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Study Of Surface Sterilization On Nodal Explants Of *Dendrocalamus Hamiltonii*

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Abstract

During In-vitro studies, there was an investigation on the effect of the use of different surface sterilizing agents on nodal explants of *Dendrocalamus hamiltonii*. Surface sterilization is the most vital step during the preparation of healthy and viable explants for the micropropagation technique. The collection of explants was done from CPTs, that were highly exposed to microbial contaminants. Most contaminants such as bacteria and fungi are discarded by surface sterilization with the help of appropriate sterilizing agents. There was the use of $HgCl_2$ and NaOCl, and Ca $(OCl)_2$ a sterilizing agent during in-vitro culture. The leaf sheath covering the nodal segments containing axillary buds (1.5-2.00cm) was carefully removed and cleaned with 70% ethanol using sterilized cotton. These segments were pre-treated with 0.5% Bavistin as fungicides for 15 min. After pre-treatment segments were rinsed with Savlon and fresh running tap water at least three times. Further surface sterilization of explants was done under a laminar airflow cabinet with different treatments of $HgCl_2$ and Ca $(OCl)_2$ at different concentrations. Two methods of surface sterilization were used under a laminar airflow cabinet, (a) the explants were treated with 0.1% $HgCl_2$ solution for 10,15, and 20 minutes. It was thoroughly washed with sterile distilled water. It was then 30sec.dip within 70% ethanol and again rinsed in sterile distilled water. (b) the explants were treated for 10, 15, and 2o min with Ca $(OCl)_2$ with a few drops of tween-20. After this treatment, explants were washed with sterile distilled water, and they were then given a 30 sec. dip in 70% ethanol and again rinsed in sterile distilled water. After these sterilizing agents were used for the satisfactory result of the aseptic culture, viability, and bud break; sterilization with 0.1% $HgCl_2$ for 20 min and 30min continuous rinsing showed 68% Aseptic buds with 52% bud break whereas Ca $(OCl)_2$ (10min.) and similarly rinsing for 30min. showed 17% Aseptic buds with 12% Bud-break.

Key-Words: Surface sterilization, *D. hamiltonii*, $HgCl_2$, bud-break, Ca $(OCl)_2$

Introduction

Dendrocalamus hamiltonii is an evergreen bamboo. It belongs to the Poaceae family and is commonly known as Tama Bans (Kigomo BN,1988; Yesmin L,2014. It grows up to 15-18m and 12-15 cm in height and diameter respectively. Stems are dull green coloured and covered with whitish brown hairs. The whitish-brown hair is present on the above and below the node. It is very useful and also known as a Poor man's Timber plant. It is not a tree; it belongs to the grass family with a variety of valuable properties. It is a non-timber forest product. It is fast fast-growing species. For improved quality and healthy culture, there is a need for micropropagation of *Dendrocalamus hamiltonii*. During tissue culture, there is a controlled environmental condition that provides a proper supply of nutrients, pH, and temperature. In-vitro culture techniques are now used to propagate several plant species and are necessary for crop improvement. For culture technique, there is a need for a collection of explants that are collected from different selected sample sites. In the field, there is the presence of microbial contaminants that are transferred to the culture room through explant entry. For microbes-free culture and better output; surface sterilizing agents must be used before and after the culture technique.

Material and Methods

The nodal explants (2.0-2.5cm) had been collected from the selected bamboo plots (*D. hamiltonii*) from Tazpur village of Samastipur district of Bihar state. Collected nodal segments were cut into single nodal explants, the laboratory work was carried out at C.M. Science LNMU Darbhanga; Bihar, India during the rainy season of 2012. It is situated between longitude $25^0 51' 46.6848''$N and $85^0 46' 51.7044''$E and is bounded by Darbhanga, Muzaffarpur, Begusarai, and some parts of Khagaria district. These explants were at first rinsed with running tap water for 7 minutes. So that all the surfaces hold contaminants washed off easily. After this, the pretreated segments were washed with an antiseptic (Savlon) solution, and then under running tap water 2-3 times, consequently for 2 minutes each. Now the basal sheath covering the axillary buds was to be removed and shaped with the help of scalpels. Thereafter, the segments were surface sterilized with 0.1% $HgCl_2$ soln. for 5minutes. The experimental contaminants were easily removed by washing them in running tap water for 30-90 minutes and disinfecting them with surface sterilizing agents such as fungicides, alcohols, $HgCl_2$, and NaOCl. The success of sterilization depends upon the concentration, duration, and antimicrobial agents (Oyebanji et al. 2009). Mercuric chloride is often used to overcome microbial contamination; however, it is considered one of the most toxic elements for the ecosystem causing a major alteration in hepatic levels of both animal and plant living systems (Lung et al. 1993). Others have used Sodium hypochlorite and Chlorine water for surface sterilization (Rao et. al.; 1985, Yeh and Chang, 1986, a,b).

Observation

The nodal explants were collected from selected CPTs of Tazpur of Samastipur district (Bihar). Surface sterilization was done with the treatment of different sterilizing agents. During my research work, there were uses of $HgCl_2$, Ca (OCl_2), and NaOCl. Applying all these agents to the explants directly affected the sprouting and survival rate. The effect of sterilizing agents is shown in the table given below duration of application

was 10.15 and 20 minutes with all the sterilizing agents. According to the collected data shown below; among all the used sterilizing agents; $HgCl_2$(20 minutes) produces a large number of aseptic plants whereas most surviving products were Ca $(OCl)_2$ for 10 minutes and $HgCl_2$(10 minutes). HgCl2 is better than the other used ones among all these sterilizing agents. Maximum bud break was shown at treatment with Ca $(OCl)_2$ for 10 minutes. And the reading was 67%.

Effect of different surface sterilization treatments on explants of D. hamiltonii collected from Tazpur(Samastipur)

Surface Sterilizing Agents	Duration of Application	Aseptic%	Survival rate%
$HgCl_2$	10minutes	34%	62%
	15 minutes	41%	49%
	20 minutes	68%	52%
Ca $(OCl)_2$	10 minutes	22%	67%
	15 minutes	23%	33%
	20 minutes	17%	12%
NaOCl	10 minutes	25%	59%
	15 minutes	24%	54%
	20 minutes	17%	15%

Conclusion

During the research work for the elimination of microbes, there was an application of surface sterilizing agents such as $HgCl_2$, NaOCl, and Ca $(OCl)_2$. According to observations and data, the HgCl2 (20 min) application was an appropriate agent. Sometimes Ca (OCl)2 application was also a good attempt.

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