Original Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

IN VITRO ZYGOTIC EMBRYO CULTURE OF AN ECONOMICALLY IMPORTANT FOREST TREE SPECIES *GMELINA ARBOREA* ROXB.

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ABSTRACT

Gmelina arborea is an economically important forest tree species. The wood of this species is very valuable for making furniture, general carpentry and constructions. This plant also has medicinal value in Ayurvedic formulations for treating various diseases. In the present investigation, the zygotic embryos of *G.arborea* were cultured on ½MS,MS,WPM and B_5 medium containing various sucrose levels (7.5-40 g/l) supplemented with different concentrations of auxins (2,4-D/IAA/IBA/NAA) and also with GA_3. Maximum percentage (95%) of zygotic embryo germination was observed at MS medium containing 30 g/l sucrose supplemented with 1.0 mg/l GA_3 compare with all other combinations and concentrations. Embryo orientation technique also effected the germination.

KEYWORDS: **Gmelina arborea, Zygotic embryo, Sucrose, Callus, in vitro, Auxins**

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INTRODUCTION

Multiplication of forest trees is essential to develop the forest eco-system. To achieve rapid multiplication of forest trees in short time, tissue culture tools can be applied for tree improvement programme[1]. *In vitro* zygotic embryo culture technique has practical applications in tree breading in rescuing embryo from overcome dormancy and overcome seed sterility. The first attempt to few zygotic embryos of angiosperms was made by Hanning[2]. He obtained viable plants from *In vitro* isolated embryos of two Bracicaceae members of *Cochleria* and *Raphanus*. Later Liabach demonstrated the practical applications of this technique by isolating and growing the embryos of interspecific cross *Linum Parenne* and L. *Austriacum*[3-4]. *Gmelina arborea,* Roxb. (Vern: Tel. Gummadi Teaku) 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[5]. The total plant parts of *G.arborea* are used as a very good medicine for treating skin diseases, fever, and stomach disorders[6]. Roots of this species are known as *"Dashamula"*, is used as Ayurvedic medicine[7]. Roots and bark are useful in treatment of hallucinations, dyspepsia, and burning sensation[8-9]. The leaves and fruits are used as fodder for animals and silkworms[10-13].

MATERIALS AND METHODS

Matured dry and fresh fruits were collected and socked for 2 days in the water. Then the seeds were separated from the fruits and washed with teepol for 5 times and surface sterilized with 0.1% $HgCl_2$ solution for 5 times followed with sterile distilled water under aseptic conditions. Finally the embryos were isolated from the sterile seeds and inoculated on ½MS,MS,WPM and B_5 medium containing various sucrose levels (7.5 g/l, 15g/l, 30g/l, 40g/l), MS medium fortified with different concentrations of GA_3(0.2-5.0 mg/l), MS medium with various concentrations (0.5-2.0 mg/l) of auxins (2,4-D,IAA,IBA,NAA (Table1-3).In addition, the effect of embryo orientation on the medium (MS+30gr/l sucrose) was investigated. This was conducted by placing the embryos upright on the medium, upright but embedded into the medium, horizontally on the medium, using embryos without cotyledons (Table-4).After inoculation, all the cultures were maintained at 25±2^{0}C under 16-h photoperiod with 2000-3000 lux intensity of light provided by white fluorescent lamps [14-16].

RESULTS

Zygotic embryos of *G. arborea* were cultured on different types of media containing various sucrose levels and also different concentrations of growth regulators showed various types of results.

(i)Effect of Different Types of Media and various Sucrose levels

The zygotic embryos were germinated on all the types of media and various levels of sucrose except on B_5 and WPM media containing 7.5 g/L sucrose. Maximum percentage of germination was observed on MS medium containing 30 g/L sucrose. More percentage of germination was found in all the sucrose levels on MS medium. Less percentage of germination was observed on MS medium containing 7.5 g/L and 40 g/L sucrose. Likewise, less percentage of germination was observed on B_5 medium followed by WPM and ½MS medium. Whereas on ½MS, MS, WPM and B_5 media at high concentration of sucrose, the embryos were converted into callus within 4 weeks of germination. Early germination with lengthy seedling height was recorded on MS medium containing 30 g/L sucrose (Table.1 & Fig.1,2)

(ii) Effect of GA_3

The zygotic embryos cultured on MS medium supplemented with 1.0 mg/L GA_3 showed early germination in comparison to other concentrations of GA_3 tested. Maximum percentage of germination and faster growth rate of seedlings was also noted at the same concentration of GA_3. The zygotic embryos were turned in to callus at high concentration of GA_3 used. Seedling height was also recorded more at 0.8 – 1.0 mg/L GA_3 (Table.2).

(iii) Effect of 2,4-D/ IAA/ IBA/ NAA

Absolute percentage of germination was observed at 0.5 mg/L IAA /2,4-D / IBA/ NAA. Less germination percentage was found at 2.0 mg/L. Low concentration of auxins showed the callus development in the basal region of seedlings whereas at higher concentrations of auxins the whole seedlings turned into callus after 4 weeks of culture. It was interesting to note that the percentage of germination was gradually reduced as the concentration of auxins increased. IAA was found to be the best auxin followed by the remaining for germination of zygotic embryos (Table.3).

(iv)Effect of Embryo Orientations

The zygotic embryos were also cultured on MS medium containing 30 g/L sucrose to know the effect of orientations at the time of culture. The zygotic embryos upright in the medium showed the maximum percentage of germination with an early emergence of seedling. The zygotic embryos embedded in the medium showed 50% of germination. The embryos horizontally placed were turned in to callus within three weeks of culture. Embryos without cotyledons showed less percentage of germination and also taken more days for germination. Healthy seedlings formation was recorded in zygotic embryos cultured upright in the medium.

4. DISCUSSION

In vitro zygotic embryo culture was successfully established in *G. arborea.* The embryos were germinated in all the types of media used with different sucrose levels and MS media with different concentrations of GA_3 and also with auxins. Highest

percentage of germination was observed at MS medium containing 30gm/l sucrose supplemented with 1.0mg/l GA_3 followed by remaining all concentrations and the embryos were converted in to callus in auxins. Similarly, Rambabu et.al., have reported the same in *Givotia rottleriformis*[17]. Asif et al., have also reported the superiority of MS medium with 5% sucrose in *Musa acuminate* sps., *malacensis*[18]. Manashi Kalita and Sharma have recorded the highest percentage of germination *in vitro* on MS medium in *Acampe lengifolia*[19]. *G.arborea* embryos embedded and placed upright in the medium were beneficial to germination. Afele and De langhe reported that germination of *Musa balbsiana* embryos were promoted when their longitudinal axes laid flat and half embedded in MS medium[20]. Recently Rambabu et al., reported same in *Givotia rottleriformis.* But Asif et.al., have reported that, embryo orientation was not effected on germination of *Musa accuminata* spp.*malaccensis.*

Plantlet establishment

The seedlings developed from *in vitro* zygotic embryo cultures were separated from media and washed with sterile distilled water and these were shifted to plastic cups containing sterile soilrite and kept into culture room for 3 weeks. These were covered with polythene bags to maintain the wanted humidity. After 3 weeks of hardening, these Plants were transferred to earthenware pots containing garden soil and maintained in the research field under shady condition.

Table 1

Effect of different types of media and various concentrations of sucrose on in vitro zygotic embryo culture in G. arborea.

Medium	Sucrose concentration (g/L)	Percentage of germination	Days for shoot germination
½ MS	7.5	30	14
	15	35	12
	30	50	10
	40	45	12
MS	7.5	35	15
	15	60	12
	30	75	12
	40	60	**
WPM	7.5	*	*
	15	50	15
	30	70	13
	40	55	**
B_5	7.5	*	*
	15	50	14
	30	65	13
	40	60	**

**** = No response; ** = Transformed to callus***

Table 2

Effect of MS medium supplemented with different concentrations of GA_3 on in vitro zygotic embryo culture in G. arborea.

Concentration of GA_3 (mg/L)	Percentage of shoot elongation	No. of days for shoot elongation
MSO*	50	10
MS + 0.2	75	10
M.S+0.4	75	09
M.S+0.6	80	09
M.S.+ 0.8	90	08
M.S + 1.0	95	07
M.S+2.0	90	08
M.S+3.0	Trans formed in to callus at based region	
M.S. +4.0	Transformed in to callus	
M.S.+5.0	Transformed in to callus	

**** = MS medium with 30 g/L sucrose; ** = Shoot length after 30 days***

Table 3

Effect of MS medium supplemented with different concentrations of auxins on in vitro zygotic embryo culture in G. arborea.

Plant growth regulator	Concentration of PG R (mg/L)	Percentage of response	No. of days for response	Morphogenesis
2.4-D	0.5	100	15	Callus at basal region
	1.0	90	12	Callus at basal region
	1.5	75	13	Transformed to callus
	2.0	65	15	Transformed to callus
IAA	0.5	100	12	Callus at basal region
	1.0	95	10	Transformed to callus
	1.5	90	14	Transformed to callus
	2.0	90	15	Transformed to callus
IBA	0.5	100	11	Callus at basal region
	1.0	80	13	Transformed to callus
	1.5	75	14	Transformed to callus
	2.0	60	11	Transformed to callus
NAA	0.5	100	12	Callus at basal region
	1.0	90	14	Callus at basal region
	1.5	85	12	Transformed to callus
	2.0	75	15	Transformed to calls

Table 4

Effect of embryo orientation on in vitro germination of zygotic embryos in G. arborea.

Sl.No.	Embryo orientation	Percentage of germination	No. of days for germination
1	Embryo embedded	50	13
2	Embryo apright	95	10
3	Embryo horizontal	Transformed to callus	--
4	Embryo without Cotyledons	35	15

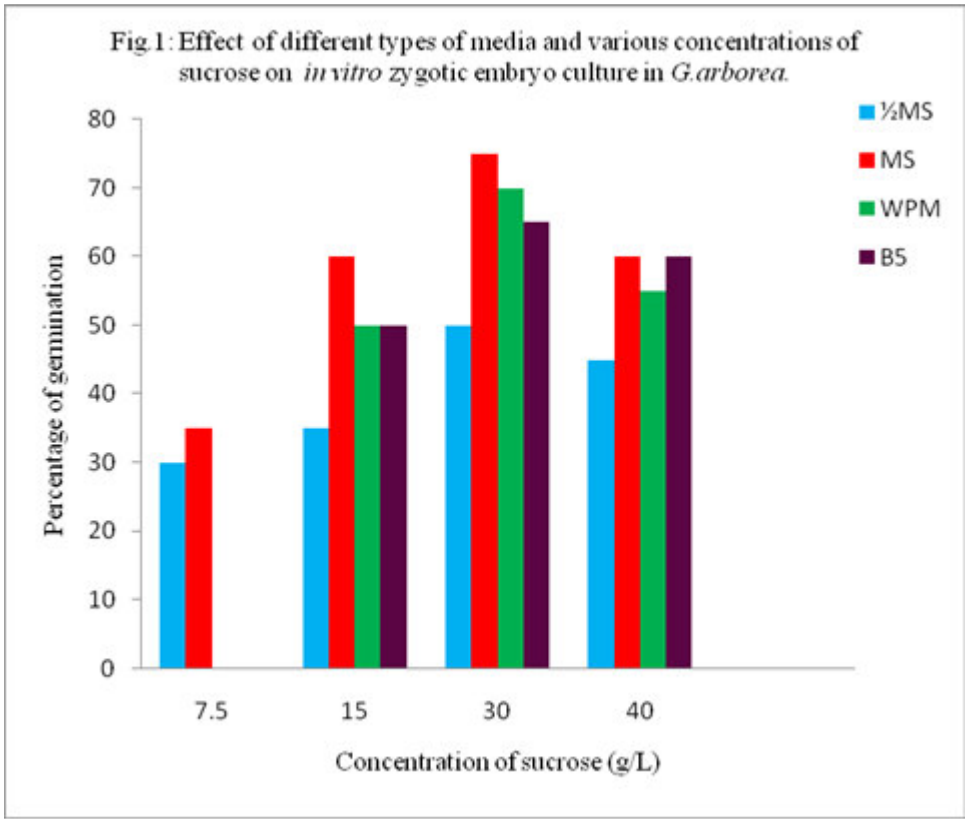
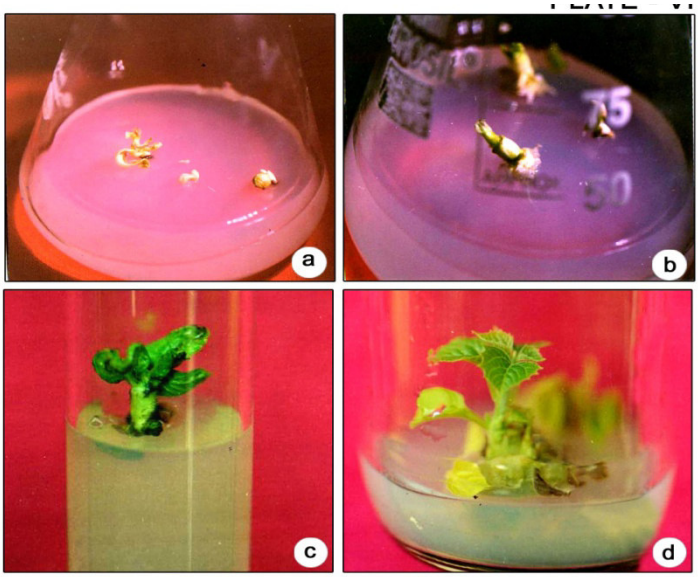


Figure 2
Zygotic embryos cultured on MS medium with 30g/l sucrose



a. embryos inoculation on the medium.
b. germination of embryos on the medium after one week of culture.
c. Elongation of germinated embryo after two weeks of culture.
d. Complete plantlet from embryo germination after four weeks of culture.

CONCLUSION

The better protocol for zygotic embryo culture is MS medium containing 30gm/l sucrose supplemented with 1.0mg/l GA_3. Embryos embedded and placed upright in the medium were beneficial to germination. This formula optimized for the zygotic embryo culture during the present studies can be used for the enhancement of germination in *G.arborea.* Thus the protocol for *in vitro* zygotic embryo culture has been established in *G.arborea* which plays a vital role in rapid multiplication of the species

ACKNOWLEDGEMENT

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

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