [3] suggested that the negative impact of BA derived plantlets might be occurred because of the accumulation of the more stable and toxic metabolite, 6-benxzylamine-9-A-D-glucosepyrons sylphurin (Werbrouckl *et al.* 1995) [28], at the basal portion of the plant, could result in slow release of BA during acclimatization and hence reduced the success.

**Conclusion**

Present investigation described an efficient regeneration system in *S. cylindrica* L. by the application of a new class of growth regulator meta-topolin (mT). The advantageous effect of mT with regards to complete plantlets development is of considerable importance over the use of other cytokinins routinely used for micropropagation.

This result, as far as literature available concerned, is the first report on successful application mT for the enhanced organogenesis and plantlets development in *S. cylindrica*. As a large number of agave species are increasingly being utilized at industrial level for the harvesting of quality fiber and as a biofuel crop, the present species is also proving worth because of being a very hardy i.e. can be propagated well with minimal water availability. Hence the developed protocol could be used for the generation of large number of clonal propagules to feed the industrial demand.

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