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ORIGINAL ARTICLE

Plantlet regeneration through indirect shoot organogenesis and somatic embryogenesis in *Justicia gendarussa* Burm. f., a medicinal plant

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Abstract A protocol for the regeneration of a large number of plantlets via indirect shoot organogenesis and somatic embryogenesis has been developed from the stem and leaf explants of *Justicia gendarussa* Burm. f. The callus was efficiently induced from the explants using Murashige and Skoog (MS) medium supplemented with α-Naphthalene acetic acid (NAA) + Benzyl amino purine (BAP) (1.0+ 0.1 mg/l). The highest number of plantlets through indirect shoot organogenesis was obtained when the callus was sub-cultured to MS medium with BAP + NAA (0.1+1.0 mg/l). The maximum number of plantlets via somatic embryos was obtained in the medium with BAP + NAA (1.0+0.1 mg/l) for stem derived calli and Kinetin (Kn) + NAA (2.0+ 0.1 mg/l) for leaf derived calli. The in vitro developed shoots were rooted well in half strength MS medium supplemented with 0.5 mg/l of Indole-3-acetic acid (IAA). The in vitro regenerated plantlets were hardened using a mixture of sterile sand:soil:manure (1:1:1). The present study is the first report on the regeneration of plants through somatic embryogenesis from stem and leaf derived calli of *J. gendarussa*.

Keywords *Justicia gendarussa* · Shoot organogenesis · Somatic embryogenesis · Histology

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Abbreviations

BAP	Benzyl amino purine
IAA	Indole 3 acetic acid
Kn	Kinetin
MS	Murashige and Skoog
NAA	Naphthalene acetic acid
TDZ	Thidiazuron (N-phenyl-N'-1-thiadiazol-5-ylurea)
Zea	Zeatin

Introduction

The plant regeneration through in vitro culture technique involves the exposure of the explants to various growth regulators, vitamins, amino acid conditions that activates a new developmental pathway, either through direct organogenesis or through somatic embryogenesis in *Pisum sativum* (Zhihui et al. 2009). The regeneration of plantlets through indirect shoot organogenesis was reported in *Ocimum gratissimum* L. (Gopi et al. 2006), *Tinospora cordifolia* (Raghu et al. 2007), *Solanum nigrum* (Linn.) (Shridhar and Naidu 2011) and through somatic embryogenesis in *Dalbergia sissoo* (Singh and Chand 2003), *Bacopa monnieri* (Jain et al. 2010) and *Pulsatilla koreana* Nakai (Lin et al. 2011) were reported. In vitro regeneration of plants through somatic embryogenesis is preferred over other methods as the embryos have single-cell origin, low frequency of chimeras and the production of a high number of regenerates (Ammirato 1983).

Justicia gendarussa Burm. f. commonly known as Aduthoda gida, Karalakki gida in Kannada is well known for its antioxidant, anti-arthritic, anti-inflammatory and analgesic properties. Other medicinal properties of the plant

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include antinociceptive, antifertility, anticancerous, hepatoprotective, larvicidal properties and also used in the treatment of rheumatism, dysuria, fever, carbuncles, jaundice, diarrhea, pains in the head, ear and paralysis (Ratnasooriya et al. 2007; Paval et al. 2009; Senthilkumar et al. 2009). The exploitation of the naturally grown plant is increasing day by day as the plant is well known in the traditional medicine and due to the pharmacological reports. Therefore, the present work is focused on the in vitro regeneration of plantlets through indirect shoot organogenesis and somatic embryogenesis.

A basic work on the direct regeneration of *J. gendarussa* using nodal explants and indirect regeneration through shoot organogenesis has been reported by Johnson et al. (2004), Agastian et al. (2006), Bushrabi et al. (2008), Bhagya and Chandrashekar (2010), Janarthanam and Sumathi (2010) and Thomas and Yoichiro (2010). However, the regeneration of plants through somatic embryogenesis and histological confirmation for the regeneration of plants through indirect shoot organogenesis and somatic embryogenesis has not been reported so far. Therefore, the present study mainly focused on the plant regeneration through indirect shoot organogenesis and somatic embryogenesis followed by their confirmation using histological studies.

Materials and methods

Plant material

J. gendarussa plant materials such as stem and leaves were collected from the natural forests of Dakshina Kannada Districts, Karnataka, India. The collected materials were identified following Flora of Madras Presidency and Dakshina Kannada (Bhat 2003; Gamble 1958) and the voucher specimen was deposited at the herbarium of Department of Applied Botany. The explants were washed in running tap water (45 min) to remove the surface debris and sterilized using 1 % Bavistine (30 min), 70 % alcohol (1 min), 0.1 % $HgCl_2$ (Mercuric chloride) (8 min) and washed in sterile distilled water three to four times. The explants with a size of ~0.5 cm were inoculated on to MS medium supplemented with NAA (α-Napthalene acetic acid) + BAP (Benzyl amino purine) (1+0.1 mg/l) for callus induction (Agastian et al. 2006). The cultures were incubated at 25±2 °C with 16 h of photoperiod and 40.0±3.0 μmol $m^{-2}s^{-1}$ light intensity.

Indirect shoot organogenesis and somatic embryogenesis

The callus obtained on MS (Murashige and Skoog 1962) medium supplemented with NAA + BAP at the concentration of 1+0.1 mg/l was used for indirect shoot organogenesis and somatic embryogenesis. For this, the stem and leaf derived calli were inoculated to full strength MS medium with varying concentrations of GR (Growth Regulator). The selected GR for indirect shoot organogenesis include BAP/Kn (Kinetin) (1.0, 2.0, 3.0 mg/l), BAP + NAA (0.1+1.0, 1.0/2.0/3.0+0.1 mg/l) and Kn + NAA (1.0/2.0/3.0+ 0.1 mg/l). The somatic embryogenesis was tested in MS medium supplemented with different GR such as BAP/Kn (1.0, 2.0, 3.0 mg/l), Zea (Zeatin)/TDZ (Thidiazuron (N-phenyl-N'-1-thiadiazol-5-ylurea) (0.5, 1.0 mg/l), BAP/Kn + NAA (1.0/2.0/3.0+0.01/0.1 mg/l) and Zea/TDZ + NAA (0.5/1.0 +0.1 mg/l). Medium without GR served as control.

The calli were repeatedly subcultured for every 40 days of incubation to their respective medium. Observations were made for indirect shoot organogenesis, different stages of somatic embryogenesis and plantlets obtained from the embryos. The number of embryogenic calli, somatic embryos, plantlet formed and the time taken for the development of these stages were also noted. The callus subcultured for indirect shoot proliferation was incubated at 25±2 °C with 16 h of photoperiod and 60.0±3.0 μmol$m^{-2}s^{-1}$ light intensity and for somatic embryogenesis the intensity of light was reduced to 40.0±3.0 μmol$m^{-2}s^{-1}$.

Histological studies

The explants showing callus induction and different developmental stages of organogenic and embryogenic calli were subjected to histological studies. The tissues were fixed in Carnoy's 'B' (6:3:1 ratio of absolute alcohol: chloroform: acetic acid) fluid for 24 h and processed through a series of graded alcohol from 70 % to absolute alcohol for 24 h in each grade and later dehydrated using absolute alcohol, alcohol:butanol and butanol to avoid the interference of moisture during infiltration and embedding of tissues in paraffin wax. The tissues fixed in paraffin wax were subjected to sectioning using Leica RM 2145 microtome with a thickness of 10 μm and stained using Toludine blue reagent. The different stages of indirect organogenesis and somatic embryogenesis were observed and the images were captured using Nikon DS-fi 1.

Root induction and hardening

For root induction, multiple shoots of~2–3 cm height were separated carefully and inoculated to half strength MS nutrient medium with 0.1, 0.5 and 1 mg/l of IAA (Indole 3 acetic acid) or 0.1, 0.5 and 1 mg/l of NAA using paper bridge method. The shoots with well developed roots were later carefully taken out of the culture tubes and subjected to hardening.

For hardening, a mixture of sterile soil:sand:manure (1:1:1) was used and the plantlets were gradually exposed to the external environment using polythene covers. The

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polythene covers with single small hole was kept upside down over the plants and the size of the hole was increased progressively after every 2 to 3 days. After 2 weeks, the polythene cover was removed and the plants were exposed to the open environment. The plants were watered using sterile distilled water for 1 week and slowly changed to distilled water and then to tap water. The percent of survival of the plantlets were calculated.

Statistical analysis

Each treatment consisted of 30 explants and the experiment was repeated twice. The statistical analysis of all the data were carried out using SAS package (version 9.0) and the treatment means were compared using Duncun's Multiple Range Test (DMRT) at a level of 5 % significance.

Results and discussion

Callus induction and regeneration

The stem and leaf explants of *J. gendarussa* cultured on MS medium and supplemented with NAA + BAP (1.0+0.1 mg/l) showed the emergence of callus from the cut ends after a week of incubation. The calli obtained were cream, light yellow and light green (Fig. 1a and b).

The callus induction and adventitious shoot regeneration from the selected explants depends on the genotype and combinations of plant GR (Zhang et al. 2004). The induction of callus using only auxin or in combination with cytokinin has been reported in *Piper nigrum* L. (Sujatha et al. 2003); *Beta vulgaris* L. (Zhang et al. 2004); *Mucuna pruriens* (L.) DC. (Vibha et al. 2009) and *P. koreana* (Lin et al. 2011). Callus induction in the members of Acanthaceae such as *Beloperone plumbaginifolia* (Jacq.) Nees. using NAA, IBA, 2, 4-D (2, 4-Dichloro phenoxy acetic acid) (Shameer et al. 2009) and *Adhatoda vasica* Nees using different types of auxins (Maurya and Singh 2010) has also been reported.

Callus induction from *J. gendarussa* stem and leaf explants and direct organogenesis from nodal explants has been reported by Agastian et al. (2006), Bhagya and Chandrashekar (2010) and Thomas and Yoichiro (2010). Agastian et al. (2006) reported the callus induction using MS medium with 2.0 mg/l of NAA. Bhagya and Chandrashekar (2010) reported the induction of callus in the medium supplemented with NAA at 1.0 mg/l or 2, 4- D at 2.0 mg/l and Thomas and Yoichiro (2010) reported the induction of callus using Kn+2,4-D at the concentrations of 14+4.5 μM. however, in the present study, the induction of callus was achieved using MS medium with NAA + BAP at 1.0+0.1 mg/l concentrations.

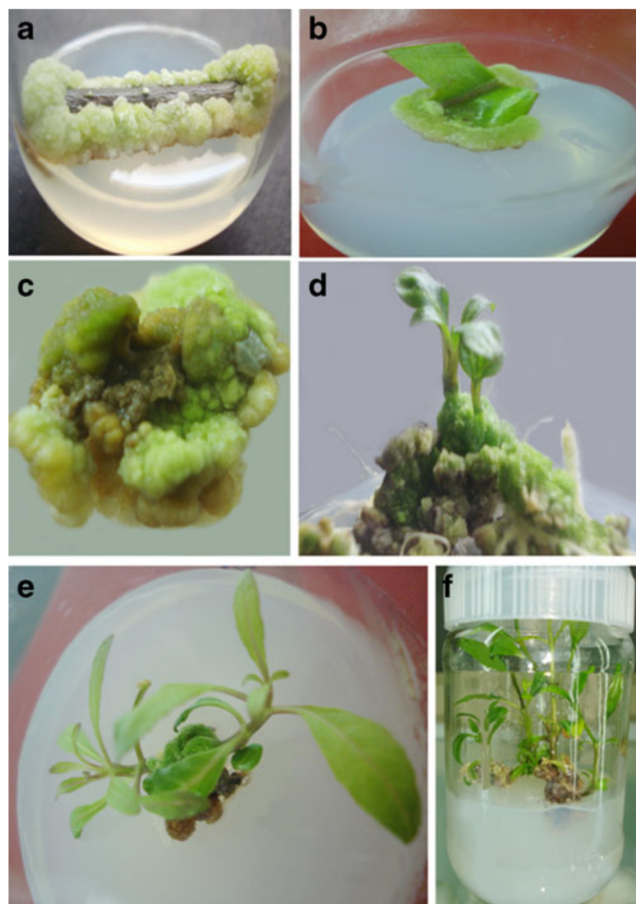

Fig. 1 Callus induction and indirect shoot organogenesis in *J. gendarussa* stem and leaf explants. **a** Callus induction from stem explant. **b** Callus induction from leaf explant. **c** Organogenic callus. **d** Indirect shoot organogenesis in stem callus. **e** Indirect shoot organogenesis in leaf callus. **f** A culture flask showing numerous indirect shoots

Indirect shoot induction

A full strength MS medium supplemented with different GR at different concentrations showed a significantly highest number of indirect shoot organogenesis in the medium supplemented with BAP + NAA at the concentrations of 0.1+1.0 mg/l with a maximum shoots per callus clump of 8.50±0.71 in stem derived callus and 7.00±1.41 in leaf derived callus (Table 1). The callus subcultured to fresh medium fortified with different concentrations of GR for shoot induction changed from light green to dark green color within 20–25 days of incubation (Fig. 1c). The emergence of indirect shoots was observed from the callus mass between 120 and 160 days of culture initiation (Fig. 1d and f). However, higher concentration of cytokinins alone (BAP / Kn at 3.0 mg/l) or in combination with NAA (BAP + NAA at 2.0+0.1, 3.0+0.1 mg/l and Kn + NAA at 3.0+0.1 mg/l) failed to induce shoots. From the present study, it was found that stem derived callus was more responsive than the leaf derived callus.

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Table 1 Effect of GR on indirect shoot organogenesis from stem and leaf callus of *J. gendarussa*

GR (mg/l)		Number of shoots per callus clump* (Mean ± SD)	
		Stem	Leaf
BAP	1.0	1.50±0.71^{def}	0.50±0.71^{b}
	2.0	1.00±1.41^{ef}	1.00±1.41^{b}
	3.0	0.00±0.00^{f}	0.00±0.00^{b}
Kn	1.0	2.50±0.71^{cde}	1.50±0.71^{b}
	2.0	3.50±0.71^{bc}	0.00±0.00^{b}
	3.0	0.00±0.00^{f}	0.00±0.00^{b}
BAP + NAA	0.1+1.0	8.50±0.71^{a}	7.00±1.41^{a}
	1.0+0.1	4.50±0.71^{b}	2.00±1.41^{b}
	2.0+0.1	0.00±0.00^{f}	0.50±0.71^{b}
	3.0+0.1	0.00±0.00^{f}	0.00±0.00^{b}
Kn + NAA	1.0+0.1	0.50±0.71^{f}	0.00±0.00^{b}
	2.0+0.1	3.00±1.41^{bcd}	2.00±1.41^{b}
	3.0+0.1	0.00±0.00^{f}	0.00±0.00^{b}
For stem: CV (%)=38.16, SE/plot=3.55			
For leaf: CV (%)=76.64, SE/plot=2.73			

* Values are Mean ± SD of two experiments. The values with the same letters are on par with each other at 5 % level

In vitro regeneration of plants through indirect shoot organogenesis has been reported in *P. nigrum* L. (Sujatha et al. 2003), *Memecylon edule* (Elavazhagan and Arunachalam 2010) and *P. koreana* (Lin et al. 2011). *Asteracantha longifolia* L. Nees, a member of Acanthaceae showed the induction of callus from stem and leaf explants in the medium fortified with varying concentrations of auxin/cytokinin. They also observed the indirect shoot proliferation when cultured on the medium supplemented with BAP + NAA at 2.0+0.5 mg/l (Mishra et al. 2006). Thomas and Yoichiro (2010) have reported the indirect shoot organogenesis from *J. gendarussa* leaf explants when cultured on MS medium supplemented with BAP + NAA at the concentrations of 17.7+5.4 μM with a maximum of 12 shoots per callus clump. Agastian et al. (2006) reported the production of 2–3 shoots per callus clump in *J. gendarussa* when cultured on MS medium fortified with NAA + BAP at 0.5+0.1 mg/l concentrations with a light intensity of 27 $\mu mol^{-2} s^{-1}$. From all these reports it is clear that BAP + NAA favour the indirect shoot organogenesis in *J. gendarussa* and *Asteracantha longifolia* and is in agreement with the present study with a maximum induction of indirect shoots from leaf and stem derived calli in the medium supplemented with BAP + NAA (0.1+1.0 mg/l).

Somatic embryogenesis

The light green and transparent white calli when subcultured to MS medium supplemented with different concentrations of GR showed the development of a dark green compact callus after a week of incubation. The compactness and the green color of the calli increased at the end of 1 month. In the subsequent subcultures, different stages of embryogenic calli were observed (Fig. 2). A significant difference among the embryogenic calli, somatic embryos and plantlets obtained in media with different GR at their varying concentrations was observed both in stem and leaf calli of *J. gendarussa* (Figs. 3 and 4). The stem derived calli showed the highest mean number of embryogenic calli (22.00±4.24 and 22.00±2.83) and somatic embryos per callus clump (19.00±5.66 and18.50±2.12) in the medium supplemented with Kn + NAA at the concentration of 2.0+0.01 and 1.0+0.1 mg/l respectively. Similarly, for leaf derived calli the maximum mean number of embryogenic calli (22.00±4.24) were observed in the medium supplemented with Kn + NAA (2.0+0.1 mg/l). The highest mean number of 20.00±1.41 and 18.00±4.24 somatic embryos was observed in the medium

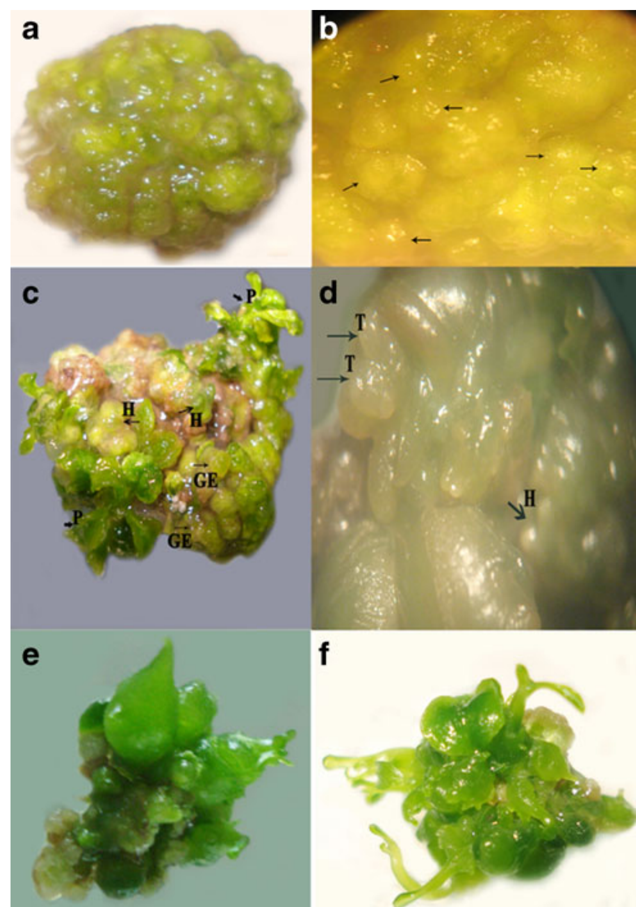

Fig. 2 Somatic embryogenesis in *J. gendarussa* explants. **a** A mass of globular embryos. **b** Globular embryos (*arrows*) under stereo microscope. **c** Mass of embryogenic callus showing globular (*GE*), heart shaped (*H*) and germinated embryo (*P*). **d** Stages of somatic embryogenesis under stereo microscope showing heart (*H*) and torpedo (*T*) shaped embryos. **e** Mature somatic embryo. **f** Germinated somatic embryos

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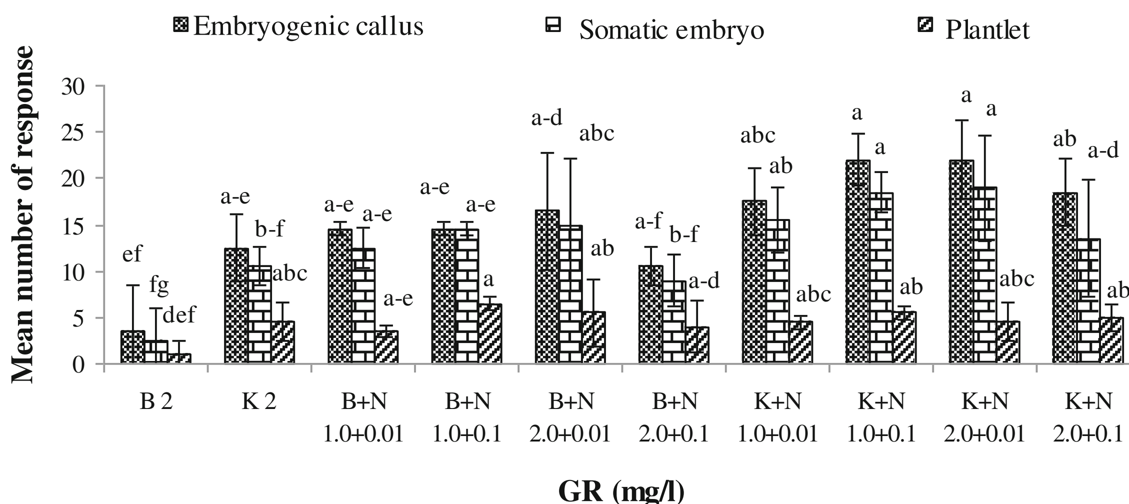

Fig. 3 Somatic embryogenesis in *J. gendarussa* stem derived callus. B—BAP, K—Kn, B + N—BAP + NAA, K + N—Kn + NAA. Values represent the Mean ± SD. Means with the same letters are on par with each other at $p<0.05$

supplemented with BAP + NAA (2.0+0.01 mg/l) and Kn + NAA (2.0+0.01 mg/l) respectively. The bipolar somatic embryos were developed with the enlargement of globular shaped cells which changed their shape to heart and torpedo shapes (Fig. 2). These bipolar structures later developed into plantlets within a week of incubation. The somatic embryos observed were green in color which was mainly due to the incubation of cultures in the presence of light. The time taken for the development of a plant from a somatic embryo was 2 to 3 months on an average with an illumination under the lower light intensity (40.0±3.0 μmol$m^{-2}s^{-1}$).

The maximum mean number of 6.50±0.70 plantlets per clump of stem derived calli was developed through somatic embryogenesis in the medium supplemented with BAP + NAA with 1.0+0.1 mg/l concentration (Fig. 3). From leaf derived somatic embryo, a maximum mean number of 5.50±0.70 plantlets was observed in the medium with Kn + NAA at the concentration of 2.0+0.1 mg/l (Fig. 4). The stem derived calli was more responsive towards the induction of plantlets through somatic embryogenesis method. However, both in stem and leaf derived calli, the GR used alone and in combinations of Zea/TDZ with NAA showed either lesser number of somatic embryo forming callus or none. Instead, the callus subcultured in these media led to repeated cell division resulting in the formation of a large mass of callus clumps.

The complete dedifferentiation of meristemoid cells lead to embryo formation followed by plant regeneration (Thorpe 1980). The regeneration of plantlets through somatic embryo has been reported in *Catharanthus roseus* (Dhandapani et al. 2008), *Mucuna pruriens* (L.) DC. (Vibha et al. 2009) and *P. koreana* (Lin et al. 2011). Shameer et al. (2009) reported the

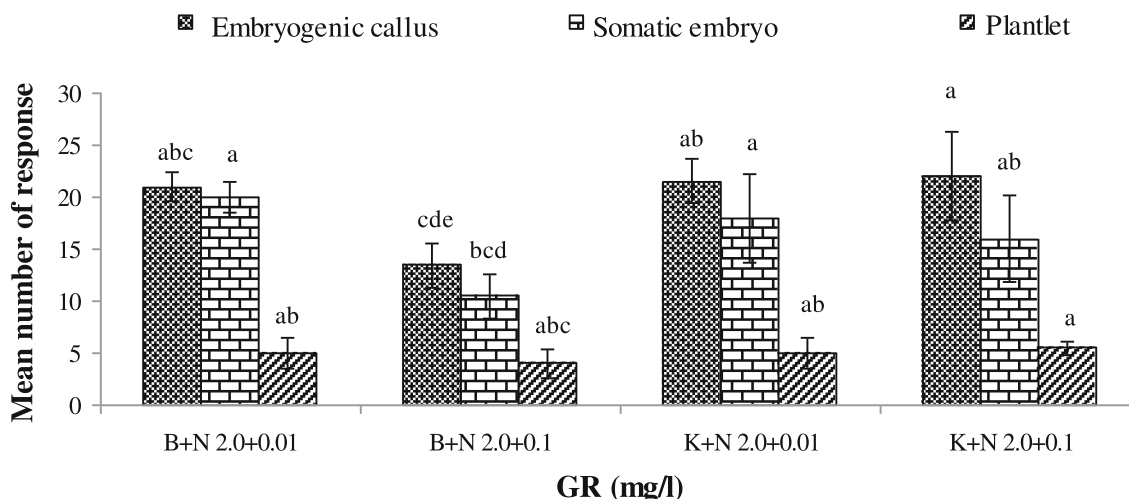

Fig. 4 Somatic embryogenesis in *J. gendarussa* leaf derived callus. B+N—BAP + NAA, K + N—Kn + NAA. Values represent the Mean ± SD. Means with the same letters are on par with each other at $p<0.05$

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regeneration of plantlets from *B. plumbaginifolia*, a member of Acanthaceae through somatic embryogenesis. In the present study also, plantlet regeneration was observed through somatic embryogenesis. However, all the somatic embryos produced did not germinate into plantlets and similar results were observed in other species like *M. pruriens* (Vibha et al. 2009) and *Crambe abyssinica* Hochst.ex R.E. Fries (Palmer and Keller 2011). The plant species such as *Tylophora indica* (Jayanthi and Mandal 2001), *C. roseus* (Dhandapani et al. 2008) and *Kalopanax septemlobus* (Moon et al. 2008) cultured under dark favoured the development of somatic embryos. Even in the present study, reduced light induced the somatic embryogenesis.

The induction of somatic embryo is dependent on various factors such as explant, genotype, GR, nitrogen source, polyamines oxygen supply etc. (Bhojwani and Razdan 2008). In *D. sissoo,* the development of somatic embryos was observed when the embryogenic calli was transferred to ½ strength MS medium without any GR (Singh and Chand 2003). High frequency somatic embryo induction was achieved using cotyledon derived calli of *M. pruriens* in the medium supplemented with 2.3 μM Kn and 5.4 μM NAA with 13.6 μM adenine sulphate (Vibha et al. 2009). Lin et al. (2011) observed the production of somatic embryo in *P. koreana* on MS medium supplemented with 0.25 mg/l Zea and 0.5 mg/l of IAA. In the present study, the development of somatic embryo was more in the medium supplemented with Kn + NAA or BAP + NAA (2.0+0.01 mg/l) indicating the role of relatively higher concentration of cytokinin and lower concentration of auxin. Similar results were reported in various medicinally and economically important plant species such as *Psophocarpus tetragonolobus* (L.) DC. (Ahmed et al. 1996), cotyledonary explants of *Arachis hypogaea* L. (Venkatachalam et al. 1999), *Psoralea corylifolia* Linn. (Sahrawat and Chand 2001) and *Digitalis lamarckii* Ivan. (Verma et al. 2011) where, MS medium supplemented with 0.5 mg/l NAA+1.0 mg/l BAP, 22.19 mM BAP+2.68 mM NAA, 1.4 μM NAA+2.2 μM BAP and 1.34 μM NAA+ 8.87 μM BAP respectively induced the frequency of somatic embryogenesis. This suggests that the induction of somatic embryo is highly species specific.

The stem explants of *Verbena officinalis* L. led to the production of enhanced plant regeneration compared to petiole explants (Turker et al. 2010). Jawahar et al. (2008) reported the induction of varying degrees of callus and shoots from the stem and leaf explants of *Cardiospermum halicacabum* L. when cultured on MS medium supplemented with varying concentrations of different GR. Lin et al. (2011) reported the enhanced frequency of shoot induction from the petiole and pedicel derived calli compared to the leaf derived calli. In the present study the stem explant was more responsive for plant regeneration. Thus it is clear that the in vitro regeneration of plants from different explants of the same plant is also depended on different factors as explained by Bhojwani and Razdan (2008).

Histological analysis

Based on the histological studies, it was clear that the callus initiation took place from the cambial region (Fig. 5a and b) with the accumulation of cytoplasmic content in the enlarged cambial cells. The induction of callus from the cambial cells, phloem and cortical cells was reported in tea (Frisch and Camper 1987), *Vigna radiata* W. (Park et al. 2002) and black pepper (Sujatha et al. 2003).

The histological studies of indirect organogenesis revealed the presence of smaller darkly stained meristemoid cells surrounded by larger parenchymatous cells (Fig. 5c and d). The sections passing through shoot organogenic callus showed the presence of shoot apical meristem with procambial strands and leaf primordium with small densely stained cells (Fig. 5d).

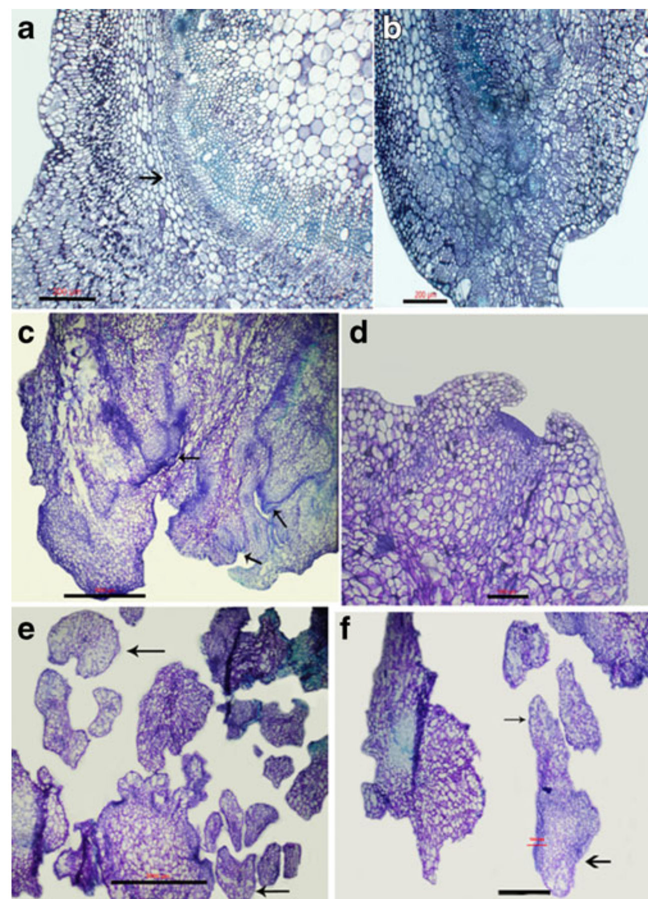

Fig. 5 Histology of *J. gendarussa* callus. **a** *J. gendarussa* stem explant showing callus induction from the cambial region (arrow) (bar=0.2 mm). **b** *J. gendarussa* leaf explant showing callus induction (bar=0.2 mm). **c** Section showing shoot apical meristems (*arrow*) (bar=5 mm). **d** Section showing single shoot apical meristem and leaf primordia (bar=1 mm). **e** Section showing heart (*arrows*) shaped embryos (bar=5 mm). **f** Section showing torpedo shaped embryo with root apex (*thin arrow*) and shoot apex (*thick arrow*) (bar=1 mm)

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Table 2 Effect of GR on root induction in *J. gendarussa* plantlets

GR (mg/l)	0.1	0.5	1.0	Mean GR
Number of roots per stem calli derived shoots*				
IAA	3.33±2.08bc	9.67±0.58a	10.00±0.00a	7.67±3.43a
NAA	2.67±0.58bcd	3.33±0.58bc	4.67±0.58bc	3.58±1.01b
Mean conc	3.00±1.41b	6.50±3.51a	7.33±2.94a	
CV (%)=17.32, SE/plot=0.97, $p<0.001$				
Number of roots per leaf calli derived shoots*				
IAA	4.67±2.08bc	9.33±0.58a	10.00±0.00a	8.00±2.74a
NAA	2.33±1.15bcd	4.33±0.58bc	5.00±1.00bc	3.89±1.45b
Mean conc	3.50±1.97b	6.83±2.79a	7.50±2.81a	
CV (%)=18.60, SE/plot=1.11				

*Values are Mean ± SD of two experiments. The values with the same letters are on par with each other at 5 % level

The shoot apical meristems were observed mainly at the periphery of the callus. Various stages of somatic embryogenesis were observed in the sections passing through the embryos (Fig. 5e and f). Small globular embryos which later developed into heart shaped embryos (Fig. 5e) and torpedo shaped embryos (Fig. 5f). The indirect shoot organogenesis and somatic embryogenesis showing different developmental stages were confirmed by histological studies in *Crocus sativus* L. (Ghareyazie et al. 2010), *Cinnamomum camphora* L. (Shi et al. 2010), *P. koreana* (Lin et al. 2011) etc.

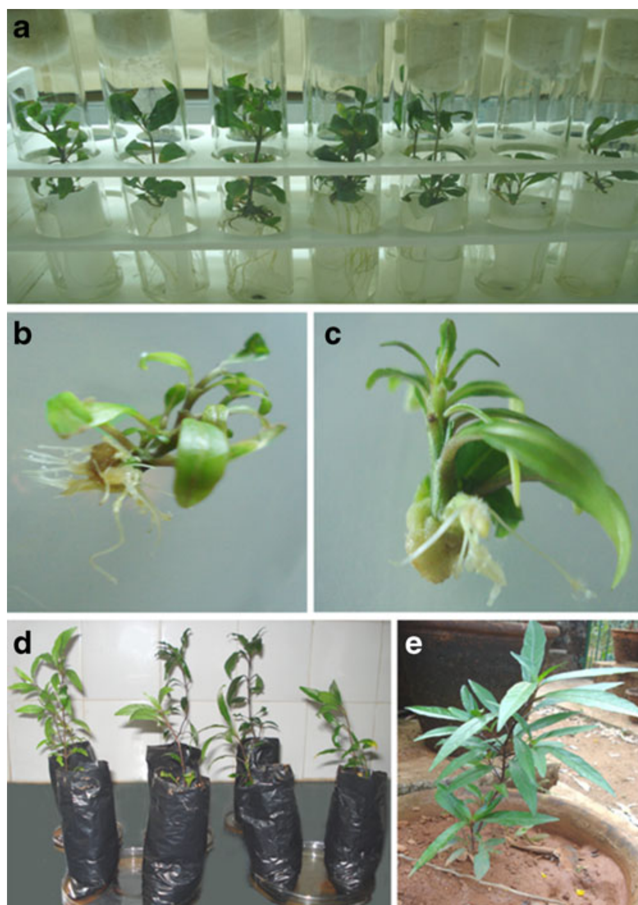

Fig. 6 Rooting and plant acclimatization. **a** Rooting of *J. gendarussa* in 1/2 MS medium supplemented using paper bridge. **b** and **c** In vitro regenerated plantlets. **d** Acclimatization of in vitro regenerated plants. **e** Hardened plant

Root induction and hardening

The optimum root induction was observed in the half strength medium supplemented with IAA at the concentration of 0.5 and 1.0 mg/l (Table 2). The roots began to emerge within 2 weeks of incubation of shoots in rooting medium (Fig. 6a), followed by the development of 2–3 lateral roots in the next 4–5 days. The mean number of roots produced in the medium supplemented with NAA was significantly lower than IAA. Auxins such as IBA and IAA are known to induce rooting in in vitro grown shoots. The use of IAA and IBA for root induction in *J. gendarussa* has been reported by Johnson et al. (2004), Bushrabi et al. (2008) and Thomas and Yoichiro (2010). The plantlets transferred to sterile sand:soil:manure mixture in polybag showed 68 % survival from indirect shoot organogenesis method and 81 % from somatic embryo derived method (Fig. 6b–e, Table 3). The percent survival of plants (Table 3) from polybag to field condition was 81 % for indirect shoot organogenesis and 92 % for somatic embryogenesis derived plants.

The present work provides a method for in vitro regeneration of plantlets via indirect organogenesis and somatic

Table 3 Percent survival of plants in the polybag and field conditions

Condition	Indirect shoot organogenesis	Somatic embryogenesis
Polybag	68 %	81 %
Field	81 %	92 %

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embryogenesis from stem and leaf explants of *J. gendarussa*. The present study forms the first report on the regeneration of plants through somatic embryogenesis and confirmation using histological studies. The study also provides a protocol for the production of a large number of in vitro regenerated plants which may help in the conservation and to further work on the isolation of bioactive compounds.

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