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TISSUE CULTURE STUDY FOR *IN VITRO* INDUCTION OF CALLUS FROM LEAF EXPLANTOF *BUTEA MONOSPERMA* (LAM) TAUB, KUNTZE.

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ABSTRACT:
Buteamonosperma (Lam), Taub, Kuntze also called as "Flame of the forest" belongs to the family *Fabaceae.* It is an important medicinal tree and different parts such as root, barks, leaves, flowers and fruits are used for the treatment of different disease. Because of over exploitation of this tree the plant is facing problem and is now considered as endangered. For this conservation and protection tissue culture techniques are the best alternative. In the present study, attempts have been made to induce callus from the leaf and internodal explant and obtained biomass of culture, so that it may be used for the extraction of different secondary metabolites, which are being extractedfromdifferent parts of the plant. In the present study, woody plant medium was supplemented with 3% sucrose, different concentrations and combinations of auxins and cytokinins and gelled with 0.8% agar. The leaf and internodal explants were inoculated in it. It was found that callusing in both the explants were found in all the five concentrations (0.5, 1.0, 1.5, 2.0, 2.5 mg/l) of BAP alone as well as along with 1.5 mg/l IBA, NAA, IAA and 2,4-D. WPM + 2.5 mg/l BAP alone induced callusing in 62% leaf explants and 73.0% in internodal explants. However, when the explants were inoculated in WPM + 2.5 mg/l BAP + 1.5 mg/l IBA, the percentage of callusing in leaf explant was 74.0 and internodal explants it was 86.0%. Similarly, WPM + 5 different concentrations of NAA induced callusing in both the explants but WPM + 2.5 mg/l BAP + 1.5 mg/l NAA induced callusing in leaf explants that was 70% while in nodal explants it was 78%. WPM + 2.5 mg/l BAP + 1.5 mg/l IAA induced callusing in leaf explants that was 64.0% and in internodal explants it was 72.0% respectively. Similarly, WPM + 2.5 mg/l BAP + 1.5 mg/l 2,4-D induced callusing in 57.0% in leaf explants and 61.0% in internodal explants respectively. Therefore, WPM + 2.5 mg/l BAP + 1.5 mg/l IBA were the best combination for induction of callus, while internodal segment was the best explants for callus induction. Growth rate of callus was also the best in the medium where higher percentage of response for callusing was found. The colour and texture of calli varied from loose white to compact white, compact white to compact brown and compact white green respectively.

KEY WORDS: *Butea monosperma* (Lam), Medicinal Tree, Callus induction, Compact white, Explants, Loose white.

INTRODUCTION:
Butea monosperma (*Fabaceae*) is commonly referred to as flame of woodland. It has much medicinal importance. *Butea monosperma* has become a gem of contemporary medicine and is widely utilized in Unani healing, Ayurvedics and Homeopathic treatment. It has been traditionally claimed to have qualities that are stringent in nature, sexual stimulant, a repellent, antiseptic, and anti asthmatic. Bark extract is used as

antifungal and antidiarrhoeal agent. Antimicrobial activity is due to presence of polyphenolics, alkaloids, cynogenic glycosides, flavonoids, terpenoids, tannins and saponins etc. (Hetal *et al*; 2024). Seeds of *Butea monosperma* are used for the treatment of different diseases such as tumors, hemoerrhaging piles, kidney stones, intestinal worms, abdominal problems and inflammation (Hetal *et al*; 2024). Further the seed extracts are also used as anthelmintic, as an antibiotic agent. Extract of flowers is used as an anticonvulsant and as anti hepatotoxic agent. It is currently endangered as a result of the damaging collection of plant parts for fire wood and medicinal purposes, the destruction of its natural habitat and ignorance of its limited availability and germination rate of seed being poor.

Plant tissue culture techniques for micropropagation, callus inductionand somatic embryogenesis are best alternative for conservation of *Butea monosperma* in its natural habitat. We get several reports regarding the micropropagation and callogenesis in tree medicinal plants. Some of them are being mentioned here such as Sehgal *et al*; (1985) reported morphogenesis and plant regeneration from cultured endosperm of *Emblica officinalis*. Furmanowa *et al*; (19997) reported effect of picloram and methyl jasmonate on growth and taxane accumulation in callus culture of *Taxus media* var Hatfieldii. Nishi *et al*; (1998) reported induction of somatic embryogenesis and plant regeneration from leaf callus of *Terminalia arjuna*. Chaturvedi *et al*; (2003) reported an efficient protocol for production of triploid plants from endosperm callus of neem (*Azadirachta indica* A. Juss.). Anjaveyalu *et al*; (2004) reported somatic embryogenesis from callus culture of *Terminalia chebula* Retz an important medicinal tree. Khalafulla *et al*; (2007) induced callus in neem (*Azadirachta indica* and observed its larvicidal activity against Anopheles mosquito). Sujanya *et al*; (2008) reported *in vitro* production of Azadictarin from cell suspension culture of *Azadirachta indica*. Anumugam *et al*; (2011) callus induction in *Terminalia indica*; Giri *et al*; (2012) callus induction *Habenaria edgeworthii*. Dhakarket *et al*; (2013) clonal propagation in *Terminalia bellirica*, Sopurza *et al*; (2014) callus induction in *Boerhaavia paniculata*. Singh *et al*; (2015); Wang *et al*; (2015); Bhosale *et al*; (2016); Goel *et al*; (2017); Gehlot *et al*; (2017) all have reported tissue culture of different medicinalplants. Mandoza *et al*; (2018) in *Thevetia peruviana*, Park *et al*; (2018) in *Butea monosperma*, reported induction of callus and its application.

Keeping all these ideas in mind present work was done to establish a suitable culture condition, for initiation of callus in different explants of *Butea monosperma*, so that it may be utilized for production of different cell suspension culture and production of secondary metabolites.

MATERIALS & METHODS:

Chemicals:

Differentingredients of Woody Plant Medium (Lloyd and McCown, 1981), Plant Growth Regulators, Ascorbic Acid and other chemicals were purchased from Hi-Media.

Preparation of Medium for callus induction:

All ingredients of woody plant medium were taken from the stock solution for the preparation of one liter medium. It was supplemented with 3% sucrose, five different concentrations of BAP and one concentration of IBA, NAA, IAA and 2,4-D, separately. Medium was gelled with 0.8% agar. 50 mg/l each of ascorbic acid and citric acid were also added. 30 ml medium was dispensed in culture flasks. The mouths were covered with cotton plugs wrapped with muslin cloth. The pH of the medium was adjusted to 5.8 and then autoclaved at 15 lb pressure for 20 min. All culture flasks were stored at low temperature for future use.

Explant Preparation:

Collection of Material:

Fresh and young branches were collected from the adult tree of *Butea monosperma* (Lam) Taub growing in the garden of University Departmentof Botany, B.R.Ambedkar Bihar University, Muzaffarpur. It was brought in the Laboratory and leaves and internodes were separated. Leaves were cut into small pieces. Both leaves and internodes were washed in running tap water for 45 min, followed by treatment with tween 20 for 20 min, and antifungal treatment with 4% Bavistin (w/v) for 20 min. and subsequent rinsed with 70% ethanol for 1 min, was followed by surface sterilization with 0.1% $HgCl_2$ solution for 2-3 min. The explants were washed thoroughly with sterilized distilled water (three rinses of approximately 15 min each) to remove all traces of sterilant. The explants were wrapped in pre-sterilized and moist muslin cloth, store in freeze for further inoculation.

Inoculation and culture conditions:

The surface sterilized explants ere blotted dry (using sterilized blotting paper). The edges of the internodal segments and the pieces of leaves were carefully trimmed using sterile surgical blades. Explants of

approximately 1 cm^2 in size for leaf and 1 to 1.5 cm long in case of internodal segments were prepared. They were separately inoculated in culture flask containing culture medium, under aseptic conditions of Laminar air flow chamber. After inoculation the flasks were incubated in culture room at a light intensity of 1000 lux, a photoperiod of 16/8 h (Light and Darkness) and a temperature of 26 ± 1^0C, moisture 65-76% respectively. Observation was done for callus induction frequency (Percentage response), proliferation and nature of callus, texture, colour. Mean of the data were tabulated after 8 week. Best medium were used in future for callus induction. Cultures showing any contamination were taken out, and destroyed after autoclaving. All experiments were done in triplicate and in each cycle 20 cultures were used. The mean of the data was tabulated in table-1.

RESULTS:

Explants both leaves and internodes started induction of callus in all the concentrations of BAP alone or different concentrations of BAP + different auxins, within two weeks of inoculation, however, texture and colour were recorded after four weeks. The maximum percentage of callus induction in internodal and leaf explants was in WPM + 2.5 mg/l BAP, which was 62% for leaf explants and 73% for internodal explants respectively. This was followed by 49% for leaf and 55% for internodal explants in WPM + 2.0 mg/l BAP alone. It was further noted that addition of 1.5 mg/l IBA with different concentrations of BAP there was increase in the percentage response for callusing in both the leaf and internodal explants. Here maximum percentage of induction of callus for leaf and internodal explants was noted in WPM + 2.5 mg/l BAP + 1.5 mg/l IBA. It was 74% for leaf explants while 86.0% for the internodal explants respectively. Although there was increase in percentage of response for callusing in WPM + 2.5 mg/l BAP + 1.5 mg/l NAA, than that of the BAP alone, but the percentage of response was less than IBA. Here for leaf explants it was 70.0 and for internodal explant 78.0 respectively. In case of WPM + 2.5 mg/l BAP + 1.5 mg/l IAA, the percentage response for callusing in leaf explant was 64.0 and for internodal it was 72.0 respectively. Similarly, the percentage response for callus induction in WPM + 2.5 mg/l BAP + 1.5 mg/l 2,4-D for leaf explant was 57.0 while for internodal explant it was 61.0 respectively.

The growth rate, colour and texture of the calli in different culture media were also observed. Here again best growth rate for both the explants was noted in WPM + 2.5 mg/l BAP + 1.5mg/l IBA. This was followed in WPM + 2.0 mg/l BAP + 1.5 mg/l IBA, where the growth rate was better. In lower concentration of BAP (0.5 to 1.0 mg/l) the growth rate was average even though 1.5 mg/l IBA was added. WPM + 2.5 mg/l BAP + 1.5 mg/l NAA induced callusing where the growth rate was better. At the similar concentrations IAA and 2,4-D along with 2.5 mg/l BAP had also better growth rate. Texture and colour of the calli were also observed. In most cases the texture was loose and the colour was white. But in certain culture the texture was compact and the colour was brown. Similarly, it was also loose and brown. The texture of calli was compact the colour was white green. In WPM + 2.5 mg/l BAP + 1.5 mg/l IBA. In case of other auxins at similar concentrations the texture was loose in case of 1.5 mg/l NAA and colour brown while in 1.5 mg/l IAA, the texture was compact and colour brown. In case of WPM + 2.5 mg/l BAP + 1.5 mg/l 2,4-D the texture was loose and colour brown.

DISCUSSION:

Butea monosperma (Lam) is an important medicinal tree. Different parts such as bark, leaves, flowers and seeds are being used in the traditional medicines. Due to damaging removal of plant parts for fire wood, medicinal purposes and other purposes, along with destruction of its natural habitat, and ignorance of its unique properties, the species is currently in danger or going endangered in nature. Therefore, plant tissue culture may be used to conserve this species. The callus may be used for isolation of desired secondary metabolites of pharmaceutical importance. In the present study the leaf and internodal explants induced callus on WPM + five different concentration of BAP alone. However, the percentage response of callusing, the growth rate of callusing varied according to increasing concentrations of BAP alone. Here lowest percentage of response and average growth of calli were noted at WPM + 0.5 mg/l BAP alone in both the explants such as the leaf and the internodes. However, on WPM + 2.5 mg/l BAP, the percentage responses for callusing and growth rate of calli were increased manifold. Above findings are in agreement with the findings of Saad and Elnour (2010); Mehta *et al*; (2012); Safari *et al*; (2013); Chiruvella *et al*; (2014). It was further noted that when WPM + different concentrations of BAP + 1.5 mg/l IBA or NAA, or IAA or 2,4-D was used for callusing, in both the explants there were many fold increase in percentage response for callusing. Here maximum percentage of response for callus induction, its growth rate etc. were noted among both the explants when cultured on WPM + 2.5 mg/l BAP + 1.5 mg/l IBA. Although there was increase in percentage of response even at low concentration of BAP (0.5 mg/l), there was increase in percentage response in comparison to similar concentration of BAP. Similar conditions were noted when WPM + different concentration of BAP +1.5 mg/l NAA was added. Here also the percentage response was higher for callusing in comparison to BAP alone at the similar concentrations. Above

findings are in accordance with the findings of Ali *et al*; (2015); Sharma *et al*; (2015); Yarra *et al*; (2016); Tjie Kok *et al*; (2017); Park *et al*; (2018); Salim (2018; Ashokhan *et al*; (2020). It was further noted that when 1.5 mg/l of IAA or 2,4-D was added with above five concentrations ofBAP in WPM, there were increase in percentage response but it was lower than that of the similar concentration of IBA and NAA used separately (Nazir *et al*; 2020; C. Kumar, 2017; Gadzovska *et al*; 2017).

It may be noted that use of cytokinin (BAP) and auxins (IBA, NAA, IAA, 2,4-D) enhanced both the percentage of response and growth rate of calli in both the explants. The interdependent balance between cytokinin and auxins encouraged cell division for initiation of callus (Nazir *et al*; 2020). Similar findings are also reported by Sasane and George (2023); C. Kumar (2017). The combination of cytokinin (BAP) and auxin NAA, has been shown to produce highest callus induction on cotyledon and hypocotyls explants of *Glycerrhira glubra* (Safari *et al*; 2013). Synergistic action of both the cytokinin and auxin therefore are much effective with respect callus induction and its growth rate (Khan and Wabi). Here texture and colour of the calli also varied according to the concentrations of the cytokinin and auxins. In most cases loose white calli were noted but compact white and compact brown calli were also observed. Similarly, compact white green callus was observed.

CONCLUSION:

In the present study leaf and internodal explants of *Butea monosperma* were used for callus induction. Here among the explants internodal explant was found more suitable than that of the leaf explant. Similarly, among the five different concentrations of BAP, 2.5 mg/l concentrations were more promising than the rest concentrations. Similarly, among the auxins IBA at 1.5 mg/l concentration was more promising than that of the other three auxins at the similar concentrations.

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TABLE-1

Cytokinin (mg/l)	Auxin (mg/l)	Explant		Growth rate of callus	Texture of Callus
		Leaf	Internode		
BAP	IBA	% Response	% Response		
0.5	0.0	25.0	28.0	+	LW
1.0	0.0	31.0	35.0	+	LW
1.5	0.0	38.0	43.0	+ +	LW
2.0	0.0	49.0	55.0	+ + +	CW
2.5	0.0	62.0	73.0	+ + +	CW
0.5	1.5	36.0	39.0	+	LW
1.0	1.5	40.0	46.0	+ +	LW
1.5	1.5	54.0	62.0	+ + +	CW
2.0	1.5	63.0	71.0	+ + +	CB
2.5	1.5	74.0	86.0	+ + + +	CWJ
BAP	NAA				
0.5	1.5	31.0	34.0	+	LB
1.0	1.5	38.0	41.0	+ +	LW
1.5	1.5	52.0	58.0	+ + +	LW
2.0	1.5	60.0	67.0	+ + +	LB
2.5	1.5	70.0	78.0	+ + +	LB
BAP	IAA				
0.5	1.5	29	31	+	LW
1.0	1.5	34	37	+	LW
1.5	1.5	47	53	+ +	LW
2.0	1.5	55	60	+ +	LW
2.5	1.5	64	72	+ + +	CB
BAP	2,4-D				
0.5	1.5	26	29	+	LW
1.0	1.5	30	34	+	LW

1.5	1.5	41	47	+ +	LW
2.0	1.5	49	53	+ + +	LB
2.5	1.5	57	61	+ + +	LB
+ Average, + + Good, + + + Better, + + + + Best, LW = Loose White, CW= Compact White, LB= Loose Brown, CB = Compact Brown, CWG= Compact White Green					

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