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HIGH FREQUENCY *in vitro* PLANTLET REGENERATION FROM LEAF, NODE AND SHOOT TIP EXPLANTS OF *Corynandra felina* (L.f.) Cochrane & Iltis, AN IMPORTANT ENDEMIC MEDICINAL PLANT IN PENINSULAR INDIA

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ABSTRACT

An efficient *in vitro* plant regeneration protocol using leaf, node and shoot tip has been developed in *Corynandra felina* (L. f.) Cochrane & Iltis., an endemic medicinal herb in peninsular India. The above explants, taken from field-grown plants, were sterilized, and then cultured on Murashige and Skoog's (MS) medium supplemented with various concentrations of 6-benzylaminopurine (BAP, 0.5-3.0 mg L^{-1}), thidiazuron (TDZ, 0.25-1.25 mg L^{-1}) and kinetin (Kn, 0.5-3.0 mg L^{-1}) either alone or in combination with indole-3-acetic acid (IAA, 0.25-1.0 mg L^{-1}). Maximum number of shoots (48.3 ± 0.59) with average shoot length of 1.4 ± 0.57 cm were regenerated directly from the leaf explants grown on MS medium supplemented with TDZ (0.5 mg L^{-1}) + IAA (0.25 mg L^{-1}). Maximum number of shoots (5.4 ± 0.38 and 6.2 ± 0.43) with shoot length (1.9 ± 0.66 and 2.2 ± 0.19 cm) were observed in node and shoot tip explants, respectively, when cultured on MS medium supplemented with TDZ (0.5 mg L^{-1}) + IAA (0.25 mg L^{-1}). Tiny shoots from leaf, node and shoot tip explants were elongated (5.6 ± 0.40) on MS medium enriched with 1.0 mg L^{-1} GA_3. The elongated shoots were rooted (90%) on half-strength MS medium fortified with 1.0 mg L^{-1} IBA. The regenerated plantlets when transferred to the field showed 89% plant survival. For the first time, we report direct plantlet regeneration in *Corynandra felin*a using leaf explants.

Keywords: *Corynandra felina*, leaf, node, regeneration, shoot tip, thidiazuron

INTRODUCTION

Corynandra felina (L. f.) Cochrane & Iltis [= *Cleome felina* L. f.] (Cochrane and Iltis, 2014), belongs to the family Cleomaceae, and is commonly called ‘cat spider flower’. It is endemic to peninsular India (Hooker and Thompson, 1872; Sundararagavan, 1993; Kundu, 2007; Rawath, 2008). The entire plant is prescribed as an astringent (Nadkarni, 1954). The vesicant seeds of this plant have been given internally to treat vermifuge (Baillon, 1874). The whole plant extracts show anti-diabetic and anti-hyperglycemic properties (Nagarajan *et al*., 2005). The plant is mentioned as ‘Swarnaksheeri’ in the ancient Indian medical text *Astanga Hrudayam*. It was used in the preparation of Kshara (alkaline substances obtained from the water soluble ash of the drugs of plant origin) for the treatment of diseases like haemorrhoids and oedema (Kodlady *et al*., 2012). Mahimadoss *et al*. (2014) reported that the ethanolic extract of *C. felina* leaves show anticancer activity. Whole plant has anticancer, anti-inflammatory, antimicrobial activities (Vijyashalini and Abirami, 2018). Due to these medicinal

properties, this plant gained much importance, and has been collected from its natural habitat by the local healers. This has led to the destruction of natural sources and the plant has now become endangered. Tissue culture is an alternative method to propagate and conserve this valuable medicinal plant. Earlier, there was one report on the establishment of *in vitro* propagation through stem explants of *C. felina* (Singh, 2020). Therefore, the present work was aimed to regenerate this plant using *in vitro* techniques.

MATERIALS AND METHODS

Plant material

One month old *Corynandra felina* was collected from Gopalpur, Hanamakonda district, Telangana, India and planted in the Departmental Botanical Garden, Osmania University, Hyderabad, India.

Preparation of explants

Leaf, node and shoot tip explants were collected from field grown two months old plants and washed under running tap water to remove surface dust particles. Later, the explants were sterilized with carbendazim (Bavistin) solution (0.1%) followed by three washes with distilled water. The explants were sterilized with $HgCl_2$ (0.01%) for 2 min. Explants were rinsed three times in distilled water to remove residual $HgCl_2$ from explants. Explants were blotted on a sterile tissue paper for absorption of water drops and then the explants were inoculated in a laminar flow cabinet.

Shoot induction, elongation and rooting

Leaf, node and shoot tip explants were cultured on Murashige and Skoog's (MS) (Murashige and Skoog, 1962) medium supplemented with thidiazuron (TDZ), 6-benzylaminopurine (BAP), kinetin (KN) for shoot induction. Tiny shoots, obtained from shoot induction were transferred onto MS medium fortified with gibberellic acid (GA_3) for shoot elongation. The elongated shoots were transferred onto ½ strength MS medium supplemented with 0.5, 1.0, 1.5 and 2.0 mg L^{-1} indole -3-butyric acid (IBA) and 0.5, 1.0, 1.5 and 2.0 mg L^{-1} indole-3-acetic acid (IAA) for root induction. Tiny shoots induce roots on ½ MS medium fortified with IAA or IBA and record the number of roots per shoot and percentage of the rooting response. Each experiment was carried out thrice and the average mean and standard error were taken.

Explant inoculation and incubation

The sterilized explants such as leaf, node and shoot tip were inoculated onto MS medium under aseptic conditions using laminar air flow cabinet. All the cultures were incubated under controlled conditions at temperature 25 ± 2°C and maintains 16/8 h photoperiod using Phillips (40 W) tube lights. The cultures were examined visually on a regular basis for morphological changes like dedifferentiation from leaf explants to emerging tiny shoot buds from the cut ends of leaf explants. Bud proliferation leads to the formation of shoots. The number of shoots and shoot length were recorded after 30 days of culture for each treatment.

Statistical analysis

All the experiments were repeated three times. The experimental data were recorded from ten cultures and mean comparison was carried out by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Shoot induction

Leaf, shoot tip and node explants were cultured on MS medium supplemented with BAP (0.5 -3.0 mg L^{-1}), TDZ (0.25-1.25 mg L^{-1}) and KN (0.5-3.0 mg L^{-1}) either alone or in combination with IAA (0.25,

Table 1: *In vitro* multiple shoots induction via direct organogenesis from leaf explants of *Corynandra felina*, cultured on MS media supplemented with BAP, KN and TDZ (0.25-1.25 mg L^{-1}) alone, after 4 weeks of culture

Hormone (mg L^{-1})		No of shoots (mean ± SE)	Shoot length (cm) (mean ± SE)
BAP	0.5	-	-
	1.0	-	-
	1.5	2.6 ± 0.67	1.8 ± 0.98
	2.0	7.9 ± 0.46	1.4 ± 0.76
	2.5	6.3 ± 0.58	1.6 ± 0.49
	3.0	2.8 ± 0.87	2.0 ± 0.84
TDZ	0.25	-	-
	0.50	5.8 ± 0.67	1.5 ± 0.86
	0.75	9.6 ± 0.76	1.2 ± 0.49
	1.00	5.3 ± 0.89	1.6 ± 0.58
	1.25	4.8 ± 0.58	1.9 ± 0.76
KN	0.5	-	-
	1.0	-	-
	1.5	-	-
	2.0	4.2 ± 0.68	2.3 ± 0.89
	2.5	6.8 ± 0.46	1.6 ± 0.67
	3.0	5.4 ± 0.29	1.8 ± 0.42

*Data represent mean of 10 cultures. Each experiment was repeated thrice. Data were scored after 4 weeks of culture.

Table 2: *In vitro* multiple shoots induction *via* direct organogenesis from leaf explants of *Corynandra felina*, cultured on MS medium supplemented with BAP, KN and TDZ in combination with IAA after 4 weeks of culture

Explant	Hormone combinations used (mg L^{-1})				No. of shoots (mean ± S.E)	Shoot length (cm) (mean ± S.E)
	BAP	TDZ	KN	IAA		
Leaf	0.5	-	-	1.0	-	-
	1.0	-	-	1.0	-	-
	1.5	-	-	1.0	8.8 ± 0.54	2.5 ± 0.65
	2.0	-	-	1.0	10.6 ± 0.21	2.0 ± 0.98
	2.5	-	-	1.0	7.9 ± 0.36	2.8 ± 0.57
	3.0	-	-	1.0	5.4 ± 0.15	3.0 ± 0.66
	-	0.25	-	0.25	9.3 ± 0.84	1.8 ± 0.54
	-	0.50	-	0.25	48.3 ± 0.59	1.4 ± 0.57
	-	0.75	-	0.25	23.4 ± 0.57	1.6 ± 0.38
	-	1.00	-	0.25	7.8 ± 0.34	2.0 ± 0.98
	-	1.25	-	0.25	4.5 ± 0.28	2.2 ± 0.84
	-	-	0.5	1.0	-	-
	-	-	1.0	1.0	-	-
	-	-	1.5	1.0	-	-
	-	-	2.0	1.0	5.8 ± 0.28	3.0 ± 0.58
	-	-	2.5	1.0	7.8 ± 0.94	2.5 ± 0.66
	-	-	3.0	1.0	6.5 ± 0.87	2.9 ± 0.49

*Data represent mean values of 10 cultures.

0.5 and 1.0 mg L^{-1}) for shoot induction. Maximum mean number of shoots (48.3 ± 0.59) with average shoot length of 1.4 ± 0.57 cm was observed on MS medium supplemented with TDZ (0.5 mg L^{-1}) in combination with IAA (0.25 mg L^{-1}) from leaf explants after three weeks of culture (Table 1 and 2; Fig. 1). Maximum mean number of shoots 5.4 ± 0.38 and 6.2 ± 0.43 with a shoot length of 1.9 ± 0.66 and 2.2 ± 0.19 cm was observed from node and shoot tip explants, respectively, when cultured on MS medium supplemented with TDZ (0.5 mg L^{-1}) in combination with IAA (0.25 mg L^{-1}) after four weeks of culture (Table 3 and 4; Fig. 2). In this study, leaf explants cultured on cytokinins in combination with auxins induced a higher number of multiple shoots than cytokinins alone. A similar report has been published in *Cleome viscosa* (Vijayakumar *et al*., 2014). TDZ, a thiadiazole-substituted phenylurea, has been shown to have higher cytokinin activity than phenyl urea derivatives and other active adenine cytokinins (Huetteman and Preece, 1993). Several investigators used it for plantlet regeneration of medicinal plants and to increase the frequency of shoot let formation (Ravi *et al*., 2012; Korra *et al*., 2017; Sreenu *et al*., 2019). It is more stable and biologically active at low concentrations than other cytokinins (Washimkar and Shende, 2016).

Shoot elongation and rooting

Small shoots obtained from leaf, shoot tip and node explants were cultured on shoot induction medium,

Table 3: Induction of multiple shoots *via* direct proliferation from nodal segments and shoot tip explants of *Corynandra felina*, cultured on MS medium supplemented with BAP (0.5-3.0 mg L^{-1}), KN (0.5- 3.0 mg L^{-1}) and TDZ (0.25-1.25 mg L^{-1}) alone in after 4 weeks of culture

Hormones used (mg L^{-1})			Nodal segments		Shoot tip	
BAP	TDZ	KN	No. of shoots (mean ± SE)	Shoot length (cm) (mean ± SE)	No. of shoots (mean ± SE)	Shoot length (cm) (mean ± SE)
1.0	-	-	-	-	-	-
1.5	-	-	-	-	-	-
2.0	-	-	1.9 ± 0.53	3.2 ± 0.19	2.4 ± 0.68	3.5 ± 0.56
2.5	-	-	3.4 ± 0.91	2.9 ± 0.72	3.9 ± 0.47	2.8 ± 0.49
3.0	-	-	2.3 ± 0.67	3.0 ± 0.38	2.8 ± 0.63	3.1 ± 0.75
-	0.25	-	-	-	-	-
-	0.50	-	2.2 ± 0.68	3.6 ± 0.89	3.2 ± 0.43	2.6 ± 0.89
-	0.75	-	4.0 ± 0.47	3.0 ± 0.79	4.3 ± 0.87	2.1 ± 068
-	1.00	-	2.5 ± 0.58	3.2 ± 0.43	3.4 ± 0.49	2.3 ± 0.43
-	1.25	-	1.7 ± 0.76	3.4 ± 0.73	2.7 ± 0.98	2.8 ± 0.81
-	-	1.0	-	-	-	-
-	-	1.5	-	-	-	-
-	-	2.0	-	-	-	-
-	-	2.5	2.4 ± 0.56	3.7 ± 0.53	3.1 ± 0.59	3.9 ± 0.57
-	-	3.0	1.9 ± 0.75	2.1 ± 0.49	2.3 ± 0.46	4.0 ± 0.29

*Data is mean of 10 cultures. Each experiment was repeated thrice. Data were scored after 2 weeks of culture

Table 4: Induction of multiple shoots *via* direct proliferation from nodal segments and shoot tip explants of *Corynandra felina*, cultured on MS medium supplemented with BAP (0.5-3.0 mg L^{-1}), KN (0.5- 3.0 mg L^{-1}) and TDZ (0.25-1.25 mg L^{-1}) combined with IAA (0.25 and 0.5 mg L^{-1}) in after 4 weeks of culture

Hormones used (mg L^{-1})				Nodal segments		Shoot tip	
BAP	TDZ	KN	IAA	No. of shoots (mean ± SE)	Shoot length (cm) (mean ± SE)	No. of shoots (mean ± SE)	Shoot length (cm) (mean ± SE)
1.0	-	-	0.50	-	-	-	-
1.5	-	-	0.50	2.6 ± 0.83	4.3 ± 0.58	3.6 ± 0.47	3.7 ± 0.43
2.0	-	-	0.50	4.2 ± 0.67	3.5 ± 0.67	5.0 ± 0.33	3.0 ± 0.76
2.5	-	-	0.50	3.3 ± 0.99	3.8 ± 0.95	4.1 ± 0.19	3.4 ± 0.38
3.0	-	-	0.25	2.4 ± 0.71	4.1 ± 0.74	3.6 ± 0.74	4.1 ± 0.55
-	0.25	-	0.25	2.6 ± 0.63	2.6 ± 0.57	3.2 ± 0.68	3.6 ± 0.43
-	0.50	-	0.25	5.4 ± 0.38	1.9 ± 0.66	6.2 ± 0.43	2.2 ± 0.19
-	0.75	-	0.25	4.1 ± 0.22	2.3 ± 0.49	4.9 ± 0.66	2.9 ± 0.85
-	1.00	-	0.25	3.6 ± 0.17	3.1 ± 0.51	4.1 ± 0.89	3.6 ± 0.48
-	1.25	-	0.25	1.8 ± 0.63	3.3 ± 0.98	2.6 ± 0.46	4.0 ± 0.37
-	-	1.0	0.50	-	-	-	-
-	-	1.5	0.50	-	-	-	-
-	-	2.0	0.50	1.8 ± 0.49	3.6 ± 0.29	2.3 ± 0.48	4.7 ± 0.58
-	-	2.5	0.50	3.6 ± 0.55	2.8 ± 0.75	4.0 ± 0.36	3.8 ± 0.44
-	-	3.0	0.50	2.2 ± 0.63	3.1 ± 0.37	3.1 ± 0.44	4.2 ± 0.79

*Data is average of 10 cultures. Each experiment was repeated thrice. Data scored after 4 weeks of culture

transferred onto MS medium fortified with GA_3 (0.5-2.0 mg L^{-1}) for shoot elongation. Maximum mean shoot length of 5.6 ± 0.40 cm was recorded on MS medium fortified with GA_3 at 1.0 mg L^{-1} after 1 week of culture (Table 5; Fig. 1 and 2). Cytokinin-amended media produce tiny shoots, but when transferred onto MS medium having GA_3 they produce adequately longer shoots. Similar results were also reported in *Cleome viscosa*. (Vijaykumar *et al.*, 2014). A further increased concentration of GA_3 did not promote shoot length. MS medium with GA_3 is known for shoot elongation (Deore and Johnson, 2008). The elongated shoots were transferred onto ½ strength MS medium supplemented

Table 5: Elongation of tiny shoots which were obtained from leaf, petiole and stem were transferred onto MS medium fortified with GA_3 in *Corynadra felina*, after 1 week of culture

GA_3 (mg L^{-1})	Shoot length (cm) (mean ± SE)
0.5	3.8 ± 0.27
1.0	5.6 ± 0.40
1.5	4.3 ± 0.33
2.0	2.6 ± 0.59

*Data is mean values of 10 cultures. Data scored after 1 week of culture

Table 6: Induction of roots from regenerated micro-shoots were cultured on ½ strength MS medium supplemented with various concentrations of IAA and IBA, in *Corynandra felina*, after 1 week of culture

Hormone (mg L^{-1})		%	No. of roots	Root length (cm)
IBA	IAA	Response	(mean ± SE)	(mean ± SE)
0.5	-	40	33.6 ± 0.68	1.6 ± 0.37
1.0	-	90	48.9 ± 0.53	2.9 ± 0.78
1.5	-	70	39.4 ± 0.64	2.2 ± 0.46
2.0	-	50	28.9 ± 0.78	2.6 ± 0.64
-	0.5	30	20.7 ± 0.83	1.5 ± 0.68
-	1.0	50	37.6 ± 0.77	1.9 ± 0.82
-	1.5	70	28.2 ± 0.67	2.1 ± 0.65
-	2.0	60	23.6 ± 0.60	2.6 ± 0.47

*Data represent average values of 10 cultures. Each experiment was repeated thrice. Data were scored after 1 week of culture

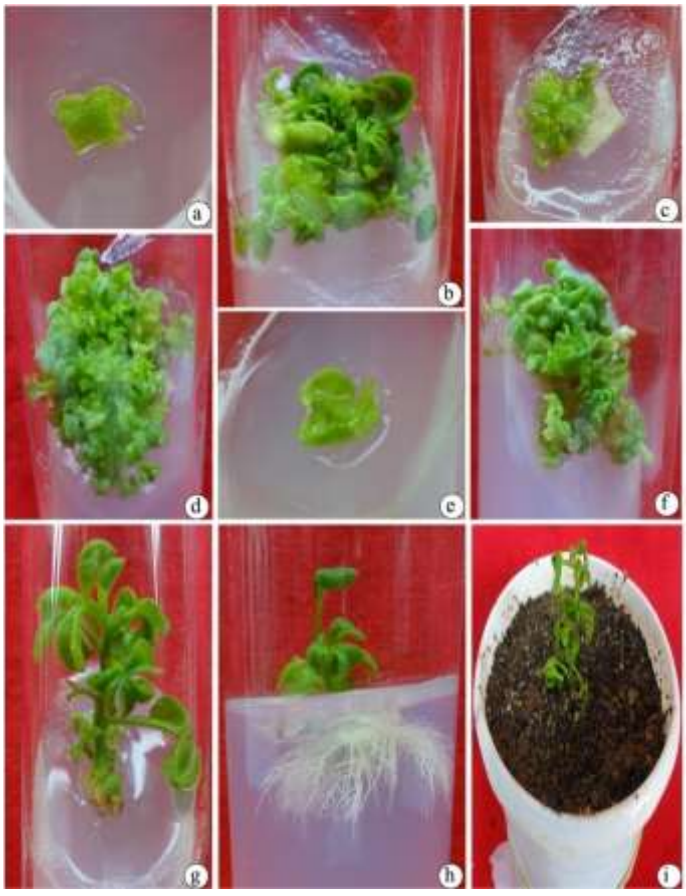

Fig. 1: *In vitro* multiple-shoot induction via direct organogenesis from *Corynandra felina* leaf explants, cultured onMS medium supplemented with BAP, KN and TDZ (alone or in combination with IAA); a) Shoot bud initiation from leaf explants cultured on MS medium + BAP (2.0 mg L^{-1}) + IAA (mg L^{-1}) after 3 days; b) Multiple shoots induced from leaf explants cultured on MS medium + BAP (2.0 mg L^{-1}) + IAA (1.0 mg L^{-1}) after 4 weeks of culture; c) Shoot bud initiation from leaf explants cultured on MS medium + TDZ (0.5 mg L^{-1}) + IAA (0.25 mg L^{-1}) after 1 week of culture; d) Multiple shoots from leaf explants cultured on MS medium + TDZ (0.5 mg L^{-1}) + IAA (0.25 mg L^{-1}) after 4 weeks of culture; e) Shoot bud initiation from leaf explants cultured on MS medium + KN (2.5 mg L^{-1}) + IAA (1.0 mg L^{-1}) after 3 days; f) Multiple shoots from leaf explants cultured on MS medium + KN (2.5 mg L^{-1}) + IAA (1.0 mg L^{-1}) after 4 weeks; g) Elongation of shoot cultured on MS medium + GA_3 (1.0 mg L^{-1}) after 1 week; h) Root induction from elongated shoots cultured on ½ strength MS medium + IBA (1.0 mg L^{-1}) after 1 week; i) Complete plantlet transferred into plastic pot for hardening after 6 weeks

with IBA or IAA (0.5-2.0 mg L^{-1}) for root induction. Maximum rooting of 90% with a mean number of 48.9 ± 0.53 roots and root length of 2.9 ± 0.78 cm per plantlet was noticed in ½ strength MS medium supplemented with 1.0 mg L^{-1} IBA. Roots were noticed after one week of culture (Table 6; Fig. 1 and 2). In our study the IBA fortified medium induce more roots than IAA fortified medium. A high concentration of IBA (2.0 mg L^{-1}) resulted in a low rooting rate. Naseem and Jha (1994, 1997) found

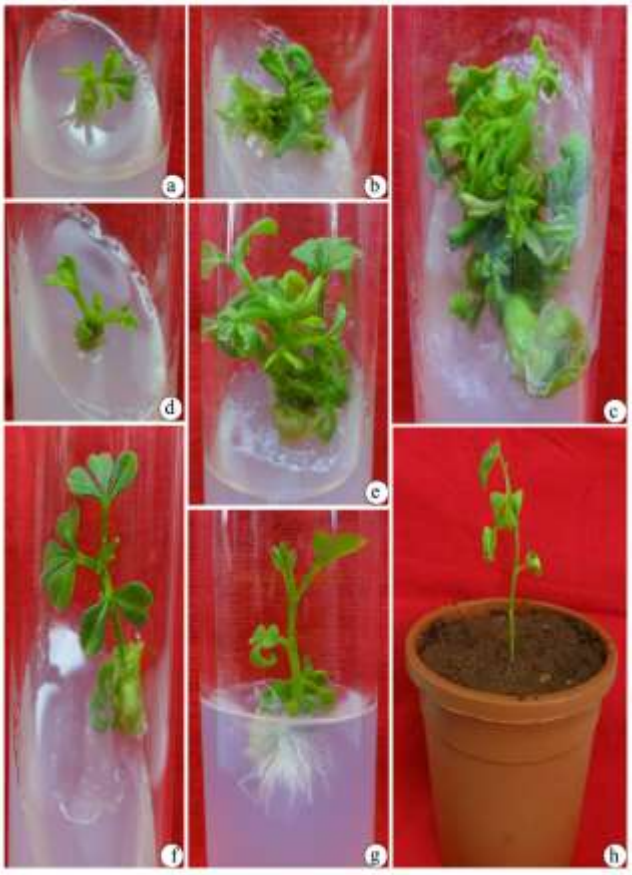

Fig. 2: Induction of multiple shoots via direct proliferation from nodal segments and shoot tip explants of *Corynandra felina* cultured on MS medium supplemented with BAP, KN and TDZ either alone or in combination with IAA after 4 weeks; a) Emerging shoot buds from nodal explants cultured on MS medium + TDZ (0.5 mg L^{-1}) + IAA (0.25 mg L^{-1}) after 1 week; b) Multiple shoots induced from nodal explants cultured on MS medium + TDZ (0.5 mg L^{-1}) + IAA (0.25 mg L^{-1}) after 2 weeks; c) Multiple shoots from nodal explants cultured on MS medium + TDZ (0.5 mg L^{-1}) + IAA (0.25 mg L^{-1}) after 4 weeks; d) Emerging shoot buds from shoot tip explants cultured on MS medium + TDZ (0.5 mg L^{-1}) + IAA (0.25 mg L^{-1}) after 1 week; e) Multiple shoots from shoot tip explants cultured on MS medium + TDZ (0.5 mg L^{-1}) + IAA (0.25 mg L^{-1}) after 4 weeks; f) Elongation of shoots transferred to MS medium + GA_3 (1.0 mg L^{-1}) after 1 week; g) Root induction from shoots cultured on ½ strength MS medium + IBA (1.0 mg L^{-1}) after 1 week; h) Complete plantlet transferred into plastic pot for hardening after 6 weeks

that IBA was essential for root formation in *Cleome viscosa* and *Cleome gynandra*. IBA is a widely used plant growth regulator for root induction in cucurbits (Sarowar *et al*., 2003). The superiority of IBA over other auxins regarding *in vitro* rooting (Karthikeyan *et al*., 2018; Rautela *et al*., 2018).

Plantlet hardening

Rooted plantlets with 3 to 4 leaves were carefully taken out from the test tubes and traces of agar adhered roots were gently removed. Plantlets were then transferred onto pots containing sterile red soil, vermicompost and sand in a 2:1:1 ratio. Each pot was wrapped with plastic bag to keep humidity and the plantlets were watered with inorganic salts once a week. The plantlets were transferred into field conditions with a survival rate of 89% (Fig. 1 and 2). Similar results were published in the reports of (Anburaj *et al*., 2011; Simões-Gurgel *et al*., 2021).

Conclusions: A reliable and reproducible regeneration protocol from leaf, node, shoot tip explants was developed. For the first time we report direct plantlet regeneration using leaf explants in *C. felina* and the protocol may be used for mass multiplication of this endemic medicinal plant and the regeneration protocol can be used for genetic transformation studies.

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