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STANDARDIZATION OF SURFACE STERILIZATION TREATMENT DURING *IN VITRO* REGENERATION OF *SIMAROUBA GLAUCA*

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ABSTRACT

Simarouba Glauca is an agro climatic plant having wide range of economical importance and new generation biofuel source, but it has a very small population in India. In order to increase its number it is necessary to propagate this plant through in vitro regeneration methods. Woody plants generally contains high amount of secondary metabolites and phenolic substances which inhibits the regeneration. Frequent sub culturing and incubation in dark are the simplest methods to reduce the browning effect up to some extent, but use of $HgCl_2$ showed higher rate of survival of the explants. Various concentrations of Mercuric Chloride have been used in this study. Pre- treatment of 2.5% Ascorbic acid along with the 0.1% $HgCl_2$ treatment for 2-3 minutes gave the effective results.

KEYWORDS: *Simarouba Glauca*, Regeneration, Phenolics, Ascorbic Acid

INTRODUCTION

Simarouba Glauca is a rain fed wasteland, evergreen tree commonly known as “paradise tree” or Laxmitaru, belongs to the family *Simarobaceae*. It is a native to Bhamas and North America but now cultivation is spread among the various states of India like Tamil Nadu, Orissa, Karnataka and Maharashtra (Cronquist, A,1994., Joshi and Joshi, 2002., Govindraju et al, 2009., and Joshi and Hiremant, 2000). It has a wide range of economical importance in the field of pharmaceuticals, ethanobotanical, phytoremidiation, manure etc. (Neelavathi et al, 2004., Govindraju et al, 2009, I., Medinilla, 1997 and Caceres et al, 1990). It is considered as a potential source of edible oil and biodiesel resource of future generation. (Shukla and Padmaja, 2013). *Simarouba Glauca* is conventionally propagated through seeds but the major problem in its large scale planting is the short viability of the seeds for 3-4 months only.(Shukla and Padmaja, 2013). Therefore an effective approach was needed to increase its population; therefore, *in vitro* regeneration is the most preferred method to propagate this plant. A few studies on *In Vitro* culture of *Simarouba* have been reported using nodal and leaf explants (Rout &Das, 1993&1994).

As browning of media prevents further progress in biotechnology of woody plants hence, it is necessary to explore a suitable protocol for successful micro propagation.(Sauls and Lampbell,1994). These plants have high amount of phenolic compounds and secondary metabolites, cause activation of oxidative enzyme when explants are excised, which causes failure of tissue culture process. It is due to the activity of Polyphenol oxidase (PPO), leads to the death of tissue. (Litz and Vijayakumar 1988). Biotic and abiotic stress also stimulates oxidative browning of explants and it is transferred to the wounded tissue during culture preparation. (Yahraus et al, 1955). These are also used in defence mechanism of the plants. (Ahamad et al, 2013). During in vitro regeneration, explants are excised leads to the leaching of phenolic compounds as a result darkening or browning of media occurs which blocks the uptake of nutrients and finally leads to the

explant death. By using different antioxidants and absorbants we can overcome this problem. (Shukla and Padmaja, 2013).

Browning and subsequent death of cultured explants is usually depended on the phenolic compounds and the quantity of total phenols also. Due to exudation of phenolics various problems such as media discoloration, contamination, explant browning and death has been reported in previous studies. (Gassman et al, 1978). In case of *S.glauca*, browning occurs within 2-3 days when explants are subjected to callus induction. Several attempts have been made to resolve this problem, including pre treatment of explants with antioxidants, frequent subculturing and incubation of cultures in dark. (Bhatt and Dhar, 2000). These manipulations may or may not be successful depending upon the type of the explants. In comparison to the previous studies we have tried to investigate the best suited pre treatment of explants to inhibit the browning effect and successful in vitro regeneration of *Simarouba Glauca*.

MATERIALS AND METHODS

Selection of Explants

The present study was conducted in the Life Science Department of ITM University, Gwalior. Nodal segments of *Simarouba Glauca* plant were collected from the 7-8 months old plants which were procured from Forest Research and Development Department (TAPOVAN), Gwalior (M. P). These explants were placed in a beaker containing tap water and washed properly for 30 minutes with the running tap water.

Surface Sterilization

Each explant was pre treated with 0.1% mercuric chloride, 0.2% mercuric chloride and 0.1% mercuric chloride along with the 2.5% of Ascorbic acid for different time intervals followed by 4-5 repeatedly washing with double distilled water under aseptic conditions. Thereafter, explants were carefully transferred to the growth medium and results were observed.

RESULTS AND DISCUSSIONS

Surface sterilization is the initial step towards *In Vitro* regeneration of a plant. Treatment with 0.1% and 0.2% Mercuric Chloride ($HgCl_2$) under various time durations (1 min, 2 min, 2.5 min, 3 min, 3.5 min and 4 minutes) were tried for the surface sterilization of explants prior to the inoculation. Further, 0.1 % $HgCl_2$ (2-3min) followed by the sterilization with 2.5% Ascorbic acid was also tried for different time durations (3, 5, 7 and 10 minutes).

There was no survival of explants when the exposure time was 1 minute while the highest survival of 80% was recorded when the explants were surface sterilized with 0.1% Mercuric Chloride, at the same time contamination level was only 20%. However, 40-60% survival was noticed with 2- 2.5 minutes exposure time. It was studied that lowest survival was recorded at treatment duration longer or lesser than 2- 3.5 minutes. (Figure-1) Whereas Dudhare et al, (2014) has reported 80% culture establishment when the exposure time was 4 minutes with the same treatment. The death of the explants during in vitro regeneration, at longer exposure duration to mercuric chloride may due to the phytotoxicity caused by Mercury. (Dhaliwal et al, 2015).

Results generated from 0.2% mercuric chloride treatment was extremely opposite as contamination rate was significantly higher with this treatment. Only 2% survival rate was observed along with the 6-8% mortality and 90-92%contamination rate when the exposure time was 3-3.5minutes. (Figure 2).

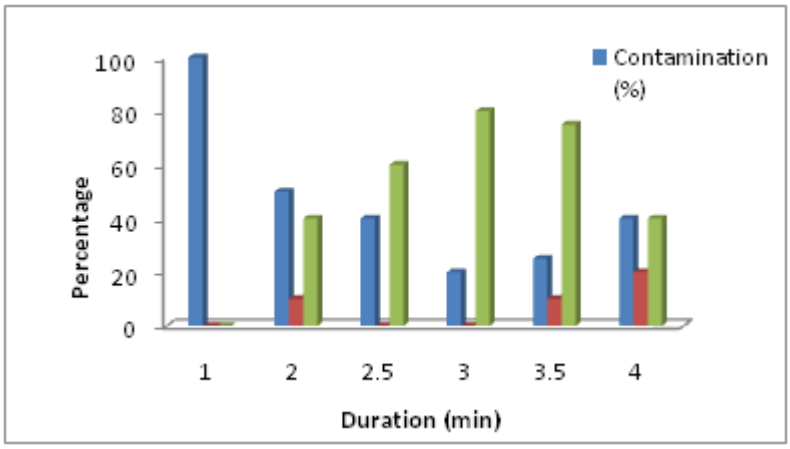

Figure 1: Surface Sterilization with 0.1% $HgCl_2$

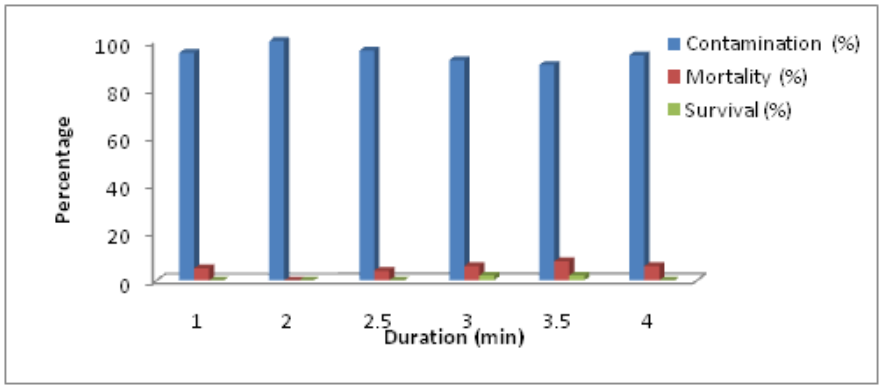

Figure 2: Surface Sterilization with 0.2% $HgCl_2$

Results were highly effective with the treatment of 0.1% Mercuric Chloride for 2-3 minutes followed by sterilization with 2.5% Ascorbic acid for 3, 5, 7 and 10 minutes. (Figure 3). Longer exposure resulted into higher survival rate. When the explants were treated for 10 minutes with Ascorbic acid, the survival percentage reached to 95% along with the only 5% contamination. (Dudhare et al, 2014). However, 3 and 5 minutes exposure gave only 50- 60 % survival rate along with the 10% mortality rate respectively. Whereas, only 20% contamination was reported with the 7 minutes exposure of ascorbic acid.

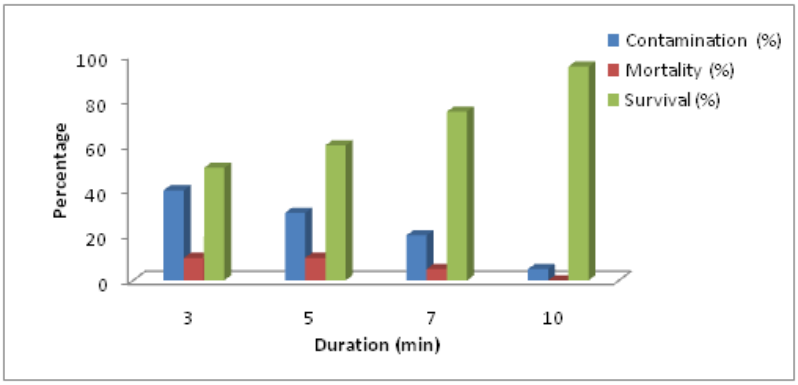

Figure 3: Surface Sterilization with 0.1% $HgCl_2$ + 2.5% Ascorbic Acid

CONCLUSIONS

In the present study, it was observed that using 0.1% mercuric chloride as a surface sterilization treatment was found to have significant response (Dudhare et al, 2014) but in combination with Ascorbic acid the results were highly effective as the survival rate was maximum because it is believed that ascorbic acid may have been absorbed by the plantlets, tarnslocated to the leaves and prevents the oxidation of phenolic compounds on the target sites. (Park et al, 2000). By far, this study on *Simarouba Glauca* can help in developing a productive protocol which can be easily adopted for its large scale micropropagation.

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