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MINI-REVIEW

Biotechnological advancements in *Catharanthus roseus* (L.) G. Don

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Abstract

Catharanthus roseus (L.) G. Don, also known as Madagascar periwinkle or Sadabahar, is a herbaceous plant belonging to the family *Apocynaceae*. Being a reservoir for more than 200 alkaloids, it reserves a place for itself in the list of important medicinal plants. Secondary metabolites are present in its leaves (e.g., vindoline, vinblastine, catharanthine, and vincristine) as well as basal stem and roots (e.g., ajmalicine, reserpine, serpentine, horhammericine, tabersonine, leurosine, catharanthine, lochnerine, and vindoline). Two of its alkaloids, vincristine and vinblastine (possessing anticancerous properties), are being used copiously in pharmaceutical industries. Till date, arrays of reports are available on in vitro biotechnological improvements of *C. roseus*. The present review article concentrates chiefly on various biotechnological advancements based on plant tissue culture techniques of the last three decades, for instance, regeneration via direct and indirect organogenesis, somatic embryogenesis, secondary metabolite production, synthetic seed production, clonal fidelity assessment, polyploidization, genetic transformation, and nanotechnology. It also portrays the importance of various factors influencing the success of in vitro biotechnological interventions in *Catharanthus* and further addresses several shortcomings that can be further explored to create a platform for upcoming innovative approaches.

Key Points

- *C. roseus yields anticancerous vincristine and vinblastine used in pharma industry.*
- *In vitro biotechnological interventions prompted major genetic advancements.*
- *This review provides an insight on* in vitro*-based research achievements till date.*
- *Key bottlenecks and prospective research methodologies have been identified herein.*

Keywords Anticancerous · Genetic transformation · Nanotechnology · Polyploidy · Secondary metabolite · Somatic embryogenesis

Introduction

Catharanthus roseus (L.) G. Don, popularly known as Madagascar periwinkle or Sadabahar, is one of the most comprehensively explored flowering plant species, owing to its wide range of medicinal properties. This species was named by Swedish naturalist Carl Linnaeus as *Vinca rosea*, which was changed to *Catharanthus roseus* by Scottish botanist George Don (Le Roux and Guéritte 2017). It is a perennial or annual herbaceous plant or small sub-shrub with diploid chromosome number 2n = 16. It is native to Madagascar Island and belongs to the family *Apocynaceae* (Omino 1996; Shala and Deng 2018). The plant has ornamental value due to its year-round flowering. Additionally, various alkaloids that are present in this plant, make it as one of the most demanding medicinal plants. The secondary metabolites of this plant are effective against several ailments, disorders, and insect-pests as well. Two dimeric alkaloids extracted from periwinkle, namely, vincristine and vinblastine possesss anticancerous properties, and therefore their demand is much higher in the pharmaceutical industries (Jaleel et al. 2009; Kalidass et al. 2010). However, a very minute quantity of these very valuable alkaloids is produced in the plant. Moreover, conventional seed propagation and external environment regulate the synthesis and accumulation of secondary metabolites,

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qualitatively and quantitatively (Pietrosiuk et al. 2007; Binder et al. 2009; Shukla et al. 2010; Rizvi et al. 2015). Therefore as a solution to the above problem, in vitro propagation of periwinkle came into the figure. Many researchers have tried and tested various plant tissue culture-based biotechnological interventions for mass clonal propagation along with improvements in the alkaloid profile of periwinkle till date. The present review article extensively analyses the various factors affecting the in vitro culture, regeneration of plants via direct and indirect organogenesis, somatic embryogenesis, enhancement of secondary metabolites, acclimatization, clonal fidelity, and also the future prospects of *C. roseus*.

Geographical distribution

Genus *Catharanthus* includes eight species, out of which seven (*C. longifolius*, *C. coriaceus*, *C. roseus*, *C. lanceus*, *C. trichophyllus*, *C. ovalis*, *C. scitulus*) are prevalent in Madagascar and only one, *C. pusillus*, is from India (Almagro et al. 2015). As the name "Madagascar periwinkle" indicates, *C. roseus* is native and endemic to Madagascar, located in the Indian ocean. *C. roseus* is localized in America, continental Africa, Asia, Southern Europe, Australia, and in quite a few islands of the Pacific Ocean (Mujib et al. 2012) (Fig. 1). It is cultivated as an ornamental plant in most of the tropical and sub-tropical areas (Hirata et al. 1994). In India, *C. roseus* is distributed along the Northwestern and Northeastern Himalayas, Western Ghats, Eastern Ghats, West Coast, East Coast, Central Deccan Plateau, and Indo-Gangetic Plain (Fig. 1). It grows well in the temperate regions as an annual plant and thrives through frost as well (Salma et al. 2018). It can survive in extreme abiotic stress due to its wide adaptability. It is distributed in parts of Maharashtra, Gujarat, Madhya Pradesh, Uttar Pradesh, Assam, Bihar, Karnataka, Andhra Pradesh, and Tamil Nadu.

Botanical description

C. roseus is a perennial or annual herbaceous plant or small sub-shrub. This plant grows up to 80–100 cm high, with year-round flowering. Because of its branched taproot system, it thrives well in drought. The sub-woody stem is solid, erect with profuse branching, which are dark purple, light pink or light green in color at the base (Fig. 2a). The leaves are petiolate, elliptic-ovate to oblong (Fig. 2b), measuring 2.5–9 cm in length and 1–3.5 cm in breadth (Das and Sharangi 2017). The phyllotaxy shows broad, unicostate reticulate, dark green, and glossy leaves that are arranged oppositely with a short petiole and a midrib. Out of the two common cultivars of *C. roseus*, one blooms pink flowers named as "Rosea" and another is "Alba" with white flowers (Fig. 2c) (Aruna et al. 2015). The inflorescence is a solitary axillary or dichasial cyme. Paired, hermaphrodite, pedicellate, actinomorphic, bracteate, hypogynous, pentamerous, and complete flowers are borne in axils with a 2.5–3 cm long cylindrical tube at the base (Fig. 2d). The calyx is polysepalous, composed of five velvet sepals, free to the base. Corolla is gamopetalous,

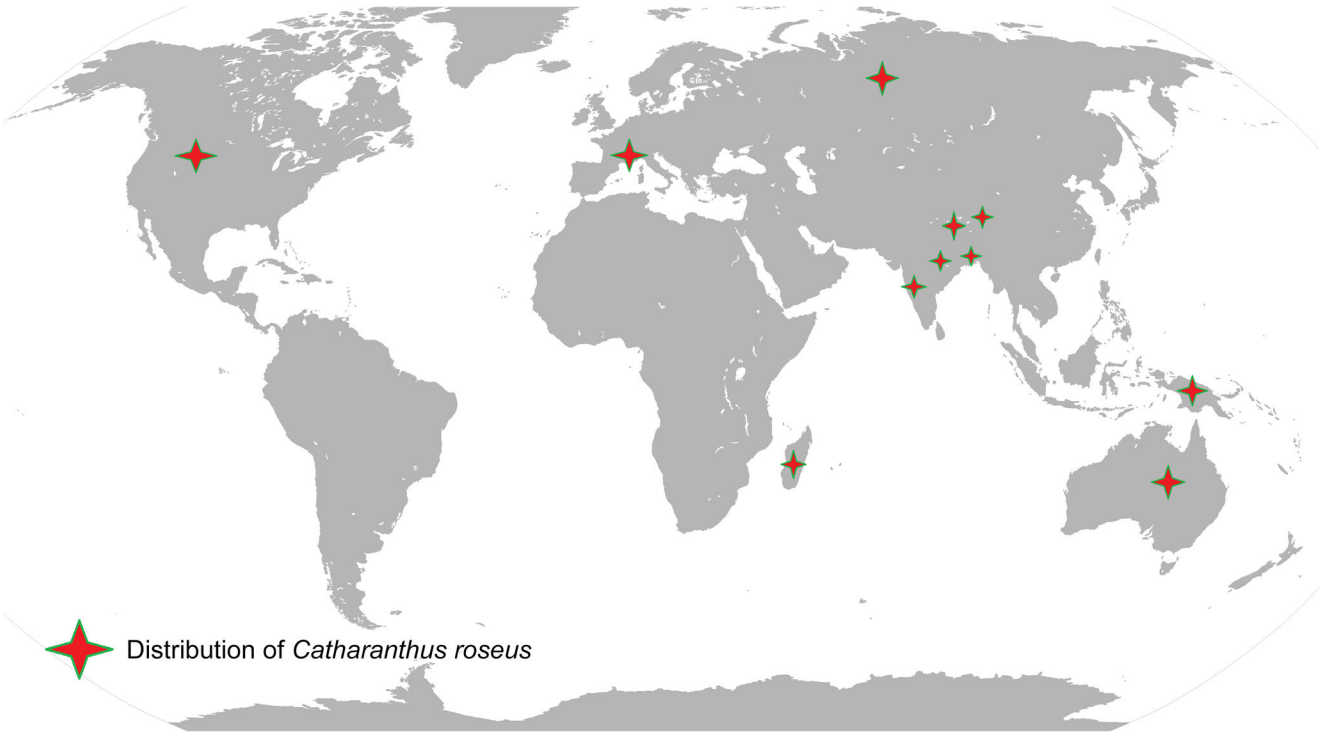

Fig. 1 Distribution of *Catharanthus roseus* around the world (Photograph is not in scale) (source: unpublished photograph of Anamika Das)

Fig. 2 Prevalent botanical characters of *Catharanthus roseus*. **a** Fully grown *C. roseus* plant at its flowering stage, **b** elliptic-ovate to oblong leaves, **c** blooming flowers of "Rosea" (pink) and "Alba" (white) cultivars, **d** development of flowers in axils on long cylindrical tube (*inset* overy and stigma head), **e** anthers and pllens (inset), **f** fruit with a pair of elongated follicles is gamopetalous, mature fruit bursted and released seeds (photographs are not in scale) (source: unpublished photographs of Anamika Das and Saikat Gantait)

made up of five petals, light to dark pink with a dark reddish-pink center, or white in color with a diameter of 2.5–5 cm. Attached to the corolla tube, stamens are five in number with short filament and free anthers (Fig. 2e). Two distinct carpels, each with about 10–30 ovules in series of two, have long and slender style and capitate stigma. The fruit consists of a pair of elongated follicles, parallel or diverging, with 10–30 small, cylindrical and oblong seeds, black (Fig. 2f) in color (Kulkarni et al. 2016).

Medicinal uses

The tropical plant, *C. roseus*, is a single one of its kind to harbor a host of medicinal uses. Being rich in more than 200 alkaloids, every part of this plant has got some medicinal properties. Since ancient times, the extracts of this plant has been used against many ailments like diabetes, high blood pressure, cancer, and insomnia in Malaysia. Its leaf and stem extracts are used to induce nausea and as a laxative, respectively, in Madagascar. In India, the juice from its leaves is applied to treat insect bites. According to some reports, the phytochemicals present in this plant have antibacterial, antioxidant, antihelminthic, and pesticidal properties as well (Aruna et al. 2015). It is also known to have been used for the treatment of digestive ailments like enteritis, diarrhea, gastritis, loss of appetite, and also for nose bleeding, muscular pain, depression, cystitis, bleeding gums, asthma, etc. (Gajalakshmi et al. 2013). The leaves contain major alkaloids, namely, vincristine, vinblastine (Fig. 3), vindoline, and catharanthine, whereas the basal stem and roots contain ajmalicine, reserpine, serpentine, horhammericine, tabersonine, leurosine, catharanthine, lochnerine, and vindoline (Kaushik et al. 2017). Antineoplastic alkaloids, vinblastine, and vinblastine are applied during the treatment of neuroblastoma, Hodgkin's disease, breast cancer, lung cancer, and chronic leukemia. Serpentine and ajmalicine are used against hypertension and cardiac disorders (Uniyal et al. 2001). The alkaloids, vincamine, and vindoline also show antiulcer properties. Out of all the alkaloids found in *C. roseus*, a few are utilized in pharmaceutical industres.

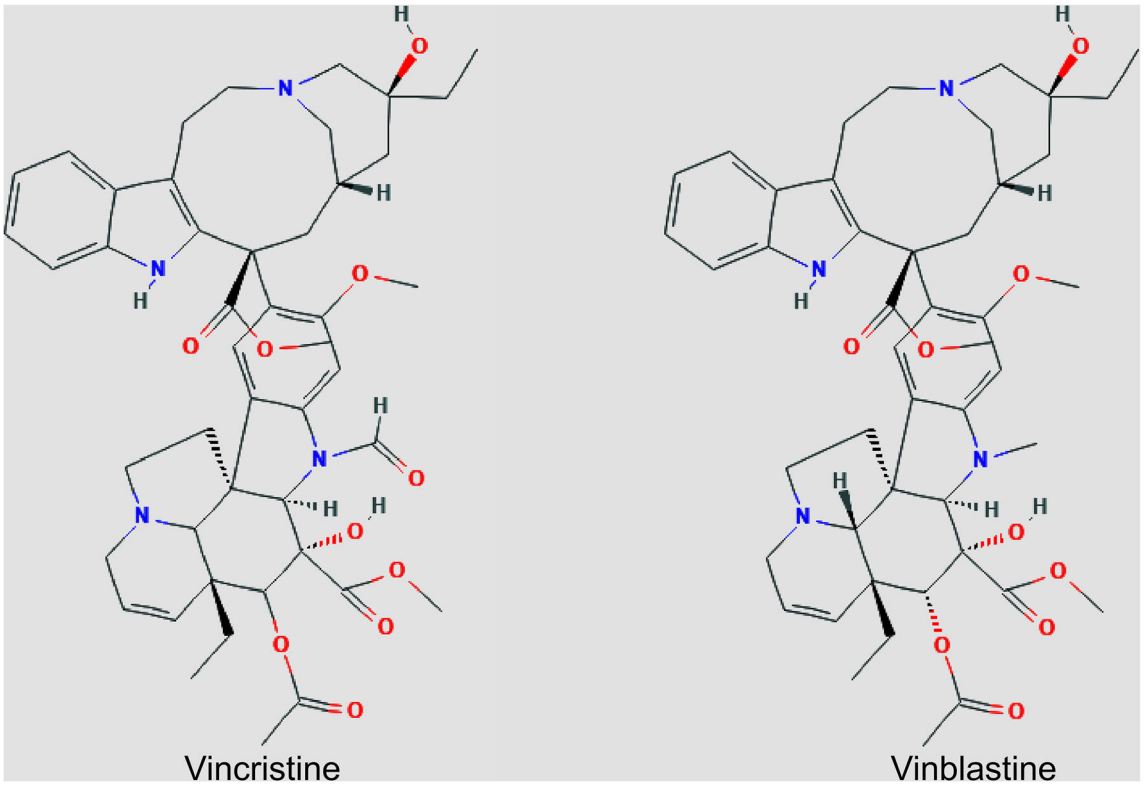

Fig. 3 Interactive 2D chemical structure of vincristine and vinblastin (structure source: PubChem)

The two key alkaloids, viz., vinblastine and vincristine(with anticancerous properties) are available in the market under the trade name Velban and Oncovin or Vincovin, respectively (Sharma et al. 2016), and the semi-synthetic alkaloids, vinorelbine (trade name Navelbine) and vindesine (trade name Eldisine and Fildesine), are used for the treatment of breast and lung cancer, and refractory lymphoma and acute lymphoblastic leukemia, respectively (Kulkarni et al. 2016). Ajmalicine (trade name Hydrosepan and Lamuran) is used for the treatment of hypertension (Van der Heijden et al. 2004).

Conventional propagation practices and its demerits

Conventionally, *C. roseus* is a seed propagated crop species. One-year-old seeds are either planted in the nursery before being (the seedlings) transferred in main field, or directly planted in the main field. In case of nursery, 500 g seeds are sufficient to transplant in 1 ha with spacing of 45 × 30 cm, whereas direct sowing takes around 2.5 kg of seeds for 1 ha, with thinning in order to maintain the same spacing. During sowing, the small sized seeds are mixed with moist, fine sand for even distribution. The sown seeds take around 10 days to germinate and then the seedlings become ready for transplantation, by around 60 days. In some cases, stem or tip cuttings are also used for the propagation of this plant. There are certain demerits associated with conventional seed propagation that includes seed health and environment, viz., low viability and vigor of seeds, and poor germination percentage. Moreover, natural outcrossing, in the long run, brings out genetic variations, which can affect the quality and quantity of desirable phytochemicals (Kulkarni et al. 2016). Therefore, to meet the demands for the valuable secondary metabolites of this plant, a rapid clonal propagation method in the form of in vitro clonal propagation, is a pre-requisite.

In vitro regeneration

A large number of true-to-type plants of a species could be developed via micropropagation in a short period of time, under a controlled and aseptic environment. Plant cell, tissue, and organ culture-based techniques are being applied for mass clonal propagation of *C. roseus* as an alternative to conventional commercial cultivation. This review provides an updated overview of biotechnological advancements in *C. roseus* in the last three decades. The different in vitro-based techniques, viz., direct organogenesis, indirect organogenesis, somatic embryogenesis, and major elicitors used for enhancement of secondary metabolites in *C. roseus*, including physical conditions and plant growth regulators (PGRs) are discussed in this review article.

Choice of explant

The choice of explant plays a decisive role in plant tissue culture. A number of plant parts can be used as an explant in *C. roseus*, such as nodal segment, axillary bud, shoot tip or apical bud, leaf, stem, anther, petiole, root, etc. Based on the availability, contamination level, response, and objective, proper explants are selected for the initiation of individual in vitro regeneration systems (Salma et al. 2018). Among the different explants used for direct organogenesis, nodal segment (node or internode) is found to be the best explant for initiation of multiple shoots and induction of roots (Mitra et al. 1998; Zárate et al. 1999; Swanberg and Dai 2008; Srivastava et al. 2009; Pati et al. 2011; Mehta et al. 2013; Rajora et al. 2013; Begum and Mathur 2014; Rahmatzadeh et al. 2014; Panigrahi et al. 2018). Shoot tip consisting of apical or axillary buds also proved to be quick responsive explant for direct organogenesis (Yuan et al. 1994; Satdive et al. 2003; Bakrudeen et al. 2011; Kumar et al. 2013; Moghe et al. 2016; Sharma et al. 2019). According to some reports, in vitro raised seedlings could be a choice explant for shoot proliferation (Hirata et al. 1990; Moreno et al. 1994; Hernández-Domínguez et al. 2004). In vitro leaf was used as explant by Verma and Mathur (2011), which resulted in giving rise to adventitious shoot buds and roots. Leaf and stem were used as explant for indirect organogenesis via callus induction by many researchers (Hilliou et al. 1999; Zhao et al. 2000a, b, 2001a, b, c, d, e; Xu et al. 2005; Xu and Dong 2005a, b; Ramani and Jayabaskaran 2008; Shukla et al. 2010; Rajora et al. 2013). Some of the researchers used hypocotyl as explant for callus induction (Datta and Srivastava 1997; Ilah et al. 2009; Singh et al. 2011; Tonk et al. 2016). Kim et al. (1994) got embryogenic callus by using anthers as explant. Segments of in vitro grown seedlings were used as explant by Filippini et al. (2000) to produce callus. Dhandapani et al. (2008) reported callus regeneration through somatic embryogenesis by using mature zygotic embryo as explant. Callus and root induction were achieved by the use of leaf petiole, as evidenced from the reports of Ataei-Azimi et al. (2008). Shoot tip was also used as explant for callus induction by Saifullah (2011). Based on the reports of multiple researchers, hypocotyl was considered to be the best explant used for somatic embryogenesis (Junaid et al. 2006, 2007a, b; Aslam et al. 2008, 2009, 2010a, b; Ilah et al. 2009; Yuan et al. 2011; Maqsood et al. 2012). Immature and mature zygotic embryos were also used as explants for somatic embryogenesis by Kim et al. (2004) and Dhandapani et al. (2008), respectively. Malabadi et al. (2012) reported callus induction, somatic embryo maturation and germination by using shoot tip as explant. The use of epicotyls as an explant for shooty teratomas with increased vincristine production was reported by Begum et al. (2009). Some of the researchers reported increased production of secondary metabolites by using leaf as explant (Kalidass et al. 2010; Shukla et al. 2010; Almagro et al. 2011; Verma et al. 2012; Guo et al. 2013). Several reports are available on the utilization of different explants for enhanced secondary metabolite production (Table 4). Nodal segments can be considered as the best explant for mass multiplication via direct organogenesis due to its ability to give rise to two lateral buds simultaneously, whereas leaves are best suited for callogenesis because of their larger surface area than any other explant (Gantait and Kundu 2017a).

Surface sterilization

After the selection of explant, proper surface sterilization is a decisive step for tissue culture. It prevents contamination, unless the selected explant itself is harboring the causal organism endogenously. Various sterilants are used after autoclaving. The concentration of sterilizing agents and duration of treatment varies with species and type of explant. According to various reports found in *C. roseus*, 70% (*v*/*v*) ethanol wash for 30 s to 1 min along with other sterilants was found to be very common for surface sterilization of explant (Hirata et al. 1990; Mitra et al. 1998; Zárate et al. 1999; Zhao et al. 2001a, b; Hernández-Domínguez et al. 2004; Junaid et al. 2007a, b; Aslam et al. 2008; Srivastava et al. 2009; Bakrudeen et al. 2011; Malabadi et al. 2012; Maqsood et al. 2012; Rahmatzadeh et al. 2014; Sharma et al. 2019). Some of the researchers used a higher concentration of ethanol ranging from 75 to 95% (Yuan et al. 1994; Yuan et al. 2011; Al-Oubaidi and Mohammed-Ameen 2014). There are few reports of 70% ethanol wash for 2–3 min (Datta and Srivastava 1997; Satdive et al. 2003; Dhandapani et al. 2008; Swanberg and Dai 2008; Verma et al. 2012). Ethanol wash was followed by treatment with sodium hypochlorite or bleach solution (for its antimicrobial property), by many researchers in varying concentrations (0.1–25%), and the duration of treatment was 5–45 min (Hirata et al. 1990; Yuan et al. 1994; Hernández-Domínguez et al. 2004; Dhandapani et al. 2008; Swanberg and Dai 2008; Yuan et al. 2011; Rahmatzadeh et al. 2014). A few researchers used the bleach solution alone (Hilliou et al. 1999; Kim et al. 2004; Ataei-Azimi et al. 2008), or the same was used with a few drops of liquid detergent Tween-20 or Tween-80 or Triton-X (Zárate et al. 1999; Pati et al. 2011; Maqsood et al. 2012; Al-Oubaidi and Mohammed-Ameen 2014). Saifullah (2011) used 95% sodium hypochlorite followed by absolute ethanol for surface sterilization of shoot tip. Even though mercuric chloride is toxic to plants, it has been used by researchers in the range of 0.04–0.5% (w/v) for 2–5 min, maximum up to 15 min (Datta and Srivastava 1997; Mitra et al. 1998; Zhao et al. 2001b; Satdive et al. 2003; Ramani and Jayabaskaran 2008; Srivastava et al. 2009; Shukla et al. 2010; Junaid et al.

2007a, b; Aslam et al. 2008, 2010b; Kalidass et al. 2010; Bakrudeen et al. 2011; Verma et al. 2012; Kumar et al. 2013; Mehta et al. 2013; Begum and Mathur 2014; Panigrahi et al. 2018; Sharma et al. 2019). There are a few reports mentioning the use of mercuric chloride alone (Begum et al. 2009; Rajora et al. 2013) or with a detergent-like Tween-20 (Ilah et al. 2009; Pati et al. 2011). A range of 0.5–1% (w/v) cetrimide solution was used by Datta and Srivastava (1997) and Srivastava et al. (2009). Other surface sterilants such as Tween-20, Tween-80, hydrogen peroxide, Teepol, Dettol, Savlon, Labolene, etc. were also used by various researchers. Some researchers treated the explant for 10–30 min with fungicide (Bavistine) and/or antibiotic solution (Cefotaxime or Streptomycin) for surface sterilization of explant to keep contamination at bay (Ramani and Jayabaskaran 2008; Srivastava et al. 2009; Singh et al. 2011; Malabadi et al. 2012; Verma et al. 2012; Kumar et al. 2013; Moghe et al. 2016; Panigrahi et al. 2018). A thorough wash in liquid detergent followed by ethanol and/or NaOCl is supposed to be sufficiently effective against contamination, but involving multiple sterilants in surface sterilization can remove maximum causal agents responsible for contamination.

Basal medium

The basal medium provides nutrition required for the explant the same way via which a plant gets nutrient from the soil. A basal medium generally consists of macro and micronutrients crucial for plant growth. The selection of suitable basal medium depends upon the objective and the type of plant species used in the experiment (Gantait and Kundu 2017a; Mitra et al. 2020). Various reports available on *C. roseus* suggest that the Murashige and Skoog (MS) medium (Murashige and Skoog 1962) is the most commonly used basal medium to this day. Full-strength MS medium was utilized as a basal medium by almost all the researchers for in vitro direct organogenesis, implying that MS medium provides the required nutrients for shoot bud induction and shoot proliferation (Table 1). Although rooting was reported with shoot induction and proliferation in full-strength MS medium (Mitra et al. 1998; Pati et al. 2011; Kumar et al. 2013; Mehta et al. 2013; Rajora et al. 2013; Begum and Mathur 2014), half-strength MS medium was also used by researchers for rooting experiments (Zárate et al. 1999; Bakrudeen et al. 2011; Verma and Mathur 2011; Rahmatzadeh et al. 2014). Swanberg and Dai (2008) reported multiple shoots from internode explants by using woody plant medium (WPM) (Lloyd and McCown 1981) as basal medium. As per the reports available in *Catharanthus*, full-strength MS medium was used for almost all the in vitro indirect organogenesis experiments (Table 2). Gamborg's B5 medium (Gamborg et al. 1968) was used as a basal medium by Filippini et al. (2000) for callus induction. Almagro et al. (2011) used liquid Linsmaier and Skoog (LS) medium (Linsmaier and Skoog 1965) for suspension cell culture. Verma et al. (2012) reported an increase in the biomass of callus and accumulation of alkaloids in *Catharanthus* by using half-strength MS medium as a basal medium.

Carbon source

Carbon is one of the macronutrients essential for plant nutrition and plays a crucial part in the growth of plants. The carbohydrates in a culture medium provide the same for explant regeneration. Different sugars are used as a carbon source in the culture medium. These sugars not only provide energy but also regulate the osmotic potential (Gantait et al. 2018). Out of all the types of sugar available, the most commonly used sugar in the culture medium is sucrose. The majority of the researchers used 3% (w/v) sucrose in culture medium for all types of in vitro experiments in *C. roseus*, whether it be direct or indirect organogenesis (Tables 1, 2, 3, and 4). Only a few researchers reported a different concentration of sucrose other than 3% sucrose to be effective. For instance, 2% sucrose for optimum shoot proliferation (Swanberg and Dai 2008), 4% sucrose for callus induction (Zhao et al. 2000b) and increase in callus biomass with 6% sucrose (Verma et al. 2012) were reported. Instead of sucrose, other sugars like glucose (Moghe et al. 2016) and maltose (Junaid et al. 2006; Aslam et al. 2008) were also used as carbon sources. However, there are no distinctive reports available based on the use of dissacharadies in the basal medium.

Physical conditions

When a plant species is grown in vitro, physical conditions, viz., temperature, light intensity, photoperiod, and relative humidity play decisive roles in the growth response of explant to the culture conditions (Mukherjee et al. 2019).

Temperature

Optimum temperature is required for the proper functioning of enzymes (Gantait and Kundu 2017b). Across all the available literatures on *Catharanthus*, the uniform growth temperature were reported to be set between 23 and 28 °C (Tables 1, 2, 3, and 4). However, a lower range of temperature between 20 and 22 °C was also described (Junaid et al. 2006; Swanberg and Dai 2008). Higher temperature ranges such as 27 ± 2 °C for multiplication of shoots (Moghe et al. 2016), 35 °C for callus induction and rooting (Ataei-Azimi et al. 2008) were also mentioned in a few reports.

Table 1 Factors influencing in vitro direct organogenesis of *Catharanthus roseus* (arranged in chronological order)

Explant	Surface sterilization	Basal media	Carbon source	Solidifying agent	PGR (type and conc., mg/l or μM*)	Additives (mg/l or g/l†)	Culture condition [T; LI (lux or μmol/m²/s PPFD#); PP; RH]	Response	Reference
IVS	70% (v/v) EtOH 1 min ➔ 1% (v/v) NaOCl 20 min	MS	3% sucrose	0.55% agarose	NM	NM	25 °C; 20 W/m²; NM; NM	Multiple shoot	Hirata et al. (1990)
IVS	NM	MS	3% sucrose	1% agarose	0.05 NAA + 2.5 Kn	NM	25 °C; 1500; 12 h; NM	Multiple shoot	Moreno et al. (1994)
Shoot segment	79% EtOH ➔ 1% NaOCl 20 min	MS	3% sucrose	0.6% agar	7 BA + 1 NAA	1† CH	26 ± 0.5 °C; 700; NM; NM	Multiple shoot	Yuan et al. (1994)
Node	1% Teepol ➔ 70% EtOH ➔ 0.12% $HgCl_2$	MS	NM	NM	2 Kn 0.2 BA + 0.1 IAA 0.005 NAA	NM	25 ± 2 °C; NM; 16 h; NM	Shoot induction Multiple shoots Root induction (80%)	Mitra et al. (1998)
Node	70% EtOH 30 s ➔ 7% (w/v) NaOCl + drops of detergent 30 min	½ MS	3% sucrose	0.7% agar	1 BA 0.5 BA + 0.125 NAA	NM	25 ± 2 °C; 1000; 16 h; NM	Bud induction Root induction (98%)	Zárate et al. (1999)
Shoot tip	Dettol 5 min➔70% EtOH 2 min ➔ 0.1% $HgCl_2$ 3 min	MS	3% sucrose	0.25% phytagel	11.42* IAA + 2.22* BA	NM	26 ± 2 °C; 50#; 16 h, NM	Multiple shoot	Satdive et al. (2003)
IVS	70% EtOH ➔ 5% NaOCl	MS	NM	NM	1 BA	NM	25 °C; NM; 16 h, NM	Multiple shoot	Hernández-Domínguez et al. (2004)
Internode	70% EtOH 2 min ➔ 20% NaOCl 15 min	WPM	2% sucrose	0.65% agar	5* BA + 5* NAA	NM	21 °C; NM; 16 h; NM	Multiple shoot	Swanberg and Dai (2008)
Nodal segment	0.5% Cetrimide 10 min ➔ 1% (bavistine + streptomycin sulfate) 30 min ➔ 0.1% $HgCl_2$ 5 min ➔ 70% EtOH	MS	3% sucrose	0.62% agar	0.1 NAA + 1 BA	500 CH	25 ± 2 °C; NM; 16 h; NM	Multiple shoot	Srivastava et al. (2009)
Axilary bud	Teepol solution 2 min ➔ 70% EtOH 1 min ➔ 0.1% $HgCl_2$ 5 min	MS ½ MS	3% sucrose	0.8% agar	4 NAA + 4 BA 4 IBA	NM	25 ± 2 °C; 60#; 16 h; 55–60%	Shoot induction Root induction	Bakrudeen et al. (2011)
Nodal segment	Tween 80 ➔0.4% NaOCl + a drop of Tween-80 25 min ➔ 0.04% $HgCl_2$ + a drop of Tween-80 7–8 min	MS	3% Sucrose	NM	5* BA 5* NAA	NM	25 ± 2 °C; 20 ± 5#; 14 h; NM	Multiple shoot Root induction	Pati et al. (2011)
In vitro leaf	NM	½ MS	NM	0.4% phytagel	7 BA + 3 NAA 3 IBA	NM	24 ± 2 °C; 3000; 16 h; NM	Adventitious shoot buds Root induction	Verma and Mathur (2011)
Apical bud	Few drops of Tween-20 ➔ 1000 ppm cefotaxime 10 min ➔ 1000 ppm bavistine 10 min ➔ 0.1% $HgCl_2$ 15 min	MS	NM	NM	1 BA + 0.2 NAA 0.25 g/l charcoal	NM	25 ± 2 °C; 35–40#; 16 h; NM	Multiple shoot Root induction	Kumar et al. (2013)

Table 1 (continued)

Explant	Surface sterilization	Basal media	Carbon source	Solidifying agent	PGR (type and conc., mg/l or μM*)	Additives (mg/l or g/l†)	Culture condition [T; LI (lux or μmol/m²/s PPFD#); PP; RH]	Response	Reference
Nodal segment	Tween-20 ➔ 0.1% $HgCl_2$ 5 min	MS	3% sucrose	0.8% agar	0.5 BA + 1 NAA 5 IBA	NM	25 ± 2 °C; 2000–2500; 16 h; NM	Multiple shoot Root induction	Mehta et al. (2013)
Nodal segment	0.1% $HgCl_2$	MS	3% sucrose	0.8–1.0% agar	6 BA 6 Kn 10 IBA	NM	25–28 °C; 100#; 16 h; NM	Bud breaking Multiple shoot Root induction	Rajora et al. (2013)
Shoot tip	95% EtOH 1 min ➔ 2% NaOCl + 2 drops of Tween-20 10 min	MS	3% sucrose	0.8% agar	2 BA + 0.2 NAA	10 thiamine HCl, 1 glycine	25 ± 1 °C; 1000; 16 h; NM	Multiple shoot	Al-Oubaidi and Mohammed-Ameen (2014)
In vitro nodal segment	70% EtOH 30 s ➔ 1% NaOCl 15 min	MS ½ MS	3% sucrose	0.8% agar	0.5 BA + 1 NAA 0.1 IBA	250 tryptophan 350 tryptophan	NM; NM; NM; NM	Multiple shoot Root induction	Rahmatzadeh et al. (2014)
Shoot tip	Liquid detergent 10–20 min ➔ 1% bavistin 15–20 min ➔ 0.1% NaOCl 5–7 min	MS	3% glucose	0.7% agar	2 BA+ 1 Kn	10 thiamine	27 ± 2 °C; NM; 16 h; NM	Multiple shoot	Moghe et al. (2016)
Nodal segment	Labolin ➔ 0.1% $HgCl_2$ 15 min	MS	3% sucrose	0.8% agar	0.5 BA +1 NAA 5 IBA	NM	25 ± 2 °C; 2000–2500; 16 h; NM	Multiple shoot Root induction	Begum and Mathur (2014)
Nodal segment	H_2O_2 2 min ➔ 2% bavistin + 2–3 drops of Tween-20 20 min ➔ 0.1% $HgCl_2$ 2 min	MS	3% sucrose	7% agar	3* BA + 3* Kn	0.1 μM $AgNO_3$	25 ± 1 °C; 40#; 12 h; NM	Multiple Shoot and precocious flowering	Panigrahi et al. (2018)
In vitro axillary shoot	70% EtOH 30 s ➔ 0.1% $HgCl_2$ 2 min	MS	3% sucrose	0.8% agar	1 BA +0.1 NAA	0.38 thymine HCl	25 ± 2 °C; 48#; 16 h; NM	Multiple Shoot	Sharma et al. (2019)

BA N^6-benzyladenine, *BAP* N^6-benzylaminopurine, *CH* casein hydrolysate, *IAA* indole acetic acid, *IBA* indole-3-butyric acid, *IVS* in vitro raised seedlings, *Kn* kinetin, *LI* light intensity, *MS* Murashige and Skoog (1962), *NAA* α-naphthalene acetic acid, *NM* not mentioned, *PGR* plant growth regulator, *PP* photoperiod, *RH* relative humidity, *WPM* woody plant medium

Table 2 Factors influencing in vitro indirect organogenesis of *Catharanthus roseus* (arranged in chronological order)

Explant	Surface sterilization	Basal media	Carbon source	Solidifying agent	PGR (type and conc., mg/l or μM*)	Additives (mg/l or g/l*)	Culture condition [T; LI (lux or μmol/m^2/s PPFD#); PP; RH]	Response	Reference
Anther	NM	MS	3% sucrose	0.4% gelrite	1 NAA + 0.1 Kn	0.4 thiamine HCl	25 °C; 3 W/m^2; 16 h; NM	Embryogenic callus	Kim et al. (1994)
In vitro hypocotyl	1% (w/v) Cetrimide 5 min ➔ 10% NaOCl 20 min ➔ 0.1% (w/v) $HgCl_2$ 5 min ➔ 70% EtOH 3 min	MS	3% sucrose	0.6% agar	2 NAA + 5 BA	1000 CH + 100 asparagine	26 ± 2 °C; 1045; 10 h; 55%	Callus induction	Datta and Srivastava (1997)
					0.1 NAA + 5 BA + 1 zeatin	100 asparagine + 100 glutamine		Shoot regeneration	
Leaf	25% NaOCl 5 min	MS	3% sucrose	0.8% agar	1 NAA + 0.1 Kn	NM	25 °C; NM; 12 h; NM	Callus initiation and proliferation	Hilliou et al. (1999)
Seedling segment	NM	B5	3% sucrose	0.8% agar	5.88* 2,4-D + 1.16* Kn + 1.34* NAA +	NM	NM	Callus (95%)	Filippini et al. (2000)
Leaf	NM	MS	3% sucrose	NM	1 2,4-D + 1 IAA + 0.5 Kn	1100 KNO_3 + 2 thiamine-HCl + 0.1 riboflavin + 0.1 biotin + 0.1 folic acid	23 ± 2 °C; dark; NM;NM	Callus induction	Zhao et al. (2000a)
Stem and Leaf	NM	MS	4% sucrose	NM	1 NAA + 1 IAA + 0.5 Kn	NM	23 ± 2 °C; dark; NM; NM	Callus induction	Zhao et al. (2000b)
Stem and leaf	NS	MS	3% sucrose	NM	5.37* NAA + 4.65* Kn	1100 KNO_3 + 12 thiamine HCl + 0.1 riboflavin + 0.1 biotin + 0.1 folic acid	23 ± 2 °C; dark with 45 min/day fluorescent light; NM; NM	Compact callus clusters	Zhao et al. (2001d)
Stem	NM	MS	3% sucrose	NM	5.37* NAA + 4.65* Kn	1100 KNO_3 + 12 thiamine HCl + 0.1 riboflavin + 0.1 biotin + 0.1 folic acid	23 ± 2 °C; dark; NM;NM	Compact callus cluster	Zhao et al. (2001e)
Stem and Leaf	NM	MS	3% sucrose	NM	2 NAA + 2 IAA + 0.1 Kn	NM	23 ± 2 °C; dark; NM; NM	Callus induction	Zhao et al. (2001c)
Leaf	70% EtOH ➔ 0.1% $HgCl_2$	MS	3% sucrose	0.65% agar	1 NAA + 1 IAA + 0.1 Kn	NM	23 ± 2 °C; dark; NM; NM	callus induction	Zhao et al. (2001b)
Stem	NM	MS	3% sucrose	NM	5.37* NAA + 4.65* Kn	1100 KNO_3 + 12 thiamine HCl + 0.1 riboflavin + 0.1 biotin + 0.1 folic acid	23 ± 2 °C; dark; NM; NM	Compact callus cluster	Zhao et al. (2001a)
					4.52* 2,4-D + 4.65* Kn 5.7* IAA + 4.45* BA			Increase in biomass Increase in TAC	
Young stem	NM	MS	3% sucrose	NM	2 NAA + 2 IAA + 0.1 Kn	NM	25 °C; dark; NM; NM	Callus induction	Xu et al. (2005)
Young stem	NM	MS	3% sucrose	NM	2 NAA + 2 IAA + 0.1 Kn	NM	25 °C; dark; NM; NM	Callus induction	Xu and Dong (2005a)

Table 2 (continued)

Explant	Surface sterilization	Basal media	Carbon source	Solidifying agent	PGR (type and conc., mg/l or μM*)	Additives (mg/l or g/l*)	Culture condition [T; LI (lux or μmol/m^2/s PPFD#); PP; RH]	Response	Reference
Young stem	NM	MS	3% sucrose	NM	2 NAA + 2 IAA + 0.1 Kn	NM	25 °C; dark; NM; NM	Callus induction	Xu and Dong (2005a)
Leaf petiole	5% NaOCl 45 min	MS	NM	NS	0.1 NAA + 0.1 Kn	NM	35 °C; dark; NM; NM 35 °C; 7 W/m^2; 24 h; NM	Callus and root	Ataei-Azimi et al. (2008)
Mature zygotic embryo	70% EtOH 2 min ➔ 1.5% NaOCl 10 min	MS	3% sucrose	0.4% gelrite	7.5* TDZ	NM	25 ± 1 °C; 60#; 14 h; NM	Callus regeneration through SE	Dhandapani et al. (2008)
Leaf	0.5% (w/v) Bavistin 20 min ➔ 0.01% (*v*/v) Tween 20 20 min ➔ 0.1% $HgCl_2$ 5 min	MS	3% sucrose	NM	2 NAA + 0.2 Kn	NM	25 ± 2 °C; 40#; 16 h; NM	Callus induction	Ramani and Jayabaskaran (2008)
Hypocotyl	0.1% $HgCl_2$ + 3 drops of Tween-20 5 min	MS	3% sucrose	0.8% agar	0.45* 2,4-D + 6.62* BA + 1.44* GA_3	NM	25 ± 2 °C; 150–200 μE m^{-2} s^{-1}; 16 h; 50–60%	Somatic embryo	Ilah et al. (2009)
In vitro shoot					5.37* NAA + 5.71* IAA			Root induction	
In vitro Leaf	Labolene ➔ Savlon ➔ 0.1% $HgCl_2$ 1 min	MS	NM	NM	1 2,4-D + 0.5 BA	NM	25 °C; 1200; 12 h; NM	Callus induction	Shukla et al. (2010)
Shoot tip	95% NaoCl 10 min ➔ 100% EtOH	MS	3% sucrose	0.8% agar	1.5 2,4-D + 0.5 Kn	NM	25 ± 1 °C; NM; 16 h; NM	Callus induction	Saifullah (2011)
Hypocotyl	Tap water + few drop of Teepol ➔ 0.4% (w/v) Bavistin 30 min	MS	NM	0.8% agar	1 BA +1 NAA	NM	26 °C; dark; NM; NM	Callus induction	Singh et al. (2011)
		½ MS			1.5 BA + 1 NAA		26 °C; 50 μ^2gm/s; 16 h; NM	Multiple shoots	
		MS			2.5 IBA + 0.5 NAA			Rooting	
					1.5 BA + 1 NAA			Shoot regeneration	
Leaf	0.1% $HgCl_2$	MS	NM	0.8–1.0% agar	4 2,4-D	NM	25–28 °C; 100#; 16 h; NM	Callus induction	Rajora et al. (2013)
Nodal segment					6 BA 6 Kn 10 IBA			Bud initiation Multiple shoot Rooting	
Nodal segment	Liquid detergent 10–20 min ➔ 1% Bavistin 15–20 min ➔ 0.1% NaOCl 5–7 min	MS	3% glucose	0.7% agar	2 NAA + 1 Kn	10 Thiamine	27 ± 2 °C; NM; 16 h; NM	Callus induction	Moghe et al. (2016)
In vitro hypocotyl	NS	MS	NM	0.8% agar	4.52* 2,4-D 6.62* BAP + 5.36* NAA	NM	25 ± 2 °C; 100#; 16 h; NM	Callus induction Embryo proliferation	Tonk et al. (2016)
					2.89* GA_3			Embryo maturation	
					2.22* BAP			Embryo germination	

2,4-D 2,4 dichlorophenoxyacetic acid, *B5* Gamborg's B5 medium (Gamborg et al. 1968), *BA* N^6-benzyladenine, *BAP* N^6-benzylaminopurine, *CH* casein hydrolysate, *GA_3* gibberellic acid, *IAA* indole acetic acid, *IBA* indole-3-butyric acid, *Kn* kinetin, *LI* light intensity, *MS* Murashige and Skoog (1962), *NAA* α-naphthalene acetic acid, *NM* not mentioned, *PGR* plant growth regulator, *PP* photoperiod, *RH* relative humidity, *TDZ* thidiazuron

Table 3 Factors influencing somatic embryogenesis of *Catharanthus roseus* (arranged in chronological order)

Explant	Surface sterilization	Basal media	Carbon source	Solidifying agent	PGR (type and conc., mg/l or μM*)	Additives (mg/l or g/l*)	Culture condition [T; LI (lux or μmol/m^2/s PPFD#); PP; RH]	Response	Reference
Anther	NM	MS	3% sucrose	0.4% gelrite	1 NAA + 0.1 Kn	0.4 thiamine HCl	25 °C; 3 W/m^2; 16 h; NM	Embryogenic callus	Kim et al. (1994)
Immature zygotic embryo	0.4% NaOCl 10 min	MS	3% sucrose	0.4% gelrite	4.52* 2,4-D	0.4 thiamine HCl	25 °C; dark; NM; NM	Somatic embryo (20%)	Kim et al. (2004)
In vitro hypocotyl	NS	MS	NM	NM	1 2,4-D	–	20 °C; dark; NM; NM	embryogenic callus induction	Junaid et al. (2006)
					1 NAA		25 °C; 100#; 16 h, NM	SE proliferation	
			3% maltose		1 GA_3			SE maturation	
			NM		0.5 BAP			SE germination	
In vitro hypocotyl	70% EtOH ➔ 0.5% $HgCl_2$ 2 min ➔ 5% H_2O_2	MS	NM	NM	1.0–2.0 mg dm^{-3} either of 2,4-D, NAA, or CPA	NM	25 ± 2 °C; 100#; 16 h, NM	Somatic embryo	Junaid et al. (2007a)
In vitro hypocotyl	70% EtOH ➔ 0.5% $HgCl_2$ 2 min ➔ 5% H_2O_2	MS	NM	NM	1.5 BA + 1 NAA	NM	25 ± 2 °C; 100#; 16 h, NM	Somatic embryo	Junaid et al. (2007b)
In vitro hypocotyl	70% EtOH ➔ 0.5% $HgCl_2$ 2 min ➔ 5% H_2O_2	MS	3% sucrose	0.8% Agar	6.78* 2,4-D 2.24* BA + 5.37* NAA	NM	25 ± 2 °C; 100#; 16 h, NM	Embryogenic callus SE proliferation	Aslam et al. (2008)
			3% maltose		2.6* GA_3			SE maturation	
			6% maltose		2.25* BA			SE germination	
Mature zygotic embryo	70% EtOH 2 min ➔ 1.5% NaOCl 10 min	MS	3% sucrose	0.4% gelrite	7.5* TDZ	NM	25 ± 1 °C; 60#; 10 h dark/14 h light; NM	Callus regeneration through SE	Dhandapani et al. (2008)
In vitro hypocotyl	70% EtOH ➔ 0.5% $HgCl_2$ 2 min ➔ 5% H_2O_2	MS	3% sucrose	NM	6.96* 2,4-D 5.37* NAA + 6.72* BA	NM	25 ± 2 °C; 100#; 16 h; NM	Callus Induction SE initiation and proliferation	Aslam et al. (2009)
			3% maltose		2.6* GA_3			SE maturation	
					2.24* BA			SE Germination	
Hypocotyl	0.1% $HgCl_2$ + 3 drops of Tween-20 5 min	MS	3% sucrose	0.8% Agar	0.45* 2,4-D + 6.62* BA + 1.44* GA_3	NM	25 ± 2 °C; 150–200 μE m^{-2} s^{-1}; 16 h; 50–60%	Somatic embryo	Ilah et al. (2009)
In vitro shoot					5.37* NAA + 5.71* IAA			Root induction	
In vitro hypocotyl	70% EtOH ➔ 0.5% $HgCl_2$ 2 min ➔ 5% H_2O_2	MS	3% maltose	NM	1 2,4-D 1 NAA + 0.5 BA	NM	25 ± 2 °C; 100#; 16 h; NM	Callusing SE initiation and proliferation	Aslam et al. (2010b)
					1 GA_3			SE maturation	
In vitro hypocotyl	75% EtOH 2 min ➔ 5.25% NaOCl 10 min	MS	3% sucrose	0.3% Gelrite	1 2,4-D + 1 NAA + 0.1 Zeatin	NM	25 ± 2 °C; 40#; 16 h; NM	Callus induction	Yuan et al. (2011)
					5 BA+ 0.5 NAA			Embryogenic callus	

Table 3 (continued)

Explant	Surface sterilization	Basal media	Carbon source	Solidifying agent	PGR (type and conc., mg/l or μM*)	Additives (mg/l or g/l*)	Culture condition [T; LI (lux or μmol/m^2/s PPFD$^{\#}$); PP; RH]	Response	Reference
Shoot tip	0.1% streptomycin 20 s ➔ 70% EtOH 50 s ➔ 0.1% HgCl 2 min	MS	3% sucrose	0.7% Agar 0.8% Agar	2* 2,4-D + 5* TRIA 5* ABA	1 g/l CH + 0.5 g/l glutamine + 250	25 ± 3 °C; dark; NM; 55–60%	Callus induction SE maturation	Malabadi et al. (2012)
		½ MS		0.7% Agar	NM	peptone + 0.2 g/l PABA + 0.1 g/l biotin		SE germination	
In vitro hypocotyl	70% EtOH 30 s ➔ 0.5% NaOCl + a drop of triton-X 5 min	MS	NM	NM	4.5* 2,4-D	NM	NM; 100$^{\#}$; 16 h; NM	Embryogenic callus	Maqsood et al. (2012)

2,4-D 2,4 dichlorophenoxyacetic acid, *ABA* abscisic acid, *BA* N^6-benzylaminopurine, *BAP* N^6-benzylaminopurine, *GA_3* gibberellic acid, *Kn* kinetin, *LI* light intensity, *MS* Murashige and Skoog (1962), *NAA* α-naphthalene acetic acid, *NM* not mentioned, *PGR* plant growth regulator, *PP* photoperiod, *RH* relative humidity, *TDZ* thidiazuron, *TRIA* triacontanol

Light intensity

The quantity of light received by the plants is termed as light intensity. Generally, light intensity is measured in lux or μmol/m^2/s or photosynthetic photon flux density (PPFD). Since, light intensity directly affects photosynthesis, poor light intensity tends to reduce the growth and development of plants (Gantait and Kundu 2017a). Whereas, high light intensity increases the rate of respiration and transpiration which again negatively affects the plant growth. Therefore, it is presumed that proper light intensity should be maintained for in vitro cultures (Gantait et al. 2018). In the early 90s, light intensity was measured in W/m^2,for instance, light intensity was reported to have been maintained at 3–7 W/m^2 by some researchers (Hirata et al. 1992; Kim et al. 1994; Ataei-Azimi et al. 2008). Hirata et al. (1990) reported a higher light intensity of 20 W/m^2 for shoot proliferation. Light intensity of 2000–3000 lx was reported by many researchers for majority of the in vitro cultures (Kalidass et al. 2010; Verma and Mathur 2011; Mehta et al. 2013; Begum and Mathur 2014). However, there are few reports where a lower light intensity of about 700–1500 lx was mentioned (Moreno et al. 1994; Yuan et al. 1994; Datta and Srivastava 1997; Zárate et al. 1999; Shukla et al. 2010; Al-Oubaidi and Mohammed-Ameen 2014). Measuring light intensity in terms of PPFD is more appropriate. Light intensity between 40 and 60 μmol/m^2/s is reported by the majority of the researchers (Satdive et al. 2003; Dhandapani et al. 2008; Ramani and Jayabaskaran 2008; Bakrudeen et al. 2011; Yuan et al. 2011; Kumar et al. 2013; Panigrahi et al. 2018; Sharma et al. 2019). Exceptionally, a lower light intensity of 15–25 μmol/m^2/s (Pati et al. 2011; Sharma et al. 2019) and higher light intensity up to 100 μmol/m^2/s (Junaid et al. 2006; Junaid et al. 2007a, b; Aslam et al. 2008, 2009, 2010b; Maqsood et al. 2012; Rajora et al. 2013; Tonk et al. 2016) were also reported in *C. roseus*. For callus induction, explants were initially kept under dark conditions (Tables 2 and 3).

Photoperiod

Photoperiod is the duration of light to which a plant is exposed in a 24-h cycle. It determines the physiological response of the plant to the relative length of light and dark conditions (Gantait et al. 2018). Almost all the reports in *C. roseus* suggests 16 h light and 8 h dark be the optimum photoperiod (Tables 1, 2, 3, and 4). However, there still exists a few reports wherein the implementation of a 10–14-h photoperiod in the culture room is also mentioned. For instance, Moreno et al. (1994), Hilliou et al. (1999), Shukla et al. (2010), and Panigrahi et al. (2018) carried out experimentations in 12 h light and 12 h dark condition.

Table 4 Factors influencing in vitro regeneration for secondary metabolite production of *Catharanthus roseus* (arranged in chronological order)

Explant	Surface sterilization	Basal media	Carbon source	Solidifying agent	PGR (type and conc., mg/l or μM*)
IVS	70% EtOH 1 min ➔ 1% NaOCl 20 min	MS	3% sucrose	0.55% agarose	NM
IVS	70% EtOH 1 min ➔ 1% NaOCl 20 min	MS	3% sucrose	0.55% agarose	NM
IVS	NM	MS	3% sucrose	1% agarose	0.05 NAA + 2.5 Kn
In vitro hypocotyl	1% (w/v) cetrimide 5 min ➔ 10% NaOCl 20 min ➔ 0.1% (w/v) $HgCl_2$ 5 min ➔ 70% EtOH 3 min	MS	3% sucrose	0.6% agar	2 NAA + 5 BA 0.1 NAA + 5 BA + 1 zeatin
Seedling segment	NM	B5	3% sucrose	0.8% agar	5.88* 2,4-D + 1.16* Kn + 1.34* NAA +
Leaf	NM	MS	3% sucrose	NM	1 2,4-D + 1 IAA + 0.5 Kn
Stem and leaf	NM	MS	4% sucrose	NM	1 NAA + 1 IAA + 0.5 Kn
Stem and leaf	NM	MS	3% sucrose	NM	5.37* NAA + 4.65* Kn
Stem	NM	MS	3% sucrose	NM	5.37* NAA + 4.65 *Kn
Stem and leaf	NM	MS	3% sucrose	NM	2 NAA + 2 IAA + 0.1 Kn
Leaf	70% EtOH ➔ 0.1% $HgCl_2$	MS	3% sucrose	0.65% agar	1 NAA + 1 IAA + 0.1 Kn
Stem	NM	MS	3% sucrose	NM	5.37* NAA + 4.65* Kn 4.52* 2,4-D + 4.65* Kn 5.7* IAA + 4.45* BA
Shoot tip	Dettol 5 min➔70% EtOH 2 min ➔ 0.1% $HgCl_2$ 3 min	MS	3% sucrose	0.25% phytagel	11.42* IAA + 2.22* BA
IVS	70% EtOH ➔ 5% NaOCl	MS	NM	NM	1 BA
Young stem	NM	MS	3% sucrose	NM	2 NAA + 2 IAA + 0.1 Kn
Young stem	NM	MS	3% sucrose	NM	2 NAA + 2 IAA + 0.1 Kn
Young stem	NM	MS	3% sucrose	NM	2 NAA + 2 IAA + 0.1 Kn
Leaf petiole	5% NaOCl 45 min	MS	NM	NM	0.1 NAA + 0.1 Kn
Leaf	0.5% (w/v) Bavistin 20 min ➔ 0.01% (v/v) Tween 20 20 min ➔ 0.1% $HgCl_2$ 5 min	MS	3% sucrose	NM	2 NAA + 0.2 Kn
In vitro hypocotyl	70% EtOH ➔0.5% $HgCl_2$ 2 min ➔ 5% H_2O_2	MS	3% sucrose 3% maltose	NM	6.96* 2,4-D 5.37* NAA + 6.72* BA 2.6* GA_3 2.24* BA
Epicotyl	0.1% $HgCl_2$ 5 min	MS	NM	0.8% Agar	NM
In vitro hypocotyl	70% EtOH ➔0.5% $HgCl_2$ 2 min ➔ 5% H_2O_2	MS	3% maltose	NM	1 2,4-D 1 NAA + 0.5 BA 1 GA_3
Leaf and stem segment	1% Savlon 5 min➔0.1% $HgCl_2$	MS	3% sucrose	6–8% agar	1* NAA + 0.5* BA
In vitro Leaf	Labolene ➔ Savlon ➔ 0.1% $HgCl_2$ 1 min	MS	NM	NM	1 2,4-D + 0.5 BA
Leaf	NM	LS	3% sucrose	NM	0.19 NAA + 0.22 2,4-D
Nodal Segment	Tween 80 ➔ 0.4% NaOCl + a drop of Tween-80 25 min ➔ 0.04% $HgCl_2$ + a drop of Tween-80 7–8 min	MS	3% sucrose	NM	5* BA 5* NAA
Shoot tip	95% NaoCl 10 min ➔ 100% EtOH	MS	3% sucrose	0.8% agar	1.5 2,4-D + 0.5 Kn
In vitro leaf	Bavistine 10 min ➔ few drops of Tween-20 ➔70% EtOH 2 min➔ 0.1% $HgCl_2$ 6–8 min	½ MS	6% sucrose	0.7% agar	0.5 2,4-D + 1 BA
Cell line C4	–	MS	3% sucrose	0.65% agar	0.5* NAA + 0.5 BA
In vitro hypocotyl	NS	MS	NM	0.8% agar	4.52* 2,4-D 6.62* BA + 5.36* NAA 2.89* GA_3 2.22* BA

Table 4 (continued)

In vitro axillary shoot	70% EtOH 30 s ➔ 0.1% $HgCl_2$ 2 min		MS	3% sucrose	0.8% agar	1 BA +0.1 NAA 1 2,4-D + 0.5 BA

Explant	Additives (mg/l or g/l*)	Elicitors	Culture condition [T; LI (lux or µmol/m^2/s PPFD#); PP; RH]	Response	Secondary metabolite estimation	Reference
IVS	NM	NM	25 °C; 5 W/m^2 + NUV 1.5 W/m^2; NM; NM	NM	HPLC; leurosine (23 µg/g FW)	Hirata et al. (1990)
IVS	NM	NM	25 °C; NUV 5 W/m^2; NM; NM	NM	HPLC; leurosine (45 µg/g FW), vinblastine (15 µg/g FW)	Hirata et al. (1992)
IVS	NM	*Pythium aphanidermatum*	25 °C; 1500; 12 h; NM	Multiple shoots	TLC and GC-MS; DHBA	Moreno et al. (1994)
In vitro hypocotyl	1000 CH + 100 asparagine 100 asparagines + 100 Glutamine	NM	26 ± 2 °C; 1045 lx; 10 h; 55%	Callus induction Shoot regeneration	HPLC; vinblastine (1.6 µg/g FW)	Datta and Srivastava (1997)
Seedling segment	NM	NM	NM	Callus (95%)	HPLC; ajmalicine and serpentine	Filippini et al. (2000)
Leaf	1100 KNO_3 + 2 thiamine-HCl + 0.1 riboflavin + 0.1 biotin + 0.1 folic acid	Cerium (CeO_2, $CeCl_3$), yttrium (Y_2O_3), and neodymium ($NdCl_3$)	23 ± 2 °C; dark; NM;NM	Callus induction	RPHPLC; ajmalicine and catharanthine	Zhao et al. (2000a)
Stem and leaf	NM	6 g/l KCl 300 mM Mannitol, 1.5% Sodium alginate, 0.2% PVP 0.3% PVP	23 ± 2 °C; dark; NM;NM	Callus induction	HPLC; catharanthine, ajmalicine Ajmalicine Catharanthine	Zhao et al., (2000b)
Stem and leaf	1100 KNO_3 + 12 thiamine HCl + 0.1 riboflavin + 0.1 biotin + 0.1 folic acid	NM	23 ± 2 °C; dark with 45 min/day fluorescent light; NM;NM	compact callus clusters	HPLC; ajmalicine, serpentine and catharanthine	Zhao et al. (2001d)
Stem	1100 KNO_3 + 12 thiamine HCl + 0.1 riboflavin + 0.1 biotin + 0.1 folic acid	250 mM mannitol, 4 g/l KCl, 60 mg/l TAB, 10 mM succinic acid, 300 mg/l tryptamine, 500 mg/l, tryptamine	23 ± 2 °C; dark; NM;NM	compact callus cluster	HPLC; catharanthine, ajmalicine, serpentine	Zhao et al. (2001a)
Stem and leaf	NM	TAB and *Aspergillum niger* Mannitol and sodium alginate	23 ± 2 °C; dark; NM;NM	Callus induction	Ajmalicine yield (63 mg/l) Catharanthine yield (26 mg/l)	Zhao et al. (2001c)
Leaf	NM	NM	23 ± 2 °C; dark; NM;NM	Callus induction	HPLC; catharanthine ajmalicine, vindoline, serpentine	Zhao et al. (2001b)
Stem	1100 KNO_3 + 12 thiamine HCl + 0.1 riboflavin + 0.1 biotin + 0.1 folic acid	NM	23 ± 2 °C; dark; NM;NM	Compact callus cluster Increase in biomass Increase in TAC	HPLC; ajmalicine, serpentine and catharanthine	Zhao et al. (2001e)

Table 4 (continued)

Shoot tip	NM	NM	26 ± 2 °C; 50#; 16 h, NM	Multiple shoot	HPLC; Ajmalicine (0.166% dry wt.)	Satdive et al. (2003)
IVS	NM	0.1 μM methyl jasmonate	25 °C; NM; 16 h, NM	Multiple shoot	HPLC; 10-fold vindoline (2 mg/g dry wt.)	Hernández-Domínguez et al. (2004)
Young stem	NM	0.5 mmol/l nitric oxide (NO) donor SNP	25 °C; dark; NM; NM	Callus induction	TLC, co-elution and HPLC-diode array detection; catharanthine (3-fold)	Xu et al. (2005)
Young stem	NM	*Aspergillus niger*	25 °C; dark; NM; NM	Callus induction	HPLC; catharanthine (3.1-fold)	Xu and Dong (2005b)
Young stem	NM	10 mmol/l nitric oxide (NO) donor SNP	25 °C; dark; NM; NM	Callus induction	HPLC; ajmalicine, catharanthine and total alkaloids (1.6-, 2.9-, and 1.8-fold)	Xu and Dong (2005a)
Leaf petiole	NM	NM	35 °C; dark; NM;NM 35 °C; 7 W/m^2; 24 h; NM	Callus and root	TLC and HPLC; vindoline, catharanthine, vincristine, vinblastine	Ataei- Azimi et al. (2008)
Leaf	NM	UV-B	25 ± 2 °C; 40#; 16 h; NM	Callus	HPLC; catharanthine (3-fold) and vindoline (12-fold)	Ramani and Jayabaskaran (2008)
In vitro hypocotyl	NM	NM	25 ± 2 °C; 100#; 16 h; NM	Callus Induction SE initiation and proliferation SE maturation SE Germination	HPLC; vincristine	Aslam et al. (2009)
Epicotyl	NM	*Agrobacterium tumefaciens*	25 ± 2 °C; NM; 16 h; 80%	Shooty teratomas	HPLC; vincristine	Begum et al. (2009)
In vitro hypocotyl	NM	NM	25 ± 2 °C; 100#; 16 h; NM	Callusing SE initiation and proliferation SE maturation	HPLC; vinblastine	Aslam et al. (2010b)
Leaf and stem segment	NM	NM	25 ± 1 °C; 2000–3000; 16 h; NM	NM	HPLC; vincristine (20.38 mg/g DW)	Kalidass et al. (2010)
In vitro Leaf	NM	*Pytium aphanidermatum*, Methyl Jasmonate	25 °C; 1200; 12 h; NM	Callus induction	HPLC; catharanthine and vindoline	Shukla et al. (2010)
Leaf	0.4 thiamine	50 mM β-CD + 100 μM MJ + UV 15 min	25 °C; Dark; NM; NM	NM	HPLC; ajmalicine	Almagro et al. (2011)
Nodal Segment	NM	NM	25 ± 2 °C; 20 ± 5#; 14 h; NM	Multiple shoots Root induction	HPLC; TIAs, vincristine and vinblastine	Pati et al. (2011)
Shoot tip	NM	NM	25 ± 1 °C; NM; 16 h; NM	Callus induction	TLC	Saifullah and Khan (2011)
In vitro leaf	NM	NM	25 ± 2 °C; NM; 16 h; NM	Increase in callus biomass	Dragendroff's reagent; total alkaloid content increased	Verma et al. (2012)
Cell line C4	NM	20 μg/l hydrogen peroxide 30 μg/l acetyl CoA	25 ± 2 °C; dark; NM; NM	Cell growth	HPLC; taberosine (9.02 mg/g DW) Vindoline (0.33 mg/g DW)	Guo et al. (2013)

Table 4 (continued)

		0.5 μmol/l benzotriazole			Vindoline (0.42 mg/g DW)		
		5 mg/l acetyl CoA + 20 μg/l H_2O_2 + 0.5 μmol/l benzotrizole + 100 mg/l tryptophan + 100 mg/l loganin + 30 mg/l cerium chloride			Vindoline (0.81 mg/g DW)		
In vitro hypocotyl	NM	0.15% *Aspergillus flavous*	25 ± 2 °C; 100#; 16 h; NM	Callus induction	HPTLC; vincristine and vinblastine	Tonk et al. (2016)	
				Embryo proliferation			
				Embryo maturation			
				Embryo germination			
In vitro axillary shoot	0.38 Thymine HCl	3 g/l KCl, 300 triptamine, 500 tryptophan, 5 g/l KCl	25 ± 2 °C; 48#; 16 h; NM; 15#; NM; NM	Multiple shoot	HPLC; vindoline, vinblastine, catharanthine	Sharma et al. (2019)	
				Callus induction			

2,4-D 2,4 dichlorophenoxyacetic acid, *B5* Gamborg's B5 medium (Gamborg et al. 1968), *BA* N^6-benzyladenine, *BAP* N^6-benzylaminopurine, *CD* cyclodextrin, *CH* casein hydrolysate, GA_3 gibberellic acid, *IAA* indole acetic acid, *Kn* kinetin, *LI* light intensity, *MS* Murashige and Skoog (1962), *NAA* α-naphthalene acetic acid, *NM* not mentioned, *PGR* plant growth regulator, *PP* photoperiod, *RH* relative humidity, *IVS* in vitro raised seedlings, *HPLC* high performance liquid chromatography, *NUV* near ultraviolet, *FW* fresh weight, *TLC* thin-layer liquid chromatography, *GC-MS* gas chromatography-mass spectrometry, *LS* Linsmaier and Skoog (1965) medium, *PVP* polyvinyl pyrrolidone, *TAB* tetramethyl ammonium bromide, *SNP* sodium nitropruside, *MJ* methyl jasmonate, *SE* somatic embryo, *DW* dry weight, *TIA* terpenoid indole alkaloid

Relative humidity

Optimum relative humidity (RH) in a culture room plays a major part in the growth of plantlets. Very high or very low relative humidity affects the transpiration process, which has detrimental effects on plantlets (Gantait et al. 2018). In spite of being an important factor for culture conditions, relative humidity was not mentioned in the majority of reports. Whereas, 50–60% RH was maintained by some researchers (Datta and Srivastava 1997; Ilah et al. 2009; Bakrudeen et al. 2011; Malabadi et al. 2012); and in only one study implementation of 80% RH (Begum et al. 2009) was reported.

PGRs

PRGs are an integral part of plant tissue culture, which influences the growth and development of plantlets in in vitro culture. Among all PGRs, the proportions of auxin and cytokinin utilized solely or together are indispensable factors for any in vitro experiment (Gantait and Kundu 2017a). Auxins and cytokinins are mainly used for in vitro experiments in plant tissue culture.

Direct regeneration

Direct regeneration refers to the induction of shoots and roots without any callogenesis, eventually leading to complete plantlet development from explant. PGRs added to the culture medium decide whether an explant will regenerate via direct or indirect regeneration (Gantait and Kundu 2017b). Various literature available on *C. roseus* indicated the combination of cytokinin and auxin to be very effectual in the case of shoot initiation and proliferation. Adequate number of shoots inducing from a single shoot tip was reported in the medium containing a higher dose of cytokinin and a lower dose of auxin (Moreno et al. 1994; Yuan et al. 1994; Mitra et al. 1998; Srivastava et al. 2009; Verma and Mathur 2011; Kumar et al. 2013; Al-Oubaidi and Mohammed-Ameen 2014; Sharma et al. 2019). Out of all the combinations of auxin and cytokinin used for shoot proliferation (Fig. 4a), N^6-benzyladenine (BA) + α-naphthalene acetic acid (NAA) was found to be the most promising one. The shootlets were induced for rooting (Fig. 4b) in auxin supplemented medium. The usage of a novel class of cytokinins namely *meta*-topolin have been seldom reported. Indole-3-butyric acid (IBA) was commonly used by many researchers (Bakrudeen et al. 2011; Verma and Mathur 2011; Mehta et al. 2013; Rajora et al. 2013; Rahmatzadeh et al. 2014; Begum and Mathur 2014). Exceptionally, Kumar et al. (2013) found rooting in PGR-free MS medium supplemented with 0.25 g/l activated charcoal.

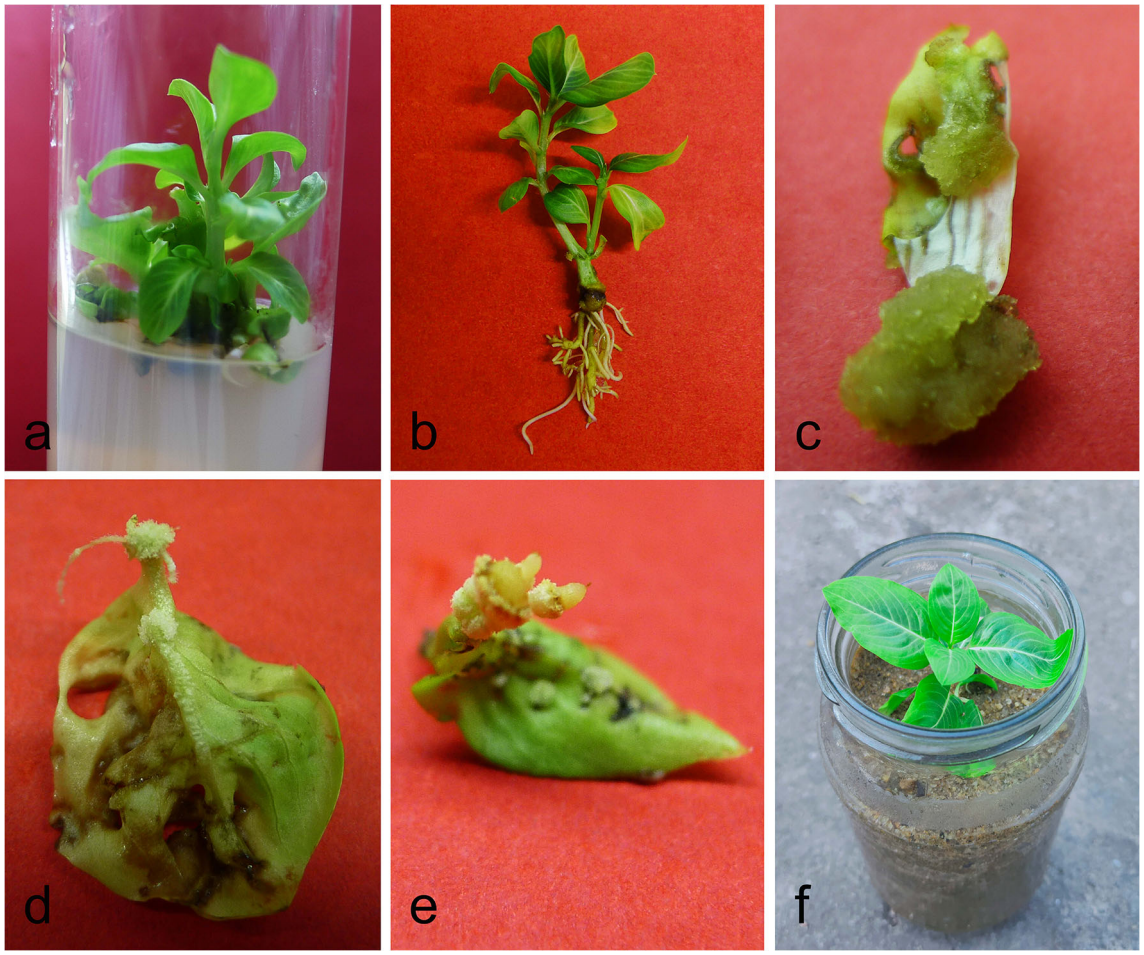

Fig. 4 In vitro regeneration of *Catharanthus roseus*. **a** Multiplication and proliferation of shoots from shoot tip explants, **b** fully grown in vitro plantlet with multiple shoots and roots, **c** development of organogenic calli from leaf explants, **d**, **e** induction of multiple adventitious shoots and roots from calli via indirect regeneration procedure, **f** acclimatized plantlet on autoclaved sand (Photographs are not in scale) (source: unpublished photographs of Anamika Das)

Indirect regeneration

Indirect regeneration indicates to the process of organogenesis via callus formation from the explant. The proper dose and type of PGRs induce callogenesis in the explant and provide direction to the morphogenesis. Generally, auxins are known for their capability to initiate cell division and meristem formation, which eventually results in the formation of callus (Gantait et al. 2018). It is evident from the reports available on *Catharanthus* that auxin in combination with cytokinin, in lower dose, induces callus (Fig. 4c) from the explant. Out of all the auxins available, NAA or 2,4-D was commonly used for callus induction (Table 2). The combination of NAA+ kinetin (Kn) proved to be effectual during callus induction, as reported by several researchers (Kim et al. 1994; Hilliou et al. 1999; Zhao et al. 2001a, b, c, d, e; Ataei-Azimi et al. 2008; Ramani and Jayabaskaran 2008; Moghe et al. 2016), whereas some researchers added indole acetic acid (IAA) along with NAA+Kn (Zhao et al. 2000b, 2001b, c; Xu et al. 2005; Xu and Dong 2005a, b) as well. Similary, Filippini et al. (2000) found that 2,4 dichlorophenoxyacetic acid (2,4-D) when used along NAA+Kn, resulted in 95% callogenesis. Datta and Srivastava (1997) found callus induction from in vitro hypocotyl on NAA+BA supplemented media, and calli regenerated into shoots on addition of zeatin with NAA+BA supplemented media. Thereafter, cytokinins were used for shoot induction and proliferation from the induced calli, and auxins were used for rooting. The use of dicamba or picloram (which mimic the activity of auxin) is not yet been reported.

Somatic embryogenesis

The process of plant regeneration from the embryos that are derived from somatic cells is known as somatic embryogenesis. In *C. roseus*, regeneration of plant via somatic embryogenesis has not been much explored by researchers. It is noticeable from Table 3 that almost all the researchers have

reported the sole use of 2,4-D or in amalgamation with other PGRs (mainly auxins) to develop the embryogenic calli (Fig. 4e). For instance, Kim et al. (2004) observed the development of 20% somatic embryos from immature zygotic embryos on 4.52 μM 2,4-D supplemented MS medium. By using in vitro hypocotyls as explant, Junaid et al. (2006) reported that 2,4-D as low as 1 mg/l was capable of inducing embryogenic calli, whereas a higher dose of 2,4-D (6.96 μM) was used by Aslam et al. (2008) for the same. Exceptionally, Ilah et al. (2009) managed to develop somatic embryos by using 2,4-D + BA+ gibberellic acid (GA_3). Dhandapani et al. (2008) reported callus regeneration through somatic embryogenesis by using thidiazuron (TDZ). The combination of BA+NAA was reported to have been used to initiate somatic embryogenesis and proliferation of somatic embryos (Junaid et al. 2007a, b; Aslam et al. 2008; Aslam et al. 2009, 2010a, b; Yuan et al. 2011). Malabadi et al. (2012) used triacontanol (TRIA) along with 2,4-D to induce calli and abscisic acid (ABA) for maturation of somatic embryos. Although, reports on direct somatic embryogenesis are still not available, microscopic studies to categorize the different stages of embryo can be considered in its place.

Media additives

A basal medium enriched with proper PGR, depending on the objective, acts as a suitable culture medium for an experiment. The response of explants in suitable media can be further boosted up by supplementing some additives to the media. These additives can be various vitamins, amino acids, inorganic salts, antibiotics, etc. Across various reports available on *C. roseus*, Yuan et al. (1994) added casein hydrolysate to the culture media in a concentration as low as 1 mg/l, whereas Srivastava et al. (2009) used a much higher concentration of 500 mg/l for induction of multiple shoots. Vitamins like thiamine or thiamine HCl, biotin, folic acid, and riboflavin are also used as additives. For instance, Zhao et al. (2001a, b, e) added thiamine HCl, biotin, folic acid, riboflavin, and inorganic salt KNO_3 to the MS medium supplemented with NAA+Kn and found clusters of compact calli. Exceptionally, precocious flowering with multiple shoots was reported with the use of $AgNO_3$ as media additive by Panigrahi et al. (2018). Amino acids like glycine, tryptophan, asparagine, and glutamine were also reported to be used as additives. Rahmatzadeh et al. (2014) reported the use of 250 mg/l tryptophan for shoot proliferation and 350 mg/l tryptophan for root induction. 1000 mg/l casein hydrolysate with 100 mg/l asparagine was used as an additive for callus induction experiment and 100 mg/l asparagine with 100 mg/l glutamine was used for shoot regeneration from the induced callus (Datta and Srivastava 1997).

Acclimatization

The complete plantlets with proper roots are taken out from the general culture conditions in the growth chamber, and subsequently acclimatized to withstand the external environment. Eventually, the success of a micropropagation experiment is decided by a successful acclimatization of plantlets in soil or potting mixture and their survival percentage (Mukherjee et al. 2019). The procedure followed for the acclimatization of in vitro grown plantlets was not specifically mentioned in the literature available on *C. roseus*. But a variety of substrates such as vermiculite, peat moss, potting mixture along with soil or sand (Fig. 4f) has been mentioned for acclimatization, with a survival percentage of 60–100%. For instance, Zárate et al. (1999) used gardening peat soil for acclimatization of plantlets and noted the survival percentage to be ~98% under high humidity conditions in the growth chamber. Potting soil was used for acclimatization of in vitro regenerated plantlets by Kim et al. (2004), Choi et al. (2004) and Yuan et al. (2011), securing 90–100% survival. Dhandapani et al. (2008) acclimatized the plantlets in sterile potting mixture in culture room, and later shifted them to the greenhouse, whereas, Swanberg and Dai (2008) also used similar potting mixture and noted 60% survival. Soil alone, as a substrate, was used for acclimatization by Verma and Mathur (2011) and Rajora et al. (2013). Many researchers used a mixture of two or more components for acclimatization process. For instance, Junaid et al. (2007a) and Aslam et al. (2008) transplanted the plantlets to sterile soil rite at first, and subsequently transferred them to soil rite: sand (1:1), before their final transfer to normal soil, which resulted in 100% survival of the plantlets. Bakrudeen et al. (2011) acclimatized the plantlets in a mixture of sterile garden soil, sand and vermiculite in equal proportions. A simple mix of sand and soil (1:1; v/v) was used by Pati et al. (2011) and Kumar et al. (2013).

Clonal fidelity assessment

The clonal fidelity assessment of in vitro regenerated plantlets is a pre-requisite in micropropagation of any crop species, since it ensures the genetic uniformity of the regenerants (Gantait et al. 2014). The genetic clonality or clonal fidelity can be validated by various methods, such as micro morphological studies, cytological studies, and molecular markers. At present, molecular markers are in use for validation of clonal fidelity of the micropropagated plants, as they are not affected by growth stages or external factors. There are various kinds of molecular markers available, but predominantly, RAPD and ISSR (Fig. 5a–c) are used for clonal fidelity assessment, since prior knowledge of sequencing is not required in their cases. In *Catharanthus*, there are not many reports available on the assessment of genetic fidelity of in vitro regenerants.

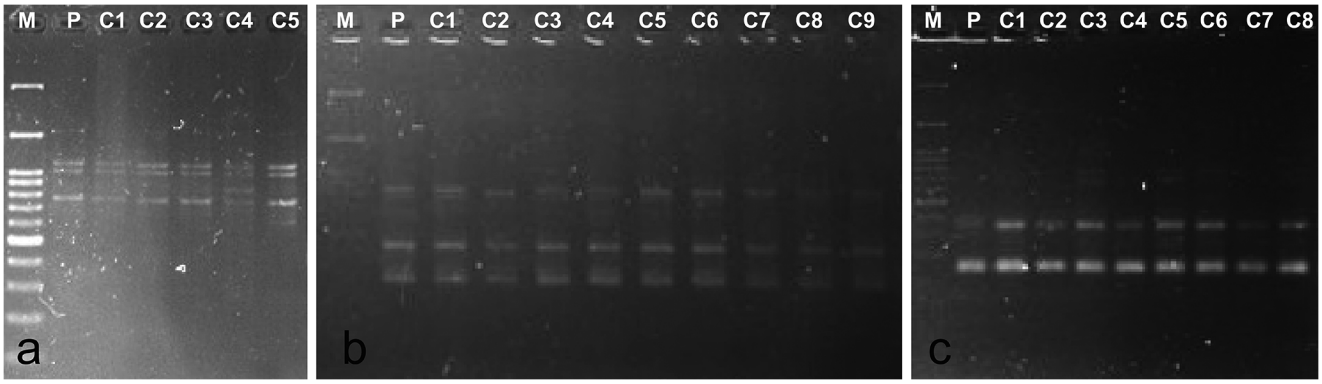

Fig. 5 Clonal fidelity of in vitro regenerated plantlets of *Catharanthus roseus* revealed by **a** RAPD (TDG-CU-5) (TGCGGCTGAG), **b**, **c** ISSR primers [18(GT) and 65(AG)] (GTGTGTGT and AGAGAGAG) (source: unpublished photographs of Anamika Das)

Srivastava et al. (2009) reported monomorphic banding showing the uniformity of regenerants by using 17 RAPD primers. Similarly, with the use of 20 RAPD markers, 21 randomly selected in vitro raised plants and the mother plant exhibited monomorphic banding patterns (Kumar et al. 2013). While checking the genetic stability of transgenic *C. roseus* after prolonged maintenance of 5 years, Verma et al. (2014) found that out of total 254 bands, 72.1% were monomorphic and 27.9% were polymorphic, by using 17 ISSR primers. As compared to RAPD primers, ISSR primers are promising in terms of detecting even low levels of genetic variations and higher reproducibility (Verma et al. 2015).

In vitro secondary metabolite production

The secondary metabolites of *C. roseus* possess some medicinal properties for which they have been in use from time immemorial. This medicinal plant produces a number of terpenoid indole alkaloids, which are used in pharmaceuticals. Some of them are vindoline, vinblastine, catharanthine, vincristine, ajmalicine, reserpine, serpentine, horhammericine, tabersonine, leurosine, and lochnerine. The extraction of these alkaloids produced in plant cells under in vitro condition assures better quality and quantity than conventionally grown plants. There are a number of reports available on the estimation of various secondary metabolites production or accumulation in *C. roseus* (Table 4). The increase in leurosine (45 μg/g fresh weight) and vinblastine (15 μg/g fresh weight) from the in vitro grown seedlings was recorded by high-performance liquid chromatography (HPLC), with the use of near UV light detector (Hirata et al. 1992). Moreno et al. (1994) found an increase in phenol, DHBA, by using fungal concentrate of *Pythium aphanidermatum*. Some of the researchers reported elevation in the accumulation of one or more secondary metabolites during in vitro callus culture and during indirect regeneration from calli (Datta and Srivastava 1997; Filippini et al. 2000; Zhao et al. 2001a, b, c). Zhao et al. (2000a) used some rare-earth elements like cerium, yttrium, and neodymium for induction of callus from leaves, and reported an increase in ajmalicine and catharanthine production. Abiotic elicitors such as KCl, mannitol, sodium alginate, and PVC were used and reported to have an impact on catharanthine and ajmalicine production in *C. roseus* (Zhao et al. 2000b). Many other elicitors were also used for the enhancement of secondary metabolites in *C. roseus* (Table 4). For instance, Hernández-Domínguez et al. (2004) reported a tenfold increase in vindoline, by carrying out multiple shoot cultures of *C. roseus* on media supplemented with methyl jasmonate. Similarly, threefold enhancement in catharanthine was recorded by the use of SNP as an elicitor (Xu et al. 2005). In some of the literature on *C. roseus*, use of biotic elicitors such as fungal concentrate was also reported for effectively enhancing secondary metabolites under in vitro conditions (Moreno et al. 1994; Zhao et al. 2001c; Xu and Dong 2005a, b; Begum et al. 2009; Shukla et al. 2010; Tonk et al. 2016). For the estimation of secondary metabolites in *C. roseus*, the majority of the researchers used HPLC (Table 4). Exceptionally, Tonk et al. (2016) estimated the enhancement in vincristine and vinblastine by using biotic elicitor *Aspergillus flavous* via high-performance thin-layer chromatography (HPTLC).

Nanotechnology

A nanometer (10^{-9}) is one billionth of a meter. Manipulation of any matter at this length scale is called nanotechnology. In recent times, nanotechnology is attracting researchers due to its wide application in the field of medicine. Nanoparticles synthesized using plant system is getting popular since it is cost effective, eco-friendly, safe, and involves single-step method when compared to the more complex chemical and physical methods of nanoparticle synthesis (Ponarulselvam et al. 2012). A number of nanoparticles were synthesized using extracts of *C. roseus*. For example, silver nanoparticles were reported to have been synthesized from leaf extracts (Ponarulselvam et al. 2012; Sheshadri et al. 2015; Ghozali

Table 5 Factors influencing hairy root culture in *Catharanthus roseus* (arranged in chronological order)

Explant	Basal medium for induction	Carbon source	*A. rhizogenes* strain	Antibiotics(g/l or mg/ml*)	Additives/elicitors/ PGR	Basal medium for maintenance	Secondary metabolite estimation	Response	References
Hypocotyls segment	SH	3% sucrose 3% fructose	strain 15,834	0.5 carbenicillin	NA	1/3 SH	Catharnathine ajmalicine	40% higher growth rate 2-fold increase in Catharanhine	Jung et al. (1992)
In vitro grown seedling	YEM agar medium	3% sucrose	ATCC 15834	0.25 cefotaxime	NA	½ B5	HPLC; ajmalicine, serpentine, catharanthine, and vindoline	3-fold increase in vidoline	Bhadra et al. (1993)
In vitro grown seedling	Yeast extract-mannitol amino acid	3% sucrose	R1000CbR	1* carbenicillin or cephatoxime	NA	½ B5	Ajmalicine and serpentine	Increased TIAs	Ciau-uitz et al. (1994)
In vitro grown seedling	YEB agar medium	3% suc rose	ATCC 15834	0.5 g carbenicillin/l	0.01 g/l *A. niger* and *Penicillium* sp.	1/3 SH	TLC and HPLC; catharnathine ajmalicine	2.5 times increase in catharanthine	Sim et al. (1994)
Leaf disks	B5	2% sucrose	Strain K599	0.5* carbenicillin	0.5 mM 2,4- -- dichlorophenoxy-acetic acid (2,4-D)	B5	TLC and HPLC/MS; ajmalicine, vindoline	Stable production of vindoline	O'Keefe et al. (1997)
Hypocotyls	Yeast extract peptone medium	3% sucrose	RI000	0.4 cefotaxime	13.32 μM BA + 5.37 μM NAA	1/3 SH	NA	Adventitious shoots from hairy roots	Choi et al. (2004)
In vitro grown seedlings	YEM	3% sucrose	15,834.	0.05 kannamycin	NA	½ B5	HPLC; tryptamine and serpentine	NM	Hughes et al. (2004)
Leaf and stem	LB ½ MS	6% sucrose	K599	0.05 kannamycin	NA	MS Cefotaxime	HPLC; vincristine and catharanthine	NM	Hanafy et al. (2016)

B5 Gamborg's B5 medium (Gamborg et al. 1968), *BA* N^6 -benzylaminopurine, *HPLC* high performance liquid chromatography, *LB* Luria Broth, *MS* Murashige and Skoog (1962), *NA* not applicable, *NAA* α-naphthalene acetic acid, *NM* not mentioned, *SH* Schenk and Hildebrandt (1972), *TLC* thin-layer liquid chromatography; *TIA* total indole alkaloids, *YEM* yeast extract mannitol, *YEB* yeast extract broth

et al. 2015; Al-Shmgani et al. 2017; Pavunraj et al. 2017), root extracts (Rajagopal et al. 2015), and flower extracts (Raja et al. 2016); and titanium dioxide nanoparticles (Velayutham et al. 2012) and chitosan nanoparticles (Nagaonkar et al. 2015), gold nanoparticles (Shittu et al. 2017) from leaf extracts, and copper oxide nanoparticles from flower extracts (Baskar et al. 2016) were also reported. The involvement of in vitro techniques in synthesis of nanoparticles in case of *C. roseus* is limited. For instance, synthesis of silver nanoparticles having antibacterial activity, using roots, leaves, and callus from in vitro-derived plants (Malabadi et al. 2012) and seed-derived callus (Osibe et al. 2018) was also carried out. Riaz et al. (2018) reported improved phytochemical production and rapid synthesis of zinc oxide nanoparticles in callus culture of *C. roseus* when melatonin was supplemented. Ghasempour et al. (2019) recorded an increase in leaf size, root length and total plant biomass by growing seeds in MS medium supplemented with multi-walled carbon nanotubes.

Hairy root cultures

Hairy root culture has emerged as an extensively used approach for the production of pharmaceutically important secondary metabolites in medicinal plants, over the past three decades. Hairy roots are neoplastic roots, capable of rapid multiplication and growth in PGR-free culture media, with stable genetic and biochemical profile (Shanks and Morgan 1999). Hairy roots are induced when a wounded plant is infected by *Agrobacterium rhizogenes,* a Gram-negative soil bacterium, which transfers the T-DNA from its *Ri* plasmid into the nuclear DNA of the infected plant. The T-DNA encodes the enzyme, which is responsible for the biosynthesis of cytokinin and auxin that eventually induces the hairy roots (Guillon et al. 2006). Till date, there are many reports available on hairy root induction in *C. roseus* for secondary metabolite production (Table 5). For hairy root culture, in vitro grown seedlings have been used as explant by the majority of researchers (Bhadra et al. 1993; Ciau-uitz et al. 1994; Sim et al. 1994; Hughes et al. 2004), whereas in some reports leaf or stem (O'Keefe et al. 1997; Hanafy et al. 2016) and hypocotyl segment (Jung et al. 1992; Choi et al. 2004) were also used as explants. In most of the reports, *A. rhizogene* strain ATCC 15834 or K599 was used for induction of hairy roots and the antibiotics used were either carbenicillin, cefotaxime or kanamycin (Table 5). The different types of basal media that were used for induction are SH, YEM, LB, B5, etc.; however, for maintenance of hairy roots, one-third strength of SH medium or half strength of B5 medium was used by majority. Molecular confirmation of *rol* A, *rol* B, *rol* C and *vir* C gene along with southern blot analysis was done by Choi et al. (2004). Hanafy et al. (2016) amplified the HPTII, GUS and GFP fragments by PCR analysis of transformed hairy roots. Till date, development of hairy root cultures for enhancement of secondary metabolites in *C. roseus* served as a better alternative for large-scale production of valuable alkaloids.

Conclusion and future scope

With the emergence of cancer, a most pronounced disease in today's world, medicinal plants with anticancerous secondary metabolites have become a source of life-saving drugs. Till date, many biotechnological interventions have been used significantly in *C. roseus* to provide its useful secondary metabolites for mankind. Still, there are not many reports in *C. roseus* for some of the biotechnological advancements which can harbor scope for future research. Despite having a number of reports on induction of somatic embryos, only Maqsood et al. (2012) have developed a method of synthetic seed production from somatic embryos derived from hypocotyls; and there is no other report on synthetic seed production, its storage and exchange in *C. roseus* till date. According to Dhawan and Lavania (1996), induced polyploidy can bring enhancement in the quality and/or quantity of secondary metabolites of plants. Therefore, it can act as a rapid means to increase the secondary metabolites having pharmaceutical importance, in medicinal plants like *C. roseus*. In the last two decades, there are fewer reports on induced polyploidy in *C. roseus*. For instance, Kobza and Qing (2000) developed tetraploid and mixoploid plants of *C. roseus* by treating seeds with 0.1–0.4% colchicine. Xiang et al. (2010) found increased ajmalicine content in callus, induced from leaves of the tetraploid plant, which were raised from seeds treated with 0.04% (*v*/v) colchicine. Xing et al. (2011) reported the use of 0.2% colchicine for 24 h to induce tetraploidy, and recorded an increase in three alkaloids, namely, vindoline, catharanthine and vinblastine in tetraploids when compared to diploid plants. Some other researchers also induced polyploidy in *C. roseus* by using colchicine, but none of them used in vitro conditions (Hosseini et al. 2013; Hosseini et al. 2018; Shala and Deng 2018). Therefore, induction of polyploidy under in vitro conditions throughout can be attempted with various antimitotic agents (such as oryzalin) other than colchicine.

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Author contributions SG conceived the idea of the review. AD and SG surveyed the literature and drafted the initial manuscript. SG, SS, and SB scrutinized and corrected the manuscript to its final version. All the authors read and approved the final version of the manuscript prior to its submission.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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

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