The Biobrio ISSN : 2393-9508 e-ISSN : 2582-4902
An International Quarterly Journal of Life Sciences
Website : www.thebiobrio.in
7 (1&2) : 495-502, 2020

Micropropagation Study on *Gmelina arborea* Roxb. under Salinity Stress

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ABSTRACT

The abiotic stress factors are the major limiting factors for the plant growth and crop yield. Among the various abiotic stress factors affecting the plant growth, salinity stress is one of most prominent and the major issue for several crop yields. The salinity stress adversely affects plant propagation by altering the photosynthetic rate and oxidative stress and the metabolic pathways. The present work was designed to determine the salt tolerant capacity and to observe the effects of salinity on the propagation rate of explants from *Gmelina arborea* Roxb. For this aim, three explants viz; node, internode and shoot apex were collected from the mature tree and were surface sterilized by mercuric chloride. The sterile explants were then cultured over the MS medium supplemented with different hormonal combinations. Along with the hormonal combinations, the explants were also subjected to different salt stress conditions that included, 0.17M, 0.34M and 0.68M sodium chloride. This was done evaluate the effect of the hormones accompanied by slat stress on the proliferation and callusing potential of the explant. The observations from every experimental setup led to the conclusion that the salt stress adversely affects propagation strategy of the explant from G. arborea by affecting the shooting potential, pigmentation and increases the oxidative stress in the growing callus. The salt tolerance potential of these plants needs to be improved by certain methodology to improve the salinity stess problem and overcome the less yield impact for any crop.

Key words - Micropropagation, *Gmelina arborea*, Salinity stress, explants.

INTRODUCTION

One of the major problems faced for the cultivation in the arid and semiarid tropics is the Salinity stress resulting from the accumulation of large amount of salt in the fertile lands. The country India acquires about 8.6 Mha of the land being affected under the salt stress (Pathak, 2000). The presence of higher concentration of salt in soil affects a broad array of the metabolic activities in plant leading to the circumstances and outcomes like stunned growth, low enzyme activity potential and also the lack of accumulation of several important biochemical constituents (Dudhane *et al.*, 2011). The plant cells when under salt stress, initially starts the accumulation of salts as free osmotica and when the cells reacha certain threshold of sodium and/or chlorine accumulation, a toxic and specific ion effect appears (Masour and Salama, 2004). When under the stress conditions the plant, cells produce some defense mechanisms for their protection against the oxidative stress. Scavenging the reactive oxygen species is one of the most important mechanism carried by the plants for protection against the abiotic stress (Vranova *et al.*, 2002). The tolerance potential against salt stress in the plants can be promoted by the antioxidant mechanisms. The enzyme catalase and peroxidase participate for the response generated against the pathogens either by reinforcement of cell wall or by the generating their antioxidant role (Dudhane *et al.*, 2011).

Gmelina arborea Roxb. is tree species belonging to the Verbenaceae family and is mainly found in the deciduous forests of southern and southeastern Asia (Sinha *et al.*, 2006). The tree is also considered as the most durable timber of India and is also called as white teak. Its timber yielding potential has made it to

be introduced as a plantation in the African and South American countries (Dvorak, 2004). The value of this tree adds up due to its potential to be used as medicinal agents in various formulations used for the treatment of ailments like stomach disorders, skin problems and fever. The tree is known to be a rich source of bioactive flavonoids and iridoid glycosides and also carries a good antioxidant potential (Naik *et al.*, 2003; Sinha *et al.*, 2006). The plant is well known for its medicinal importance in Ayurveds where the roots, leaves, fruits and bark of the plants are used for the treatment of various ailments by the traditional formulations. It is used for treating the scorpion sting, snake bites and also diabetes (Nadkarni, 2000 and Khan and Khanum, 2005). The plant is also used for treating hallucinations, excess thirst, piles, burning sensations and gastrointestinal issues (Kulkarni *et al.*, 2013). The bark decoction of G. arborea is successfully employed for the control of glycemia and complications of diabetes mellitus (Ediriweera and Ratnasooriya, 2009).

Naturally the tree germinates by the seeds and cuttings and the propagation of this plant is affected by the source and it affects the crop management and productivity (Gatti *et al.*, 2011). The micropropagation of this tree based on the use of explant obtained from the adult trees and this technique act as an alternate to improve the genetic stability, uniformity, quality and also a continuous source of new plant crops (Nguyen and Kozai, 2001). In the present study, the micropropagation of three different explants, that is node, internode and shoot tip, from the adult tree was studied under the salt stress condition. The study was devised to determine the potential of the explants to survive under the salinity conditions, in order to determine whether the tree could be cultivated on the soil with higher salt concentration.

Materials and Methodology

In the present study the experimental plant, Gmelina arborea Roxb. was subjected to the micropropagation setup under the salt stress condition where variable concentrations of sodium chloride were used for the salt stress environment that include, 017M, 0.34M and 0.68M. The experimental setup was designed with the aim to determine and evaluate the response of the explant of G. arborea for micropropagation under different hormonal concentration along with the salt stress. The experimental setup to elucidate the outcome was designed in the following manner.

Preparation of culture media

The culture media used for the micropropagation of explant was Basal medium given by Murashige and Skoog (1962). The pH of medium was set at 5.8pH before sterilization and this culture media was also supplemented with various growth regulators that include, IBA, NAA, 2,4-D, Kn and BA.

Collection and sterilization of explants

The explants used under the study include, stem segment that is node and internode and the shoot tip segment. The explants segments were collected from the in-vivo grown mature tree of G. arborea of 12-14yrs old. The emerging young shoots after the leaf fall were used as the material for explant. The explants collected from the mature tree were properly surface sterilized before use for the micropropagation. For the surface sterilization of the explant, after washing them thoroughly with tap water followed by the distilled water, they were treated with a 1% solution of Cetavelon (20% cetramide in 10% of isopropyl alcohol). Next the explants were thoroughly washed with distilled water followed by their surface sterilization using 0.2% of the mercuric chloride solution for 5minutes. After this the explants were washed thoroughly 4-5times with distilled water and then finally they were transferred into the sterile petri-dishwhere the explants were trimmed to the size of 8-10mm using sterile forceps or blades to be used for the inoculation into sterile culture medium.

Inoculation and transfer of explant

The explants hence prepared after surface sterilization were then transferred aseptically on the culture medium under laminar air flow, pre-

illuminated with UV light for 40minutes. The explants viz, node, internode and shoot tip segment were inoculated over the culture medium containing different hormonal combinations that includes, 5mg of 2,4-D; 2mg of Kn; 2+2mg of NAA+Kn; 2+1mg of 2,4-D and Kn and 2+2mg of 2,4-D and Kn. Each hormonal combination of the medium was also supplemented with three different concentration of sodium chloride that is 0.17M, 0.34M and 0.68M.

Maintenance and subculture of cultures

The cultures were incubated at 25±2ºC in the culture room with a relative humidity of about 60% under the continuous fluorescent light (2000 lux, cool and white). For the maintenance of aeration in the growth culture the cultures were inoculated on the semi-solid medium and they were in direct contact with the ambient air passing through the porous cotton plugs. The growing callus was maintained by sub-culturing it on the pre-sterile medium, for which the callus was cut into pieces of equal size and transferred aseptically on the medium with the similar combination of growth regulators and salt concentration as per the previous culture. The quantitative measurement of the callus growth was determined by measuring weight of proliferating callus by placing it on the pre-dried whatmann filter paper. For each experimental setup 20 replicates were prepared and repeated once while the observations were recorded on the weekly basis.

Result and Discussion

The explants (node, internode and shoot tip segment) were kept for propagation on the MS medium supplemented with different hormonal combinations and each hormonal combination was added up with three different concentration (Molar) of sodium chloride to provide the salt stress. The characteristic properties of the callus and the overall observation during such treatment during the incubation time period was noticed and recorded for each setup. The experimentation for each setup was kept in 20 replicates, where the response of the culture is depicted in percentage and the callus weight is given in milligrams alongwith some characteristic's observations of the callus.

From the observation for effect of hormonal combinations on shoot multiplication under different salt stress on the nodal culture, it was observed that the Kn showed the best results at 2mg/L concentration giving the shoot generation on two different concentration of sodium chloride that is 0.17M and 0.34M giving the number of shoots per culture as 4.4±0.5 and 2.3±0.4 shoots respectively. Although, at other hormonal combinations the culture response was seen but none of the combination under the salt stress was able to initiate the shoot multiplication over the incubation period of 21 days. The shoot formation was only initiated by Kn and that too under 0.17M and 0.34M and not under 0.68M. On the other hand, the rest hormonal combinations were showing the promising response on callusing even under the salt stress, while at the highest salt stress of 0.68M either there was no growth in the explant or the explant turned brown after slight multiplication over the incubation period. The results for the same are summarized in the table no.1.

For the effect of hormonal combinations on callus biomass and organogenesis in the internodal culture, the findings suggest that for every hormonal combination used viz., 2,4-D (mg/L), NAA+Kn (1+2mg/L and 2+2mg/L) and 2,4-D+Kn (1+2mg/L and 2+2mg/L), the response to callusing in the internodal culture is being affected by the increasing salt concentration. The increasing salt concentration is increasing the number of days for callusingresponse by the culture, while in case of 0.68M salt stress on 2,4-D+Kn (1+2mg/L) hormonal combination there was no callusing observed throughout the incubation period. The callus in every hormonal combination showed browning at the 0.17 M (except for2,4-D (mg/L) and NAA+Kn (1+2mg/L and 2+2mg/L), 0.34M (except for 2,4-D (mg/L), and 0.68M salt stress. The results for the same are depicted in the table no. 2. The studies carried out on the shoot tip explant for the same salt stress conditions, the culture response

for the shoot multiplication was better than other two explants as the hormonal combinations of Kn (2mg/L) and 2,4-D + Kn (1+2mg/ l and 2+2mg/L) both showed the shoot generation at 0.17M and 0.34M salt stress, while at the highest salt stress of 0.68M no response was seen in any hormonal combination. Although the initial observation of the callus and shoot were normal for some cases, but eventually either the callus showed browning or the shoot became yellow after certain period of incubation. The best callus response among this criterion was observed for NAA + Kn (1+2 and 2+2 mg/L) combination at the lowest 0.17M salt stress. The salt stress for shoot apex explant was not favorable at all. The summarization of the results for the same are depicted in the table no. 3.

The crop productivity and quality across the globe are majorly limited by the salinity of soil. The negative effect of salt stress includes the reduction in plant growth rate and biomass, smaller leaf size, osmotic effects, nutritional deficiency and also the mineral disorders (Hoorn *et al.*, 2002). The salinity stress prominently affects the photosynthesis and cell growth (Munns *et al.*, 2006), and this eventually leads to reduction in the shoot and root growth. Various studies conducted on different plant species have shown that the shoot development gets limited by the susceptible abiotic stress while the growth of plant considered as tolerant remains unaltered (de Abreu *et al.*, 2008 and Amara *et al.*, 2013). The similar findings are observed where the callusing attribute of the culture is promising while their shooting property is adversely affected by the abiotic slat stress. The current study reported that the higher salinity level are adversely affecting the culture characteristics although not in all cases but at some hormonal combinations the slat stress was tolerable. The similar type of outcome was shown by Dudhane*et al.*, (2011) in Gmelinaarborea Roxb. and Ghollarata andRaiesi, (2007) showed that in clover plant.

Conclusion

The present study overall forms the findings concludes that the abiotic stress generated by the salinity conditions adversely affected the growth of the cultured explants. The lack of shooting ability in the callus under hormonal effect depicts the negative action of salt stress on plant growth. The yellowing of the shoot tip if generated in some cases indicates the reason behind the lack of photosynthesis, whereas the browning of callus indicated the oxidative stress in the growing callus. The salinity conditions are adversely affecting the plant propagation and hence there is a need to look over the methodology and technique that could improve the salinity tolerance in the micro propagated plants, and there is a need to develop such tolerant strains to overcome the problem that's affecting the worldwide production of some potential crops. The incorporation of mycorrhizae as suggested by some studies could be helpful for improving the tolerance capacity.

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Table 1: Effect of different concentrations of NaCl and effective combination of growth hormones on shoot multiplication and callus growth in nodal culture of G. arborea*

Hormones (MS Medium)	Hormonal concentration (mgl-1)	NaClconc. (M)	% culture showing response	Number of shoots per culture	Other response
2,4-D	5.0	0.17 M	71.4	---	Callusing (1800 ± 43.48)
		0.34 M	45.3	---	Callusing (1250 ± 20.84)
		0.68 M	---	---	Hypertrophy, explant turned brown
Kn	2	0.17 M	72.4	4.4 ± 0.5	Shoots, callus (351 ± 9.20)
		0.34 M	48.4	2.3 ± 0.4	Multiple shoots, callus (198 ± 9.02)
		0.68 M	---	---	Explant turned brown
NAA + Kn	2 + 2	0.17 M	78.0	---	Vigorous callus (2890 ± 28.23)
		0.34 M	52.8	---	Profuse callus (2020 ± 21.15)
		0.68 M	---	---	No growth of explant
2,4-D + Kn	2 + 1	0.17 M	72.0	---	Excellent & green callus (2580 ± 67.12)
		0.34 M	48.0	---	Moderate callus (1732 ± 29.32)
		0.68 M	---	---	Explant turned brown
	2 + 2	0.17 M	71.6	---	Excellent & green callus (2470 ± 23.20)
		0.34 M	47.0	---	Moderate callus (1678 ± 78.12)
		0.68 M	---	---	Explant turned brown

*The data obtained is from the mean of 20 replicates over the growth period of 21 days and in the figures in parentheses indicate fresh callus weight in mg.

Table 2: Response of effective combinations of growth regulators and different concentrations of NaCl on callus biomass and organogenesis in internodal culture of G. arborea*

Hormones (MS medium)	Hormonal conc. (mgl^{-1})	NaCl conc. (M)	Callusing day	Callus biomass (mg)	% culture showing response	Other response
2,4-D	5.0	0.17 M	9	3920 ± 38.75	80.7	Callus white and crystalline
		0. 34 M	13	2410 ± 75.12	62.5	Callus green, white and crystalline
		0.68 M	15	870 ± 19.0	---	Browning
NAA + Kn	1 + 2	0.17 M	8	3421 ± 24.15	83.5	Callus green and crystalline
		0. 34 M	12	2571 ± 8.20	56.2	Do
		0.68 M	17	---	---	Browning
	2 + 2	0.17 M	9	4035 ± 21.27	86.5	Callus green
		0. 34 M	11.5	3575 ± 38.03	42.5	Do
		0.68 M	14.5	---	---	Poor callus growth, Browning
2,4-D + Kn	1 + 2	0.17 M	9	3150 ± 42.12	80.2	Callus finally turned brown
		0. 34 M	11	2180 ± 72.68	42.7	Do
		0.68 M	---	---	---	Browning
	2 + 2	0.17 M	8.5	2580 ± 75.02	83.6	Callus white, finally turned brown
		0. 34 M	10.5	2010 ± 15.30	56.2	Callus white, finally turned brown
		0.68M	---	---	---	Browning of the explant

*The data obtained is from the mean of 20 replicates over the growth period of 21 days

Table 3: Frequency of shoot multiplication and callus induction from shoot-apex culture of G. arborea on effective combinations of growth regulators and different concentrations of NaCl*

Hormones (MS medium)	Hormonal conc. (mgl^{-1})	NaCl conc. (M)	No. of shoots per culture	% culture showing response	Other response
2, 4-D	5	0.17M	---	82.5	Culture finally turned brown
		0.34M	---	48.7	Do
		0.68M	---	---	Explant turned brown
Kn	2	0.17M	3.2 ± 0.6	80.5	Shoots turned yellow after 25 days
		0.34M	2.8 ± 0.2	46.8	Do
		0.68M	---	---	Browning of explant
NAA + Kn	1 + 2	0.17M	---	81.5	Excellent callus
		0.34M	---	49.6	Moderate callus
		0.68M	---	---	Browning
	2 + 2	0.17M	---	80.2	Excellent callus
		0.34M	---	47.8	Moderate callus
		0.68M	---	---	Browning
2, 4-D + Kn	1 + 2	0.17M	1.7 ± 0.3	79.6	Yellowing of shoots
		0.34M	1.5 ± 0.6	48.3	Do
		0.68M	---	---	Browning of explant
	2 + 2	0.17M	1.7 ± 0.6	81.6	Yellowing of shoots
		0.34M	1.4 ± 0.9	49.8	Do
		0.68M	---	---	Browning of explant

*The data obtained is from the mean of 20 replicates over the growth period of 21 days