Role of *Meta*-topolin on in Vitro Shoot Regeneration: An Insight

12

Saikat Gantait and Monisha Mitra

Contents

Abstract

Meta-topolin [6-(3-hydroxybenzylamino)purine)] is an aromatic cytokinin bearing a benzyl ring substituted by a hydroxyl group at *meta*-position. It was first isolated from the leaves of poplar tree. *Meta*-topolin (*m*T) has immense potential for shoot regeneration. Its other beneficial attributes include delaying senescence, preventing shoot-tip necrosis, and evading the effects of hyperhydricity. The concise results obtained from different studies, conducted over the past one decade, distinctively show that the effects of *m*T basically include in vitro shoot induction, shoot proliferation, and increase in shoot length. When used in

S. Gantait (✉)
Crop Research Unit (Genetics and Plant Breeding), Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India
e-mail: saikatgantait@bckv.edu.in

M. Mitra
Department of Agricultural Biotechnology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

N. Ahmad, M. Strnad (eds.), *Meta-topolin: A Growth Regulator for Plant Biotechnology and Agriculture*, https://doi.org/10.1007/978-981-15-9046-7_12

combination with auxins, it exhibits an ability to induce regenerative callus. Based on all the beneficial attributes of *m*T, it can be regarded as a potent aromatic cytokinin that can be utilized in micropropagation. Considering its significant application in plant tissue culture, the present chapter intricately describes the nature, usage, and advantages of *m*T on shoot regeneration, in particular.

Keywords

Auxins · Callus · Cytokinin · Hyperhydricity · *Meta*-topolin · Micropropagation · Senescence

12.1 Introduction

Cytokinins are a major class of plant growth hormones that induce cell division in tissues (Koshimizu and Iwamura 1986). Its major properties include impeding the senescence process mainly by preventing protein degradation that further increases the activity of RNase (Hall 1973). The primary function of cytokinins is suppressing the apical dominance and thus culminating a way for the development of buds at lateral positions (Leopold and Kriedemann 1975). Considering the extensive uses of cytokinins, there has been an increase in demand on the usage of synthetic cytokinins having analogous effects to naturally derived cytokinins. A novel class of cytokinins, i.e., topolins, is recently being used in plant tissue culture. Chemically, they are regarded as aromatic cytokinins (ARCKs), and structural studies showed that topolins consist of a hydroxylated benzyl that is attached at N^6-position of adenine (Aremu et al. 2012a). *m*T was first isolated from the leaves of *Populus* × *canadensis*. The nomenclature of the hormone has been derived from the Czech word 'topol' that signifies the plant poplar from where it was first isolated (Strnad et al. 1997). Furthermore, the cytokinin activity depends on the N1 position which should remain free since in the case of hydroxyl at the *ortho*-position, hydrogen bonding occurs between this group and the N1 atom, which in turn makes the *meta*-substituents highly potent (Holub et al. 1998). Henceforth, the name *meta*-topolin [6-(3-hydroxybenzylamino)purine)] is clearly justified. Generally, *m*T serves as substrate for *O*-glucosyltransferase (ZOG1 enzyme found in *Zea mays* and *Phaseolus lunatus*). Additionally, from the reports on correlation between the activity of *m*T and their ability to serve as substrate for ZOG1 enzyme, it can be concluded that there is a similarity between the receptors and the binding sites of *m*T (Mok et al. 2005). The physical attributes of *m*T are, namely, 'solid form' and 'off white' to 'white' colour. During preparation of stock solution, *m*T is readily soluble in water, but other solvents like KOH and dimethyl sulfoxide (DMSO) are also equivalently effective. *m*T also has many multifaceted properties like evading the ill effects of shoot-tip necrosis and hyperhydricity and causing delay in senescence that eventually facilitates multiple shoot regeneration and proliferation (Malá et al. 2013).

12.2 Principles of Regeneration via In Vitro Shoots

The development and regeneration of new organs from different explants are based on the phenomena known as 'totipotency', which means that a single cell can develop into a functional organ. The entire process of shoot organogenesis spans entirely over three broad aspects, which are competence, determination, and morphogenesis (Sugiyama 1999). Competence is regarded as the initial step of shoot organogenesis where cell signalling induced by hormones leads to dedifferentiation of cells (Howell et al. 2003). The next step is determining the identity of the organ fixed by the proportion of plant growth hormone; in case of shoot organogenesis, it is possible through cytokinins (Gahan and George 2008). Morphogenesis is the ultimate step where shoot induction finally occurs (Sugiyama 1999).

Furthermore, the phenomena of shoot induction can be categorized into two pathways, namely, direct and indirect pathway. The direct pathway involves the formation of shoot bud, and there is no formation of callus (Yancheva et al. 2003), whereas indirect pathway involves an intermittent step where formation of callus occurs and from adventitious shoots induces from the respective callus (Gahan and George 2008).

12.3 In Vitro Shoot Regeneration in Plant System

The regeneration of shoots from desired explants, mediated via direct or indirect organogenesis approach, serves as a steadfast methodology for micropropagation (Gahan and George 2008). The regeneration of shoots in multiple numbers depends on the type of explant utilized and also on an array of factors like the nutrients utilized in basal media, the environmental factors, and the type and dosage of plant growth hormone used (Gantait et al. 2014).

12.3.1 Influence of Explant

The fundamental criteria before establishment of in vitro culture is the proper selection of explant that should be free from contamination and can be imparted by physical factors like dust or dirt and biological factors like bacteria, fungi, and other microorganisms (Gantait and Kundu 2017). There are innumerable types of explants utilized in micropropagation for inducing shoot directly. Shoot tips and axillary buds are predominantly used explants in any micropropagation experiment since it is easily available and it maintains the genetic integrity in the in vitro-derived plantlets from the mother plant (Rout et al. 2006). Histological studies showed that the shoot-tip region contains a zone comprising of meristematic cells that exhibit the phenomena called 'totipotency' and possess the ability of accumulating plant growth hormones at an anticipated level (Akin-Idowu et al. 2009). Another desirable explant utilized is nodal segment, which has the capability to generate multiple axillary buds, since bud break can be easily induced in a short period of time with the application of

desired level of plant hormones (Gantait et al. 2009). Some other explants apart from shoot tip and nodal segment such as leaf, corm, bulb, epicotyl, hypocotyledon, cotyledon, root, zygotic embryos, and tillers exhibited promising results and displayed a positive correlation with shoot regeneration (Meyer et al. 2009; Nas et al. 2010; Niedz and Evens 2010; Valero-Aracama et al. 2010; De Diego et al. 2011, Moyo et al. 2011; Niedz and Evens 2011a; Baskaran et al. 2012; Aremu et al. 2014; Moyo et al. 2014; Masondo et al. 2014; Ncube et al. 2015; Aremu et al. 2016; Chiancone et al. 2017; Baskaran et al. 2018a, b; Behera et al. 2018; Chauhan and Taylor 2018; Ahmad and Anis 2019).

12.3.2 Influence of Basal Media

In any micropropagation setup, the basal media enriched with macro- and micronutrients is essential for proper regeneration of explant. The popularly used basal media is Murashige and Skoog (MS) (Murashige and Skoog 1962) medium for shoot regeneration (Table 12.1). There are also other types of media apart from MS media, where the results achieved were quite promising, such as Le Poivre (LP) basal medium (Cortizo et al. 2009); Woody Plant (WP) medium (De Diego et al. 2010; Lattier et al. 2014; Mirabbasi and Hosseinpour 2014; Wen et al. 2016; Rakrawee et al. 2018; Tongsad et al. 2018; Nowakowska et al. 2019), comprising low concentration of nitrate and ammonium ions (Bosela and Michler 2008); Nas and Read medium (NMR) (Nas et al. 2010); Murashige and Tucker (MT) medium (Niedz and Evens 2011b); Quoirin and Lepoivre (QL) (Quoirin and Lepoivre 1977) medium where calcium nitrate is the sole nitrogen source (Lattier et al. 2013); and Driver and Kuniyuki (DKW) medium (Driver and Kuniyuki 1984) that possess minimum proportions of ammonium ions with calcium nitrate serving as nitrogen sources (Gentile et al. 2017; Nacheva et al. 2015; Stevens and Pijut 2018).

12.3.3 Influence of Carbon Source and Concentration

The explants that require regeneration under in vitro conditions possess partial autotrophic condition; hence, carbon source is mandatory (Van Huylenbroeck and Debergh 1996). Carbon source, mainly sugars, is required for fine-tuning the osmotic regulation (Hazarika 2003). Majority of research reports indicated the use of optimum amount of carbon source (2–3% sucrose), which is prevalently used (Table 12.1). However, as low as 0.57% sucrose was used to assess its effect on multiple shoot induction from shoot tip of *Asparagus officinalis* that eventually resulted in elongated multiple shoots (Hudák et al. 2013). It is noteworthy to mention that there is a single instance wherein glucose was used as a carbon source instead of sucrose (Nacheva et al. 2015).

Table 12.1 Influence of *meta*-topolin (*m*T) on induction, proliferation, and elongation of in vitro multiple shoots

Plant species	Explant	Basal media	Carbon source	Physical factors (temp/LI/PP/RH)	*m*T (mg/L* or μM)	Supplement	Gelling agent	References
Harpagophytum procumbens	NS	MS	3% (w/v) sucrose	25 ± 1 °C; 45 μmol/m^2/s; 16 h; NM	5	None	0.9% (w/v) Agar	Bairu et al. (2009a)
Aloe ferox Mill	ST	MS	3% sucrose	24 ± 1 °C; 45 μmol/m^2/s; 16 h; NM	5	0.01% (w/v) MI	1% Agar	Bairu et al. (2009b)
Pinus pinea L.	NS	LP	3% sucrose	22 °C; 120 μmol/m^2/s; 16 h; NM	50	None	0.4% (w/v) Gelrite; 0.02% (w/v) Difco-Bacto agar	Cortizo et al. (2009)
Hypericum	L	MS	3% sucrose	23 ± 1 °C; dark; NA; NM	15	1.25 μM IAA	0.8% Agar	Meyer et al. (2009)
Saccharum officinarum	Apical dome	MS	NM	22 ± 1 °C; 100 μmol/m^2/s; 16 h; NM	10	None	0.8% Agar	Vinayak et al. (2009)
Pinus sylvestris L.	AB	DCR, LP, WPM	3% sucrose	22 °C; 120 μmol/m^2/s; 16 h; NM	50	None	1% Difco-Bacto agar	De Diego et al. (2010)
Lycaste aromatica	Pseudo bulb	MS	3% sucrose	25 ± 1 °C; 50 μmol/	8.87	2 mg/L Glycine; 0.01% MI	0.3% (w/v) Gellan gum	Mata-Rosas et al. (2010)

(continued)

Table 12.1 (continued)

Plant species	Explant	Basal media	Carbon source	Physical factors (temp/LI/PP/RH)	*m*T (mg/L* or μM)	Supplement	Gelling agent	References
				m^2/s; 16 h; NM				
Prunus microcarpa	Cotyledon, hypocotyl, root	NRM	3% sucrose	23 ± 1 °C; 80 μmol/m^2/s; 16 h; NM	12.5	None	0.55% Microbiological agar	Nas et al. (2010)
Citrus reticulata × *Poncirus trifoliata*	Epicotyl		NM	27 °C; 30–35 μmol/m^2/s; 16 h; NM		None	0.8% Agar	Niedz and Evens (2010)
Uniola paniculata	Tiller	MS	87.6 μM sucrose	24 ± 1 °C; 40 μmol/m^2/s; 16 h; 58%	10	0.56 μM MI, 1.2 μM thiamine	0.8% Agar	Valero-Aracama et al. (2010)
Pelargonium sp.	ST	MS	3% sucrose	24 ± 1 °C; 40 μmol/m^2/s; 16 h; NM	1	None	0.55% Microbiological agar	Wojtania (2010)
Telekia speciosa (Schreb.) Baumg.	In vitro seedling	MS	3% sucrose	22 ± 2 °C; 105 μmol/m^2/s; 16 h; NM	5	KNO_3 (500 mg/L); casein (500 mg/L);	0.7% Agar	Csabai et al. (2011)
Pinus pinaster Ait.	Zygotic embryos	MS	3% sucrose	22 °C; 100 μmol/m^2/s; 16 h; NM	2.2	0.2% AC	1% Difco-Bacto agar	De Diego et al. (2011)

Sclerocarya birrea	ST, hypocotyl, epicotyl	MS	3% sucrose	25 ± 2 °C; 40 µmol/ m^2/s; 16 h; 58%	8	0.01% MI, 3 g/L polyvinylpyrrolidone	NM	Moyo et al. (2011)
Citrus sinensis	Epicotyl	MT	NM	27 °C; 30 µmol/ m^2/s; 16 h; NM	0.25	0.75 µM BA	0.8% Agar	Niedz and Evens (2011b)
Aloe arborescens	ST	MS	3% sucrose	25 ± 2 °C; 40 µmol/ m^2/s; 16 h; NM	15	0.01% MI	0.8% Agar	Amoo et al. (2012)
Musa spp. AAA cv. 'Williams'	ST	MS	3% sucrose	25 ± 2 °C; 45 µmol/ m^2/s; 16 h; NM	30	None	0.3% Gelrite	Aremu et al. (2012b)
Musa spp. AAA cv. 'Williams'	ST	MS	3% sucrose	25 ± 2 °C; 45 µmol/ m^2/s; 16 h; NM	30	None	0.3% Gelrite; INCYDE	Aremu et al. (2012c)
Merwilla plumbea (Lindl.) Speta	L	MS	NM	25 ± 2 °C; 40 µmol/ m^2/s; 16 h; NM	0.42	Trimethoprim (TMP), yeast extract (YE), and yeast malt broth	NM	Baskaran et al. (2012)
Pistacia vera L.	NS	MS	3% sucrose	22 ± 1 °C; 40 µmol/ m^2/s; 16 h; NM	2	0.01% Inositol, 500 mg/L casein hydrolysate	NM	Benmahioul et al. (2012)
Pelargonium sidoides	ST	MS	3% sucrose	25 ± 2 °C; 40 µmol/	2	0.01% (w/v) MI, 180 mg/L ascorbic acid	0.8% Agar	Moyo et al. (2012)

(continued)

Table 12.1 (continued)

Plant species	Explant	Basal media	Carbon source	Physical factors (temp/LI/PP/RH)	*m*T (mg/L* or µM)	Supplement	Gelling agent	References
				m^2/s; 16 h; NM				
Magnolia 'Ann'	ST	MS	3% sucrose	23 ± 2 °C; 70 µmol/m^2/s; 16 h; NM	8	0.01% inositol, MES monohydrate	0.8% Agar	Parris et al. (2012)
Romulea sabulosa	ST	MS	3% sucrose	NM	5	None	0.8% Agar	Swart et al. (2012)
Pelargonium × *hortorum* cv. 'Grand Prix'	ST	MS	3% sucrose	20 °C; 40 µmol/m^2/s; 16 h; NM	1	0.01% MI	0.55% Agar	Wojtania and Węgrzynowicz-Lesiak (2012)
Huernia hystrix	ST	MS	3% sucrose	25 ± 2 °C; 40 µmol/m^2/s; 16 h; NM	20	0.01% MI	0.8% Agar	Amoo and van Staden (2013)
Barleria argillicola	ST, NS	MS	3% sucrose	25 ± 2 °C; 40 µmol/m^2/s; 16 h; NM	7	0.01% MI	0.8% Agar	Amoo and van Staden (2013)
Agapanthus praecox	ST	MS	3% sucrose	25 ± 2 °C; 40 µmol/m^2/s; 16 h; NM	4.1	22.2 µM BA	0.8% Agar	Baskaran and van Staden (2013)
Asparagus officinalis	ST	MS	0.57% sucrose	22 ± 2 °C; 105 µmol/	2	25 mg/L Fe-EDTA, 0.01% MI	0.8% Agar	Hudák et al. (2013)

				m^2/s; 16 h; NM				
Acer platanoides	ST	MS, WPM, QL	3% sucrose	22 ± 2 °C; 75 µmol/ m^2/s; 16 h; NM	2*	0.01% MI, MES (100 mg/ L),	0.75% Agar	Lattier et al. (2013)
Aloe arborescens	ST	MS	3% sucrose	25 ± 2 °C; 30 µmol/ m^2/s; 16 h; NM	10	0.01% MI	0.8% Agar	Amoo et al. (2014)
Merwilla plumbea	L	MS	3% sucrose	25 ± 2 °C; 40 µmol/ m^2/s; 16 h; NM	1	0.01% MI	0.8% Agar	Aremu et al. (2014)
Coleonema pulchellum	ST	MS	3% sucrose	25 ± 2 °C; 40 µmol/ m^2/s; 16 h; NM	2.1	13.6 µM BA	0.8% Agar	Baskaran et al. (2014)
Scutellaria barbata	ST, NS	MS	3% sucrose	25 ± 2 °C; 40 µmol/ m^2/s; 16 h; NM	5	34.2 g D-maltose; 18 g fructose; 10 g D-mannose; 10 g MI, 18 g D-mannitol; 0.1 µM NAA	0.7% Agar	Brearley et al. (2014)
Paulownia elongate	AB	MS	3% sucrose	NM	1	None	0.3% Agar	Clapa et al. (2014)
Coleonema album	ST	MS	3% sucrose	25 ± 2 °C; 30 µmol/ m^2/s; 16 h; NM	5	0.01% MI, 2 µM IBA	0.8% Agar	Fajinmi et al. (2014)
Lonicera kamtschatica	ST	MS	3% sucrose	23 ± 3 °C; 35 µmol/	1	None	5% (w/v) wheat starch	Al et al. (2014)

(continued)

Table 12.1 (continued)

Plant species	Explant	Basal media	Carbon source	Physical factors (temp/LI/PP/RH)	*m*T (mg/L* or μM)	Supplement	Gelling agent	References
				m^2/s; 16 h; NM				
Prunus rootstock (Torinel)	AB	MS	3% sucrose	24 ± 2 °C; 37.5 μmol/m^2/s; 16 h; NM	2.1	None	0.55% Agar	Gentile et al. (2014)
Ocimum basilicum	In vitro seedling	½MS	3% sucrose	22 °C; 36 μmol/m^2/s; 16 h; NM	2.07	10 mg/L Meso-inositol	0.62% Agar	Kőszeghi et al. (2014)
Cornus 'NCCH1'	ST	WPM	3% sucrose	NM	2.5	0.01% MI, MES (100 mg/L),	0.75% Agar	Lattier et al. (2014)
Eucomis autumnalis	ST	MS	3% sucrose	25 ± 2 °C; 45 μmol/m^2/s; 16 h; NM	2	0.01% MI, 10 μM NAA	0.3% Gelrite	Masondo et al. (2014)
Ulmus glabra	AB	WPM	3% sucrose	24 ± 2 °C; 2000 lux; 16 h; NM	0.2*	100 mg/L Sequestrene, 10 mg/L glutamine	0.57% Agar	Mirabbasi and Hosseinpour (2014)
Hypoxis hemerocallidea	Corm	MS	3% sucrose	25 ± 2 °C; 45 μmol/m^2/s; 16 h; NM	10	0.01% MI	0.8% Agar	Moyo et al. (2014)
Dioscorea trifida	ST	MS	3% sucrose	27 ± 1 °C; 50 μmol/	25	0.2% AC, 0.23 μM GA	0.7% Agar	Engelmann-Sylvestre and

				m^2/s; 12 h; NM				Engelmann (2014)
Pelargonium hortorum	ST	MS	3% sucrose	21 °C; 40 µmol/m^2/s; 12 h; NM	2.07	0.01% MI, 3.78 µM ABA	0.6% Agar	Wojtania and Skrzypek (2014)
Malus domestica	ST	MS	3% sucrose	23 ± 2 °C; 5500 lux; 16 h; NM	1	None	0.25% (w/v) Phytagel	Al et al. (2015)
Syringa vulgaris	ST	MS	2% sucrose	23 ± 1 °C; 35 µmol/m^2/s; 12 h; NM	5	None	0.8% Agar	Ilczuk and Katarzyna (2015)
Cyrtanthus guthrieae	Bulbs	MS	3% sucrose	25 ± 2 °C; 60 µmol/m^2/s; 12 h; NM	5	0.01% MI	0.8% (w/v) Bacto-agar	Ncube et al. (2015)
Pelargonium × *hortorum* 'Bergpalais	ST	MS	3% sucrose	23 °C; 40 µmol/m^2/s; 16 h; NM	2.07	0.01% MI	0.6% Bacto-agar	Wojtania et al. (2015)
Eucomis autumnalis	L	MS	3% sucrose	25 ± 2 °C; 45 µmol/m^2/s; 16 h; NM	5	5 µM NAA	0.8% Agar	Aremu et al. (2016)
Pistacia vera	ST	MS	3% sucrose	22 ± 1 °C; 40 µmol/m^2/s; 16 h; NM	2*	0.01% MI, 500 mg/L casein hydrolysate	0.7% Agar	Benmahioul et al. (2016)

(continued)

Table 12.1 (continued)

Plant species	Explant	Basal media	Carbon source	Physical factors (temp/LI/PP/RH)	*m*T (mg/L* or μM)	Supplement	Gelling agent	References
Dendrobium nobile	NS	MS	3% sucrose	25 ± 2 °C; 35–50 μmol/m^2/s; 12 h; NM	1*	0.8 mg/L putrescine	0.8% Agar	Bhattacharyya et al. (2016)
Mixed diploid Banana (*Musa* AB)	ST	MS	3% sucrose	28 ± 2 °C; NM; 14 h; NM	8.89	None	0.8% Agar	Bohra et al. (2016)
Nardostachys jatamansi	ST	MS	3% sucrose	22 ± 1 °C; 50 μmol/m^2/s; 14 h; NM	1*	None	0.8% Agar	Bose et al. (2016)
Pyrus communis Rootstock OHF-333	ST	MS	3% sucrose	22 ± 2 °C; 40 μmol/m^2/s; 14 h; NM	9*	None	0.65% (w/v) Phyto-agar	Dimitrova et al. (2016)
Withania coagulans	NS	MS	3% sucrose	25 ± 2 °C; 35 μmol/m^2/s; 14 h; NM	2.5*	0.1 mg/L NAA + 50 mg/L AdS	0.8% Agar	Joshi et al. (2016)
Clerodendrum phlomidis	NS	MS	3% sucrose	25 ± 2 °C; 35 μmol/m^2/s; 16 h; NM	8.28*	271 μM AdS	0.8% Agar	Kher et al. (2016)
Cannabis sativa	NS	MS	3% sucrose	25 ± 2 °C; 52 μmol/	2	None	0.8% Agar	Lata et al. (2016)

				m^2/s; 16 h; NM				
Allium achoenoprasum	ST	MS	2% sucrose	25 ± 2 °C; 100 µmol/m^2/s; 16 h; NM	10	0.01% MI, 2 mg/L adenine	0.7% Agar	Tubić et al. (2016)
Silk Banana var. Nanjanagud Rasabale (*Musa* AAB)	Sucker	MS	3% sucrose	28 ± 2 °C; 40 µmol/m^2/s; 14 h; NM	35.48	None	0.6% Agar	Waman et al. (2016)
Paeonia × *lemoinei* 'High Noon'	AB	WPM	3% sucrose	25 ± 1 °C; 25 µmol/m^2/s; 14 h; NM	5	1.5 µM GA_3	0.7% Agar	Wen et al. (2016)
Pistacia vera	AB	MS	3% sucrose	NM; 40 µmol/m^2/s; NM; NM	2	0.01% MI, 500 mg/L casein hydrolysate	0.7% Agar	Benmahioul (2017)
Ansellia africana	NS	MS	3% sucrose	25 ± 2 °C; 40 µmol/m^2/s; 12 h; 80%	10	None	0.8% Agar	Bhattacharyya et al. (2017)
Citrus sinensis [L.] *Osbeck* × *Poncirus trifoliata* [L.] Raf.	Epicotyl segment	MS	3% sucrose	27 ± 1 °C; 35 µmol/m^2/s; 16 h; NM	1*	500 mg/L ascorbic acid, 500 mg/L malt extract	0.85% Agar	Chiancone et al. (2017)
Corylus colurna	ST	DKW	3% sucrose	24 ± 1 °C; 37.5 µmol/m^2/s; 16 h; NM	8.2	None	0.7% Agar	Gentile et al. (2017)

(continued)

Table 12.1 (continued)

Plant species	Explant	Basal media	Carbon source	Physical factors (temp/LI/PP/RH)	*m*T (mg/L* or µM)	Supplement	Gelling agent	References
Cannabis sativa	ST	MS	3% sucrose	24 ± 1 °C; NM; 18 h; NM	0.5*	0.1% AC	0.7% Agar	Grulichova et al. (2017)
Eriocephalus africanus	ST	MS	3% sucrose	25 ± 2 °C; 40–50 µmol/m^2/s; 16 h; NM	5	0.01% MI	0.8% Agar	Madzikane-Mlungwana et al. (2017)
Prunus domestica	ST	MS	3% sucrose	24 ± 2 °C; 37.5 µmol/m^2/s; 16 h; NM	2.1	None	0.55% Agar	Monticelli et al. (2017)
Merwilla plumbea	ST	DKW	3% glucose	22 ± 2 °C; 40 µmol/m^2/s; 16 h; NM	5.5	None	0.65% Agar	Nacheva et al. (2015)
Scadoxus puniceus	L	MS	NM	25 ± 2 °C; 30 µmol/m^2/s; 16 h; NM	5.5	None	–	Naidoo et al. (2017)
Dendrocalamus asper	NS	MS	3% sucrose	24 ± 2 °C; 40–50 µmol/m^2/s; 16 h; NM	20	None	0.25% Phytagel	Ornellas et al. (2017)

Cotula bipinnata, *Chironia linoides*	ST	MS	3% sucrose	24 ± 1 °C; 30 µmol/ m^2/s; 16 h; NM	0.3*	None	0.7% Agar	Sacco et al. (2017)
Ceratonia siliqua	ST	MS	3% sucrose	24 ± 2 °C; 50 µmol/ m^2/s; 16 h; NM	10	None	0.8% Agar	Shahzad et al. (2017)
Carthamus tinctorius (NARI-H-15)	ST, NS	MS	3% sucrose	25 ± 2 °C; 15 µmol/ m^2/s; 16 h; NM	3	0.5 µM CPPU	0.8% Agar	Vijayakumar et al. (2017)
Ficus carica	AB	MS	3% sucrose	NM; 35 µmol/ m^2/s; 16 h; NM		None	0.65% Agar	Yahyaoui et al. (2017)
Santalum album			NM			None		Akhtar and Shahzad (2019)
Epilobium canum garretti	NS	MS	3% sucrose	25 ± 1 °C; 38 µmol/ m^2/s; 16 h; NM	8.8	2 mg/L thiamine	0.7% Agar	Alosaimi et al. (2018)
Urginea altissima	ST	MS	3% sucrose	25 ± 2 °C; 50 µmol/ m^2/s; 16 h; NM	10	2 µM IAA	0.8% Agar	Baskaran et al. (2018a)
Eucomis autumnalis	L	MS	3% sucrose	25 ± 2 °C; 40 µmol/ m^2/s; 16 h; NM	10	None	0.8% Agar	Baskaran et al. (2018b)

(continued)

Table 12.1 (continued)

Plant species	Explant	Basal media	Carbon source	Physical factors (temp/LI/PP/RH)	*m*T (mg/L* or μM)	Supplement	Gelling agent	References
Hedychium coronarium	Rhizome	MS	NM	25 ± 1 °C; 50 μmol/m^2/s; 16 h; NM	3	None	–	Behera et al. (2018)
Dendrobium aphyllum	NS	MS	3% sucrose	25 ± 2 °C; 50 μmol/m^2/s; 16 h; NM	15	10 μM $AgNO_3$, 10 μM TDZ		Bhattacharyya et al. (2018a)
Ansellia africana	NS	MS	3% sucrose	25 ± 2 °C; 40 μmol/m^2/s; 12 h; NM	7.5	None	0.8% Agar	Bhattacharyya et al. (2018b)
Cassava	L	MS	2% sucrose	NM	7.5	None	0.8% Agar	Chauhan and Taylor (2018)
Kadsura heteroclita	NS	MS	3% sucrose	25 °C; NM; 16 h; NM	0.5*	None	0.26% Gellan gum	Jedoroh et al. (2018)
Mammillaria dixanthocentron	ST	MS	3% sucrose	25 ± 1 °C; 55 μmol/m^2/s; 16 h; NM	2.2	0.1% AC	0.8% Agar	Lázaro-Castellanos et al. (2018)
Mammillaria hernandezii, *M. lanata*	ST	MS	3% sucrose	25 ± 1 °C; 55 μmol/m^2/s; 16 h; NM	4.4	None	0.8% Agar	Lázaro-Castellanos et al. (2018)
Rose cv. *Frisco*	NS	MS	4% sucrose	24 ± 1 °C; 60 μmol/	2.1*	None	0.7% agar	Mahmood et al. (2018)

				m^2/s; 16 h; NM				
Amelanchier alnifolia	In vitro shoots	MS	3% sucrose	24 ± 1 °C; 40 µmol/ m^2/s; 16 h; NM	20	0.01% MI	NM	Moyo et al. (2018)
Stylosanthes hamata cv. *Verano*	In vitro seedling	MS	3% sucrose	25 ± 1 °C; 27 µmol/ m^2/s; 16 h; NM	3*	None	0.26% Phytagel	Ngoenngam et al. (2018)
Malus × *domestica Borkh*	ST	MS	3% sucrose	21 °C; 30 µmol/ m^2/s; 16 h; NM	8	None	0.6% Agar	Podwyszyńska and Cieślińska (2018)
Gluta usitata (217 Mae Ka)	ST	WPM	3% sucrose	25 ± 2 °C; NM; 16 h; NM	2*	None	0.2% Phytagel	Rakrawee et al. (2018)
Gluta usitata (Napong3)	NS	WPM	3% sucrose	25 ± 2 °C; NM; 16 h; NM	0.5*	0.2% AC	0.26% Gellan gum	Rakrawee et al. (2018)
Juglans nigra	NS	DKW	3% sucrose	24 ± 2 °C; 80 µmol/ m^2/s; 16 h; NM		None	0.22% Phytagel	Stevens and Pijut (2018)
Tectona griandis	ST	WPM	3% sucrose	25 ± 2 °C; NM; 16 h; NM	0.25*	None	0.26% Gellan gum	Tongsad et al. (2018)
Pterocarpus marsupium	Cotyledonary node	MS	3% sucrose	24 ± 2 °C; 50 µmol/	7.5	14 µM NAA	0.8% Agar	Ahmad and Anis (2019)

(continued)

Table 12.1 (continued)

Plant species	Explant	Basal media	Carbon source	Physical factors (temp/LI/ PP/RH)	*m*T (mg/L* or μM)	Supplement	Gelling agent	References
				m^2/s; 16 h; NM				
Stylosanthes hamata cv. Verano	In vitro seedling	MS	3% sucrose	25 ± 1 °C; 27 μmol/ m^2/s; 16 h; NM	3*	None	0.26% Phytagel	Ngoenngam et al. (2019)
Daphne mezereum	NS	MS	3% sucrose	NM	1*	0.01% MI	Bacto-agar	Nowakowska et al. (2019)
Daphne mezereum	NS	WPM	3% sucrose	NM	2*	None	Bacto-agar	Nowakowska et al. (2019)

2,4-D 2,4-Dichlorophenoxy acetic acid, *AB* axillary buds, *AC* activated charcoal, *BA* N^6-benzyladenine, *DKW* Driver and Kuniyuki walnut medium (Driver and Kuniyuki 1984), *IAA* indole-3-acetic acid, *IBA* indole-3-butyric acid, *INCYDE* 2-Chloro-6-(3-methoxyphenyl)aminopurine, *L* leaf, *LI* light intensity, *LP* Le Poivre basal medium, *MI* myo-inositol, *MS* Murashige Skoog medium (Murashige and Skoog 1962), *MT* Murashige and Tucker medium (Murashige and Tuker 1969), *NA* not applicable, *NAA* α-naphthalene acetic acid, *NM* not mentioned, *NRM* Nas and Read medium (Nas and Read 2004), *NS* nodal segments, *PP* photoperiod, *QL* Quoirin and Lepoivre, *RH* relative humidity, *ST* shoot tip, *TDZ* thidiazuron, *Temp* temperature, *WPM* Woody plant medium
* indicates that the plant growth regulator doses are in mg/L

12.3.4 Influence of Physical Environment

Light and temperature are the indispensable parameters for successful establishment of any in vitro culture. Light is dependent upon two factors, namely, intensity and duration. The source of light utilized in any in vitro culture room is fluorescent lamp that can be 'cool' or 'warm' (Gantait and Kundu 2017). Based on the research reports of past one decade, it was observed that the optimum light intensity ranging between 40 and 300 μmol/m^2/s was maintained for successful in vitro regeneration (Table 12.1). Additionally, temperature also plays an obligatory role for normal growth of in vitro culture, and it is mainly dependent on the basic design and structural organization of laboratory. The temperature regime adopted during establishment of in vitro multiple shoot culture protocols is summarized specifically in Table 12.1. Relative humidity (RH) also serves as a regulatory factor in any commercial tissue culture laboratory. Lower levels of RH result in instilling a positive growth in explants, since it strikes a perfect balance with the transpiration rate, whereas higher levels of humidity cause degenerative disorders in explants (Ghashghaie et al. 1992). Generally, the optimum level of RH recurrent in all the relevant literatures scrutinized is around 60%.

12.3.5 Influence of *Meta*-topolin

There are ~100 reports on the usage of *meta*-topolin as a plant growth hormone for shoot regeneration that have been documented and illustrated precisely in this chapter. For shoot induction and multiplication, proper dosage of cytokinins is essential (Gahan and George 2008). *m*T proved to be an effective cytokinin for multiple shoot culture in a number of medicinal, ornamental, and aromatic plants (Fig. 12.1). In majority of the cases, *m*T alone was sufficient to induce multiple shoots and also aid in shoot proliferation in many species summarized in Table 12.1. A range of 5–10 μM *m*T was employed to induce multiple shoot proliferation in various plant species through direct and indirect regeneration systems (Bairu et al. 2009a; Vinayak et al. 2009; Nas et al. 2010; Swart et al. 2012; Clapa et al. 2014; Al et al. 2014; Mukherjee et al. 2020). It was observed that even at a concentration lower than 5 μM, *m*T resulted in successful shoot induction (Wojtania and Węgrzynowicz-Lesiak 2012; Hudák et al. 2013; Aremu et al. 2014; Al et al. 2015; Lata et al. 2016).

12.3.6 Influence of *m*T in Combination with Other PGRs or Additives

*m*T was also coupled with various classes of plant hormones and also growth additives that gave fruitful results (Table 12.1). *m*T when used in combination with auxins provided positive results towards shoot induction; however, the formation of regenerative callus was observed when it was exogenously used with auxins like indole-3-acetic acid (IAA) (Meyer et al. 2009). Alternatively, multiple shoot was observed in *Coleonema album* when combined with indole-3-butyric acid

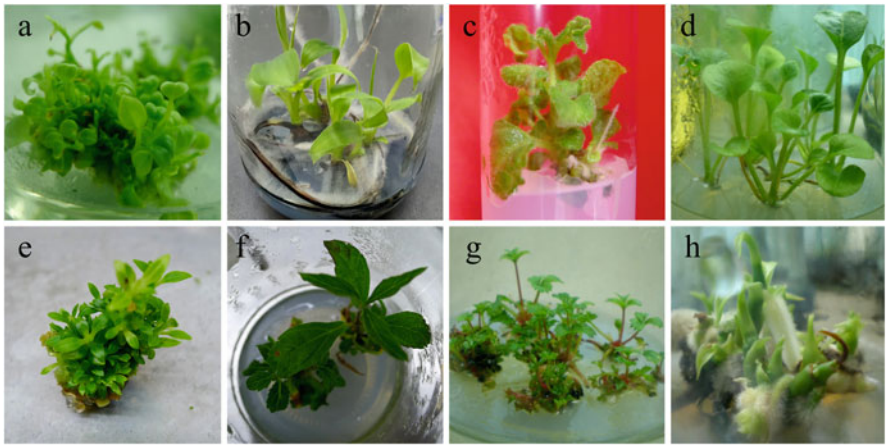

Fig. 12.1 Influence of *meta*-topolin (10 μM in semi-solid MS medium with 3% sucrose incubated at 25 ± 1 °C temperature, 50 μmol/m^2/s PPFD light intensity, 16 h photoperiod with 60% RH) on in vitro multiple shoot proliferation of several plant species. Multiple shoot cultures of (**a**) *Anthurium andreanum*, (**b**) *Musa* sp. cv. Grande Nine, (**c**) *Coleus forskohlii* Briq., (**d**) *Gerbera jamesonii* Bolus, (**e**) *Rauwolfia serpentina* (L.) Benth. ex Kurz., (**f**) *Sphagneticola* sp., (**g**) *Fragaria* × *ananassa* Dutch, (**h**) *Vanilla planifolia* Andrews (figures are not in scale). (*Source*: Unpublished photographs of Saikat Gantait)

(IBA), where myo-inositol (MI) was used as an additive (Fajinmi et al. 2014). Surprisingly, in combination with N^6-benzyladenine (BA), shoot induction occurred along with the formation of callus in the basal end, when shoot tips of *Coleonema pulchellum* were cultured (Baskaran et al. 2014). In combination with other cytokinins, a high synergistic effect was observed with *m*T. A high frequency of multiple shoot formation was observed when *m*T was used in combination with BA (Baskaran and van Staden 2013). Even the use of abscisic acid (ABA) along with *m*T gave positive results in multiple shoot regeneration in *Pelargonium hortorum* (Wojtania and Skrzypek 2014). Combination of *m*T with gibberellic acid (GA_3) resulted in shoot multiplication with increased shoot length (Engelmann-Sylvestre and Engelmann 2014; Wen et al. 2016). Similarly, with the application of additives like MI and casein hydrolysate along with *m*T resulted in longer shoot length in *Pistacia vera* (Benmahioul 2017). Additives like trimethoprim, yeast extract, and yeast malt broth when used in the medium together with *m*T also resulted in an increase in nodal length of multiple shoots in *Merwilla plumbea* (Baskaran et al. 2012). Synthetic cytokinins like thidiazuron (TDZ) when used in combination with *m*T and an additive $AgNO_3$ exhibited better results during in vitro culture of nodal segments of *Dendrobium aphyllum* (Bhattacharyya et al. 2018a). Activated charcoal serves as a potent additive for in vitro culture since it has the capability of absorbing hazardous phenolic compounds that are generated during in vitro regeneration (Gantait et al. 2009). Activated charcoal gave promising results with high frequency of shoot multiplication when used in combination with *m*T (De Diego et al. 2011; Engelmann-Sylvestre and Engelmann 2014; Grulichova et al. 2017).

12.4 Conclusion and Future Prospect of *m*T Use

In this chapter, the extensive use of *mT* in various plant species along with their appropriate doses has been scrutinized and elaborately described. The usage of *m*T in various in vitro cultures has shown promising results when used alone or in combination with other plant growth hormones or additives. The other facets of *m*T apart from effective shoot induction and multiplication include increase in shoot length, fresh weight, and increase in photosynthetic capacity of in vitro-derived plantlets (Ahmad and Anis 2019). It also has an additional capability of inducing regenerative callus when coupled with some auxins, thus highlighting the efficiency of *m*T. Considering these positive attributes, we can consider *m*T as an upcoming plant growth regulator that can be utilized commercially. The need for a comprehensive and reproducible protocol on in vitro culture of plantlets using *m*T is an utmost necessity for commercialization of plant tissue culture system globally. However, the utility of this compound needs to be harnessed in plants having medicinal and aesthetic value.

Acknowledgement Authors acknowledge the e-library assistance from Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India. We are further thankful to the anonymous reviewers and the editor of this chapter for their critical comments and suggestions on the manuscript.

In Compliance with Ethical Standards *Conflict of Interest*: none

References

Ahmad A, Anis M (2019) Meta-topolin improves in vitro morphogenesis, rhizogenesis and biochemical analysis in *Pterocarpus marsupium* Roxb.: a potential drug-yielding tree. J Plant Growth Regul 38:1007–1016

Akhtar R, Shahzad A (2019) Morphology and ontogeny of directly differentiating shoot buds and somatic embryos in *Santalum album* L. J For Res 30:1179–1189

Akin-Idowu PE, Ibitoye DO, Ademoyegun OT (2009) Tissue culture as a plant production technique for horticultural crops. Afr J Biotech 8:3782–3788

Al F, Clapa D, Cristea V, Plopa C (2014) In vitro propagation of *Lonicera kamtschatica*. Agric Sci Pract 1-2:89–90

Al F, Simu M, Vlaicu B, Clapa D (2015) Aspects regarding the in vitro propagation of 'Royal Gala' apple cultivar. Bull Univ Agric Sci Vet Med Cluj Napoca Hortic 72:342–349

Alosaimi AA, Tripepi RR, Love SL (2018) Micropropagation of *Epilobium canum garretti* (Firechalice) by axillary shoot culture. HortSci 53:62–66

Amoo SO, van Staden J (2013) Influence of plant growth regulators on shoot proliferation and secondary metabolite production in micropropagated *Huernia hystrix*. Plant Cell Tissue Organ Cult 112:249–256

Amoo SO, Aremu AO, van Staden J (2012) In vitro plant regeneration, secondary metabolite production and antioxidant activity of micropropagated *Aloe arborescens* Mill. Plant Cell Tissue Organ Cult 111:345–358

Amoo SO, Aremu AO, Moyo M et al (2014) Physiological effects of a novel aromatic cytokinin analogue in micropropagated *Aloe arborescens* and *Harpagophytum procumbens*. Plant Cell Tissue Organ Cult 116:17–26

Aremu AO, Bairu MW, Doležal K et al (2012a) Topolins: a panacea to plant tissue culture challenges? Plant Cell Tissue Organ Cult 108:1–16

Aremu AO, Bairu MW, Szüčová L et al (2012b) Shoot and root proliferation in 'Williams' banana: are the topolins better cytokinins? Plant Cell Tissue Organ Cult 111:209–218

Aremu AO, Bairu MW, Novák O et al (2012c) Physiological responses and endogenous cytokinin profiles of tissue-cultured 'Williams' bananas in relation to roscovitine and an inhibitor of cytokinin oxidase/dehydrogenase (INCYDE) treatments. Planta 236:1775–1790

Aremu AO, Plačková L, Bairu MW et al (2014) How does exogenously applied cytokinin type affect growth and endogenous cytokinins in micropropagated *Merwilla plumbea*? Plant Cell Tissue Organ Cult 118:245–256

Aremu AO, Plačková L, Pěnčík A et al (2016) Auxin-cytokinin interaction and variations in their metabolic products in the regulation of organogenesis in two *Eucomis* species. New Biotechnol 33:883–890

Bairu MW, Jain N, Stirk WA et al (2009a) Solving the problem of shoot-tip necrosis in *Harpagophytum procumbens* by changing the cytokinin types, calcium and boron concentrations in the medium. S Afr J Bot 75:122–127

Bairu MW, Kulkarni MG, Street RA et al (2009b) Studies on seed germination, seedling growth, and in vitro shoot induction of *Aloe ferox Mill*., a commercially important species. HortSci 44:751–756

Baskaran P, van Staden J (2013) Rapid in vitro micropropagation of *Agapanthus praecox*. S Afr J Bot 86:46–50

Baskaran P, Ncube B, van Staden J (2012) *In vitro* propagation and secondary product production by *Merwilla plumbea* (Lindl.) Speta. Plant Growth Regul 67:235–245

Baskaran P, Moyo M, van Staden J (2014) *In vitro* plant regeneration, phenolic compound production and pharmacological activities of *Coleonema pulchellum*. S Afr J Bot 90:74–79

Baskaran P, Kumari A, van Staden J (2018a) *In vitro* propagation via organogenesis and synthetic seeds of *Urginea altissima* (Lf) Baker: a threatened medicinal plant. 3 Biotech 8:18

Baskaran P, Kumari A, van Staden J (2018b) Analysis of the effect of plant growth regulators and organic elicitors on antibacterial activity of *Eucomis autumnalis* and *Drimia robusta* ex vitro-grown biomass. Plant Growth Regul 85:143–151

Behera S, Kamila PK, Rout KK et al (2018) An efficient plant regeneration protocol of an industrially important plant, *Hedychium coronarium* J. Koenig and establishment of genetic & biochemical fidelity of the regenerants. Ind Crop Prod 126:58–68

Benmahioul B (2017) Factors affecting in vitro micropropagation of Pistachio (*Pistacia vera* L.). Agric For J 1:56–61

Benmahioul B, Dorion N, Kaid-Harche M et al (2012) Micropropagation and ex vitro rooting of pistachio (*Pistacia vera* L.). Plant Cell Tissue Organ Cult 108:353–358

Benmahioul B, Kaïd-Harche M, Daguin F (2016) In vitro regeneration of *Pistacia vera* L. from nodal explants. J For Sci 62:198–203

Bhattacharyya P, Kumaria S, Tandon P (2016) High frequency regeneration protocol for *Dendrobium nobile*: a model tissue culture approach for propagation of medicinally important orchid species. S Afr J Bot 104:232–243

Bhattacharyya P, Kumar V, van Staden J (2017) Assessment of genetic stability amongst micropropagated *Ansellia africana*, a vulnerable medicinal orchid species of Africa using SCoT markers. S Afr J Bot 108:294–302

Bhattacharyya P, Paul P, Kumaria Set al. (2018a) Transverse thin cell layer (t-TCL)-mediated improvised micropropagation protocol for endangered medicinal orchid *Dendrobium aphyllum* Roxb: an integrated phytomolecular approach. Acta Physiol Plant 40:137

Bhattacharyya P, Kumar V, van Staden J (2018b) In vitro encapsulation based short term storage and assessment of genetic homogeneity in regenerated *Ansellia africana* (Leopard orchid) using gene targeted molecular markers. Plant Cell Tissue Organ Cult 133:299–310

Bohra P, Waman AA, Sathyanarayana BN et al (2016) Influence of different growth regulators on in vitro multiplication of mixed diploid Banana (*Musa* AB). Proc Natl Acad Sci India Sect B 86:179–185

Bose B, Kumaria S, Choudhury H et al (2016) Assessment of genetic homogeneity and analysis of phytomedicinal potential in micropropagated plants of *Nardostachys jatamansi*, a critically endangered, medicinal plant of alpine Himalayas. Plant Cell Tissue Organ Cult 124:331–349

Bosela MJ, Michler CH (2008) Media effects on black walnut (*Juglans nigra* L.) shoot culture growth in vitro: evaluation of multiple nutrient formulations and cytokinin types. In Vitro Cell Dev Biol Plant 44:316–329

Brearley TA, Vaidya BN, Joshee N (2014) Cytokinin, carbon source, and acclimatization requirements for in vitro propagation of *Scutellaria barbata* D. Don and *Scutellaria racemosa* Pers. Am J Plant Sci 5:3662–3672

Chauhan RD, Taylor NJ (2018) Meta-topolin stimulates de novo shoot organogenesis and plant regeneration in cassava. Plant Cell Tissue Organ Cult 132:219–224

Chiancone B, Martorana L, Gianguzzi V et al (2017) The effects of meta-Topolin and benzyladenine on in vitro organogenesis from epicotyl cuttings of Troyer citrange (*Citrus sinensis* [L.] Osbeck × *Poncirus trifoliata* [L.] Raf.). Acta Hortic 1155:185–192

Clapa D, Fira A, Simu M et al (2014) Improved in vitro propagation of Paulownia elongata, P. fortunei and its interspecific hybrid P. *elongata* x P. *fortunei*. Bull Univ Agric Sci Vet Med Cluj Napoca Hortic 71:422–427

Cortizo M, de Diego N, Moncaleán P et al (2009) Micropropagation of adult stone pine (*Pinus pinea* L.). Trees 23:835–842

Csabai J, Nagy Z, Mándy A (2011) In vitro shoot proliferation of *Telekia speciosa* (Schreb.) Baumg. Induced by different cytokinins. Acta Biol Hung 62:453–462

De Diego N, Montalbán IA, Moncaleán P (2010) In vitro regeneration of adult *Pinus sylvestris* L. trees. S Afr J Bot 76:158–162

De Diego N, Montalbán IA, Moncaleán P (2011) Improved micropropagation protocol for maritime pine using zygotic embryos. Scand J For Res 26:202–211

Dimitrova N, Nacheva L, Berova M (2016) Effect of *meta*-topolin on the shoot multiplication of pear rootstock OHF-333 (*Pyrus communis* L.). Acta Sci Pol-Hortorum 15:43–53

Driver JA, Kuniyuki AH (1984) In vitro propagation of paradox walnut rootstock. HortSci 19:507–509

Engelmann-Sylvestre I, Engelmann F (2014) Effect of various growth regulators on growth of yam (*Dioscorea trifida* L.) in vitro shoot tips. Afr J Bot 13:1645–1649

Fajinmi OO, Amoo SO, Finnie JF et al (2014) Optimization of in vitro propagation of *Coleonema album*, a highly utilized medicinal and ornamental plant. S Afr J Bot 94:9–13

Gahan PB, George EF (2008) Adventitious regeneration. In: George EF, Hall MA, De Klerk GJ (eds) Plant propagation by tissue culture, 3rd edn. Springer, Dordrecht, pp 355–401

Gantait S, Kundu S (2017) *In vitro* biotechnological approaches on *Vanilla planifolia* Andrews: advancements and opportunities. Acta Physiol Plant 39:196

Gantait S, Mandal N, Bhattacharyya S, Das PK, Nandy S (2009) Mass multiplication of *Vanilla planifolia* with pure genetic identity confirmed by ISSR. Int J Dev Biol 3:18–23

Gantait S, Sinniah UR, Das PK (2014) *Aloe vera*: a review update on advancement of in vitro culture. Acta Agric Scand B Soil Plant Sci 64:1–12

Gentile A, Gutiérrez MJ, Martinez J et al (2014) Effect of meta-topolin on micropropagation and adventitious shoot regeneration in Prunus rootstocks. Plant Cell Tissue Organ Cult 118:373–381

Gentile A, Frattarelli A, Nota P et al (2017) The aromatic cytokinin meta-topolin promotes in vitro propagation, shoot quality and micrografting in *Corylus colurna* L. Plant Cell Tissue Organ Cult 128:693–703

Ghashghaie J, Brenckmann F, Saugier B (1992) Water relations and growth of rose plants cultured in vitro under various relative humidities. Plant Cell Tissue Organ Cult 30:51–57

Grulichova M, Mendel P, Lalge AB et al (2017) Effect of different phytohormones on growth and development of micropropagated *Cannabis sativa* L. MendelNet 24:618–623

Hall RH (1973) Cytokinins as a probe of developmental processes. Annu Rev Plant Physiol 24:415–444

Hazarika BN (2003) Acclimatization of tissue cultured plants. Curr Sci 85:1705–1712

Holub J, Hanus J, Hanke DE, Strnad M (1998) Biological activity of cytokinins derived from *ortho*- and *meta* hydroxybenzyladenine. Plant Growth Regul 26:109–115

Howell SH, Lall S, Che P (2003) Cytokinins and shoot development. Trend Plant Sci 8:453–459

Hudák I, Magyar-Tábori K, Zsombik L (2013) Effect of aromatic cytokinins and explant position on shoot multiplication of *Asparagus officinalis* L. Int J Hortic Sci 19:67–70

Ilczuk A, Katarzyna JK (2015) The effect of plant growth regulators and sucrose on the micropropagation of common lilac (*Syringa vulgaris* L.). Ann Warsaw Univ Life Sci SGGW Hortic Landsc Architect 36:3–12

Jedoroh N, Laipasu P, Chareonsap PP et al (2018) Induction of shoot and root from nodes of *Kadsura heteroclite*. Int J Agric Technol 14:1287–1292

Joshi H, Nekkala S, Soner D et al (2016) In vitro shoot multiplication of *Withania coagulans* (stocks) Dunal. Plant Tissue Cult Biotechnol 26:187–195

Kher MM, Soner D, Srivastava N et al (2016) Micropropagation of *Clerodendrum phlomidis* LF. J Hortic Res 24:21–28

Koshimizu K, Iwamura H (1986) Cytokinins. In: Takahashi N (ed) Chemistry of plant hormones. CRC Press, Florida, pp 153–159

Kőszeghi S, Bereczki C, Balog A et al (2014) Comparing the effects of benzyladenine and meta-topolin on sweet basil (*Ocimum basilicum*) micropropagation. Not Sci Biol 6:422–427

Lata H, Chandra S, Techen N et al (2016) 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. J Appl Res Med Aromat Plants 3:18–26

Lattier JD, Touchell DH, Ranney TG et al (2013) Micropropagation and polyploid induction of *Acer platanoides* 'Crimson Sentry'. J Environ Hortic 31:246–252

Lattier J, Touchell DH, Ranney TG (2014) Micropropagation of an interspecific hybrid dogwood (*Cornus* 'NCCH1'). Propag Ornam Plants 14:184–190

Lázaro-Castellanos JO, Mata-Rosas M, González D et al (2018) In vitro propagation of endangered *Mammillaria* genus (Cactaceae) species and genetic stability assessment using SSR markers. In Vitro Cell Dev Biol Plant 54:518–529

Leopold AC, Kriedemann CE (1975) Plant growth and development. McGraw & Hill, New York

Madzikane-Mlungwana O, Moyo M, Aremu AO et al (2017) Differential responses to isoprenoid, N^6-substituted aromatic cytokinins and indole-3-butyric acid in direct plant regeneration of *Eriocephalus africanus*. Plant Growth Regul 82:103–110

Mahmood S, Reza MR, Hossain MG et al (2018) Response of cytokinins on in vitro shoot multiplication of Rose cv. Frisco. Res Rev J Agric Sci Technol 5:8–12

Malá J, Máchová P, Cvrčková H et al (2013) The role of cytokinins during micropropagation of wych elm. Biol Plant 57:174–178

Masondo NA, Aremu AO, Finnie JF et al (2014) Plant growth regulator induced phytochemical and antioxidant variations in micropropagated and acclimatized *Eucomis autumnalis* subspecies *autumnalis* (Asparagaceae). Acta Physiol Plant 36:2467–2479

Mata-Rosas M, Baltazar-García RJ, Moon P et al (2010) In vitro regeneration of *Lycaste aromatica* (Graham ex Hook) Lindl. (Orchidaceae) from pseudobulb sections. Plant Biotech Rep 4:157–163

Meyer EM, Touchell DH, Ranney TG (2009) In vitro shoot regeneration and polyploid induction from leaves of Hypericum species. HortScience 44:1957–1961

Mirabbasi SM, Hosseinpour B (2014) Prevention of shoot tip necrosis, hyperhydricity and callus production associated with in vitro shoot culture of *Ulmus glabra*. J Novel Appl Sci 3:683–689

Mok MC, Martin RC, Dobrev PI et al (2005) Topolins and hydroxylated thidiazuron derivatives are substrates of cytokinins O-glucosyltransferase with position specificity related to receptor recognition. Plant Physiol 137:1057–1066

Monticelli S, Gentile A, Frattarelli A et al (2017) Effects of the natural cytokinin meta-Topolin on in vitro shoot proliferation and acclimatization of *Prunus* spp. Acta Hortic 1155:375–380

Moyo M, Finnie JF, van Staden J (2011) Recalcitrant effects associated with the development of basal callus-like tissue on caulogenesis and rhizogenesis in *Sclerocarya birrea*. Plant Growth Regul 63:187–195

Moyo M, Finnie JF, van Staden J (2012) Topolins in *Pelargonium sidoides* micropropagation: do the new brooms really sweep cleaner? Plant Cell Tissue Organ Cult 110:319–327

Moyo M, Amoo SO, Aremu AO et al (2014) Plant regeneration and biochemical accumulation of hydroxybenzoic and hydroxycinnamic acid derivatives in *Hypoxis hemerocallidea* organ and callus cultures. Plant Sci 227:157–164

Moyo M, Aremu AO, Plačková L et al (2018) Deciphering the growth pattern and phytohormonal content in Saskatoon berry (*Amelanchier alnifolia*) in response to in vitro cytokinin application. New Biotechnol 42:85–94

Mukherjee E, Sarkar S, Bhattacharyya S, Gantait S (2020) Ameliorated reserpine production via in vitro direct and indirect regeneration system in *Rauvolfia serpentina* (L.) Benth. ex Kurz. 3 Biotech 10:294

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15:473–495

Murashige T, Tuker DPH (1969) Growth factor requirement of citrus tissue culture. In: International citrus symposium, Riverside, vol 1. Riverside: University of California, pp 1155–1169

Nacheva L, Gercheva P, Ivanova V et al (2015) *Meta*-topolin improves lateral bud proliferation in micropropagation of *Ginkgo biloba* L. Acta Hortic 1155:355–360

Naidoo D, Aremu AO, van Staden J et al (2017) In vitro plant regeneration and alleviation of physiological disorders in *Scadoxus puniceus*. S Afr J Bot 109:316–322

Nas MN, Read PE (2004) A hypothesis for the development of a defined tissue culture medium of higher plants and micropropagation of hazelnuts. Sci Hortic 101:189–200

Nas MN, Bolek Y, Sevgin N (2010) The effects of explant and cytokinin type on regeneration of *Prunus microcarpa*. Sci Hortic 126:88–94

Ncube B, Finnie JF, van Staden J (2015) In vitro regeneration of *Cyrtanthus* species: ornamental plants with medicinal benefits. In Vitro Cell Dev Biol Plant 51:42–51

Ngoenngam L, Pongtongkam P, Arananant J et al (2018) In vitro effect of plant growth regulators (PGRs) for callus induction and plant regeneration from suspension of Hamata (*Stylosanthes hamata* cv. Verano). Int J Agric Technol 14:1515–1524

Ngoenngam L, Pongtongkam P, Arananant J et al (2019) In vitro effect of gamma irradiation and plant growth regulators (PGRs) for induction and development of *Stylosanthes hamata* cv. Verano. Int J Agric Technol 15:63–74

Niedz RP, Evens TJ (2010) The effects of benzyladenine and meta-topolin on in vitro shoot regeneration of a *Citrus citrandarin* rootstock. Res J Agric Biol Sci 6:45–53

Niedz RP, Evens TJ (2011a) Mixture screening and mixture-amount designs to determine plant growth regulator effects on shoot regeneration from grapefruit (*Citrus paradisi* macf.) epicotyls. In Vitro Cell Dev Biol Plant 47:682–694

Niedz RP, Evens TJ (2011b) The effects of benzyladenine and meta-topolin on in vitro shoot regeneration of sweet orange. ARPN J Agric Biol Sci 6:64–73

Nowakowska K, Pacholczak A, Tepper W (2019) The effect of selected growth regulators and culture media on regeneration of *Daphne mezereum* L. 'Alba'. Rend Lincei Sci Fis Nat 30:197–205

Ornellas TS, Werner D, Holderbaum DF et al (2017) Effects of vitrofural, BAP and meta-Topolin in the in vitro culture of *Dendrocalamus asper*. Acta Hortic 1155:285–292

Parris JK, Touchell DH, Ranney TG et al (2012) Basal salt composition, cytokinins, and phenolic binding agents influence in vitro growth and ex vitro establishment of Magnolia 'Ann'. HortScience 47:1625–1629

Podwyszyńska M, Cieślińska M (2018) Rooting shoots of apple varieties and their tetraploids obtained by the in vitro technique. Acta Sci Pol Hortorum Cultus 17:49–62

Quoirin M, Lepoivre P (1977) Improved medium for in vitro culture of *Prunus* sp. Acta Hortic 78:437–442

Rakrawee R, Kittibanpacha K, Chareonsap PP et al (2018) Efficiency of antioxidant and absorbent on browning and the optimal factors of plant regeneration from young seed of *Gluta usitata* (217 Mae Ka) by tissue culture. Int J Agric Technol 14:911–922

Rout GR, Mohapatra A, Jain S (2006) Tissue culture of ornamental pot plant: a critical review on present scenario and future prospects. Biotechnol Adv 24:531–560

Sacco E, Mascarello C, Pamato M et al (2017) In vitro propagation of *Cotula bipinnata* Thunb and *Chironia linoides* L., South African ornamental species suitable for cultivation in Mediterranean area. Acta Hortic 1155:625–630

Shahzad A, Akhtar R, Bukhari NA et al (2017) High incidence regeneration system in *Ceratonia siliqua* L. articulated with SEM and biochemical analysis during developmental stages. Trees 31:1149–1163

Stevens ME, Pijut PM (2018) Rapid in vitro shoot multiplication of the recalcitrant species *Juglans nigra* L. In Vitro Cell Dev Biol Plant 54:309–317

Strnad M, Hanuš J, Vanek T et al (1997) Meta-topolin, a highly active aromatic cytokinin from poplar leaves (*Populus* × *canadensis* Moench., cv. Robusta). Phytochemistry 45:213–218

Sugiyama M (1999) Organogenesis in vitro. Curr Opin Plant Biol 2:61–64

Swart PA, Kulkarni MG, Bairu MW et al (2012) Micropropagation of *Romulea sabulosa* Schltr. ex Beg. - a potential ornamental plant. Sci Hortic 135:151–156

Tongsad P, Laipasu P, Chareonsap PP et al (2018) The effect of plant growth regulator and in vitro conservation of teak (*Tectona grandis* L.) by tissue culture. Int J Agric Technol 14:2171–2180

Tubić L, Savić J, Mitić N et al (2016) Cytokinins differentially affect regeneration, plant growth and antioxidative enzymes activity in chive (*Allium schoenoprasum* L.). Plant Cell Tissue Organ Cult 124:1–14

Valero-Aracama C, Kane ME, Wilson SB et al (2010) Substitution of benzyladenine with meta-topolin during shoot multiplication increases acclimatization of difficult-and easy-to-acclimatize sea oats (*Uniola paniculata* L.) genotypes. Plant Growth Regul 60:43

Van Huylenbroeck J, Debergh PC (1996) Impact of sugar concentration in vitro on photosynthesis and carbon metabolism during ex vitro acclimatization of *Spathiphyllum* plantlets. Physiol Plant 96:298–304

Vijayakumar J, Ponmanickam P, Samuel P et al (2017) Influence of *meta*-Topolin on efficient plant regeneration via micropropagation and organogenesis of safflower (*Carthamus tinctorius* L.) cv. NARI-H-15. Am J Plant Sci 8:688

Vinayak V, Dhawan AK, Gupta VK (2009) Efficacy of non-purine and purine cytokinins on shoot regeneration in vitro in sugarcane. Indian J Biotechnol 8:227–231

Waman AA, Bohra P, Sathyanarayana BN (2016) Effect of various cytokinins and auxins on in vitro regeneration of plantlets from isolated bud clumps of Silk Banana var. Nanjanagud Rasabale (*Musa* AAB). Ind J Plant Physiol 21:64–69

Wen S, Cheng F, Zhong Y (2016) Micropropagation of tree peony (*Paeonia* × *lemoinei* 'high noon') and the assessment of genetic stability by SSR analysis. Propag Ornam Plants 16:19–27

Wojtania A (2010) Effect of meta-topolin in vitro propagation of *Pelargonium* × *hortorum* and *Pelargonium* × *hederaefolium* cultivars. Acta Soc Bot Pol 79:101–106

Wojtania A, Skrzypek E (2014) Effects of cytokinins on antioxidant enzymes in in vitro grown shoots of *Pelargonium hortorum* LH Bayley. Acta Agrobot 4:33–42

Wojtania A, Węgrzynowicz-Lesiak E (2012) Ethylene and cytokinin interaction in the morphogenesis of *Pelargonium* × *hortorum* LH Bailey in vitro. Acta Physiol Plant 34:2407–2412

Wojtania A, Węgrzynowicz-Lesiak E, Dziurka M et al (2015) Sucrose and cytokinin interactions in relation to ethylene and abscisic acid production in the regulation of morphogenesis in *Pelargonium* × *hortorum* LH Bailey in vitro. Acta Biol Cracov Ser Bot 57:62–69

Yahyaoui E, Casamento D, D'Onghia et al (2017) Fig virus-free production and survival rate improvement using meristem tip culture technique associated with the encapsulation technology. Acta Hortic 1224:81–90

Yancheva SD, Golubowicz S, Fisher E et al (2003) Auxin type and timing of application determine the activation of the developmental program during in vitro organogenesis in apple. Plant Sci 165:299–309