International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Micropropagation through Nodal Culture of an Economically Important Forest Tree Species *Gmelina Arborea* Roxb

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Abstract: ***Micropropagation technique plays a vital role in tree improvement program. The species Gmelina arborea (Verbanaceae) is an economically and medicinally important forest tree. In the present investigation, the nodal explants containing single node were cultured on MS medium containing 30 g/L sucrose supplemented with 0.2 - 5.0 mg/L BAP/Kn and also without plant growth regulators. Maximum percentage of response and high frequency number of multiple shoots formation was observed at 0.6mg/L BAP compare with Kn in the specie G.arborea.***

Keywords: micropropagation, *in vitro, Gmelina arborea,* auxins, cytokinins, BAP, Kn.

1. Introduction

Micropropagation through proliferation of axillary buds is a common technique in *in vitro* multiplication of forest trees. Axillary bud proliferation approach typically results in many fold increase in shoot number. Each culture passage makes feasible to obtain as many as possible propagules from a single explant and they are true-to-type. The induction of axillary bud proliferation seems to be applicable as a means of micropropagation in many woody forest trees. Proliferation of these axillary buds from tree species may be difficult due to contamination, phenolic exudation and tissue maturity (Bonga, 1982a, b). Rapid clonal propagation has gained considerable importance as a promising tool for multiplication of woody plants. So there are few reports of timber trees of Combretaceae successfully established by tissue culture technology (Joshi *et al.,* 1991). Commercially important forest tree species have been multiplied by using this technique. They are: *Eucalyptus* (Lakshmisita, 1979; Grewal *et al.,* 1980, Gupta *et al.,* 1981); *Melia azaderach* (Raghuraman and Ramanujam, 1998); *Celastrus paniculatus* (Arya *et al.,* 2002); *Maytenus emerginata* (Rathore *et al.,* 1992); *Zizyphus mauritiana* (Sudershan *et al.,* 2000); *Balanites aegyptica, Citrus lemon, Syzygium cuminii* (Rathore *et al.,* 2004a, b); *Swietenia mahagoni* (Nagarajan *et al.,* 2006).

Gmelina arborea, Roxb. (Vern: Tel. Gummadi Teaku) belongs to Verbanaceae is a large to medium sized, fast grown, deciduous tree up to 40m tall and 140cm in diameter. The wood is yellow-grayish or reddish-white with soft and light. It is one of the best timber for making furniture, constructions, plywood, black doors, general carpentry and packages. It is also used in carriages, carvings, musical instruments and ornamental work etc. (Alam et al., 1988).The total plant parts of *G.arborea* were used as a very good medicine. Roots of this species are one of the constituents of *"Dashamula"*, is well known Ayurvedic formulation. Roots and bark are useful in treatment of hallucinations, fever, dyspepsia, stomachagalgia and burning sensation(Tiwari, 1995; Hartwell, 1967-1971; Shirwaiker et.al., 2003). Leaf Juice is used for ulcers. Flowers and fruits are used for treating leprosy, anemia, constipation, and also known to inhibit platelet aggregation. The leaves are harvested for fodder for animals and silkworms (Sharma et.al., 2001; Duke, 1984; Faiza and Dharakhshanda, 1996, 1998; Little, 1983).

2. Materials Methods

Young and healthy nonwoody branches from two years old plants of *G.arborea* were used as explants. Shoots were excised free of leaves, trimmed into (3cm) segments containing a single node. These explants were washed for 30 minutes under running tap water. Then treated with 5% (w/v) Teepol for 5 minutes, followed by rinsing in distilled water for five times. Surface sterilization was carried out with 0.1% (w/v) $HgCl_2$ for 5 minutes followed by five rinses in sterile distilled water. These sterilized explants were dried on sterile tissue paper. The nodal segments were trimmed into 1.0-2.0 cm long having one node and were used as explants.

The sterilized nodal segments containing single node were inoculated on MS medium containing 30 g/L sucrose without growth regulators (MSO) and also supplemented with different concentrations (0.2-5.0 mg/L) of BAP/Kn.

After inoculation, all the cultures were incubated under cool white-fluorescent lights at an intensity of 40-60 μ mol m^{-2} s^{-1} for 16 hrs photoperiod at 25 ± 2^0C.

3. Results

The initial response exhibited by the nodal explants was an enlargement and subsequent breaking of the axillary bud. The time taken for bud breaking was found to be varied depending on the concentrations and combinations of growth regulators used. New shoots were developed after

two weeks of culture and attained height of 2 cm after 3 weeks. Maximum percentage of response (90%) was recorded at 0.6 mg/L BAP followed by 0.8 mg/L(Table-1). Average number of shoots per explant was also recorded at the same(Fig.1). As the concentration of BAP increased, lowest percentage of response was observed.Nodal segments cultured on MS medium fortified with different concentrations of Kn, showed varied results. Maximum percentage (80%) of response was observed at 0.6 mg/L Kn. Lowest percentage of response was recorded at high concentrations of Kn.

4. Discussion

Axillary bud proliferation and elongation was observed in all the concentrations and combinations of growth regulators used except on MS basal medium in *G.arborea*. Early bud breakage and shoot emergence was observed at 0.6 mg/L BAP/Kn. Maximum percentage of response and high frequency number of multiple shoots formation were found at the same concentration of BAP. It was also showed superiority in inducing more number of multiple shoots in comparison to Kn. Similarly Purohit and Kukda (2004) have reported the multiple shoots formation from nodal explants of forest tree *Wrightia tinctoria* on MS medium with 2.0 mg/L BAP.

Anitha and Pullaiah (2002) found that BAP was the best cytokinin in enhancing the shoot buds proliferation in *Sterculia foetida* than to Kn and TDZ. Sunnichan *et al.,* (1998) have recorded the highest frequency number of shoots from axillary bud on MS + 6.62 µm BAP than to Kn in *Sterculia urens.* At higher concentration of BAP and Kn reduced markedly the number of shoots as found in the present investigation. These finding are similar to our present observations that BAP showed superiority over Kn.. Superiority of BAP was reported in many other woody tree species too (Joshi *et al.,* 1991; Gupta *et al.,* 1993; Purohit and Dave, 1996; Sunnichan *et al.,* 1998). Whereas Tiwari *et al.,* (2004) have reported the multiple shoots induction from nodal culture on MS/B_5 medium supplemented with 0.2 mg/L IBA in an endangered forest tree *Pterocarpus marsupium.* Sunnichan *et al.,* (1998) have reported the multiple shoots induction on BAP/Kn in gum-karaya *(Sterculea urens)* and Jagdish Chandra *et al.,* (1999) in *Pisonia alba* as found in *G.arborea*. But Appa Rao (2004) had observed the synergistic effect of both cytokinins BAP and Kn combination by enhancing the shoot buds proliferation in *Sapindus trifoliatus.* Similarly Kathiravan and Ignacimuthu (1999) found the efficiency of both the cytokinins BAP and Kn together in the medium in *Canavalia virosa.*

The size of the nodal explant was also found to play an important role in initiation, proliferation and elongation of the shoots. Sharon and D'souza (2000) and D'souza and Sharon (2001) and Rama Swamy *et al.,* (2004) have reported that the smaller explants could initiate more multiples than that of longer nodal explants. Similar findings were also noted in *Sapindus trifoliatus* (Appa Rao, 2004). Nodal segment orientation at the time of culture also influences on the proliferation of shoots. Vertical orientation of *Wrightia tinctoria* explants was found better than horizontal orientation in terms of number of proliferated shoots (Purohit *et. al.,* 2004) as observed in *G.arborea*.

5. Future Scope

The species *Gmelina arborea* is a very good economic and medicinal important forest tree. This protocol for *in vitro* nodal culture has been established in *G.arborea* which plays a vital role in rapid multiplication of the species

6. Acknowledgement

The corresponding author would like thanks to University Grants Commission for providing financial assistance

Table 1: Effect of BAP/Kn on *in vitro* nodal culture in *G.arborea*

Concentration of PGR (mg/L)	% of response	Average No. of shoots/explant ± (SE)[a]	Mean length of shoot (cm) ± (SE)[a]
MSO	b	b	b
BAP			
0.2	75	2.5 ± 0.5	5.0 ± 0.15
0.4	80	3.5 ± 0.10	6.0 ± 0.5
0.6	90	5.0 ± 0.01	6.5 ± 0.5
0.8	84	3.5 ± 0.05	6.0 ± 0.13
1	72	3.0 ± 0.20	5.5 ± 0.10
2	70	2.5 ± 0.02	5.0 ± 0.12
3	65	1.5 ± 0.13*	4.0 ± 0.01
4	60	1.0 ± 0.15*	3.5 ± 0.20
5	52	1.0 ± 0.13*	3.0 ± 0.10
Kn			
0.2	65	2.0 ± 0.10	3.5 ± 0.10
0.4	75	3.0 ± 0.5	4.0 ± 0.12
0.6	80	3.5 ± 0.15	5.0 ± 0.50
0.8	70	2.5 ± 0.10	4.5 ± 0.12
1	65	2.0 ± 0.13	4.0 ± 0.01
2	60	2.0 ± 0.01	3.0 ± 0.05
3	50	1.0 ± 0.15*	2.0 ± 0.20
4	40	1.0 ± 0.10*	1.5 ± 0.15
5	40	1.0 ± 0.02*	1.0 ± 0.13

[a]SE = Standard Error; * = With callus induction; b=no response

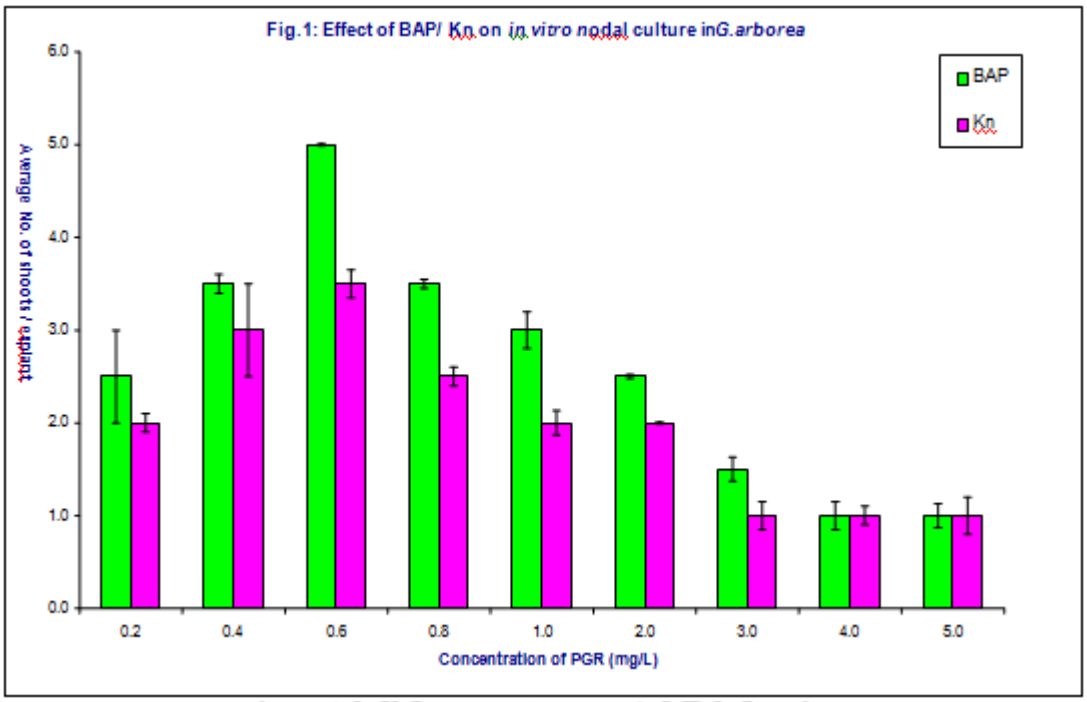

Figure 2

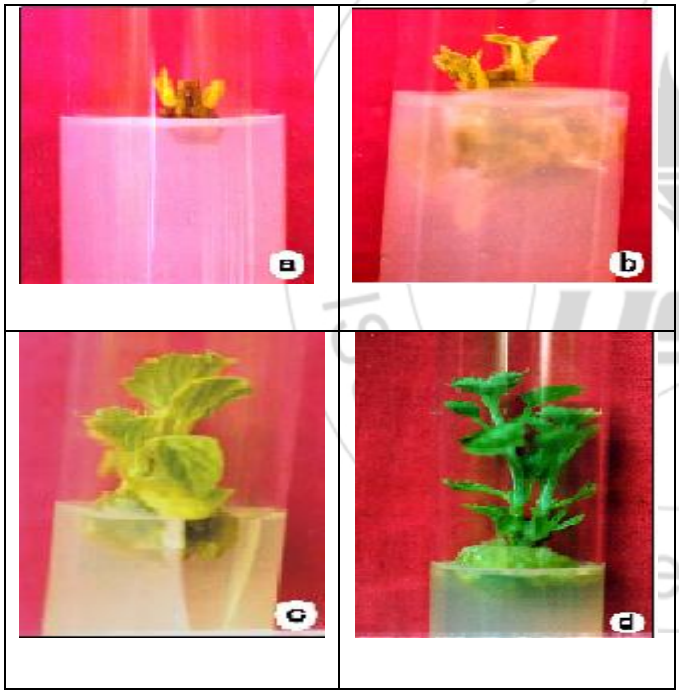

Figure 2: Nodal explants were cultured o MS medium supplemented with 0.6 mg/L BAP

a) single node containing two axillary buds cultured on the medium
b) sprouting of axillary buds after two weeks of culture
c) multiple shoots formation after four weeks of culture
d) elongation of multiple shoots after two months of culture

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