Physiol Mol Biol Plants (April–June 2014) 20(2):241–247
DOI 10.1007/s12298-014-0229-3

RESEARCH ARTICLE

Synergistic effect of BAP and GA_3 on in vitro flowering of *Guizotia abyssinica* Cass.-A multipurpose oil crop

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Received: 30 August 2013 / Revised: 3 October 2013 / Accepted: 18 March 2014 / Published online: 3 April 2014

Abstract Apical and axillary buds of *Guizotia abyssinica* Cass., isolated from seedlings raised in vitro, were cultured. High frequency of shoot regeneration was achieved on MS medium with BAP (1 mgl^{-1}). Effect of BAP, Kn and GA_3 applied successively in culture on shoot regeneration and flower bud formation has been studied. The shoots differentiated in cultures elongated on this medium. These rooted subsequently on half strength MS medium. The shoots flowered in vitro on MS medium with a combination of BAP (0.1mgl^{-1}) + GA_3 (0.1 mgl^{-1}). The plantlets thus formed were successfully hardened with 90 % survival.

Keywords Niger · In vitro flowering · Plant growth regulators · Biodiesel · *Guizotia abyssinica*

Introduction

Ramtil, Kalatil or niger (*Guizotia abyssinica* Cass.) belonging to the family Asteraceae is mainly cultivated in India and Ethiopia (Bhat and Murthy 2008) (Figs. 1a, b). The seeds of this plants are edible containing 27–50 % of yellow coloured oil having mild odour and pleasant nut like taste (Marini et al. 2003) (Fig. 1c). It is used in pharmaceuticals, food industries, as green manure and as cattle and bird feed. The predominant fatty acid in niger seed is linoleic acid (LA) present in rich amounts (54–85 %) (Asilbekova et al. 2005) and is known to prevent cardiovascular disorders and is, therefore, highly valuable. Recently it has also emerged as an alternative to fuel and being used as biodiesel. Despite being nutritionally rich and economically important it has by and large remained a neglected crop (Bhandari et al. 2009). Besides, the plant suffers from low yield due to self incompatibility, lodging, shattering, indeterminate growth habit and instability at higher temperature.

Successful utilization of plant biotechnology for plant improvement requires the development of an efficient shoot regeneration system from cultured cell or tissues (Stockigt et al. 1995). Micropropagation is the best method available for the production of high quality clonal plants which are free from any disease and pests ensuring the maximum production potential of varieties (Raven et al. 1999).

Plant tissue culture can especially be beneficial in oil crops. Oil production is based on seeds. The formation of seeds requires flowering phase in such commercial oil crops. Therefore, in vitro propagation along with in vitro flowering will not only help in production of better quality of oil plants but will also ensure shortening of the breeding cycle (Sivanesan and Jeong 2007; Kielkowska and Havey 2012; Haque and Ghosh 2013a) and studying the developmental pattern of flowers from vegetative to floral phase (Huang et al. 2009).

In vitro culture provides an ideal experiment system to study molecular mechanisms of flowering. However, the in vitro flower bud induction is still rare since it occurs only under special conditions. This induction depends on several features such as genetic, hormonal and trophic factors and seems to be the result of the repression of growth genes and the activation of those responsible for flowering process (Rkhis et al. 2006).

A number of protocols for plant regeneration and in vitro flowering through tissue culture has been reported in the past in *Guizotia abyssinica* (Sarvesh et al. 1996), *Ocimum basilicum* (Sudhakaran and Sivasankari 2002), *Vernonia cinerea* (Maheshwari and Kumar 2006), *Anthemis xylopoda* (Erdag and Emek 2009), *Scoparia dulcis* (Alieni et al. 2011), *Arachis paraguariensis* (Aina et al. 2012) and *Bacopa chamaedryoides* (Haque and Ghosh 2013b).

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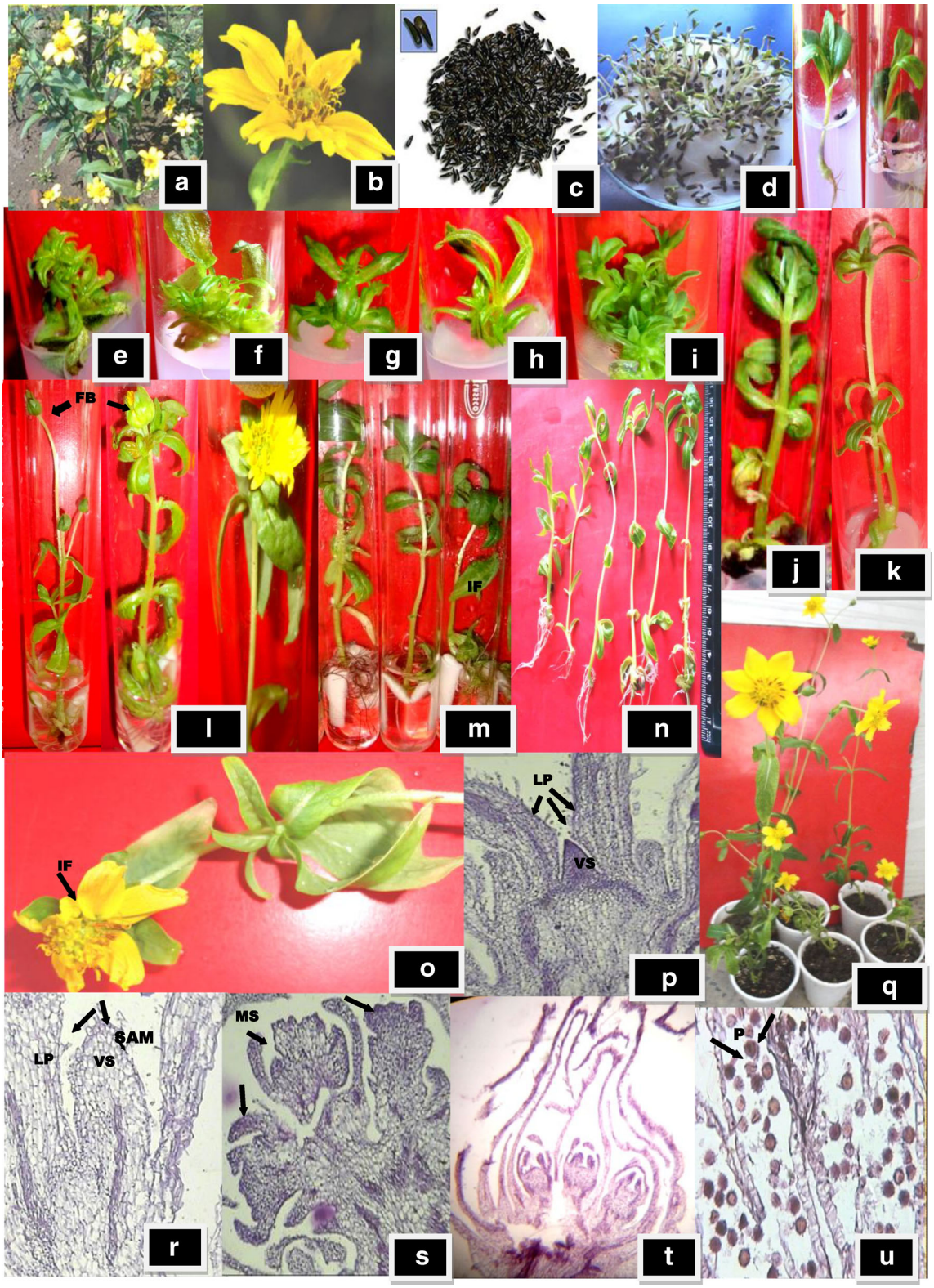

The main objective of this research was to study the effect of plant growth regulators such as BAP, Kn and GA_3 for clonal multiplication and in vitro flowering of *G. abyssinica* through apical and axillary bud explants.

Materials and methods

Seeds of niger var. JNC 6 were obtained from Jawaharlal Nehru Krishi Vishwavidyalaya (JNKVV) Jabalpur. Seeds

◀ **Fig. 1** In vitro Regeneration and flowering in *Guizotia abyssinica*. **a**–**c** Natural Plant, Flower and Seed of *Guizotia abyssinica*. **d**. Seed germination on Filter paper and Basal medium. **e**. Shoot initiation with BAP (1mgl^{-1}) 2 week. **f**. Multiple shoot formation with BAP (1mgl^{-1}) 3 week. **g**–**h**. Multiple shoot formation with Kn (0.5 mgl^{-1}). **i**. Multiple shoot formation with BAP + GA_3 (0.5+5 mgl^{-1}) 3 week from Axillary explants. **j**–**k**. Shoot elongation with Kn (0.5 mgl^{-1}) 4 week from Apical and Axillary bud explants. **l**–**m**. Flowering of in vitro regenerated plantlets. **n**. Rooting of shoots in ½ strength MS medium. **o**. Plantlets with well developed roots. **p**. Hardened in vitro regenerated plants in poly cup. **q**–**r**. Histological section showing shoot initiation and development. **s**. Histological section showing multiple shoot formation. **t**. Histological section showing carpel development **u**. Histological section showing Pollen development. *SAM* shoot apical meristem, *LP* leaf primordium, *VS* provascular stand, *MS* multiple shoots, *IF* in vitro flower, *FB* flower bud, *P* pollen

were soaked in water for 24 h and washed by wetting agent labolene (1 %) and then rinsed in running water (1 h). They were then surface sterilized using (w/v) 0.1 % $HgCl_2$ for 6–8 min followed by three rinses with sterile distilled water. Seeds were then inoculated on filter paper and MS (Murashige and Skoog 1962) medium (Fig. 1d).

Explant preparation

Three types of explants viz. apical, axillary buds (1.0 cm each) and root (0.8 to 1 cm) were isolated from 3 weeks old in vitro germinated seedling and inoculated on MS Basal medium.

Culture medium and condition

MS medium was supplemented with phytohormones BAP (6-Benzylaminopurin, Kn (6-Furfurylaminopurine) and GA_3 (Gibberellic acid) (0.1 to 5 mgl^{-1}), individually or in combinations. Sucrose (3 %) was used as the carbon source and gelled with 0.8 % agar. The pH of the medium was adjusted between 5.6 and 5.8 before autoclaving at a pressure of 15 lbs at 121 °C for 15 min.

All the cultures were maintained at 25 °C±1 under white fluorescent light of 16/8 h photoperiod. Explants were subcultured into appropriate medium after 15–20 days of inoculation. For root induction the in vitro regenerated shoots (4–6 cm long) were transferred to ½ strength MS medium containing 3 % (w/v) sucrose.

Hardening and acclimatization of plantlets

In vitro regenerated plantlets (3–4 months) possessing well developed shoot and root system were washed with tap water to remove adhering agar without damaging the delicate root system and treated with 1 % (w/v) fungicidal (Bavistin) solution for 5 min. The plants were then transferred to plastic pots containing sterilized sand, soil and farm yard manure (1:1:1). To maintain humidity, hardened plantlets were completely covered with plastic bags, and irrigated regularly with distilled water. After 2 weeks the plastic covers were perforated with small holes. The bags were removed intermittently (5–10 min) to aid adaptation of in vitro plantlets to normal environment conditions. Subsequently exposure time was increased in the following weeks and after 7–8 weeks plastic covers were removed completely. After 8 weeks of hardening the plantlets were transferred to the field.

Histological studies

In vitro behaviour of explants was evaluated through visual observations and histological studies of the cultured tissue. The tissues were fixed in Formalin-Aceto-alcohols (FAA) solution (90 ml of 70 % ethyl alcohol, 5 ml of glacial acetic acid and 5 ml of formalin) (Berlyn and Miksche 1976; Tisserat and De Mason 1985) for 18 h, washed for 30 min with tap water, dehydrated by transferring through an ethanol–xylol series and then embedded in paraffin. Tissues were sectioned at 10 μm thickness with microtome, mounted on glass slides, and stained with 0.5 % safranin. The slides were dehydrated with successive grades of ethanol and counter stained with 0.5 % fast green (O'Brien and Mccully 1981) (fast green dissolved in 1:1 clove oil, and absolute ethanol (v/v). These slides were further differentiated in absolute ethanol and xylol series and cleared with pure xylol and finally mounted with Canada balsam. Histological evaluation of the sectioned materials were made using Olympus CH20i light microscope and photographed.

Experimental design, data collection and analysis

The experiments were set according to completely randomized design and all experiments were performed in triplicates with 12 treatments. The data were recorded within 25–30 days of inoculation. The data were analyzed using analysis of variance (ANOVA) and means were separated at $p=0.05$ level of significance using Duncan's multiple range test (DMRT) using the statistical software IBM SPSS 20.

Results and discussion

Shoot initiation and multiplication

The different responses of the explant types are probably due to endogenous hormonal balance in plant tissues (Asghari et al. 2012). Both apical and axillary bud explants of *G. abyssinica* responded within a week of culturing with basal swelling, however the root explants failed to produce shoots, and only profuse rooting was observed. Bud breakage and development of shoots from apical and axillary bud explants is known to be a function of cytokinin activity. In this study maximum frequency of shoot induction was obtained from

apical bud than axillary bud. Apical bud is known to grow and regenerate more quickly into plantlets than cultured tissue from other sources (Walkey 1980). It is probable that the higher suitability of apical bud for regenerative response may be attributed to the predominant presence of diploid cells in them which undergo higher meristematic activity as compared to axillary bud (Lakshmi Sita 1991; Piccioni and Standardi 1995; Gomes and Canhoto 2003). The effectiveness of apical bud on highest shoot multiplication than axillary bud explants has been also reported in *Pterocarpus santalinus* (Arokiasamy et al. 2000) in *Cardiospermum halicacabum* (Jayaseelan 2001) and *Heliotropium indicum* (Kumar and Rao 2007).

On the other hand root explants only produce callus found to lack the organogenic competence and which failed to differentiate into shoots similar to the past report in this plant (Bhandari et al. 2009).

Multiple shooting was found to be more pronounced when MS media was supplemented with BAP (1 mgl^{-1}) (Figs. 1e, f and Table 1). According to Buising et al. (1994) a single exposure to a low concentration of BAP reprograms the development throughout the shoot apex and causes cells that would normally remain quiescent to instead divide repeatedly and give rise to supernumerary vegetative buds.

The naturally occurring ribosides and nucleotides in the cytokinin BAP are relatively more stable in comparison to other cytokinins (Letham and Palni 1983), one of the possible explanations for the improved response obtained. In contrast, higher concentration of BAP beyond the optimal level (1 mgl^{-1}) inhibited the overall shoot sprouting frequency, number of shoot and shoot length. Reduction in the regeneration potential appeared to be due to detrimental effect of high concentration on the cells predetermined to form vegetative buds. Similar results were obtained in this plant earlier by different workers (Jadimath et al. 1998; Bhandari et al. 2009).

Kinetin supplemented media on the other hand were less responsive. The superiority of BAP over other cytokinins in shoot bud regeneration has previously been reported in this plant (Nikam and Shitole 1993; Bhandari et al. 2009). The medium supplemented with Kn (5 mgl^{-1}) showed best morphogenetic response from both apical bud and axillary bud explants (Figs. 1g, h) (Table 1).

MS supplemented with GA_3 was unable to exert promotive influence on mean shoot number induced per explant and subsequent shoot development from both apical and axillary buds both at low and high concentrations (1 mgl^{-1}) (Table 1). However, there was a progressive increase in mean shoot length on increasing GA_3 concentrations. Similar results were obtained in *Tylophora indica* (Rani and Rana 2010). GA_3 has been reported to be conducive for in vitro shoot regeneration (Chakraborty et al. 2000), promotion of growth, biomass production and xylem fibre length (Ericksson et al. 2000). Furthermore, GA_3 can act as a replacement for auxin in shoot induction and thus a ratio of cytokinin- GA_3 is decisive for differentiation of certain plant tissues (Sekioka and Tanaka 1981).

A combination of BAP (0.5 mg/l) and GA_3 (5 mg/l) was optimum for not only induced a faster bud break (within 7 days) but also enhanced the frequency of mean shoot number from axillary bud explants in the present study (Fig. 1i) (Table 1). A combination of BAP- GA_3 has been reported to show a synergistic influence on multiple shoot formation in *Ocimum basilicum* (Sahoo et al. 1997).

Shoot elongation

Both apical and axillary buds provided high mean shoot lengths in media supplemented with Kn (Figs. 1j, k). Similar results were reported earlier in this species by Bhandari et al. (2009) and in *Matthiola incana* (Hesar et al. 2011). This is corroborated by earlier report where shoot induction frequency is decreases along with rhizogenesis (Rauf et al. 2004; Gailite et al. 2010).

In vitro rooting

The in vitro regenerated shoots (3–4 cm) from both explants were transferred to ½ MS medium without auxin (Figs. 1m, n). Root formation from the basal cut portion of the shoots were observed after 1 week and rooting frequency gradually increased overtime and reached maximum (100 %) after 2–3 weeks of culture.

The success of in vitro regeneration relies on the rooting percentage and survival of the plantlets in the field conditions. The incidence of root formation on auxin free medium may be due to the availability of endogenous auxin in in vitro shootlets (Minocha 1987). Similar results were obtained in *Dendrobium microbulbon* (Sharma et al. 2007), in *Stevia rebaudiana* (Sai Murali et al. 2011) and in *Bacopa chamaedryoides* (Haque and Ghosh 2013a, 2013b).

In vitro flowering

Flower bud initiation started on the apex and axils of some branches of in vitro regenerated shoots after 3 weeks on a combination of lower concentrations of BAP (0.1 mg/l^{-1}) + GA_3 (0.1 mg/l^{-1}). This exogenous hormonal supply might have been added up to the endogenous contents, raising the hormonal level required for triggering the flowering.

In the present study the frequency of flowering was found to be 70 %. The maximum number of flower buds (15–20) initiated in plantlets which developed on GA_3 supplemented media in combination with BAP (Fig. 1l, o). This result is therefore an improvement over the earlier published report (Sarvesh et al. 1996) which showed much less (40 %) in vitro flowering.

Table 1 Effect of BAP, KN and GA_3 (alone) and in combination on shoot proliferation in apical and axillary bud explants of *Guizotia abyssinica* Cass

PGRs	Conc. (mg/l^{-1})	FSI		MSN		MSL	
		ApB	AxB	ApB	AxB	ApB	AxB
Control		80.55^{b}	63.88^{b}	1.02±0.02^{a}	6.11±0.11^{b}	2.99±0.20^{b}	1.97±0.09^{a}
BAP	0.1	86.10±4.53^{b}	69.44±2.27^{b}	2.57±0.08^{b}	2.55±0.08^{b}	3.10±0.16^{d}	2.13±0.04^{b}
	0.5	86.10±2.26^{b}	69.44±4.53^{b}	3.41±0.08^{c}	2.44±0.07^{b}	2.51±0.05^{c}	2.04±0.09^{b}
	1	**94.44±2.27^{b}**	**88.88±2.26^{c}**	**4.08±0.12^{d}**	**3.55±0.04^{c}**	1.55±0.37^{b}	2.98±0.06^{c}
	5	27.77±2.26^{a}	27.77±2.26^{a}	1±0.04^{a}	1±0.04^{a}	0.33±0.04^{a}	0.15±0.04^{a}
Kinetin	0.1	58.33±3.93^{a}	47.21±4.54^{a}	1.49±0.04^{c}	1.05±0.02^{a}	4.97±0.14^{b}	**6.80±0.14^{d}**
	0.5	77.77±4.53^{b}	61.11±2.27^{ab}	1.02±0.02^{a}	1.31±0.06^{b}	**7.15±0.2^{d}**	5.46±0.12^{c}
	1	88.88±2.26^{bc}	86.10±2.26^{c}	1.16±0.04^{b}	1.24±0.04^{b}	5.64±0.03^{c}	5.05±0.01^{b}
	5	91.66±0.72^{c}	72.22±9.08^{bc}	2.38±0.02^{d}	1.63±0.02^{c}	2.62±0.02^{a}	1.26±0.01^{a}
GA_3	0.1	61.10±2.77^{ab}	61.10±2.77^{b}	1.27±0.02^{a}	1.19±0.02^{a}	4.46±0.23^{b}	4.27±0.19^{b}
	0.5	63.88±2.77^{c}	58.33±4.81^{b}	1.36±0.11^{a}	1.44±0.02^{b}	3.77±0.09^{a}	2.65±0.09^{a}
	1	52.77±2.78^{a}	52.77±2.77^{ab}	1.38±0.02^{a}	1.52±0.02^{b}	4.41±0.20^{b}	3.77±0.19^{b}
	5	44.44±2.79^{a}	44.44±2.78^{a}	1.19±0.02^{a}	1.86±0.07^{c}	**6.30±0.17^{c}**	**6.29±0.17^{c}**
BAP + GA_3	0.1+0.1	61.10±4.53^{hij}	72.22±2.27^{hij}	2.61±0.12^{fgh}	2.55±0.02^{hi}	5.27±0.11^{n}	4.69±0.25^{m}
	0.1+0.5	69.44±2.27^{jkl}	75.00±1.36^{ij}	2.86±0.07^{ghi}	2.88±0.15^{ij}	5.01±0.13^{n}	4.94±0.23^{m}
	0.1+1	77.11±1.72^{lm}	63.88±2.26^{fghi}	2.41±0.33^{efg}	2.24±0.08^{efgh}	2.63±0.41^{k}	2.66±0.25^{ij}
	0.1+5	72.22±2.26^{klm}	55.55±2.26^{efg}	2.27±0.14^{ef}	2.19±0.05^{defgh}	3.77±0.26^{l}	3.78±0.08^{l}
	0.5+0.1	69.44±2.27^{jkl}	66.66±3.93^{ghij}	2.66±0.12^{fgh}	2.91±0.33^{ij}	1.33±0.10^{gh}	1.34±0.10^{def}
	0.5+0.5	58.33±2.26^{ghi}	55.55±2.26^{efg}	2.61±0.11^{fgh}	2.27±0.28^{fgh}	0.61±0.09^{abcde}	0.67±0.12^{bc}
	0.5+1	63.88±2.26^{ijk}	69.44±2.27^{hij}	3.05±0.43^{hi}	2.49±0.38^{ghi}	1.04±0.19^{efgh}	0.93±0.03^{cd}
	0.5+5	72.22±2.27^{klm}	61.10±2.26^{fgh}	**3.25±0.56^{i}**	**3.88±0.26^{k}**	1.32±0.04^{gh}	2.27±0.41^{hi}
	1+0.1	69.44±4.53^{jkl}	72.22±2.27^{hij}	2.38±0.11^{efg}	2.30±0.27^{fgh}	0.72±0.10^{bcdef}	1.24±0.12^{de}
	1+0.5	80.55±2.27^{m}	77.77±1.36^{j}	3.24±0.12^{i}	3.02±0.20^{j}	0.93±0.08^{defg}	0.54±0.02^{abc}
	1+1	63.88±1.72^{ijk}	52.77±2.26^{ef}	2.44±0.05^{efg}	1.72±0.14^{abcd}	0.33±0^{abc}	0.32±0.01^{ab}
	1+5	41.66±2.26^{de}	38.21±2.26^{d}	2.47±0.11^{efg}	1.66±0.12^{abc}	0.39±0^{abcd}	0.34±0.02^{ab}
	5+0.1	22.22±2.27^{c}	19.44±3.93^{c}	1.30±0.05^{ab}	1.38±0.05^{ab}	0.32±0^{abc}	0.29±0.02^{ab}
	5+0.5	16.66±2.26^{bc}	13.85±2.26^{bc}	1.22±0.02^{ab}	1.69±0.02^{abcd}	0.19±0.01^{ab}	0.18±0.01^{ab}
	5+1	11.10±2.26^{ab}	8.33±2.27^{ab}	1.02±0.02^{a}	1.55±0.05^{abc}	0.13±0^{ab}	0.13±0^{ab}
	5+5	5.55±2.27^{a}	2.77±2.26^{a}	1.33±0.02^{ab}	1.35±0.02^{a}	0.10±0^{a}	0.00
Kn + GA3	0.1+0.1	50.00±1.36^{efg}	47.22±2.27^{de}	1.58±0.04^{ab}	1.55±0.13^{abc}	1.22±0.10^{fgh}	1.30±0.32^{def}
	0.1+0.5	55.55±2.26^{fghi}	61.10±2.26^{fgh}	1.55±0.02^{ab}	1.80±0.19^{abcdef}	1.96±0.07^{ij}	1.62±0.03^{efg}
	0.1+1	47.21±4.54^{ef}	61.10±2.26^{fgh}	1.02±0.02^{a}	1.99±0.09^{cdef}	4.29±0.27^{m}	3.72±0.16^{l}
	0.1+5	77.77±4.54^{lm}	55.55±4.53^{efg}	3.26±0.18^{i}	2.27±0.1^{fgh}	3.29±0.14^{l}	3.40±0.08^{kl}
	0.5+0.1	69.44±2.27^{jkl}	63.88±2.26^{fghi}	1.99±0.19^{cde}	1.74±0.08^{abcde}	2.62±0.24^{k}	3.16±0.5^{ijk}
	0.5+0.5	61.10±2.26^{hij}	61.10±2.27^{fgh}	1.46±0.05^{ab}	1.74±0.04^{abcde}	2.72±0.11^{k}	1.76±0.33^{efgh}
	0.5+1	72.22±2.27^{klm}	47.22±2.27^{de}	1.74±0.04^{bcd}	1.94±0.05^{cdef}	2.52±0.35^{k}	1.29±0.03^{def}
	0.5+5	52.77±2.26^{fgh}	44.44±2.27^{de}	1.74±0.12^{bcd}	1.97±0.12^{cdef}	3.48±0.42^{l}	3.08±0.32^{jk}
	1+0.1	36.10±2.26^{bc}	52.77±4.54^{ef}	1.58±0.04^{ab}	1.75±0.02^{abcde}	1.56±0.02^{hi}	1.77±0.01^{efgh}
	1+0.5	69.44±2.27^{jkl}	63.88±2.26^{fghi}	2.13±0.02^{def}	1.94±0.02^{cdef}	2.20±0.23^{jk}	1.94±0.04^{gh}
	1+1	47.21±4.54^{ef}	41.66±2.26^{d}	1.22±0.02^{a}	1.69±0.07^{abc}	0.67±0.06^{abcdef}	1.69±0.09^{efgh}
	1+5	52.77±4.54^{fgh}	55.55±4.53^{efg}	1.47±0.02^{ab}	1.99±0.04^{cdef}	2.77±0.3^{k}	1.99±0.02^{gh}
	5+0.1	52.77±2.27^{fgh}	61.10±2.26^{fgh}	1.02±0.02^{a}	1.85±0.02^{abcdef}	0.27±0.01^{ab}	1.85±0^{fgh}
	5+0.5	63.88±2.26^{ijk}	66.66±2.27^{ghij}	1.10±0.02^{a}	1.88±0.05^{bcdef}	0.52±0.01^{abcde}	1.88±0^{fg}h
	5+1	63.88±2.27^{ijk}	66.66±2.27^{ghij}	1.44±0.2^{ab}	1.55±0.05^{abc}	1.44±0.22^{ghi}	1.55±0.13^{efg}
	5+5	61.10±2.26^{hij}	69.44±2.27^{hij}	1.55±0.05^{ab}	2.02±0.02^{cdefg}	0.88±0.04^{cdefg}	2.02±0.02^{gh}

Experiment was repeated thrice with 12 replicates. Values represent mean ± standard error. Mean values followed by different superscript letters is significantly different at $P \leq 0.05$, as determined by Duncan's Multiple range Test. The bold data showed the best results

The gibberellins are known to be involved in several physiological processes such as seed germination, flower induction and its development as well as shoot elongation (Ameha et al. 1998). The significance of GA_3 to induce in vitro flowering has also been reported in *Coriandrum sativum* L. (Stephan and Jayabalan 1998).

The positive role of BAP and GA_3 in morphogenesis, especially floral morphogenesis was evident in the present work. The role of cytokinins is to initiate mitosis and that of gibberellins is to affect elongation in axial organs such as stems and flower pedicels. In vitro flowering was obtained with BAP and GA_3 combination which is in accordance with previous reports in, *Pharbitis nil* (Galoch et al. 2002), *Panax ginseng* (Chang and Hsing 1980; Lin et al. 2003). The floral morphology of the in vitro plantlets was normal and identical to the donor plants. Gradual processes of flower bud initiation to complete flower formation was studied histologically (Figs. 1t, u). The different stages observed during floral morphogenesis in vitro were evidenced by similar stages in mother plant.

It is known that during the change from the vegetative to the flowering state, the growth correlations within the apical meristem of a shoot are changed, which leads to the loss of apical dominance (Bernier et al. 1981). Apical dominance is under hormonal control with auxins, cytokinins and gibberellins having a sequential role (Ali and Fletcher 1970). In vitro flowering serves as an important tool in studying flower induction, initiation and the floral developmental process by utilizing plant growth regulators such as cytokinins, gibberellins and auxins (Zivand and Naor 2006). In vitro flowering can also reduce the influence of environmental factors and can clarify the key influences affecting the flowering process by controlling environmental factors and the application of plant growth regulators (Zhang et al. 2008).

Plant tissue culture system coupled with histology offers the possibility of identifying the point determination of cell differentiation leading to morphogenesis (Fukuda and Komamine 1985).

Histological studies were carried at different stages of shoot development. After 1 week of culture, cell division started in the cell of the adaxial epidermis. Subsequent division resulted in formation of new meristematic centers along the epidermis (Figs. 1p, r). After 2 weeks, the meristematic domes were formed on the surface of explants which developed into multiple shoots (Fig. 1s) with primordial leaves and were embedded in surrounding vascular system.

The histological studies with *G. abyssinica* clearly showed that plant regeneration occurred via *de novo* multiple shoot buds differentiation.

Acclimatization of tissue culture raised plants

Well developed plantlets were transferred to poly cups containing sterile sand, soil and farm yard manure (1:1:1) under high humidity. After 4–5 weeks approximately 90 % plantlets survived upon hardening. The hardened plantlets were transferred to field after 2 months.

Conclusion

A competent and reliable protocol for micropropagation and flower induction in *G. abyssinica*, from apical and axillary bud explants was developed. However, further studies need to be conducted for the evaluation of molecular mechanism of cytokinin and GA_3 behind in vitro flowering. The protocol developed in this study will certainly help in accelerating the niger breeding programs for the production of novel hybrid clones, and more reliable culture regimes need to be elucidated in future. The flowering plantlets described in the present study may have practical values in wide hybridization and fertilization studies of *G. abyssinica* Cass.

Acknowledgments One of us (S. Baghel) acknowledges funding received under the scheme Rajiv Gandhi National Fellowship (RGNF) of University Grants Commission (UGC), New Delhi India for carrying out this research.

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