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MINI-REVIEW

Biotechnology of camptothecin production in Nothapodytes nimmoniana, Ophiorrhiza sp. and Camptotheca acuminata

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Abstract

Cancer is probably the deadliest human disease in recent years. In the past few years, rapid clinical progress has been made in the field of anticancer drug development. Plant secondary metabolites have been noted as extremely efficacious as promising natural source for anticancer therapy for many years. Camptothecin (CPT) is one of the popularly used anti-tumor drugs possessing clinically proven properties against a plethora of human malignancies that include ovarian and colorectal cancers. For the first time, CPT was obtained from the extracts of a Chinese medicinal tree, *Camptotheca acuminata* Decne. from the family *Cornaceae*. Subsequently, CPT was also isolated from the bark of *Nothapodytes foetida* (Wight) Sleumer (*Icacinaceae*). However, the availability of enough natural sources for obtaining CPT is a major constraint. Due to overexploitation and harvesting, loss of habitat, excessive trading, and unfavorable environmental factors, the natural source of CPT has become extinct or extremely limited and hence they are red listed under endangered species. Conventional propagation has also failed to meet the ever-expanding demand for CPT production. With this, biotechnological toolkits have constantly been used as a boon to produce sustainable source, utilization, and ex situ conservation of medicinal plants. The approaches serve as a supplement to traditional agriculture in the mass production of plant metabolites with potent bioactivities. Non-availability of enough anticancer medicine and the requirement to satisfy current demands need a sustainable source of CPT. With this background, we present a comprehensive review on CPT discovery, its occurrence in the plant kingdom, biosynthesis, phytochemistry, pharmacological properties, clinical studies, patterns of CPT accumulation, and biotechnological aspects of CPT production in three plants, viz., *N. nimmoniana*, *Ophiorrhiza s*pecies, and *C. acuminata.*

Key points

- *Biotechnological approaches on production of camptothecin from Nothapodytes nimmoniana, Ophiorrhiza species, and Camptotheca acuminata*
- *In vitro propagation of camptothecin-producing plants*
- *Genetic diversity and transgenic research on camptothecin-producing plants*

Keywords Cancer · Medicinal plant · Micropropagation · Cell culture · Biosynthesis · Biotechnology

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Introduction

Cancer is one of the leading causes of death in recent years throughout the world. In the past few decades, rapid clinical advancements have been witnessed in the field of anticancer drug development. However, cancer still remains as the second most common cause of death around the world after cardiovascular diseases (Ferlay et al. 2018). There has been an enormous demand for plant materials as a source for drug development. Plants are a major source of natural products, and there is always a search for novel compounds with potential therapeutic significance. Plant secondary metabolites have been proven to be an effective natural source for anticancer cure for several years. About three-quarters of anticancer drug molecules are natural based or their derivatives (Demain and Vaishnav 2011). Some of the major anticancer drug–yielding plants include *Catharanthus roseus*, *Camptotheca acuminata*, *Nothapodytes foetida, Taxus brevifolia, Taxus chinensis, Podophyllum peltatum, Betula alba, Erythroxylum pervillei, Centaurea schischkinii, Curcuma longa*, and many others (Nirmala et al. 2011; Das et al., 2021). Some of the clinically approved anticancer compounds derived from plant sources includes vinblastine (Velban), vincristine (Oncovin), etoposide, teniposide, Taxol (paclitaxel), Navelbine (vinorelbine), Taxotere (docetaxel), camptothecin (CPT) (Camptosar, Campto), topotecan (Hycamtin), and irinotecan (Demain and Vaishnav 2011). Among these, CPT is one of the widely used anti-tumor drugs having clinically proven activity against many human malignancies including ovarian cancer and colorectal cancer (Thomas et al. 2004; Prakash et al. 2016). For the first time, CPT was isolated from the extracts of a Chinese medicinal tree, *Camptotheca acuminata* (Figure 1a), belonging to the family *Cornaceae* (Wall et al. 1966). Later, CPT was also observed in the bark of *Nothapodytes foetida* (Wight) Sleumer (*Icacinaceae*), a native tree of Indo-Malaysia and Indo-China. Many other plant species, such as *Merriliodendron megacarpum, Eravatamia heyneana*, and *Ophiorrhiza* species such as *O. mungos, O. pumila, O. rugosa, O. liukiuensis, O kuroiwai, O. alata*, and *O. prostrata,* also possess CPT compound (Kaushik et al. 2015). However, *C. acuminata* and *N. foetida* are the major sources of CPT as they produce higher contents compared to any other plant species.

CPT and its chemical analogs are known for their potential anticancer activities. Several key molecules of CPT derivatives are already in clinical trials targeted against various forms of malignancies. As chemotherapeutic agents, topotecan and irinotecan (CPT analogs) are widely employed for cancer therapy in the modern medical practices (Ashfaq et al. 2018, Bailly 2019). In addition, studies have witnessed that CPT inhibits viral activity by various ways against adenoviruses, papovaviruses, herpes viruses, parvoviruses, and, Enterovirus 71 (Pantaziz et al. 1999; Prakash et al 2016; Wu and Chu 2017). Therefore, the demand for CPT compound and its analogs has certainly increased in the global market. In 2008 alone,

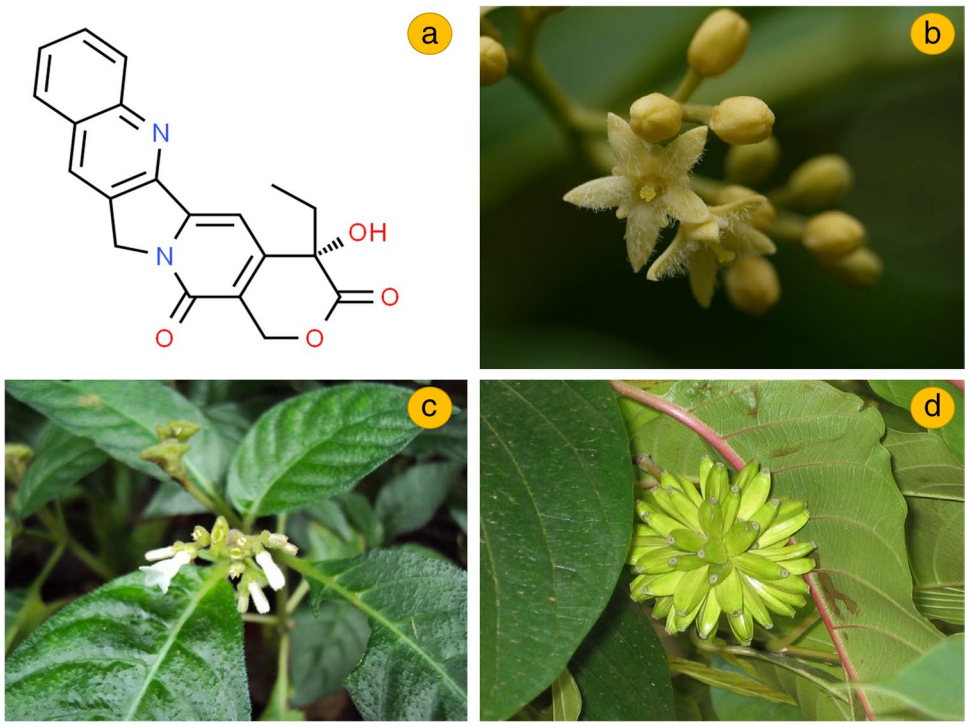

Figure 1 **a** Structure of camptothecin (obtained from www.Chemspider.com). **b** *Nothapodytes nimmoniana* (obtained from Wikimedia Commons under the Creative Commons Attribution-Share Alike 2.0 Generic license). **c** *Ophiorrhiza mungos* (obtained from Wikimedia Commons under the Creative Commons Attribution-Share Alike 3.0 Unported license). **d** *Camptotheca acuminata* (obtained from Wikimedia Commons under the Creative Commons Attribution-Share Alike 2.0 Generic license)

CPT trade was about the worth of US$ 2.2 billion (Sankar 2010). According to Watase et al. (2004), nearly more than tons of raw materials are required every year in order to meet this global demand for CPT. Globally, the annual production of CPT from plant sources is only about 600 kg, while the demand is estimated to be roughly 3000 kg per annum in the world market. This demand is expected to increase further as the cases of cancer incidence are in an alarming stage at the current situation. However, the availability of enough natural sources for obtaining CPT is a major concern in the present scenario. Due to unscientific exploration, loss of habitat, excessive trading, and unfavorable environmental conditions, the natural sources of CPT have become extinct and hence they are red listed under endangered species. Furthermore, *C. acuminata* and *N. foetida* are tree species and limited to subtropical climatic conditions and require more than 10 years to reach their maturity (Li et al. 2005; Sankar 2010). *Ophiorrhiza* species are herbs and yield only low quantity of CPT in their organs (Kaushik et al, 2015). Though conventional propagation has proved to be an alternative, however it failed to meet the demand for CPT production (Sankar 2010). Some of the factors such as root rot and fungal diseases affect the growth of these plant species (Li et al. 2005). In this aid, biotechnological approach has constantly been a boon to produce sustainable source, utility, and conservation of medicinally valuable plants (Nandy et al., 2020; Pandey et al. 2021). The potential of these approaches is used as a supplement to traditional agriculture in the mass production of bioactive plant metabolites. Non-availability of enough anticancer drugs and the demand to satisfy current needs require a sustainable source of CPT.

Earlier, *N. nimmoniana*, *Ophiorrhiza s*p., and *C. acuminata* have been reviewed for their ethnobotany, phytochemistry, and pharmacology (Taher et al., 2020, Khan et al., 2013; Li et al., 2018). Some older citations also depicted the biotechnological aspects of CPT production from *N. nimmoniana* (Isah and Mujib, 2015). However, a comprehensive account on the latest development in the biotechnology of CPT production has not been done yet. With this background and also considering the immense biomedical and pharmaceutical potential of CPT as a promising anticancer drug, we report a comprehensive review on CPT discovery, its distribution in the plant kingdom, biosynthesis, phytochemistry, pharmacological activities, clinical studies, patterns of CPT accumulation, and biotechnological aspects of CPT production from three major CPT yielding plants.

Plant sources of CPT and current problems

CPT was originally discovered from the Chinese tree, *C. acuminata* (*Cornaceae*), during the time of screening several plant extracts for anticancer activity in the 1960s (Wall et al. 1966). Since then, CPT was the only source for chemotherapy against cancers. Due to high demand from pharmaceutical industries, *C. acuminata* and *N. nimmoniana* wild populations were harvested unscientifically which has led to their endangered status. In this regard, the systematic studies aimed at drug discoveries resulted in the identification of several plant anticancer compounds. Interestingly, they also found the occurrence of CPT in other plant species including *Ervatamia heyneana* (*Apocynaceae*), *Nothapodytes nimmoniana* Syn. *N. foetida* (*Icacinaceae*), *Chonemorpha grandiflora, Tabernaemontana heyneana, Ophiorrhiza rugosa, O kuroiwai, O. alata,* and *O. pumila* (*Rubiaceae*) (Tafur et al. 1976; Gunasekera et al. 1979; Roja and Heble 1994; Kulkarni et al. 2010; Kaushik et al. 2015; Martino et al. 2017). However, the yield of CPT in plant sources is very less. For instance, the dried shoots of *C. acuminata* contains only 0.042% of CPT, while its dried roots contain about 0.051% of CPT. There is a high demand for CPT in the world market. As reported earlier in in 2008, the demand for CPT and its derived molecules was about US$ 2.2 billion and is increasing continuously (Prakash et al. 2016). Considering this demand, nearly tons of raw materials are required every year. CPT is a very unique molecule used in developing novel chemical derivatives against cancers. Thus, these unfulfilled features have influenced researchers to search for alternative sources for a feasible production of CPT. Moreover, the dependency on these natural plant resources might lead to habitat loss and severely affect the biodiversity. Therefore, alternative approaches using plant cell and tissue cultures will be more useful. In this regard, biotechnology played a significant role in co-effective approach to produce CPT without deteriorating the environment. In addition, biotechnological tools such as genetic transformation, biochemical engineering and pathway management influenced to enhance the CPT content in plant tissues. In the following section, different types of in vitro cultures established successfully for producing CPT are discussed.

In vitro culture approaches for CPT production

Plant cell can be grown in vitro to produce a wide range of secondary metabolites. Plant tissue culture approaches are generally established under controlled sterile conditions using different explants, namely, stem, leaf, root, and meristem. Plant tissue culture is employed with the aim to propagate in large scale as well as for obtaining useful secondary metabolites. In vitro production of plant metabolites have several advantages such as controlled environment, high yield, cost-effective, and fast recovery of the products. As most of the industrially valued compound-producing plant species have become endangered, it is obvious to adopt in vitro approaches as alternatives to natural habitat. This

will be useful in restoring the vulnerable plant species and conserve the plant diversity in nature. Many previous studies have reported the successful application of cell, tissue, and organ cultures to produce CPT from different plant species. The following section is mainly focused on the production and yield of CPT through different in vitro culture approaches (Tables 1, 2, 3).

***Nothapodytes nimmoniana* (J. Graham) Mabb. Syn. *Nothapodytes foetida* (Wight) Sleumer** Various biotechnological methods of producing CPT from *N. nimmoniana* are given in Table 1. Using immature embryos as explants, tissue cultures of *N. foetida* were established on Murashige and Skoog (MS) media containing cytokinins and auxins (Roja and Heble 1994). Callus was initially developed from the embryo on media supplemented with 6-benzylaminopurine (BA) and 2,4-dichlorophenoxyacetic acid (2,4-D). Later supplementation of BA and naphthaleneacetic acid (NAA) to the medium promoted the multiple shoot formation. Plantlets formed with the use of kinetin (Kn), NAA, or indole-3-acetic acid (IAA) in the medium. The regenerated plantlets contained considerably higher quantities of 9-methoxycamptothecin (0.0007% DW) compared to the undifferentiated calli (0.0001% DW). However, only traces of the CPT were observed in the cultures, while in the shoots of the mature tree, about 0.075% (DW) CPT and 0.013% (DW) 9-methoxycamptothecin were recorded. Lately, Prakash et al. (2016) established a clonal propagation protocol for *N. nimmoniana* using different kinds of plant growth regulators. Multiple shoots were induced from the explant's regenerated axillary buds derived from embryos in vitro. MS media supplemented with BA at 0.2 mg/l and IBA at 0.1 mg/l effectively differentiated multiple shoots (10.24) after 56 days of inoculation. The excised micro-shoots readily rooted in ½ MS medium added with activated charcoal (0.05 %), NAA (0.1 mg/l), and IBA (0.1 mg/l). The regenerated plants leaves, stem, and roots were detected to contain CPT content of 0.0013, 0.0260.12, and 0.12 % (w/w), respectively.

Fulzele and Satdive (2003) reported somatic embryogenesis, plant regeneration, and evaluation of CPT from *N. nimmoniana.* MS media supplemented with 6-benzyladenine showed better plant regeneration, and the plantlets were acclimatized with a high success rate (90%). Somatic embryos contained 0.011% (DW) CPT and 0.0028% (DW) 9-methoxycamptothecin, whereas about 0.2% (DW) CPT and 0.097% (DW) 9-methoxycamptothecin was reported in 2-years-old field-grown plant roots obtained from somatic embryos of *N. nimmoniana.* In another study, an efficient in vitro regeneration protocol for *N. nimmoniana* was established and quantified the levels of CPT in the regenerated plants (Dandin and Murthy 2012). They observed the induction of callus from the nodal explants on BAP/KN/2-iP-supplemented medium. Adventitious shoots regenerated from calli masses on MS semisolid media containing 2μM BA. Shoots were readily rooted on MS semisolid media with

Table 1 Production of CPT in cell, tissues, and organs of *N. nimmoniana* cultured in vitro

In vitro culture type	Explant used	Media + growth regulators	CPT (% DW)	References
Regenerated plantlets	Zygotic embryos	BA+2,4-D for callus induction; BA + NAA for multiple shoot induction; Kn + IAA for rooting	Traces	Roja and Heble (1994)
	Zygotic embryos	BA (0.2 mg/l) + IBA (0.1 mg/l) for shoot multiplication; Activated charcoal (0.05 %) + NAA and IBA at 0.1 mg/l	Leaves: 0.0013; Stem; 0.026; Roots: 0.12	Prakash et al. (2016)
Callus	Zygotic embryos	-	0.95	Veeresham and Shuler (2000)
	Leaves	Picloram (1 mg/l) + TDZ (1 mg/l) + GA_3 (4 mg/l)	2.893	Sundravelan et al. (2004)
	Leaves	Picloram (1 mg/l) + TDZ (1 mg/l) + GA_3 (4 mg/l)	0.0057	Namdeo et al. (2010)
	Leaves	2,4-D (9.05 μM) + BA (2.25 μM)	0.014	Singh et al. (2012)
	Leaves	NAA (10.74μM) + Kin (2.32 μM)	0.015	Karwasara et al. (2012)
	Zygotic embryo	BAP (2.22μM) + NAA (10.74 μM)	0.4903	Karadi et al. (2008)
	-	Picloram (2 mg/l) + sucrose 3 % (w/v)	0.0095	Ciddi and Shuler (2000)
	-	NAA (10.74μM) + Kin (2.32 μM) + Gamma irradiation (20 Gy)	0.098	Fulzele et al. (2015)
Suspension cultures	Leaves (callus)	BA (2.22 μM) + NAA (10.74 μM)	0.035	Fulzele et al. (2001)
	Leaves (callus)	BA (2.22 μM) + NAA (10.74 μM)	0.0051	Karwasara and Dixit (2013)
Untransformed hairyroots	Zygotic embryos	BA (2.22 μM) + NAA (10.74 μM)	0.01	Fulzele et al. (2002)
Transformed hairy roots	Stem and leaves	Grew on PGR-free	0.1555	Chang et al. (2014)

Table 2 Production of CPT in cell, tissues, and organs of *Ophiorrhiza* species cultured in vitro

In vitro culture type and *Ophiorrhiza* species	Explant used	Media + growth regulators	CPT (% DW)	References
Regenerated plantlets				
O. mungos	Fruits	Callus induction: MS + BA (2 ppm) + IAA (2 ppm) + GA (1 ppm); Shoot induction: MS + picloram (1 ppm) + TDZ (2 ppm) + BA (1 ppm); Rooting: MS + IBA (4 ppm) + BA (1 ppm) + GA_3 (2 ppm)	0.0768	Namdeo et al. (2012)
O. mungos	Axillary and terminal buds	Shoot induction: MS + BA (0.25 mg/l) + Kn (0.25 mg/l); Shoot elongation: MS + GA_3 (1mg/l); Rooting: ½ MS + 100 mg/l activated charcoal	0.0438	Kaushik et al. (2015)
O. rugosa	Axillary meristems	Shoot induction: MS + BA (4.0 mg/l) and NAA (0.05 mg/l); Rooting: ½ MS	Leaves: 0.090% DW; Stems:0.011% DW; Roots: 0.002% DW; Floral parts: 0.015%	Roja (2008).
O. alata	Leaves	Kn (9.30 µM) and NAA (0.54 µM); Rooting: ½ MS	Leaves: 94 µg/g DW; Roots: 556 µg/g DW	Ya-ut et al. (2011)
O. decumbens	Axillary meristems	MS + BA (4 mg/l) + NAA (0.05 mg/l) + 3% sucrose (w/v)	0.056% DW	Gopalakrishnan and Shankar (2014)
O. kuroiwai	Leaves	MS + 1% sucrose + NAA (0.5 µM) + Kn (5 µM)	Shoot: ~18 µg/tube (DW) Root: ~3 µg/ tube (DW)	Asano et al. (2004)
O. liukiuensis	Seeds	½ MS + 1% sucrose	Shoot: ~2 µg/ tube (DW) Root: ~0.3 µg/ tube (DW)	Asano et al. (2004)
O. pumula	Seeds	½ MS + 1% sucrose	Shoot: ~10.5 (DW) µg/ tube (DW) Root: ~4.4 (DW) µg/ tube (DW)	Asano et al. (2004)
O. rugosa	Hairy roots	MS + light	0.012% DW	Kamble et al. (2011)
Hairy roots				
O. kuroiwai	Stems (seedling derived)	B5 media + 2 % sucrose + cefotaxime (200mg/l)	219.3 µg/g DW	Asano et al. (2004)
O. liukiuensis	Stems (seedling derived)	B5 media + 2 % sucrose + cefotaxime (200mg/l)	83 µg/g DW	Asano et al. (2004)
O. pumula	Stems (seedling derived)	B5 media + 2 % sucrose + cefotaxime (200mg/l)	788.5 µg/g DW	Saito et al. (2001)
O. rugosa	Shoots	MS	0.009% DW CPT	Kamble et al. (2011)
O. alata	Leaves	½ MS media	785 µg/g DW	Ya-ut et al. (2011)
O. pumila	-	B5 media + 2 % sucrose (used 3l capacity bioreactor)	22mg/g DW	Sudo et al. (2002)
Adventitious roots				
O. mungos	Leaf segments (seedling derived)	MS + (0.25 mg/l) and NAA (0.25 mg/l)	-	Deepthi and Satheeshkumar (2017a)
O. mungos	Leaf segments (seedling derived)	½ MS	0.25 mg/g DW	Deepthi and Satheeshkumar (2017a)

Table 2 (continued)

In vitro culture type and *Ophiorrhiza* species	Explant used	Media + growth regulators	CPT (% DW)	References
O. mungos	Leaf segments (seedling derived)	½ MS + 5 % sucrose + IBA (0.5 mg/l) + NAA (0.25 mg/l) + GA_3 (0.1 mg/l) + 2 g/l (FW) inoculum size	0.25-0.65 mg/g DW	Deepthi and Satheeshkumar (2017a)
Callus and cell suspension cultures				
O. mungos	Leaves derived callus	½ MS + 3 % sucrose + NAA (3 mg/l) + 2,4-D (1 mg/l) + Kn (0.5 mg/l)	0.006 mg/g DW	Deepthi and Satheeshkumar (2016)
O. mungos	Leaves derived callus	½ MS + 3 % sucrose + NAA (3 mg/l) + 2,4-D (1 mg/l) + Kn (0.5 mg/l) + Yeast extract (50 mg/l)	0.8 mg/g DW	Deepthi and Satheeshkumar (2016)
O. mungos	Leaves derived callus	½ MS + 3 % sucrose + NAA (3 mg/l) + 2,4-D (1 mg/l) + Kn (0.5 mg/l) + Yeast extract (50 mg/l)	0.095 mg/g DW	Deepthi and Satheeshkumar (2017b)
O. mungos	Leaves derived callus	½ MS + 3 % sucrose + NAA (3 mg/l) + 2,4-D (1 mg/l) + Kn (0.5 mg/l) + Yeast extract (50 mg/l)	0.095 mg/g DW	Deepthi and Satheeshkumar (2017b)
O. mungos (OMC3 cell lines)	Leaves derived callus	½ MS + 3 % sucrose + NAA (3 mg/l) + 2,4-D (1 mg/l) + Kn (0.5 mg/l) + Yeast extract (50 mg/l) + jasmonic acid (50 µM)	1.12 mg/g DW	Deepthi and Satheeshkumar (2017b)

2.4 µM indole-3-butyric acid (IBA) and 5.7 µM IAA. The plantlets were successfully acclimatized in a growth chamber. Furthermore, HPLC analysis showed the presence of 0.043% and 0.013% CPT content in the leaves and stems of the mother plant, respectively, while *in vitro* plantlets' leaves and stem recorded 0.037% and 0.082% CPT, respectively, cultured in the semisolid medium.

Later, Ciddi and Shuler (2000) established callus cultures from the embryos of *N. foetida* on MS media containing 2 mg/l picloram and 3% (w/v) sucrose. Using various analytical techniques such as TLC (thin-layer chromatography), HPLC (high-performance liquid chromatography), and electrospray ionization mass spectrometry (ESI-MS), the callus cultures were recorded to contain about 9.5 µg/g (DW) of CPT content. *N. foetida* stems were cultured on MS medium fortified with different plant growth regulators initiated callus formation (Fulzele et al. 2001). Later, suspension cultures were established using NAA and 2,4-D in the MS liquid medium. Culture media containing BA (2.22 µM) and NAA (10.74 µM) produced higher cell biomass (31.3 g/l DW) after 20 days in shake flasks. HPLC and TLC analysis showed the occurrence of the maximum content of CPT (0.035 mg/ml) and 9-methoxycamptothecin (0.026 mg/ml) in the media added with NAA, while supplementation of 2,4-D in the media significantly reduced the accumulation of CPT. In a study, high nitrate in the suspension culture medium supported the cell biomass, while high ammonium augmented the production of CPT (Karwasara and Dixit 2013). The optimized suspension cultures derived from leaf calli recorded CPT content of 0.0051 ± 0.0003%. Likewise, Veeresham and Shuler (2000) reported the occurrence of about 0.95% (DW) of CPT in callus cultures of *N. foetida* established from the excised embryos. Later, Sundravelan et al. (2004) established *N. foetida* callus cultures using leaves as explants on MS media supplemented with BA (1 mg/l), picloram (2 mg/l), and gibberellic acid (GA_3) (1 mg/l). The extraction and analysis of alkaloids from the calli biomass showed the occurrence of CPT (2.893 ± 2.38%) and 9-methoxycamptothecine (0.4 ± 0.4%). In another study, Karadi et al. (2008) reported the occurrence of 0.4903% (DW) CPT from the calli established from zygotic embryos cultured on MS media supplemented with BAP (2.22µM) and NAA (10.74 µM). Namdeo et al. (2010) reported a higher CPT content (5.74%) in the methanolic extract of *N. foetida* callus as compared to the mature tree fruits (3.56%), stems (1.19%), leaves (1.56%), and roots (1.11%) solvent extracts.

In an interesting study by Fulzele et al. (2015), low doses of gamma irradiation (5 to 30 Gy) were exposed to callus cultures of *N. foetida* in order to enhance CPT production. They witnessed higher production levels of CPT and 9-methoxy camptothecin. A dose of 20 Gy influenced cell growth up to 2-fold and produced 0.098% CPT and 0.0043%

Table 3 Production of CPT in cell, tissues, and organs of *C. acuminata* cultured in vitro

In vitro culture type	Explant used	Media + growth regulators	CPT (% DW)	References
In vitro seedlings/ Somatic embryos/ Regenerated Plantlets	Shoot tips (seedling derived)	Nitsch and Nitsch + IBA (0.5 mg/l) + kinetin (0.1 mg/l)	0.119 % DW	Wiedenfeld et al. (1997)
	Shoot tips and nodal stems (seedling derived)	Shoot induction: WPM + NAA (0.01 mg/l) + BA (0.5 mg/l); Rooting: WPM + IBA or NAA (1 mg/l)	-	Chang et al. (2005)
	Shoot tips (seedling derived)	½ MS + BA (2mg/l) + IAA (0.1 mg/l)	1.96 mg/g DW	Sankar-Thomas and Lieberei (2011)
	Shoot tips (seedling derived)	Solid MS + BA (0.5 mg/l) + 3 % sucrose in TIS	2.5 mg/g DW	Sankar-Thomas and Lieberei (2011)
	Shoot tips (seedling derived)	Liquid MS + BA (0.5 mg/l) + 3 % sucrose in TIS	2.2 mg/g DW	Sankar-Thomas and Lieberei (2011)
Callus and cells suspention cultures	-	MS + 2, 4-D (0.1mg/l) + kn (3mg/l) + GA_3 (0.05mg/l)	$2.54*10^{-4}$ % DW	Sakato et al. (1974a).
Cell lines, CAS1,2,3&4	Stem	MS + B5 vitamins + NAA (1mg/l) or 2,4-D (0.4mg/l) or Kn (0.5mg/l)	$0.85\text{-}3.6*10^{-3}$ % DW	van Hengel et al. (1992)
	-	MS + NAA (4mg/l)	$4*10^{-3}$ %	van Hengel et al. (1994)
	Shoot tips/hypocotyl (seedling derived)	MS + NAA (1 mg/l) + kn (1 mg/l) + 6 % sucrose	0.236 to 0.149 % DW	Wiedenfeld et al. (1997)
	Leaves	MS + NAA (5 mg/) + BA (0.5 mg/l) + 2,4-D (0.3 mg/l) + 3 % sucrose	0.007mg/g DW	Pi et al. (2010).
	Young stems	Schenk and Hildebrandt + kn (1.0mg/l) + 2,4-D (1.0mg/l)	$30*10^{-5}$ mg/l DW	Song and Byun (1998)
	Hypocotyls (seedling derived)	MS + NAA (0.5 mg/) + BA (0.5 mg/l) + 2,4-D (0.1 mg/l) + 3 % sucrose + NH_4^+/NO_3^- (70mM)	2.5 mg/l DW	Pan et al. (2004)
	Hypocotyls (seedling derived)	MS + NAA (0.5 mg/) + BA (0.5 mg/l) + 2,4-D (0.1 mg/l) + 3 % sucrose + NH_4^+/NO_3^- (70mM)	12.8 mg/l DW	Pan et al. (2004)
	Hypocotyls/ Leaves (seedling derived)	WPM + NAA or 2,4-D (1-3 mg/l)	Traces to 0.03 % DW	Chang et al. (2006)
	Shoots (seedlings derived)	½ MS + BA (2mg/l) + IAA (0.1 mg/l)	0.87 mg/g DW	Sankar-Thomas and Lieberei (2011)
Hairy root cultures	Leaves	B5 + 3 % sucrose	1 mg/g DW	Lorence et al. (2004)
	Leaves	B5	0.14 mg/g DW	Liu and Cui (2007)
Cambial meristematic cells	Leaves and stem	MS + 2,4-D (0.1 mg/l) + kn (0.1 mg/l) + NAA (0.1 mg/l)	2.55 µg/g DW	Zhang et al. (2017)
Dedifferentiated cells	Leaves and stem	MS + 2,4-D (0.1 mg/l) + kn (0.1 mg/l) + NAA (0.1 mg/l)	1.65 µg/g DW	Zhang et al. (2017)

9-methoxy camptothecin. This was nearly 20-fold higher than that observed in callus cultures without irradiation.

Fulzele et al. (2002) reported the production of CPT from the untransformed root cultures of *N. nimmoniana* established from immature zygotic embryos. MS medium containing BA (2.22 µM) and NAA (10.74 µM) induced multiple lengthy roots. However, the highest content of CPT (0.01% DW) and 9-methoxycamptothecin (0.0016% DW) were observed in untransformed roots grown on MS medium containing BA (8.87 µM) and NAA (71.36 µM), while NAA (71.36 µM)- and Kn (9.29 µM)-supplemented media produced callus combined with roots where only 0.00017% (DW) CPT and 0.000058% (DW) 9-methoxy-camptothecin were detected.

Hairy root cultures were established by infecting leaf and stem sections with *Agrobacterium rhizogenes* strains (AR1600, AR281, and ATCC15834) (Chang et al. 2014). The strain ATCC15834 showed maximum transformation capability. After 40 days of culturing, hairy root growth index was observed to be between 2.6 and 3 and accumulated CPT contents in the range of 0.0537 to 0.1555% DW.

Recent studies showed use of different elicitors to increase the CPT yield in in vitro cultures. Isah et al. (2021) had found 14.7-fold enhancement in CPT production by using 25 mM $CaCl_2$ as elicitor in solid medium cultivation of callus, while an 11.48-fold increase in CPT production was found by 10% chitin in suspension culture system (Keshavan et al. 2022).

***Ophiorrhiza* species** In this section, CPT production through the exploration of the biotechnological prospectives from several *Ophiorrhiza* species are discussed and briefly highlighted in Table 2. In vitro plantlets of *O. rugosa* were effectively regenerated using axillary meristems cultured on MS media supplemented with BA (4.0 mg/l) and NAA (0.05 mg/l). Half-strength MS media devoid of plant growth regulators readily induced rooting of in vitro shoots. All micropropagated plants showed similar morphological features and chemical contents compared to that of the normal plants. HPLC analysis of the regenerants established in the field recorded 0.090% DW in the leaves, 0.011% DW in the stems, 0.002% DW CPT in the roots, and 0.015% in the floral parts (Roja 2008). Namdeo et al. (2012) developed a clonal propagation method for *O. mungos* using fruits as explants. They observed higher callus fresh weight and dry weight when explants were cultured on MS media supplemented with BA (2 ppm) + IAA (2 ppm) + GA (1 ppm). The addition of picloram, TDZ, and GA_3 (1:2:1) to MS media induced maximum shoots (25) with an average height of 6.5 cm after 4 weeks of inoculation. Interestingly, the whole micropropagated plantlets yielded maximum CPT content (0.0768 % w/w). While 0.0026 % (w/w) was observed in the adventitious buds, the natural *O. mungos* plants produced 0.0030% (w/w) CPT. This clearly evidenced that in vitro–propagated *O. mungos* plants produce relatively a higher quantity of CPT compared to field-grown natural plants. Kaushik et al. (2015) regenerated *O. mungos* by using in vitro–derived seedlings' terminal and axillary buds as explants. MS media fortified with 0.25 mg/l BA and 0.25 mg/l Kn exhibited superior regeneration rate (84%), higher multiple shoots (63.1), and shoot length (2.8 cm) after 4 weeks. Adding 1.0 mg/l GA_3 further elongated the shoots by 2.3-fold. One-half MS medium supplemented with 100 mg/l activated charcoal was found to be the best medium for inducing in vitro roots. The acclimatized plantlets showed about 95% survivability. HPLC analysis showed that micropropagated plants had 0.0438% CPT content which was comparable to the quantity observed in the mother plants (0.043%). In another study, axillary meristems of *O. decumbens* were used to induce multiple shoot cultures on MS media fortified with BA (4 mg/l), NAA (0.05 mg/l) and 3% sucrose (w/v). Multiple shoot cultures produced high quantities of CPT (0.056% DW) as compared to the mother plant that was determined with only 0.002% (DW) of CPT content (Gopalakrishnan and Shankar 2014).

Deepthi and Satheeshkumar (2016) have reported the production of 0.06 mg/g DW CPT content from the cell suspension cultures of *O. mungos* established from leaf segments obtained from the field-grown plants. The use of ½ MS + 3% sucrose + NAA (3 mg/l) + 2,4-D (1 mg/l) + Kn (0.5 mg/l) was found to be optimum for inducing cell suspension cultures. However, yeast extract at 50 mg/l added on the 10th day of incubation relatively increased both the cell biomass (16.5 g/flask) and CPT (0.8 mg/g DW). Likewise, adding $AgNO_3$ (2.5 µM) on the 10th day elicited the biomass accumulation (14.25 g/flask) and CPT yield (0.52 mg/g DW). Recently, a high-yielding *O. mungos* cell line, OMC3 was selected from cell suspension cultures based on the method of cell aggregate cloning method to establish a cell suspension cultures (Deepthi and Satheeshkumar 2017a). It was found that OMC3 cell suspension cultures produced considerably a higher cell biomass of 9.25 g/flask FW and yielded 0.095 mg/g DW of CPT content as compared to the primary cell suspension cultures. The inoculum size of 14 g/l FW, 5% sucrose and nitrate/ammonium concentration (20/40 mM) favored the production of CPT, whereas 3% sucrose, nitrate/ammonium concentration (40/20 mM), and phosphate (1.25 mM) enhanced the biomass accumulation. On the other hand, addition of jasmonic acid, salicylic acid, and chitin elicited the production of CPT in the original cell suspension cultures. The highest CPT production was noticed with the addition of jasmonic acid (50 µM). In OMC3 cell suspension cultures, addition of 50 µM jasmonic acid resulted in 1.12 mg/g DW of CPT.

Using the aseptic leaf explants, adventitious roots were induced in *O. mungos* and established an efficient root culture (Deepthi and Satheeshkumar 2017b). MS media supplemented with of IBA (0.25 mg/l) and NAA (0.25 mg/l) recorded the highest root induction frequency (90%) and root numbers (70.4) with an average length of 2.5 cm. However, CPT production was influenced by various parameters including inoculum size, incubation duration, salt strength, and elicitors. The use of ½ MS medium recorded about 56 g/l (FW) of biomass with a growth index (GI) of 5.2 and CPT content of 0.25 mg/g (DW) after 4 weeks of incubation. There were no major variations in CPT contents with respect to changes in the inoculum size (0.1 g/flask FW to 0.5 g/flask FW). However, a decreased trend in the CPT accumulation was noticed as the inoculum size was above

0.5 g/flask FW. The use of IBA (0.5 mg/l), NAA (mg/l), and GA_3 (0.1 mg/l) induced higher biomass of 88.3 g/l after 4 weeks and recorded with 0.42 mg/g DW of CPT content. The supplementation of 5.0% sucrose resulted in 104 g/l FW of biomass and yielded CPT content of 0.5 mg/g DW. Also, the addition of 50:10 mM (NH_4^+/NO_3^-) concentration significantly influenced the root biomass (126 g/l FW) with a higher CPT content (0.65 mg/g DW). Likewise, phosphate concentration (1.25 mM) was observed to be the best to induce higher biomass (116 g/l FW) and CPT content (0.413 mg/g DW). Overall, it was concluded that ½ MS with 5% sucrose, IBA (0.5 mg/l), NAA (0.25 mg/l), and GA_3 (0.1 mg/l) and about 2 g/l FW inoculum size are well suited to produce higher root biomass and CPT yield.

O. pumila aseptic plants (stems) were infected with *A. rhizogenes* strain 15834 and induced hairy root cultures which propagated well in B5 media. The growth was increased by nearly 16-fold within 5 weeks of culture in liquid B5 media and produced up to 0.1% DW CPT (Saito et al. 2001). Also, they stated that hairy roots secreted CPT into the medium in substantial quantities. Further, CPT content in the media was improved upon adding Diaion HP-20 (a polystyrene resin) which can absorb CPT. Later, Asano et al. (2004) established aseptic plantlets of *O. liukiuensis* by inoculating seeds on ½ MS media containing 1% sucrose. The juvenile leaves of *O. kuroiwai* were used to induce multiple shoots on MS media containing 1% sucrose, 0.5 µM NAA, and 5 µM Kn. From these aseptic plants, stems were excised and infected with *Agrobacterium rhizogenes* strain 15834 to induce hairy roots. After 4 weeks, transgenic roots were sub-cultured on B5 media containing 2% sucrose on a continuous shaker (80 rpm). The highest CPT content was found in aseptic plants shoots as compared to roots. *O. kuroiwai* plants produced the highest content of CPT (~ 18 µg/tube DW) in shoots, while in roots it was detected to be ~ 3 µg/ tube (DW). The hairy roots of *O. liukiuensis* had 83 µg/g DW, while *O. kuroiwai* hairy roots produced the highest content (219.3 µg/g DW). Interestingly, addition of methyl jasmonate as elicitor increased the CPT content (1.3-fold) in *O. liukiuensis* hairy roots. Likewise, Kamble et al. (2011) used *A. rhizogenes* strain LBA9402 to induce and establish hairy roots in *O. rugosa*. Remarkably, these transformed roots when cultured under light regenerated shoots. Later, the alkaloid analysis revealed the occurrence of 0.009% DW CPT content in hairy roots, while in the in vitro–derived transformed shoots it was observed to be 0.012% DW.

Likewise, the use of *O. alata* leaf explants when cultured on ½ MS media supplemented with Kn (9.30 µM) and NAA (0.54 µM) induced the highest number of multiple shoots (1.7±0.7). Furthermore, incubating the individual in vitro shoots on ½ MS media devoid of any plant growth regulators induced the proliferated adventitious roots (Ya-ut et al. 2011). Later, they also established hairy root cultures of *O. alata* by transfecting in vitro–grown nodal explants with a wild-type *A. rhizogenes* strain LBA9402. Comparison of the CPT contents in various parts of plants from different sources suggested that soil-grown *O. olata* contained about 83 and 388 µg/g DW in leaves and roots, respectively. However, leaves and roots of in vitro cultures had 94 and 556 µg/g DW of CPT, while the hairy roots of *O. olata* recorded the highest CPT content of 785 µg/g DW.

In an attempt to produce CPT on a large scale, hairy roots of *O. pumila* were cultured in a modified bioreactor of 3 l capacity produced about root biomass of 0.0085% FW with total yield of 22mg/g DW CPT after 8 weeks of culture. They also observed the secretion of nearly 3.6mg/g DW (17%) of the total CPT into the liquid culture media (Sudo et al. 2002).

Yamazaki et al. (2003) cloned and characterized cDNAs that encode for 2 major enzymes; tryptophan decarboxylase (TDC) and strictosidine synthase (STR) in hairy roots of *O. pumila.* The results showed increased expression of TDC and STR cDNAs in stems, roots, and hairy roots which were closely interrelated with the accumulation of TDC and STR proteins as detected by immunoblotting. This confirmed the involvement of these 2 key enzymes in the biosynthesis of CPT. Moreover, the plant stress compound, salicylic acid inhibited the expression of TDC and STR cDNAs, suggesting a coordinated regulation of these genes in the biosynthesis of CPT. Later, Lu et al. (2009) adopted the metabolic engineering strategy to increase the content of CPT in *O. pumila* hairy roots. STR and geraniol 10-hydroxylase (GH) genes isolated from *Catharanthus roseus* were introduced into *O. pumila* hairy roots, either alone or together. The overexpression of the GH gene individually enhanced CPT production compared to STR, while both genes when expressed simultaneously showed the synergistic action to improve the production of CPT. In another study, co-overexpression of STR and GH genes has increased the yield of CPT compared to non-transgenic hairy root cultures of *O. pumila* (Cui et al. 2015). In a study, CPT biosynthetic genes encoding secologanin synthase (SLS) and tryptophan decarboxylase (TDC) enzymes that are involved in the early steps of CPT biosynthesis were suppressed in the hairy roots of *O. pumila* using RNA interference (RNAi). The results revealed the occurrence of metabolic modifications of secondary metabolites in the hairy roots. This suggested the possible involvement of TDC genes in the biosynthesis of CPT (Asano et al. 2013). Likewise, deep transcriptomic and metabolomic data sets facilitated in the identification of several candidate genes and intermediates involved in the biosynthesis of CPT in *O. pumila* (Yamazaki et al. 2013).

Elicitation strategies were also used in case of *Ophiorrhiza* sp. A 7-fold increase in CPT production was found in *O. mungos* var. *angustifolia* by 150 µM methyl jasmonate elicitation for 72 h in shoot cultures (Krishnan et al. 2018).

An increase of 1.8- and 2.6-fold in root and shoot CPT production was also found by 50 µM methyl jasmonate, 12-h elicitation in *O. ridleyana* (Pisitpaibool et al., 2021).

***Camptotheca acuminata* Decne.** Wiedenfeld et al. (1997) established in vitro plantlets of *C. acuminata* through indirect organogenesis. MS media containing 1 mg/l NAA, 1 mg/l kn, and 60 g/l sucrose induced the highest callus induction frequency and growth. In vitro regenerated plant tissues and organs were detected with CPT and 10-hydroxycamptothecin. The content of CPT in callus ranged from 0.236 to 0.149% DW of callus, while the content of 10-hydroxycamptothecin was between traces and 0.08% DW of callus. The regenerated plantlets produced CPT of about 0.086 to 0.119% DW of the whole plant. Sankar-Thomas and Lieberei (2011) used temporary immersion system (TIS) to culture *C. acuminata* in vitro. Initially, embryogenic calli and somatic embryos were obtained on ½ MS media with 2 mg/l BA and 0.1 mg/l IAA by culturing in vitro shoots derived from seedlings. The shoot tips obtained were excised and subcultured in TIS and solid media using full-strength MS salts supplemented with 0.5 mg/l BA and 3% sucrose. A significant difference in the content of CPT was observed in shoots derived from TIS and the solid medium with an average of 2.5 and 2.2 mg/g DW, respectively. The in vitro seedlings had 1.96 mg/g, while somatic embryos at the cotyledonary stage and in vitro regenerants were detected with 0.87 and 1.23 mg/g DW, respectively.

Chang et al. (2005) used shoot tips and nodal stems derived from in vitro seedlings as explants to micropropagate *C. aucminata*. A higher shoot induction rate and multiple shoot formation were recorded from shoot tip explants compared to stem explants. The shoot tip explants inoculated on woody plant media (WPM) supplemented with NAA (0.01 mg/l) and BA (0.5 mg/l) produced higher number of shoots (6.4) per explant within 6 weeks of culture. The use of lower levels of BA (0.1 mg/l) promoted shoot elongation, and these individual shoots successfully rooted on WPM medium fortified with IBA or NAA (1 mg/l). The leaves of micropropagated plants recorded with higher amount of CPT content compared to the in vitro seedlings. In another study, CPT production from *C. acuminata* seedlings were increased when treated with elicitors, namely, abscisic acid, methyl jasmonate, and salicylic acid. CPT content yield ranged between 1.33 and 1.81 mg/g DW, while 10-hydroxycamptothecin content ranged from 2.25 to 2.60 m/g DW (Kai et al. 2014).

For the first time, an attempt was made to induce callus and suspension cultures of *C. acuminata* by Sakato et al. (1974 a,b). The use of MS basal medium containing 2,4-D (0.1mg/l), kn (3mg/l) and GA_3 (0.05mg/l) was found to be optimal for inducing high cell growth in the suspension culture. Further, adding 23.5 mg/l L-tryptophan to the medium stimulated cell growth remarkably in the suspension culture. About 2.5 µg/g DW CPT content was determined from the cells after 15 days of harvest. Later, van Hengel et al. (1992) reported the production of CPT ranging from 0.85 to 4 µg/g DW from the cell suspension cultures of *C. acuminata* cell lines, CAS1-4 cultured in liquid media containing different combinations of MS media supplemented with B5 vitamins, NAA (1mg/l), 2,4-D (0.4mg/l), and Kn (0.5mg/l). Later, van Hengel et al. (1994) reported the production of $4*10^{-3}$ % DW CPT in the cell suspension cultures of *C. acuminata* grown in MS media containing 4 mg/l. Likewise, young stems induced calli on Schenk and Hildebrandt (SH) medium containing 5 mg/l NAA, 0.2 mg/l BA, 2% sucrose, and 0.5% agar, while cell suspension cultures were established better on media supplemented with 0.5 mg/l kn and 1.0mg/l 2,4-D (Song and Byun 1998). About $30*10^{-5}$ mg/l DW CPT was recorded in cells after 10 days of cell culturing. Furthermore, the use of elicitors such as methyl jasmonate, jasmonic acid, yeast extract, cupric sulfate, and ferulic acid increased the CPT content in the cell biomass up to 11-fold. Callus was induced from the leaf explants of *C. acuminata* on MS medium added with NAA (5 mg/), BA (0.5 mg/l), 2,4D (0.3 mg/l), and 3% sucrose. The analytical study revealed the occurrence of nearly 0.007mg/g DW of CPT after 10 days of culture (Pi et al. 2010). Pan et al. (2004) reported that the addition of nitrate (70 mM) in MS medium during the cell suspension cultures produces a higher cell biomass and CPT yield (2.5 mg/l DW). However, 5:1 ratio of NH_4^+/NO_3^- produced the highest CPT yield. They used a 2-stage flask culture system which increased the dry weight of cell biomass (0.36 mg/g) with the yield of 12.8 mg/l CPT. Callus cultures of *C. acuminata* were established using leaf and hypocotyl explants excised from 2-month-old in vitro plants. WPM medium containing NAA or 2,4-D (1-3 mg/l) induced callus. However, a friable calli was observed with the use of 2,4-D in the medium. CPT analysis showed that different callus lines had significantly varied levels (traces to 0.03%) of CPT and 10-hydroxycamptothecin. The CPT and HCPT contents of the superior cell lines showed the presence of 0.002% and 0.03%, respectively. The content of CPT remained stable between 0.01 and 0.014% even after culturing for a long period up to 6 months (Chang et al. 2006).

Transgenic hairy roots were established from *C. acuminata* tissues transfected with *A. rhizogenes* strains R-1000 and ATCC 15834. They also reported the occurrence of 1 and 0.15 mg/g DW of CPT and 10-hydroxycamptothecin, respectively, in the root biomass (Lorence et al. 2004). Likewise, Liu and Cui (2007) reported the occurrence of about 0.14 mg/g DW of CPT in the hairy root cultures. Ni et al. (2011) reported the enhanced production of CPT from hairy roots of *C. acuminata.* They used ORCA3, a

jasmonate-responsive APETALA2-domain transcription factor sequestered from *C. roseus*, to over-express several major genes that are involved in the terpenoid indole alkaloid biosynthetic pathway. The integrated gene expressed and enhanced CPT content up to 1.5-fold when compared to the control (1.12 mg/g DW).

In an interesting study, cambial meristematic cells (CMCs) and dedifferentiated cells (DDCs) from *C. acuminata* were successfully isolated and cultured suing leaves and stem explants (Zhang et al. 2017). The irregular CMCs were moved to MS medium containing 0.1 mg/l 2,4-D, 0.1 mg/l kn, and 0.1 mg/l NAA for further dedifferentiation. They observed the occurrence of relatively higher levels of CPT in CMCs with 2.55 µg/g DW which was nearly 1.5-fold higher compared to that detected in DDCs (1.65 µg/g DW). Likewise, 2.50-fold higher concentration of 10-hydroxycamptothecin was recorded in CMCs (25.5 µg/g DW) in comparison to that found in DDCs (10.2 µg/g DW). In addition, they also suggested the downregulation of HMGR2 and HMGR3 and upregulation of IPI, G10H, ASA1, TSB, TDC1, TDC2, and STR genes which were correlated to the accumulation of higher CPT contents in CMCs compared to DDCs.

Genetic diversity studies

Nothapodytes nimmoniana

In an approach to understanding the genetic fidelity of artificially generated *N. nimmoniana* plants through comparing the banding profiles of 60 inter-simple-sequence repeat (ISSR) primers, it was found that the propagated plants maintained a genetic similarity with the mother plant. All the genetic rearrangements and the elite genotypes were well maintained in the regenerated plants. The reason for such great genetic fidelity might be a result of well-maintained hormonal level or in vitro stress caused by the addition of biochemical or different nutritional conditions (Prakash et al., 2016; Razaq et al., 2013; Singh et al., 2013). Similar ISSR markers were also used for the study of genetic diversity among the 12 populations of *N. nimmoniana* from Western Ghats, India. The intra-population diversity (73%) was significant, while the inter-population (27%) diversity was comparatively lower. The reasoning for such a phenomenon might be genetic drift caused by restricted gene flow. However, environmental influence (inappropriate environmental condition for seed germination) and physiological liability (polygamo-monoecious) have constructed the genetic structure of the plant population (Abdul Kareem et al., 2011).

Ophiorrhiza sp.

Similar experiments like *N. nimmoniana* were also performed on *Ophiorrhiza* grown in in vitro condition to check the clonal fidelity using molecular markers. In one such experiment, ten RAPD primers were used in which four of which produced scorable bands. Data from RAPD analysis between the mother plant and artificially regenerated plantlets of *Ophiorrhiza mungos* showed no significant polymorphism in the micropropagated plants and also they maintained a pronounced genetic stability among them (Kaushik et al., 2015). By comparing the amino acid sequence chloroplast *matK* genes (used to classify *Ophiorrhiza* species) and nuclear *TopI* genes (point mutation responsible for resistance to CPT) of eight *Ophiorrhiza* species from Thailand, it was revealed that CPT producing *Ophiorrhiza* had two-point mutation leucine-530 to isoleucine and asparagine-722 to serine in the *TopI* amino acid sequence. This study confirms the CPT producing ability of a certain plant was determined by its genetic code rather than its habitat (Viraporn et al., 2011).

Camptotheca acuminata

RAPD markers were used to investigate the genetic diversity of the *Camptotheca* population from both China and USA which comprised of three species—*C. acuminata, C. lowreyana,* and *C. yunnanensis.* Data suggested that all the populations have maintained significant genetic diversity due to restricted gene flow causing genetic drift and inbreeding depression. Although it has been broadly planted in southern China and numerous different areas on earth, *C. acuminata* has generally low CPT and also has less variety among populations. Thinking about both low genetic variety and low CPT yield, the species is not the ideal possibility for a major improvement in CPT production. In contrast, *C. lowreyana* ought to be considered as the board focus in both CPT yield and germplasm protection because the species does not just have higher genetic variety, also has higher CPT fixations than the other taxa (Wang et al., 2015).

Conclusion and future prospects

Overall, the use of in vitro cell, tissue, and organ cultures determines a means for sustainable production of CPT without using the field-grown plant resources or field cultivation. In vitro regenerated plants and calli produced low quantities of CPT. However, cells when cultured in suspensions secreted higher levels of CPT. Moreover, compared to untransformed plant roots, CPT accumulated maximum in genetically transformed hair roots when cultured in vitro.

This further encourages to utilize cloning and gene manipulation approaches to develop high-yielding transgenic plants. Further, the use of genomics and transcriptomics will help to characterize specific genes encoding enzymes responsible for the biosynthesizing CPT. Moreover, genetic information will be useful for deciphering the biosynthetic pathways in CPT-containing plants. Developing efficient bioreactors for an established culture technology might certainly reduce the cost of production and yield. In addition, down-stream processing and the purification steps will be simpler if bioreactors are used allowing the production of CPT on a large scale. This approach needs further manipulations in the culture lines or conditions to improve the biomass and yield of CPT so that it's ever increasing industrial demand can be achieved.

Author contribution MKS prepared the primary draft. SN and SP revised it and designed the figures. NKJ and BP prepared the tables and contributed to the discussion part. KCR edited the figures and wrote the discussion part and AD edited and supervised the entire work. All authors read and approved the manuscript.

Declarations

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

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