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Mini Review

Meta-topolin in Micropropagation of Medicinal Plants

Remya Mohanraj*

Houston Community College, Houston, TX, USA

***Corresponding Author:** Remya Mohanraj, Houston Community College, Houston, TX, USA.

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Abstract

Tissue culture of valuable medicinal plants is paramount in conservation efforts and is also a prerequisite for genetic transformation. During micropropagation, cytokinins play a very critical role. *Meta*-topolin(mT), a natural aromatic cytokinin is gaining significance in plant tissue culture and has been reported to be beneficial not only in improving morphogenesis but also in overcoming tissue culture induced abnormalities. In addition, it could also aid in boosting secondary metabolite production. This article provides a comprehensive review of the utilization of meta-topolin in micropropagation of medicinal plants.

Keywords: *Meta*-topolin; Micropropagation; Medicinal Plants; Shoot Regeneration; Root Regeneration; Secondary Metabolites

Introduction

Micropropagation could be effectively used for the conservation of valuable medical plants that have become vulnerable due to overexploitation and habitat destruction. In addition, micropropagation could also be used in the production of genetically uniform and disease free plantlets as well as beneficial active compounds [1]. Standardizing protocols for *in vitro* regeneration opens new avenues for additional improvements via ploidy manipulations, mutation treatments, and transgenic applications [2].

Efficiency of *in vitro* regeneration is dependent on culture conditions and composition of the culture medium [3-5]. Hill and Schaller, (2013) note that *in vitro* regeneration is influenced by phytohormones such as auxins and cytokinins. Among these, cytokinins play a crucial role in shoot organogenesis [6]. However, in some species cytokinins like N6-benzyl adenine (BA) can cause hyperhydricity of plant shoots and can negatively affect the rooting process [7].

A novel cytokinin, 6-(*o*-hydroxybenzylamino)-9-β-*d*-ribofuranosylpurine, was isolated from leaves of *Populus x robusta* by Horgan., *et al.* (1975) [8]. Miroslav., *et al.* (1997) isolated the aromatic cytokinin N^6 -(meta-hydroxybenzyl) adenine from the leaf extracts of poplar (*Populus × canadensis* Moench., cv. *Robusta*) and proposed *meta*-topolin as a trivial name for the compound [9]. Various studies carried out using *meta*-topolin and its derivatives have revealed that *meta*-topolin is more effective for morphogenesis than other cytokinins [10]. Werbrouck., *et al.* (1996) found it to be better than BA for *in vitro* root formation and post *vitro* rooting in *Spathiphyllum floribundum* [11]. It can reduce abnormalities [12] physiological defects induced *in vitro* in tissue culture [13] and can be efficiently used in micropropagation to achieve *in vitro* shoot induction, shoot proliferation, increased shoot length [14], better acclimatization, rooting and improved secondary metabolite production [12]. Furthermore, *meta*-topolin does not inhibit root formation as observed with the use of high concentrations of BAP [11,15,16]. The structure of *meta*-topolin is given in figure 1.

Tissue culturing serves as an excellent and reliable technique to conserve the germplasm of valuable medicinal plants. Besides,

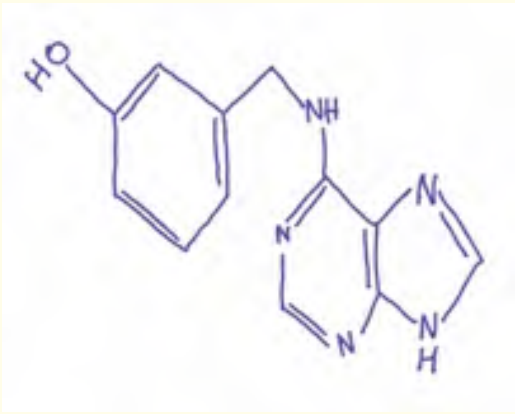

Figure 1: Structure of *meta*-topolin.

it could help increase secondary metabolite production and form the basis of genetic transformation experiments. As the choice of growth regulators is a key step during micropropagation, the above discussed advantages provided by *meta*-topolin, over other cytokinins make it an important addition to tissue culture media. This article aims at providing a comprehensive review on the utilization of *meta*-topolin for organogenesis in medicinal plants.

Meta-topolin in shoot regeneration

Salvi., *et al.* (2002) studied the effect of cytokinins including *meta*-topolin on shoot multiplication of *Curcuma longa* [17]. Agnieszka Wojtania (2010) reported that *meta*-topolin was the most efficient growth regulator for regeneration and axillary multiplication in *Pelargonium* cultivars. While BAP induced lower regeneration potency and decreased shoot quality, *meta*-topolin had stimulating effects [18]. Meyer., *et al.* (2009) observed that treatment with BA or *meta*-topolin was able to promote calli capable of regeneration and shoot differentiation in *Hypericum* sp [2].

Ptak., *et al.* (2013), reported that in *Leucojum aestivum,* maximum regeneration was achieved from somatic embryos cultured using *meta*-topolin and BA. Also, a maximum number of plants with normal development were observed in a combination of thidiazuron and *meta*-topolin [19]. Studies by Lata., *et al.* (2016) revealed that highest number of shoots, percentage of shoot producing explants and maximum shoot length in *Cannabis sativa* could be obtained by using *meta*-topolin [20]. Vijaykumar., *et al.* (2017) observed that *meta*-topolin promoted formation of adventitious shoots from shoot-tip explants and organogenesis from immature leaf calli in *Carthamus tinctorius* [21].

Chauhan and Taylor (2018) reported using a medium containing *meta*-topolin and 2,4-dichlorophenoxyacetic acid (2,4-D), followed by sub culturing in a medium with elevated levels of *meta*-topolin, to be beneficial for the induction of multiple shoots in cassava. The authors reported that this system is more favorable as compared to the use of *meta*-topolin alone and that *meta*-topolin is advantageous in initiating the regeneration of shoots from somatic embryos as well as cotyledon explants [22].

In *Pterocarpus marsupium*, preretreating nodal explants with thidiazuron in half-strength liquid MS medium before inoculating onto full-strength MS medium fortified with various doses of *meta*-topolin was found to be very effective in inducing the most number of shoots and achieving a high regeneration frequency by Naseem and Mohammad (2018) [23]. Dhandapani., *et al.* (2019) studied the efficacy of *meta*-topolin during different stages of regeneration in *Sesamum indicum* and observed that multiple shoots induced in medium containing *meta*-topolin, were amenable to shoot elongation, rooting and *in vitro* acclimatization [24].

Khanam., *et al.* (2020) developed a micropropagation protocol for *Allamanda cathartica* from nodal explants using *meta*-topolin. Higher number of shoots and good shoot length was observed on MS medium supplemented with *meta*-topolin [25]. Shekhawat., *et al.* (2021) reported an *in vitro* regeneration system from nodal segment cultures of *Scaevola taccada* using *meta*-topolin in liquid medium [26].

Meta-topolin in root regeneration

Werbrouck., *et al*. (1996) observed that in *Spathiphyllum floribundum, meta*-topolin induced good shoot production along with *in vitro* roots. Also, during acclimatization, plantlets that were cultured with *meta*-topolin higher than or equal to 10 µM exhibited better rooting than those that were on medium containing similar concentrations of BA [11].

Bairu., *et al.* (2007) investigated the utility of *meta*-topolin and its derivatives in *Aloe polyphylla* and noted that *meta*-topolin aided in high multiplication rate and rooting. Spontaneous rooting was also observed eliminating the need for an extra rooting step

[27]. Van der Westhuizen (2014) conducted trials to determine the effect of *meta*-topolin on the regeneration, hyperhydricity, and rooting of *Eucalyptus* species and found it to have beneficial effects in *in vitro* rooting [7].

Agnieszka Wojtania (2010) observed that *meta*-topolin neither produced any undesirable effect on the growth nor inhibited formation of roots in *Pelargonium* cultivars [18]. In *Prunus* rootstocks, maximum root length was obtained in plants from *meta*-topolin. Also, leaves obtained from shoots regenerated using *meta*-topolin were the only ones that demonstrated adventitious regeneration [28].

Studies on *Syzygium cumini* by Naaz., *et al.* (2019) revealed that in addition to promoting good shoot bud induction and proliferation, *meta*-topolin also increased rhizogenic competency of the shoots. The authors also reported increased activities of superoxide dismutase, catalase, glutathione reductase, and ascorbate peroxidase during acclimatization indicating plantlets regenerated using *meta*-topolin were more tolerant to *ex vitro* stress [29]. Erişen., *et al.* (2020) found that in comparison to BAP, kinetin and thidiazuron, *meta*-topolin was beneficial for both shooting and rooting in *Salvia sclarea* [30].

Various studies that used *meta*-topolin for organogenesis have been indicated in the following table.

Name of the Plant	Plant Material	Regeneration	Reference
Curcuma longa	Young vegetative buds	Shoot	[17]
Pelargonium cultivars	Axillary buds and shoot tips	Shoot and root	[18]
Hypericum sp	Leaves	Shoot	[2]
Leucojum aestivum	Somatic embryos	Whole plant	[19]
Cannabis sativa	Nodal explants	Shoot and root	[20]
Carthamus tinctorius	shoot-tip explants	Shoot	[21]
Pterocarpus marsupium	Nodal explant	Shoot	[23]
Sesamum indicum	Cotyledonary node	Shoot and Root	[24]
Cassava	Leaf, petiole, stem internode, somatic embryos and cotyledon	*Shoot*	[22]
Allamanda cathartica	Nodal explant	Shoot	[25]
Scaevola taccada	Nodal segment	Shoot	[26]
Aloe polyphylla	*In vitro* plantlets	Root	[27]
Eucalyptus species	-	Shoot and Root	[7]
Spathiphyllum floribundum	Nodal explants	Shoot and Root	[29]
Withania somnifera	Leaf explant	Shoot	[31]
Salvia sclarea	Seeds	Shoot and Root	[30]

Table

Effect of *Meta*-topolin in secondary metabolite production:

Medicinal plants possess a rich array of useful secondary metabolites. Augmentation of valuable secondary metabolites production could be achieved in tissue culture by using growth hormones [32].The production of secondary metabolites through *in vitro* culture is influenced by culture medium and its components [33]. *Meta*-topolin could be added as an elicitor to boost the production of secondary metabolites and to aid in industrial production [34]. The various effects of *meta*-topolin on the production of secondary metaboilites are explored in this section.

Amoo and Van Staden., *et al.* (2013), observed that as compared to BA, *meta*-topolin was more effective in promoting shoot proliferation and phenolic production in micropropagated

Huernia hystrix [35]. Aremu., *et al.* (2012) noted that total phenolic content was maximum in areal parts of plantlets treated with 10 µM *meta*-topolin and the same treatment resulted in the underground parts producing higher proanthocyanidins than the control plants. The findings of this study suggest that topolins have potential to promote phenolic compounds accumulation during micropropagation [36]. Masondo (2014) studied the influence of various plant growth regulators including *meta*-topolin and reported higher total phenolics and flavonoids in treatments free of plant growth regulators [37].

Nowakowska and Pacholczak (2020) reported that *meta*-topolin has a positive effect on the levels of various compounds in *Daphne mezereum* [38]. Khanam., *et al.* (2020) reported higher flavonoids and quercetin content from the leaves of plantlets raised on medium supplemented with *meta*-topolin [25].

Future Prospects

Having thrown some light on the importance of *meta*-topolin in the tissue culture of medicinal plants, we could now reflect upon the future direction of research using this cytokinin. Further research is warranted towards a better understanding of the exact mechanism through which *meta*-topolin acts. This would enable researchers to standardize efficient protocols for *in vitro* plant regeneration depending on the species. Studies related to structural modifications of the compound to improve efficiency could also be a future focus area of research. Also, detailed studies related to the role of *meta*-topolin in the production of secondary metabolites would be a valuable addition.

Conclusions

From the review presented here, the significant role played by *meta*-topolin during the various stages of micropropagation of medicinal plants could be well appreciated. It has been established through multiple studies that, the use of *meta*-topolin could enable overcoming the negative effects caused by regular cytokinins. *Meta*-topolin could therefore be a choice of cytokinin for many other researchers who are working towards standardizing a protocol for *in vitro* regeneration of valuable medicinal plants.

Bibliography

1. Chatterjee T and Ghosh B. "Micropropagation of Medicinal Plants: a Review". *International Journal of Economic Plants* 7 (2020): 66-72.
2. Meyer EM., *et al*. "*In vitro* shoot regeneration and polyploidy induction from leaves of *Hypericum* species". *HortScience* 44 (2009): 1957-1961.
3. Gantait S., *et al*. "Aloe vera: a review update on advancement of in vitro culture". *Acta Agriculturae Scandinavica, Section B — Soil and Plant Science* 64 (2014) :1-12.
4. Gantait S and Mandal N. "Tissue culture of *Anthurium andreanum*: a significant review and future prospective". *International Journal of Botany* 6 (2010): 207-219.
5. Rezal NI., *et al*. "The effects of different strength of MS media in solid and liquid media on in vitro growth of Typhonium flagelliforme". *Asian Pacific Journal of Tropical Biomedicine* 7 (2017): 151-156.
6. Hill K and Schaller G E. "Enhancing plant regeneration in tissue culture: a molecular approach through manipulation of cytokinin sensitivity". *Plant Signaling and Behavior* 8 (2013): e25709.
7. van der Westhuizen A. "The Use Of Meta-Topolin As An Alternative Cytokinin In The Tissue Culture Of *Eucalyptus* Species". *Acta Horticulturae* 1055 (2014): 25-28.
8. Horgan R., *et al*. "A new cytokinin from Populus x robusta". *Phytochemistry* 14 (1975): 1005-1008.
9. Miroslav S., *et al*. "Meta-topolin, a highly active aromatic cytokinin from poplar leaves (Populus × canadensis Moench., cv. Robusta)". *Phytochemistry* 45 (1997): 213-218.
10. Krishna Vrundha CP., *et al*. "The Role of *Meta*-topolin in Plant Morphogenesis In Vitro". In: Ahmad N., Strnad M. (eds) Meta-topolin: A Growth Regulator for Plant Biotechnology and Agriculture. Springer, Singapore (2021).
11. Stefaan PO., *et al*. "Meta-topolin, an alternative to benzyladenine in tissue culture?". *Physiogia Plantarum* 98 (1996): 291-297.
12. Koç E. "Effects of *Meta*-topolin on the Growth, Physiological and Biochemical Parameters in Plant Tissue Culture". In: Ahmad N., Strnad M. (eds) Meta-topolin: A Growth Regulator for Plant Biotechnology and Agriculture. Springer, Singapore (2021).
13. Aremu AO., *et al*. "Topolins: A panacea to plant tissue culture challenges?". *Plant Cell, Tissue and Organ Culture (PCTOC)* 108 (2012a): 1-16.

14. Gantait S and Mitra M. "Role of *Meta*-topolin on in Vitro Shoot Regeneration: An Insight". In: Ahmad N., Strnad M. (eds) Meta-topolin: A Growth Regulator for Plant Biotechnology and Agriculture. Springer, Singapore (2021).

15. Strnad M. "The aromatic cytokinins". *Physiology Plant* 101 (1997): 674-688.

16. Dolezal K., *et al*. "New cytokinin derivatives - their discovery, development and use for micropropagation of endangered tree species". *BMC Proceedings* 5 (2011): O46.

17. Salvi ND., *et al*. "Micropropagation and field evaluation of micropropagated plants of turmeric". *Plant Cell, Tissue and Organ Culture (PCTOC)* 68 (2002): 143.

18. Agnieszka W. "Effect of meta-topolin on in vitro propagation of Pelargonium × Hortorum and Pelargonium × Hederaefolium cultivars". *Acta Societatis Botanicorum Poloniae* 79 (2010): 101-106.

19. Ptak A., *et al*. "Leucojum aestivum plants propagated in in vitro bioreactor culture and on solid media containing cytokinins". *Engineering in Life Sciences, Wiley-VCH Verlag,* 13.3 (2013): 261-270.

20. Lata H., *et al*. "In vitro mass propagation of Cannabis sativa L.: A protocol refinement using novel aromatic cytokinin meta-topolin and the assessment of eco-physiological, biochemical and genetic fidelity of micropropagated plants". *Journal of Applied Research on Medicinal and Aromatic Plants* 3 (2016): 18-26.

21. Vijayakumar J., *et al*. "Influence of Meta-Topolin on Efficient Plant Regeneration via Micropropagation and Organogenesis of Safflower (Carthamus tinctorius L.) cv. NARI-H-15". *American Journal of Plant Sciences* 8 (2017): 688-705.

22. Chauhan RD and Taylor NJ. "Meta-topolin stimulates de novo shoot organogenesis and plant regeneration in cassava". *Plant Cell, Tissue and Organ Culture* 132 (2018): 219.

23. Naseem A and Mohammad F. "Preconditioning of nodal explants in Thidiazuron supplemented liquid media improves shoot multiplication in Pterocarpus marsupium (Roxb)". Anees Ahmad, Naseem Ahmad, Mohmad Anis in Thidiazuron: From Urea Derivative to Plant Growth Regulator (eds) Springer (2018).

24. Dhandapani E., *et al*. "Meta-Topolin (mT) enhances the in vitro regeneration frequency of Sesamum indicum (L.)". *Biocatalysis and Agricultural Biotechnology* 21 (2019): 101320.

25. Khanam MN., *et al*. "Meta-Topolin induced *in vitro* regeneration and metabolic profiling in *Allamanda cathartica* L". *Industrial Crops and Products* 145 (2020): 111944.

26. Shekhawat MS., *et al*. "Meta-topolin and liquid medium enhanced in vitro regeneration in Scaevola taccada (Gaertn.) Roxb". *In Vitro Cellular and Developmental Biology – Plant* 57 (2021): 296-306.

27. Bairu MW., *et al*. "Optimizing the micropropagation protocol for the endangered *Aloe polyphylla*: Can meta-topolin and its derivatives serve as replacement for benzyladenine and zeatin?" *Plant Cell, Tissue and Organ Culture* 90 (2007): 15-23.

28. Gentile A., *et al*. "Effect of meta-topolin on micropropagation and adventitious shoot regeneration in Prunus rootstocks". *Plant Cell, Tissue and Organ Culture* 118 (2014): 373-381.

29. Naaz A., *et al*. "*Meta*-topolin improved micropropagation in *Syzygium cumini* and acclimatization to *ex vitro* conditions". *Biologia plantarum* 63 (2019): 174-182.

30. Erişen E., *et al*. "*In vitro* propagation of *Salvia sclarea* L. by meta-Topolin, and assessment of genetic stability and secondary metabolite profiling of micropropagated plants". *Industrial Crops and Products* 157 (2020): 112892.

31. Kaur K., *et al*. "In vitro shoot multiplication using meta-Topolin and leaf-based regeneration of a withaferin A rich accession of *Withania somnifera* (L.) Dunal". *Industrial Crops and Products* 171 (2021): 113872.

32. Thakur M., *et al*. "Improving production of plant secondary metabolites through biotic and abiotic elicitation". *Journal of Applied Research on Medicinal and Aromatic Plants* 12 (2019): 1-12.

33. Cardoso J., *et al*. "Advances and challenges on the in vitro production of secondary metabolites from medicinal plants". *Horticultura Brasileira* 37 (2019): 124-132.

34. Turkyilmaz Unal B. "The Use of Meta-topolin in Cell and Tissue Cultures for Increasing Production of Secondary Metabolites". In: Ahmad N., Strnad M. (eds) Meta-topolin: A Growth Regulator for Plant Biotechnology and Agriculture. Springer, Singapore (2021).

35. Amoo S O and Van Staden J. "Influence of plant growth regulators on shoot proliferation and secondary metabolite production in micropropagated *Huernia* hystrix". *Plant Cell, Tissue and Organ Culture* 112 (2013): 249-256.

36. Aremu AO., *et al*. "Assessment of the role of *meta*-topolins on *in vitro* produced phenolics and acclimatization competence of micropropagated 'Williams' banana". *Acta Physiologiae Plantarum* 34 (2012 b): 2265.

37. Masondo, Nqobile Andile. "In vitro techniques for the improvement of growth and secondary metabolite production in Eucomis autumnalis subspecies autumnalis". MSc., Thesis, College of Agriculture, Engineering and Science University of KwaZulu-Natal Pietermaritzburg, South Africa (2014).

38. Nowakowska K and Pacholczak A. "Comparison of the Effect of Meta-Topolin and Benzyladenine during Daphne mezereum L. Micropropagation". *Agronomy* 10 (2020): 1994.