



Micropropagation of endemic *Corynandra chelidonii* var. *pallae* (Cleomaceae) through nodal explants and validation of their genetic integrity by ISSR markers

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Abstract

Micropropagation through nodal explants was attempted for a rare and endemic taxon *Corynandra chelidonii* var. *pallae* (Cleomaceae) in view of its medicinal uses, bioactive compounds, declining natural populations due to intermittent sterility, low seed set and erratic seed germination. Multiple shoots were regenerated directly from the nodal explants on Murashige and Skoog's medium containing different concentrations and combinations of cytokinins and auxins. High-frequency multiple shoots of 8.15 ± 0.249 with 1.63 ± 0.031 shoot length (cm) were obtained on MS medium augmented with the combination of 0.5 mg/L of thidiazuron and 1.0 mg/L of indole-3-acetic acid. Directly regenerated shoots were rooted with 95% root induction on a half-strength MS medium supplemented with 1.0 mg/L of indole-3 butyric acid. The in vitro raised plantlets were hardened in plastic pots and acclimatized initially in the greenhouse conditions and later transferred to the field conditions with a 78% of survival rate. The genetic fidelity of in vitro generated and field transferred plantlets with that of the mother plant was checked by carrying ISSR marker-based polymerase chain reaction method. This protocol on direct organogenesis and genetic fidelity analysis in *Corynandra chelidonii* var. *pallae* can be successfully employed for large-scale multiplication and conservation.

Keywords *Corynandra chelidonii* var. *pallae* · Genetic fidelity analysis · ISSR · Organogenesis · Plant regeneration

Abbreviations

BAP	6-Benzyl amino purine
cm	Centimeter
DMRT	Duncan's multiple range tests
HCl	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
ISSR	Inter simple sequence repeats
Kn	Kinetin
mg/L	Milligram per liter
MS	Murashige and Skoog

NaOH	Sodium hydroxide
PCR	Polymerase chain reaction
PGR	Plant growth regulators
TDZ	Thidiazuron

Introduction

Corynandra chelidonii (L.f.) Cochrane & Iltis (Cleomaceae) is as an important medicinal plant with its multiple usages in the traditional medicine to treat bronchitis, cough, diarrhea, flu, fever, headache, inflammation, liver diseases, malaria, nephritis, skin diseases, and snakebite (Chi and Hop 2002). In India, this medicinal species is used to treat dysentery, otitis, and rheumatism (Nguyen et al. 2017), while the seeds are used as a condiment by local people (Sirangi et al. 2020).

The pharmacological investigations on *C. chelidonii* has revealed its potential as anti-helmenthic (Rekha et al. 2019), anti-hyperglycemic (Trilochana et al. 2017), anti-inflammatory (Ethadi et al. 2013; Juarez-Vazquez et al. 2019), anti-microbial (Sridhar et al. 2014), hepatoprotective activity (Begum and Kiran 2016; Phan et al. 2021), anti-nociceptive

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(Sheeba et al. 2019), anti-oxidant (Sumitha and Gurulakshmi 2015; Nguyen et al. 2017) and anti-pyretric (Pari-malakrishnan et al. 2007). The phytochemical investigation of *C. chelidonii* has led to finding the bioactive compounds like anthraquinones, flavonoid glycosides (cleomeside A, B), glucocapparin, glucocleomin, glucosinolates, quercetins, isoquercetins, and kaempferol, reducing sugars, sterols, and saponins (Barakat et al. 1991; Songsak and Lockwood 2002, 2004; El-Sayed et al. 2010; Quang et al. 2011; Wei et al. 2011; Minh et al. 2015; Phan et al. 2015).

Cleome chelidonii var. *pallae* Reddy & Raju is an endemic taxon which is identified and described from the fringes of Pakhal reservoir, Mahabubabad district, Telangana state, India (Reddy and Raju 2001; Sirangi et al. 2020), based on morphology and molecular markers, clearly established that it is a distinct taxon as a variety under *Corynandara*, as *C. chelidoniin* var. *pallae* (C.S.Reddy & V.S.Raju) V.S.Raju. The seeds of this taxon are being used as a condiment by the local traditional people like Koyas. The natural population of this medicinal plant species is declining due to intermittent sterility, low seed set, and erratic seed germination (Sirangi et al. 2020). According to the literature, so far there are no reports on its vegetative propagation of this rare medicinal plant by stem cuttings or otherwise.

Tissue culture-based propagation is a viable alternative to propagate and conserve this medicinally important plant species by using less space, low cost, and less labour (Devarumath et al. 2007). In vitro based micropropagation protocols are being employed increasingly for the multiplication and conservation of medicinally important plant species. In view of its limited natural spread and advantages concerned with in vitro micropropagation technique, the present study was undertaken to micropropagate the medicinally important local variety, i.e., *Corynandra chelidonii* var. *pallae*, using nodal explants. The genetic uniformity among the regenerated and field acclimatized plantlets was confirmed with ISSR marker-based molecular analysis.

Materials and methods

Plant material and explant preparation

The plant material of *Corynandra chelidonii* var. *pallae* was collected from the Pakhal Reservoir (17.9526° N; 80.0060° E) of Mahabubabad district, Telangana state, India. The taxon was identified and authenticated by Dr. Vatsavaya S. Raju, Department of Botany, Kakatiya University, Warangal. The voucher specimen was deposited in KUNW. Two to three centimeter-long nodal segments, each with a single node, were used as explants. They were washed thoroughly under running tap water for 5 min to remove the dust. These explants were treated with 0.1% aqueous solution of Bavistin

(w/v) (systemic fungicide procured from BASF, India Ltd.) for 5–7 min and rinsed with distilled water for several times. Final surface sterilization of explants was carried with 0.1% (w/v) HgCl₂ (Hi-Media, India) inside the laminar airflow chamber for 6 min. The traces of mercuric chloride of explants were removed by washing continuously with sterile distilled water.

Growth media and culture conditions

The MS basal medium (Murashige and Skoog 1962), added with 3% sucrose, 0.8% agar, 100 mg/L myoinositol (Hi-Media, India), was used for all the experiments. All the basic salts, vitamins, sucrose, and plant growth regulators used for the preparation of the culture medium were of analytical grade and procured from Hi-Media, India. The pH of the culture medium was adjusted to 5.80 ± 0.05 . The basal medium was supplemented with different concentrations and with combinations of plant growth regulators. Culture tubes (Borosil, India) were filled with 10 mL of prepared medium and autoclaved with 1.1 kg/cm² pressure at 121 °C for 15–20 min. The incubation room was maintained at 26 ± 2 °C temperature, and the inoculated cultures were kept in the culture room for a photoperiod of 16/8 h (light/dark), under 50 μmol/m²/s² irradiance with fluorescent tubes while 50% of humidity was maintained. The ISSR primers used for genetic fidelity analysis were procured from GCC Biotech, India.

Bud breaking and elongation of shoots

For bud breaking from nodal explants, the sterilized explants were inoculated and cultured on MS medium supplemented with cytokinins such as BAP, Kn and TDZ ranging from 0.5 to 2.0 mg/L alone or combined with 0.5–2.0 mg/L of IAA.

Rooting and acclimatization

The rhizogenesis stage is very important for proper rooting since root development directly influences the further growth of in vitro raised plantlets. The well-developed and elongated shoots of 6-week-old (length 2–3 cm) were excised aseptically and cultured on auxin-rich MS media to induce rhizogenesis. Two types of auxins, IAA and IBA with different concentrations (0.5, 1 and 2.0 mg/L) in half strength of MS and full strength of MS, were tested separately. The mean number of roots per explant and root length were calculated after 4-week culture. The in vitro rooted plantlets were carefully taken out from the culture tubes and washed gently under running tap water in order to remove traces of adhering medium and transferred them to sterilized coco peat containing pots covered with the polythene bags for maintaining optimum relative humidity. The hardened

plantlets were shifted and maintained for 2 weeks in a greenhouse under partially controlled temperature and light conditions. The plants were finally transferred to earthen pots containing 1:1:1 soil, peat and vermicompost. The percentage survival of the hardened plants was recorded after 4 weeks of transfer to the pots.

Analysis of genetic fidelity using ISSR markers

The genomic DNA was isolated from the mother plant and randomly selected regenerated field transferred plantlets using modified cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1987). The quality of DNA was checked on 0.8% agarose gel by 1X TAE buffer, and the concentration of isolated DNA was adjusted with TE buffer to 50 ng/μL and stored at – 20° C. The genetic fidelity analysis of regenerants was performed with 10 ISSR primers. The PCR amplification reaction was carried out by using a total volume of 20 μL containing 50 ng/μL DNA, 0.4 Mm of each dNTP, 4 Mm MgCl₂, 10 p mole of ISSR primer, 1X PCR master mix (Thermo scientific) contains 0.05 U/μL of *Taq* DNA polymerase. The amplification reaction was carried out for 35 cycles in a thermal cycler (Eppendorf, India). The initial denaturation of DNA was carried at 94 °C for 5 min, followed by denaturation at 94 °C for 30 s, annealing at 50 °C for 45 s, extension at 72 °C for 2 min, final extension at 72 °C for 10 min and cool down at holding temperature of 4 °C. After amplification, the PCR products were subjected to 1% agarose gel electrophoresis, and the size of amplicons was estimated with a 1 kb DNA ladder (Thermo Scientific). The reproducibility nature of ISSR primer-based PCR reactions was checked by repeating the experiment thrice.

Data analysis

The data for number of multiple shoots, roots induced, and their length were recorded regularly at the end of every fourth week. The mean values of the repeated experiments were compared by DMRT test at 5% level of significance using statistical software SPSS Ver.23.

Results and discussion

Direct organogenesis from nodal segments

On MS basal medium, nodal explants of *Corynandra chelidonii* var. *pallae* did not show any response for morphogenesis but a slight outgrowth was observed from the basal portion of nodal explants. The multiple shoot induction, mean number of shoots, and shoot length per explant were affected by various concentrations of BAP, Kn, and TDZ alone or in combination with IAA.

When nodal explants of *Corynandra chelidonii* var. *pallae* were cultured on different concentrations of individual cytokinins of BAP supplemented MS medium, the highest mean number of shoots of 7.23 ± 0.33 and mean length of shoots (cm) of 1.13 ± 0.042 per explant was observed at 2 mg/L concentration of BAP, after 4 weeks of culture (Table 1). On individually supplemented Kn or TDZ supplemented MS media, though multiple shoots were induced from nodal explants, they were low in number when compared to the results of BAP supplemented media (Table 1). A similar observation of BAP as more effective cytokinin than Kn or TDZ on inducing of multiple shoots was reported in the cases of other plant species (Sahoo et al. 1997; Hiregoudar et al. 2003).

Similarly, regeneration via direct organogenesis was also reported from nodal and internodal segments of axenic

Table 1 Effect of plant growth regulators on multiple shoot induction from nodal explants of *C. chelidonii* var. *pallae*

Concentration of PGR (mg/mL)				Nodal explant	
BAP	KN	TDZ	IAA	Mean no. of Shoots/explants ± SE	Mean shoot Length (cm) ± SE
0.5	–	–	–	4.54 ± 0.268 ^c	1.03 ± 0.0133 ^d
1.0	–	–	–	5.46 ± 0.312 ^b	1.04 ± 0.0130 ^b
1.5	–	–	–	5.69 ± 0.237 ^a	1.22 ± 0.061 ^c
2.0	–	–	–	7.23 ± 0.33 ^b	1.13 ± 0.042 ^c
2.0	–	–	0.5	5.31 ± 0.208 ^b	1.23 ± 0.032 ^c
2.0	–	–	1.0	8.08 ± 0.211 ^a	1.08 ± 0.116 ^b
2.0	–	–	1.5	6.31 ± 0.263 ^a	1.16 ± 0.023 ^c
2.0	–	–	2.0	5.08 ± 0.265 ^c	1.06 ± 0.024 ^d
–	0.5	–	–	1.08 ± 0.178 ^d	1.15 ± 0.076 ^{cd}
–	1.0	–	–	1.00 ± 0.160 ^d	1.07 ± 0.030 ^b
–	1.5	–	–	1.23 ± 0.201 ^d	1.06 ± 0.026 ^c
–	2.0	–	–	1.15 ± 0.191 ^e	1.22 ± 0.046 ^c
–	1.0	–	0.5	4.62 ± 0.180 ^c	1.22 ± 0.0579 ^c
–	1.0	–	1.0	5.08 ± 0.211 ^b	1.06 ± 0.0241 ^b
–	1.0	–	1.5	6.15 ± 0.222 ^a	1.33 ± 0.054 ^b
–	1.0	–	2.0	8.08 ± 0.211 ^a	1.28 ± 0.046 ^{bc}
–	–	0.5	–	4.23 ± 0.231 ^c	1.80 ± 0.039 ^a
–	–	1.0	–	4.23 ± 0.231 ^c	1.66 ± 0.026 ^a
–	–	1.5	–	3.62 ± 0.213 ^c	1.58 ± 0.022 ^a
–	–	2.0	–	2.85 ± 0.154 ^d	1.38 ± 0.038 ^b
–	–	0.5	0.5	6.38 ± 0.140 ^a	1.40 ± 0.034 ^b
–	–	0.5	1.0	8.15 ± 0.249 ^a	1.63 ± 0.031 ^a
–	–	0.5	1.5	4.54 ± 0.183 ^b	1.57 ± 0.020 ^a
–	–	0.5	2.0	5.08 ± 0.179 ^c	1.50 ± 0.022 ^a

Values are mean ± SE from 13 replicates per treatment and all the experiments were repeated thrice. Means followed by different letters within PGR alone and combinations are significantly different from each other (p=0.05). Comparison by using Duncan’s multiple range test. Data were recorded after 4 weeks of cultures

plants of *Tarenaya spinosa* (Jacq.) Raf (*Cleome spinosa*: Rodriguez et al. 1990; Albarello et al. 2008) and *Tarenaya rosea* (Vahl ex DC.) Soares Neto & Roalson (*C. rosea* Simoes et al. 2004). Direct organogenesis from nodal explants on MS media supplemented with individual cytokinins was also reported in *Gynandropsis gynandra* (L.) Briq. (*Cleome gynandra*: Nasseem and Jha 1997). Nodal explants-based micropropagation was also reported from the related species such as *Corynandra viscosa* (L.) Cochrane & Iltis (*Cleome viscosa*: Anburaj et al. 2011) and *Cleome droserifolia* (Forssk.) Delile (Hassan 2014).

In the present study, along with individually supplemented cytokinins (BAP, KN, and TDZ), combinations of cytokinin amended MS media were also tested for multiple shoots induction from nodal explants of *C. chelidonii* var. *pallae*. A combination of plant growth regulators (PGR) supplemented MS media; multiple shoots were induced from nodal explants on all combinations. The combination of BAP + IAA, Kn + IAA and TDZ + IAA supplemented MS media has showed multiple shoot induction with maximum values at 2.0 mg/L + 1.0 mg/L, 1.0 mg/L + 2.0 mg/L and 0.5 mg/L + 1.0 mg/L, respectively (Table 1).

Overall, among all the combinations, on 0.5 mg/L of TDZ and 1.0 mg/L of IAA, the highest mean number of multiple shoots (8.15 ± 0.249) with mean shoot length (cm) of 1.63 ± 0.31 was observed after 4 weeks of culture (Fig. 1A–D). A combination of BAP + IAA, Kn + IAA, and TDZ + IAA was also reported to induce multiple shoot induction from nodal explants of several plant species. In a previous study, on BAP + IAA supplemented MS media, maximum multiple shoots were reported as induced from the nodal explants of *Corynandra* (*Cleome*) *felina* (Chinnappan 2020). As per another report, the combination of Kn + IAA has resulted in the highest shoot multiplication in *Dioscorea alata* (Supriya et al. 2013). Similarly, the combination of TDZ (3.0 mg/L) and IAA (0.7 mg/L) amended MS medium has shown multiple shoot induction in *Corynandra viscosa* (Vijayakumar et al. 2014).

Root regeneration from in vitro raised shoots

Rooting of in vitro regenerated shoots and transplantation of the plantlets to the field conditions is the most crucial and essential step for successful micropropagation. Regenerated shoots (about 2–3 cm long) induced from nodal explants were excised and cultured separately on half strength and full-strength MS medium supplemented with various concentrations of IAA and IBA (Table 2). The directly regenerated shoots were rooted with 95% root induction on half-strength MS medium fortified with 1.0 mg/L of IBA (Fig. 2). Root initiation started after 10–12 days of inoculation on auxin supplemented media. The maximum number of roots (3.92 ± 0.309) (Fig. 3) with 2.48 ± 0.076 cm root length

(Fig. 4) were observed on a half-strength MS medium containing 1 mg/mL IBA, after 4 weeks of culture (Fig. 1E). Generally, IBA has been reported to induce a good rooting response over IAA. Ludwig-Muller (2000) has reported that the stimulatory effects of IBA on root development may be due to several factors such as its preferential uptake, transport, and stability over other auxins and subsequent gene activation. In the species of *Gynandropsis*, *Luffa* and *Morus* also, IBA was reported to show a good rooting response (Rathore et al. 2013; Sujatha et al. 2013; Rohela et al. 2020).

Hardening of regenerated plantlets

The ultimate success of in vitro micropropagation depends on reliable hardening and acclimatization protocols, ensuring low-cost production and high survival rates of raised plantlets. After the complete development, the in vitro plantlets of *Corynandra chelidonii* var. *pallae* were transferred to plastic cups (Fig. 1F) containing sterilized coco peat and covered with the polythene bags in order to maintain optimum relative humidity. The hardened plantlets were shifted to the greenhouse and maintained for 2 weeks with partially regulated temperature and light. Later, the plants were finally transferred to pots containing soil, peat, and vermicompost in the ratio of 1:1:1 (Fig. 1G). Acclimatized plantlets were observed after 4 weeks with the emergence of new leaves. The survival rate was 78%, and the plantlets were phenotypically same as the mother plant.

PCR analysis for clonal fidelity

Confirmation of clonal fidelity of regenerated plantlets is a major step in the commercial micropropagation of medicinal plants using in vitro culture methods. Genetic fidelity is an important prospect of determining the level of genetic homogeneity between micropropagated plants and the mother plant (Rohela et al. 2018). In vitro regenerated plants are usually susceptible to genetic changes due to culture stress and might exhibit somaclonal variation (Larkin and Scowcroft 1981). Therefore, it should be a common practice to assure the trueness of tissue culture plants after regeneration. In the present study, the genetic fidelity of micropropagated plantlets was confirmed by ISSR marker-based PCR amplification. Among the ten ISSR primers screened (Table 3), ISSR2 and ISSR9 primers showed a good intensity of DNA bands and did not show any polymorphism. The remaining primers also produced monomorphic DNA bands. With the ISSR2 primer, good amplification was carried and produced six monomorphic bands ranging from 300 bp to 2 kb (Fig. 5). As the ISSR profile obtained through amplification of genomic DNA of in vitro raised plants is similar to that of the mother plant, the genetic homogeneity of micropropagated plants was confirmed as genetically similar.

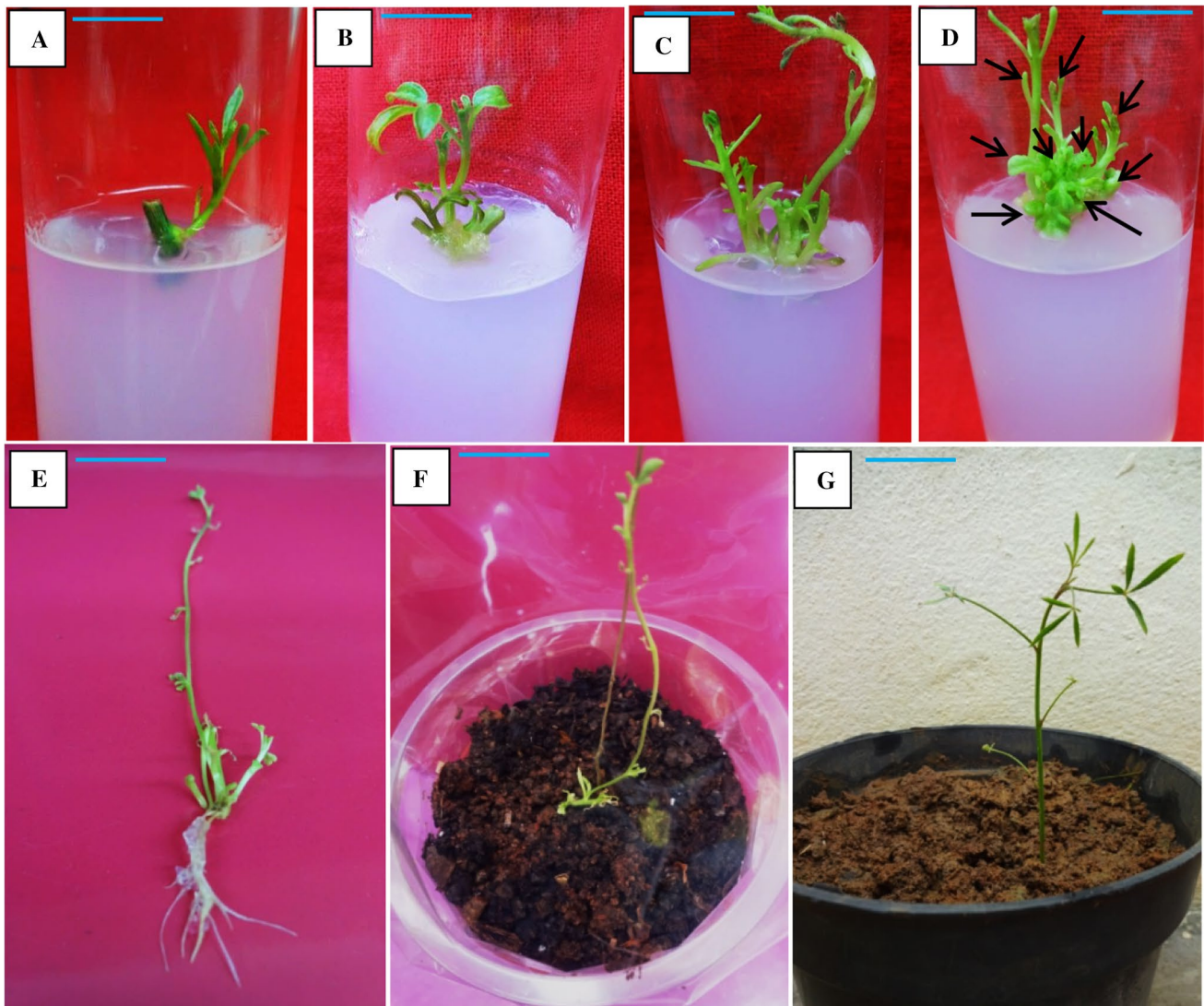


Fig. 1 In vitro micropropagation of *Corynandra chelidonii* var. *palae* through nodal explants. (Bar = 10 mm in **A–D**; bar = 18 mm in **E** and **F**; bar = 28 mm in **F**). **A** Shoot initiation from a single node on MS media supplemented with 0.5 mg/L of TDZ and 1.0 mg/L IAA after 1 week of culture. **B** Multiple shoot induction from nodal explant on MS media supplemented with 0.5 mg/L of TDZ and 1.0 mg/L IAA after 2 weeks of culture. **C** Multiple shoot induction from nodal explant on MS media supplemented with 0.5 mg/L

of TDZ and 1.0 mg/L IAA after 3 weeks of culture. **D** Multiple shoot induction from nodal explant on MS media supplemented with 0.5 mg/L of TDZ and 1.0 mg/L IAA after 4 weeks of culture. **E** Root induction on half-strength MS medium containing 1 mg/ml IBA after 4 weeks of culture. **F** Hardening in plastic cups containing sterilized coco peat. **G** Acclimatized plantlet in a pot containing soil, peat, and vermicompost in 1:1:1 ratio

Inter simple sequence repeats primers are broadly used to confirm the genetic homogeneity of micropropagated plantlets because ISSR primers have the characteristics of high accuracy with good reproducibility, and there is no need to develop specific primers (Mukherjee et al. 2010; Pendli et al. 2019). The ISSR marker-based genetic fidelity studies of micropropagated plants were reported successfully in diverse plant species (Savitikadi et al. 2020). The ISSR marker-based genetic fidelity establishment was made in various micropropagated plants, viz. *Bacopa monnieri* (Faisal et al. 2018), *Artemisia vulgaris* (Jogam et al. 2020),

Corallocarpus epigaeus (Vemula et al. 2020), *Annona reticulata* (Kudikala et al. 2020), and *Origanum majorana* (Sandhya et al. 2021).

Conclusions

The micropropagation of *Corynandra chelidonii* var. *palae* by using nodal explants is the first report of its kind. Among the various combinations of plant growth regulators tested, a high frequency of multiple shoots were induced

Table 2 Effect of plant growth regulators (PGR) on root induction from regenerated shoots of *C. chelidonii* var. *pallae*

Strength of MS media	Concentration of PGR (mg/mL)		Root induction (%)	Mean no .of Roots/shoot \pm SE	Mean root Length (cm) \pm SE
	IAA	IBA			
Half strength	0.5	–	62 \pm 1.542 ^e	0.69 \pm 0.208 ^b	1.17 \pm 0.003 ^d
	1.0	–	85 \pm 2.432 ^b	0.61 \pm 0.140 ^c	1.07 \pm 0.003 ^c
	2.0	–	81 \pm 2.230 ^b	0.84 \pm 0.153 ^c	1.22 \pm 0.004 ^c
Half strength	–	0.5	69 \pm 1.002 ^d	2.07 \pm 0.769 ^a	1.94 \pm 0.007 ^a
	–	1.0	95 \pm 3.102 ^a	3.92 \pm 0.309 ^b	2.48 \pm 0.076 ^a
	–	2.0	70 \pm 1.852 ^c	1.46 \pm 0.243 ^{ab}	2.17 \pm 0.028 ^b
Full strength	0.	–	72 \pm 1.984 ^c	0.85 \pm 0.153 ^b	1.18 \pm 0.004 ^c
	1.0	–	80 \pm 2.543 ^b	0.53 \pm 0.143 ^c	1.18 \pm 0.004 ^b
	2.0	–	84 \pm 2.024 ^b	0.84 \pm 0.153 ^c	1.17 \pm 0.005 ^d
Full strength	–	0.5	65 \pm 1.024 ^{de}	1.61 \pm 0.212 ^a	2.24 \pm 0.005 ^a
	–	1.0	87 \pm 3.102 ^{ab}	1.30 \pm 0.212 ^b	2.38 \pm 0.005 ^a
	–	2.0	74 \pm 2.046 ^c	1.15 \pm 0.153 ^{ab}	2.54 \pm 0.007 ^a

Values are mean \pm SE from 13 replicates per treatment and all the experiments were repeated thrice. Means followed by different letters within PGR alone and combinations are significantly different from each other ($p=0.05$). Comparison by using Duncan's multiple range test. Data were recorded after 4 weeks of cultures

Fig. 2 Effect of IAA/IBA on in vitro rooting of shoots derived from nodal explants of *C. chelidonii* var. *pallae*

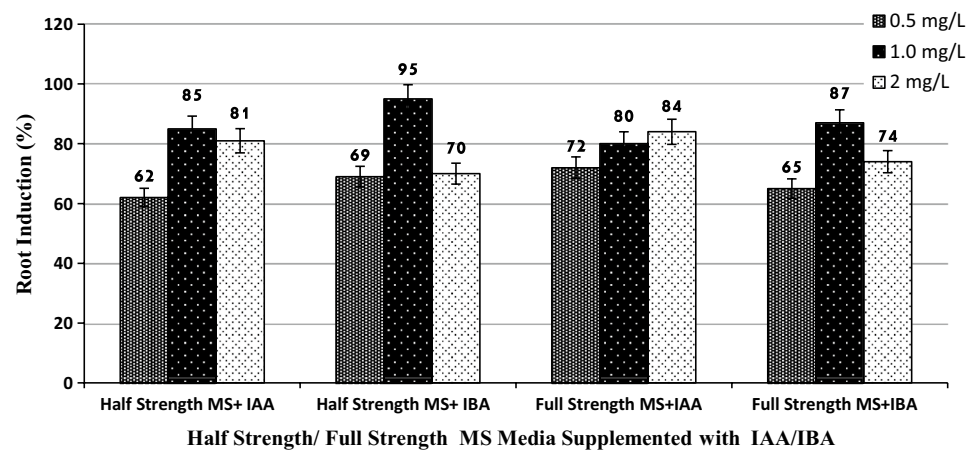


Fig. 3 Effect of IAA/IBA on mean number of roots induced from regenerated shoots of *C. chelidonii* var. *pallae*

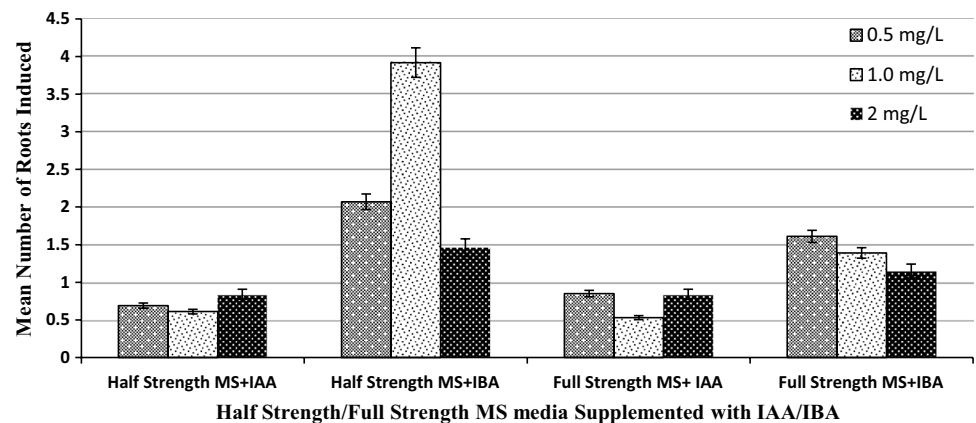


Fig. 4 Effect of IAA/IBA on mean root length (cm) of induced roots from regenerated shoots of *C. chelidonii* var. *pallae*

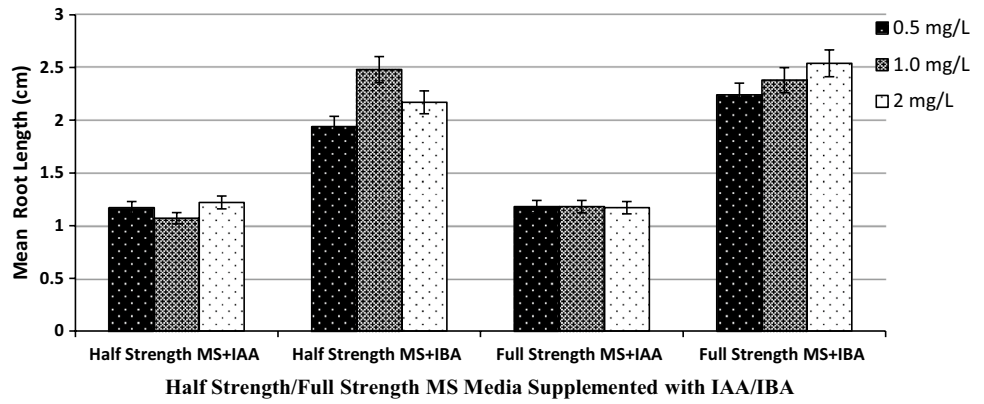
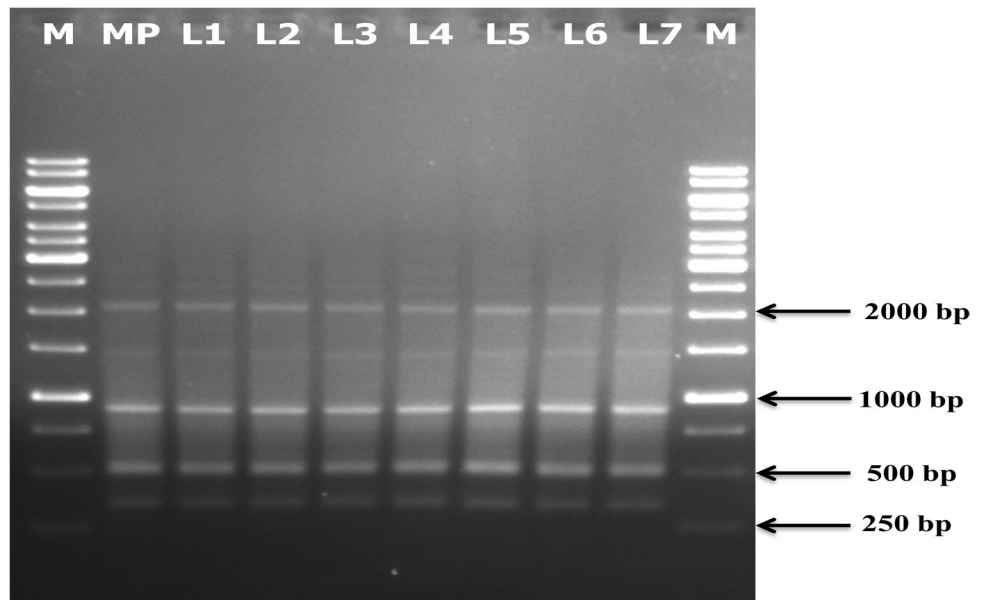


Table 3 List of ISSR markers used for genetic fidelity analysis of *Corynandra chelidonii* var. *pallae*

S. no.	Primer code	Primer sequence	Number of bands	Range of DNA bands (bp)
1	ISSR1	5' AGAGAGAGAGAGAGGC 3'	6	400–1900
2	ISSR2	5' AGAGAGAGAGAGAGAGG 3'	6	300–2000
3	ISSR3	5' GAGAGAGAGAGAGAGAT 3	7	700–1800
4	ISSR4	5' TCTCTCTCTCTCTCC 3'	2	700–1500
5	ISSR5	5' TCTCTCTCTCTCTCG 3'	4	600–1700
6	ISSR6	5' AGAGAGAGAGAGAGATC 3'	9	300–1800
7	ISSR7	5' TCTCTCTCTCTCTCRT 3'	3	600–1200
8	ISSR8	5' TCTCTCTCTCTCTCRG 3'	2	500–1000
9	ISSR9	5' TGTGTGTGTGTGTGRA 3'	9	300–2000
10	ISSR10	5' ACTGCTAGAGAGAGAGAGAG 3'	4	400–1100

Fig. 5 ISSR2 primer-based PCR amplified products obtained from *Corynandra chelidonii* var. *pallae*. Lane M: 1 kb DNA marker (Thermo scientific); Lane MP: DNA bands of mother plant amplified with ISSR2 primer; Lanes 1 to 7: Monomorphic DNA bands across the in vitro propagated plantlets amplified with ISSR2 primer



from nodal explants on MS medium augmented with TDZ (0.5 mg/L) and IAA (1.0 mg/L). The regenerated shoots were rooted with 95% root induction on a half-strength MS

medium supplemented with 1.0 mg/L of IBA. Furthermore, the regenerated plantlets were confirmed as genetically identical to the mother plant by ISSR marker-based molecular

analysis; as shown by the monomorphic nature of DNA bands across randomly selected *in vitro* raised plantlets and mother plant. The developed protocol is efficient and reliable that could help multiply large-scale production and conservation of genetically true clones of *C. chelidonii* var. *pallae*.

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Authors contributions SS and PJ conducted the experiments, GKR assisted in preparing the manuscript, VSR and RA have conceived the idea, designed the experiments, and supervised them. All authors have read and approved the manuscript.

Declarations

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

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