Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.4036

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (5):** 40-47 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

In vitro Regeneration in Callus Culture of Gliricidia [*Gliricidia sepium* (Jacq.) Steud.]

Kaushlya Choudhary, M. L. Jakhar*, Aparna, Ravi Kumar and Hari Ram Jat

Department of Plant Breeding and Genetics,

SKN College of Agriculture, Jobner (Jaipur) 303329

*Corresponding Author E-mail: mljakhar@yahoo.com

Received: 12.06.2017 | Revised: 20.07.2017 | Accepted: 29.07.2017

ABSTRACT

An efficient protocol was developed for callus induction and shoot regeneration in Gliricidia [Gliricidia sepium (Jacq.) Steud.]. Callus induction was observed at majority levels of plant growth regulators, however, profuse callus induction was observed on MS medium supplemented with 0.25 and 0.5 mg/l BAP alone and in combination 0.5 mg/l (BAP) + 2.5 mg/l (IAA). The shoot morphogenesis was observed in callus when incubated at 0.5 mg/l (BAP) + 2.5 mg/l (IAA), upon subculture on same levels of plant growth regulator. De novo shoot organogenesis from callus cultures were observed with 50 – 60 % frequency. Photoperiod regime 14:10 was found best for shoot bud and callus induction. Highest root induction was observed on 0.5 mg/l IAA under in vitro proliferated shoot with 100 % frequency.

Key words: *Gliricidia, callus induction, regeneration, plant growth regulator*.

INTRODUCTION

Gliricidia [G*liricidia sepium* (Jacq.) Steud., 2n =22] is a fast growing, medium size, semi deciduous, multipurpose forage tree belonging to the family Fabaceae. It is an introduced forage tree in India. Central America and possibly South America are believed to be native place of this forage tree[8,10]. The genus *Gliricidia* has three main species namely *Gliricidia sepium, Gliricidia maculata* and *Gliricidia brenningii*[8,11]. The genus name *Gliricidia* is usually associated with *Gliricidia sepium* because it is the most widely known and cultivated species. The plant is used for timber, fuel wood, hedge, medicinal purpose, live-fences, plantation shades, poles, soil stabilization and as green manure. *Gliricidia* is widely used to provide shade for crops like cacao, coffee, and other shade loving crops, living fence post for pasture and properly boundaries and as a fallow tree to improve degraded land. Its ease of propagation by seed, small and large cuttings make it a very easy tree for farmers to multiply quickly. It is probably the most widely cultivated multipurpose agro forestry tree after *Leucaena leucocephala*[14]. It has tremendous scope in Rajasthan for forage and green manure. However, problem of seed setting and germination of this plant is not at considerable level.

Cite this article: Choudhary, K., Jakhar, M.L., Aparna, Kumar, R. and Jat, H.R., *In vitro* Regeneration in Callus Culture of Gliricidia [*Gliricidia sepium* (Jacq.) Steud.], *Int. J. Pure App. Biosci.* **5(5):** 40-47 (2017). doi: http://dx.doi.org/10.18782/2320-7051.4036

Copyright © Sept.-Oct., 2017; IJPAB

In addition to this breeding work in this crop is very limited and variability is also a limiting factor for improvement of the crop. Thus the present study be carried out to explore *in vitro* potential of plant cell culture techniques and regeneration protocol for the improvement of this crop.

MATERIALS AND METHODS

The planting material (soft nodal stem segments) was obtained from Herbal Garden maintained under Department of Plant Breeding and Genetics, S.K.N. College of Agriculture, Jobner. Soft nodal stem segment explants of Gliricidia were surface sterilized with 0.1% mercuric chloride for 2 minutes then washed thoroughly by sterilized double distilled water for 4-5 times.The scalpel, inoculation needles and forceps were kept in rectified spirit and flamed before use. Then explants transferred aseptically to sterilized culture bottles/phyta jars, each dispensed with 40 ml containing Murashige and Skoog[13] (MS) medium. The aseptic inoculation was done in a laminar air flow chamber. The chamber was sterilized by ultraviolet irradiation for about 30 minutes. All the cultures were incubated in a growth chamber maintained at 25 ± 2 ^{0}c temperature and 3000 lux obtained from fluorescent tubes. The distance between light source and culture vessels was kept constant. A photoperiod of 14 :10 hrs was maintained.

Each experiment was conducted on a particular growth regulator at different concentration of auxin (IAA/2, 4-D) and cytokinin (BAP/Kn). These PGR added alone to MS medium in different concentration ranged from 0.2 – 4.0 mg/l and in two combination treatments at the concentration of 0.5 and 2.5 mg/l each, were replicated ten times and repeated twice. Culture vessels having soft nodal stem segment explants treatment were kept in growth chamber (culture room). For rooting *in vitro* regenerated shoots were transferred aseptically with help of sterile forceps into new glasswares on MS medium supplemented with IAA (0.2-4.0 mg/l). Cultures were observed periodically and at the end of 30th day of each experiment and observation were taken for callus induction, initiation of shoot/root formation and *de novo* organogenesis.

RESULT AND DISCUSSION

In the current investigation both the cytokinins, when incorporated singly in the basal medium, induced multiple shoot buds at all levels of BAP/Kn with 60 -100% frequency, however, highest shoot buds (3.3) were observed when soft nodal stem segments inoculated on basal medium containing 0.5 mg/l BAP (Table 1) with 100% frequency. After 8-10 days of incubation slight creamish, compact callus was observed at the base of explants. There was a decrease in callus differentiation as the level of BAP increased and maximum callus proliferation was observed at 0.25 and 0.50 mg/l BAP with the 100 per cent frequency (Fig. 3). Callus induction completely inhibited at higher concentrations (3.0, 3.5 and 4.0 mg/l) of BAP. This result was in accordance with results obtained by Bai *et al*[1]., and Kumari[9] with respect to plant growth regulator (BAP). They observed maximum rate of shoot multiplication at 0.5 mg/l BAP in *Sesbania speciosa* and *Bauhinia variegate*, respectively. Thangjam[17] and Bonyanpour and khosh-khui[3], reported role of BAP in induction of callus in *Parkia timoriana* and pomegranate, respectively.

In Kn supplemented medium, the soft nodal stem segment explants started to grow within 10 -12 days of incubation, predominantly shoot multiplication, callus and roots induction were observed at all the levels. Maximum shoot bud induction (2.2) was observed at 0.75 mg/l with 80 per cent frequency (Table 1). As like BAP, mean number of shoot declined with increasing the level of Kn. Medium callus differentiation was observed at 0.5 to 1.50 mg/l level with the 60 per cent frequency. Callus induction frequency declined with the increasing level of Kn. Callus induction was completely inhibited at 3.0, 3.50 and 4.0 mg/l concentration of Kn.

Fig. 1: Shoot induction in *Gliricidia sepium* on MS supplemented medium with 0.50 mg/l. BAP

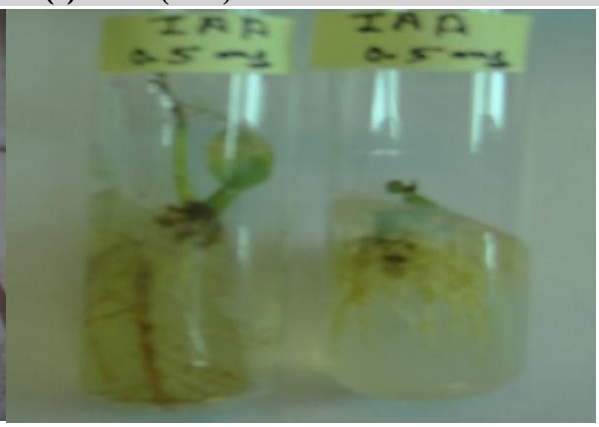

Fig. 2: Root induction in *Gliricidia sepium* on MS medium supplemented with 0.50 mg/l IAA

Table 1: Morphogenetic effect of cytokinins (BAP/Kn) added singly in the MS medium in *Gliricidia sepium*

Concentration (mg/l)	BAP			
	Callus induction	Frequency (%)	No. of shoot/ explants±SE N=10	Frequency (%)
0.25	+++	100	2.0 ±0.14	80
0.50	+++	100	3.3 ±0.15	100
0.75	+++	80	1.2 ±0.13	60
1.00	++	60	1.5 ±0.22	60
1.50	++	60	2.1 ±0.23	60
2.00	++	60	1.3 ±0.15	60
2.50	+	40	1.4 ±0.22	60
3.00	-	-	1.1±0.10	40
3.50	-	-	1.2 ±0.13	40
4.00	-	-	1.1 ±0.10	40
Concentration (mg/l)	**Kn**			
	Callus Induction	**Frequency (%)**	**Multiple shoot induction±SE N=10**	**Frequency (%)**
0.25	++	60	1.2 ±0.20	40
0.50	++	60	1.4 ±0.22	40
0.75	++	60	2.2 ±0.28	80
1.00	++	60	1.4 ±0.21	60
1.50	++	60	1.2 ±0.13	60
2.00	+	20	1.4 ±0.16	60
2.50	+	20	1.5 ±0.17	60
3.00	-	-	1.3 ±0.22	40
3.50	-	-	1.2 ±0.21	40
4.00	-	-	1.1 ±0.10	40

(+) = Slight callus (++) = Medium callus (+++) and = Profuse callus

Auxin (IAA/2,4- D) predominantly induce roots and multiple shoots from soft nodal stem segment explants. IAA mainly induces roots from soft nodal stem segment explants, whereas explants did not show any morphogenetic response at all levels (0.2 to 4.0 mg/l) of the 2, 4-D except swelling in the explants (Table 2). Auxins are mostly used for rooting and callus induction. Rooting response of shoot was reported to be controlled by growth regulators in the medium[2], basal salt composition[6,15,19]. Borthakur *et al*[4]., reported highest rooting frequency in *in vitro* grown shoots of *Albizia chinensis* on MS medium supplemented with IAA with an average of 2 roots/explant which was similar to the present investigation where rooting was observed at 0.5 mg/l IAA in *Gliricidia sepium*. Variation in concentration in both the study is due to differences in the genera.

Multiple shoot induction was observed in soft nodal stem segment in 8-10 days after incubation at the levels 0.50, 0.75, 1.00 and 1.50 mg/l of IAA. Soft nodal stem segment induced roots at all the levels (0.25 to 4.0 mg/l) of IAA. Highest root (4.4) induction was observed at 0.50 mg/l with 100 per cent frequency (Table 2).

Fig. 3: Profuse callus induction in *Gliricidia sepium on* MS mediumsupplemented with 0.50 mg/l BAP

Fig. 4: Profuse callus induction in *Gliricidia sepium on* MS medium supplemented with BAP (0.50mg/l) + IAA(2.5 mg/l)

In the present study auxins (IAA/2,4-D 0.5-2.5mg/l) and cytokinins (BAP/Kn 0.5-2.5mg/l) when added together in culture medium induced shoot bud alongwith either callus or roots or both from soft nodal stem segment explants. shoot bud induction, however, proliferated from soft nodal stem segment explants along with the induction of callus which was always more on BAP/Kn+IAA (Table 3) than BAP/Kn+2,4-D (Table 4).

Combination of cytokinins and auxins have been reported to induce organogenesis from various explants in plant species. Profuse callus was observed on 0.5 mg/l BAP + 2.5 mg/l IAA with 80 per cent frequency (Table 3) and (Fig. 4). Callus proliferation was completely inhibited on the base of soft nodal stem segment in the medium supplemented with combination BAP/Kn + 2,4-D.

Table 2: Morphogenetic effect of auxins (IAA/2, 4-D) added singly in the MS medium in *Gliricidia sepium*

Concentration (mg/l)	IAA					
	Callus induction	Frequency (%)	Multiple shoot induction±SE N=10	Frequency (%)	No. of roots/explant mean±SE	Frequency
0.25	-	-	-	-	2.1 ±0.20	60
0.50	+	20	1.2 ±0.13	20	4.4 ±0.29	100
0.75	+	20	1.3 ±0.21	20	4.1 ±0.27	80
1.00	+	20	1.2 ±0.12	-	3.1 ±0.25	60
1.50	++	60	1.1 ±0.11	-	2.2 ±0.20	60
2.00	++	60	-	-	2.1 ±0.20	60
2.50	++	60	-	-	2.6 ±0.16	40
3.00	++	60	-	-	3.2 ±0.25	40
3.50	++	60	-	-	2.5 ±0.22	40
4.00	++	60	-	-	2.2 ±0.20	40
Concentration (mg/l)	**2, 4-D**					
	Callus induction	**Frequency (%)**	**Multiple shoot induction**	**Frequency (%)**	**No of root/ explant±SE N=10**	**Frequency (%)**
0.25	-	-	-	-	-	-
0.50	+	20	-	-	2.1±0.23	40
0.75	+	20	-	-	1.4±0.16	40
1.00	+	20	-	-	1.2±0.13	20
1.50	+	20	-	-	1.3±0.15	20
2.00	-	-	-	-	1.1±0.10	20
2.50	-	-	-	-	-	-
3.00	-	-	-	-	-	-
3.50	-	-	-	-	-	-
4.00	-	-	-	-	-	-

(+) = Slight callus and (++) = Medium callus

Table 3: Morphogenetic effects of cytokinins (BAP/Kn) and auxin (IAA) added in combination in the MS medium in *Gliricidia sepium*

Concentration (mg/l)	IAA(0.5 mg/l)					
	Callus induction	Frequency (%)	No. of shoot bud/explants mean ±SE N=10	Frequency (%)	No. of root /explants mean ±SE N=10	Frequency (%)
BAP						
0.5	++	60	2.9 ±0.20	60	3.3 ±0.30	60
2.5	++	60	1.5 ±0.16	40	2.4 ±0.22	50
Kinetin						
0.5	++	60	1.2 ±0.13	40	1.6 ±0.16	40
2.5	++	60	1.1 ±0.10	20	1.4 ±0.15	20
	IAA (2.5 mg/l)					
	Callus induction	**Frequency (%)**	**No. of shoot bud/explants mean ±SE N=10**	**Frequency (%)**	**No. of root /explants mean ±SE N=10**	**Frequency (%)**
BAP						
0.5	+++	80	1.2 ±0.13	20	2.4 ±0.27	60
2.5	+++	80	-	-	2.1 ±0.22	60
Kinetin						
0.5	++	60	-	-	1.2 ±0.13	40
2.5	++	60	-	-	1.1 ±0.11	40

(+) = Slight callus, (++) = Medium callus and (+++) = Profuse callus

Table 4: Morphogenetic effects of cytokinins (BAP/Kn) and auxin (2, 4-D) added in combination in the MS medium in *Gliricidia sepium*

Concentration (mg/l)	2, 4-D (0.5 mg/l)					
	Callus induction	Frequency (%)	No. of shoot bud /explants mean ±SE N=10	Frequency (%)	No. of root /explants mean ±SE N=10	Frequency (%)
BAP						
0.5	-	-	2.1 ±0.27	60	1.3 ±0.16	60
2.5	-	-	1.4 ±0.16	60	-	-
Kinetin						
0.5	+	20	1.3 ±0.15	60	1.1 ±0.10	20
2.5	+	20	1.1 ±0.10	40	-	-
	2, 4-D (2.5 mg/l)					
	Callus induction	**Frequency (%)**	**No. of shoot bud /explants mean ±SE N=10**	**Frequency (%)**	**No. of root /explants mean ±SE N=10**	**Frequency (%)**
BAP						
0.5	-	-	1.2 ±0.13	40	1.2±0.13	40
2.5	-	-	-	-	-	-
Kinetin						
0.5	+	20	1.1 ±0.10	40	-	-
2.5	+	20	-	-	-	-

This observation was contrary with the findings of Thirupathy *et al*[18]., for callus induction in *Tefrosia hookeriana* from leaf, node and internode explants in MS medium supplemented with 0.25 mg/l BAP+ 2.0 mg/l 2,4-D This is might be due to difference in genera and kind of explants used in the particular study. The general concept given by Skoog and Miller[16], that organ differentiation in plants is regulated by interplay of auxins and cytokinins, is universally applicable and basically it is the interaction of both endogenously as well as exogenously levels of growth regulators, which direct the morphogenesis.

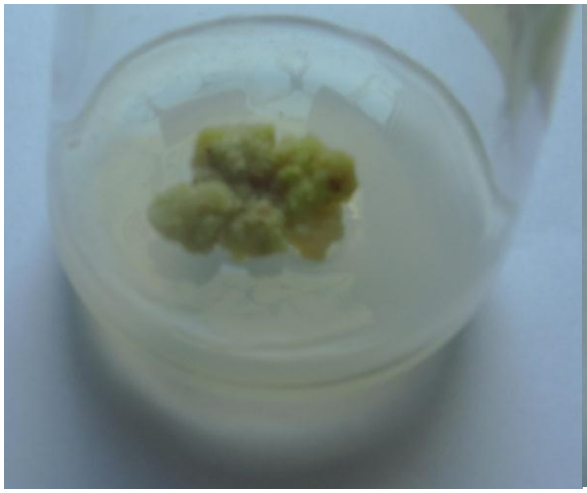

Fig. 5: Subculture of *Gliricidia sepium* callus on 0.5 mg/l BAP

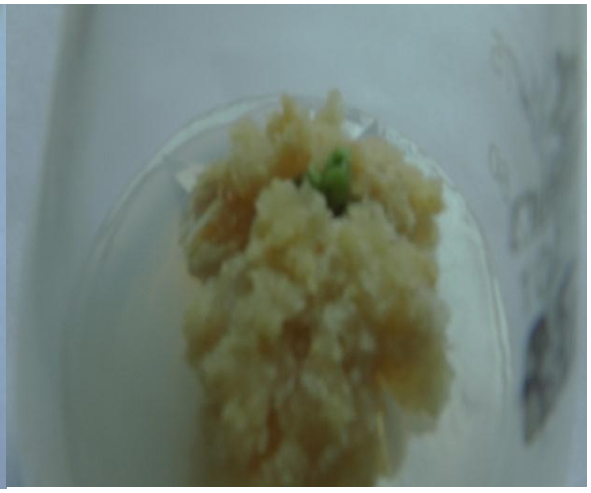

Fig. 6: Regeneration in callus culture on MS medium supplemented with 0.5 mg/l BAP + 2.5 mg/l IAA

To establish stock callus cultures, soft nodal stem segment explants were incubated on MS medium supplemented with various cytokinins (BAP/Kn 0.25– 4.0 mg/l) and auxins (IAA/2,4-D 0.25– 4.0 mg/l) added singly in the medium (Table 1, 2) and also in combination (Table 3, 4). Cytokinins and auxins incorporated singly/combination in the medium to induced slight to profuse callus at the base of explants after 8-10 days of

incubation. 0.5 mg/l BAP was found to be the best for differentiation of stock cultures from soft nodal stem segments, because this callus remained potent during subculture. The callus cultures maintained through serial subculture at the interval of 25 -30 days on the same level of plant growth regulator (Fig. 5).

Semi friable**,** light green callus proliferating from the base of soft nodal stem segment on MS medium supplemented with 0.5 mg/l BAP did induce *de novo* shoots from callus upon subculture on the MS medium supplemented with 0.5 mg/l BAP+ 2.5 mg/l IAA (Table 3 and Fig 6). The reproducibility of the event was 50-60 per cent. These results were similar to the observation of Mouhamad *et al*[12]., they showed up to 70 per cent regeneration activity in MS medium supplemented with combination of cytokinin and auxin (MS+ 0.5 mg/l BAP+ 0.2 mg/l IAA) in *Sesbania grandiflora*.

Hazzena and Sulekha[7], also reported shoot regeneration response from the callus in *Aegle marmelous* on MS medium containing BAP + IAA. Chakravathy and Negi[5] reported enhanced *in vitro* regeneration from seedling explants of a leguminous tree *Albizia libbeck.*

Fig. 7: Root induction in *in vitro* proliferated shoot of *Gliricidia sepium*

Fig. 8: Complete plant of *Gliricidia sepium*

De novo developed shoots from callus on medium 0.5 mg/l BAP +2.5 mg/l IAA, when separated and subcultured on 0.5 mg/l IAA induced roots at the base and complete plants were obtained (Fig 8) these regenerated complete plants were successfully established in green house with 55% success.

REFERENCES

1. Bai, V.N., Rani, S.U. and Tamilselvi M., Callus induction and organogenesis from seedling explants of *Sesbania speciosa* Taubert ex Engl. Proceedings of the National Academy of sciences India section B (Biological Sciences) **67(1):** 39-42 (1997).
2. Bhojwani, S.S. and Razdan, M.K., In plant tissue culture Theory and Practice. Elsevier *Amsterdam London New York Tokyo* (1992).
3. Bonyanpour, A. and Khosh-khui, M., Callus induction and plant regeneration in *Punica granatum* L *Journal of Central European Agriculture* **14(3)**: 928-936 (2013).
4. Borthakur, A., Das, C.S., Kalita, C.M. and Sen, P., An *in vitro* regeneration system for conservation of the leguminous tree *Albizzia chinensis* (Osbeck) Merr *Advances in Applied Scien Research* **3(3):** 1727-1732 (2012).
5. Chakravarthy, V.S.K. and Negi, P.S., Enhanced *in vitro* regeneration from seedling explants of a medicinally important leguminous tree (*Albizia lebbeck* Benth) *International Journal Current Microbiology Applied Science* **3(9):** 65-73 (2014).

6. Garland, P. and Stolz, L.P., Micropropagation of *Pissrdi plum*. Ann. Bot., **48:** 387-389 (1981).
7. Hazeena, M.S. and Sulekha, G.R., Callus induction and plantlet regeneration in *Aegle marmelos* (L) Corr using cotyledon explants. *Journal of Tropical Agriculture*, **46(1-2):** 79–84 (2008).
8. Hughes, C.E., Biological considerations in designing a seed collection strategy for *Gliricidia sepium* (Jacquin) Steudel (Leguminosae) *Common Wealth Forestry Review* **66** 31-45 (1987).
9. Kumari, M., Evaluation of ethanolic extracts of *in vitro* grown *Bauhinia variegata* L for antibacterial activities *International Journal of Pharma and Bio Sciences* **3(4):** 43-50 (2012).
10. Lavin, M. and Sousa, M., Phylogenetic systematic and biogeography of the tribe Robinieae(Leguminosae) *Systematic Botany Monagraphs* **45:** 1 – 160 (1995).
11. Lavin, M., Mathews, S. and Hughes, C., Intraspecific chloroplast DNA variation in *Gliricidia sepium* (Leguminosae) intraspecific phylogeny and tokogeny *Amererican Journal of Botany* **78:** 1576-1585 (1991).
12. Mouhamad, R.S., Yousir, S.A., Fadhel, A.S., Taha, D.I. and Iqbal, M. *In vitro* culture and plant regeneration of *Sesbania grandiflora. International Journal of Chemical and Biochemical Sciences* **6:** 45-49 (2014).
13. Murashige, T. and Skoog, Plant propagation through tissue culture *Annual Review of Plant Physiology* **25:** 135-166 (1974).
14. Simons, A.J. and Stewart, J.I. *Gliricidia sepium* a multipurpose forage tree legume *Forage Tree Legume in Tropical Agriculture CAB International Oxon UK* (1994).
15. Skirvin, R.M. and Chu, M.C. *In vitro* propagation of "Forever yours" rose *Horticultural science* **14:** 608 – 610 (1979).
16. Skoog, F. and Miller, C.O. Chemical regulation of growth and organ formation in plant tissue culture *in vitro Symposium of Society of Experimental Biology* **11:** 118 – 131 (1957).
17. Thangjam, R. Induction of callus and somatic embryogenesis from cotyledonary explants of *Parkia timoriana* (DC) Merr a multipurpose tree legume *Journal of Food Agriculture & Environment* **4(2):** 335-339 (2006).
18. Thirupathy, S., Sisubalan, N. and Ghouse Basha, M. Callus induction from a wild medicinal plant *Tephrosia hookeriana* (weight and arn) *International Journal of Recent Scientific Research* **5(6):** 1027-1030 (2014).
19. Zimmerman, R.H. and Broome, O.C. Phloroglucinol and *in vitro* rooting of apple cultivar cuttings *Journal of the American society for Horticultural Science* **106:** 648 – 652 (1981).