IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 8, May 2024

Study of Effectiveness of Surface Sterilized Methods in Plant Tissue Culture of *Curcuma longa* (Haldi)

Javid Khan and Chapekar Amreen
Department of Zoology
Anjuman Islam Janjira Degree College of Science, Murud-Janjira, Raigad, Maharashtra, India
Corresponding author: javidkhan.maniyar@aijdegreecollege.edu.in

Abstract: *A highly effective micropropagation method for turmeric (Curcuma longa Linn.) using rhizome bud explants has been developed. The woody plant was supplemented with various concentrations of BAP alone to initiate shoots in MS medium. Additionally, the explants were treated with different concentrations of bavistin (1%, 2%, and 3%). Among the different concentrations tested, 3% bavistin showed the highest survival rate (90%), followed by 2% (70%), while the control explants showed no survival. The impact of mercuric chloride ($HgCl_2$) on the explants was also studied, using a 0.1% concentration for different time intervals. Explants treated for 10 and 8 minutes with 0.1% $HgCl_2$ showed the highest survival rates (100% and 70% respectively). Browning was observed in explants treated for 8-10 minutes, so 6 minutes was determined as the optimal treatment time. Contamination-free explants were then inoculated onto MS media containing 2 mg/l of BAP to initiate shoot growth. After 28 days of inoculation, shoots were successfully initiated from the explants.*

Keywords: Micropropagation, *Curcuma longa,* PTC, Bavistin, $HgCl_2$, MS media

I. INTRODUCTION

Turmeric, classified under the genus *Curcuma* within the Zingiberaceae family, has a rich history of medicinal use (Dosoky and Setzer, 2018), being composed of approximately 120 species. Among the various species of *Curcuma*, *Curcuma longa* L. (Turmeric) is the most renowned. It is a cultivated plant, thriving in warm climates across numerous regions worldwide. Turmeric is a perennial, erect, leafy plant with large, lily-like leaves that can grow up to 12 meters long. It produces funnel-shaped yellow flowers with pointed leaves. The rhizome, the portion of the plant used medicinally, is typically boiled, dried, and processed into a yellow-colored powder. Known for its vibrant yellow hue, turmeric is often referred to as the "Golden spice" and is widely used for culinary, food coloring, and medicinal purposes
(Rathaur et al., 2012). Rhizomes are commonly used in Ayurveda and Chinese medicine (Sharifi-Rad et al., 2020). Turmeric is helpful in many pathological conditions as a preventive and curative crop (Shrishail et al., 2013).
Traditional turmeric propagation involves using underground rhizomes, a slow process that typically yields only 7-8 plants from a single rhizome. To overcome this limitation, micropropagation using rhizome bud explants can be employed. In vitro propagation methods enable rapid multiplication, producing high-quality, disease-free tissue culture seedlings in large quantities. These regenerated plantlets can then be successfully cultivated in field conditions (Seran, 2013).

II. MATERIALS AND METHODS

Collection of Explants

Rhizomes of turmeric (*Curcuma longa L*) were collected in a month of October 2022, from turmeric farm field, Jalgaon.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 8, May 2024

Sterilization of explant

Healthy, disease-free rhizomes from 2-month-old turmeric plants were collected. The explants were thoroughly washed under running tap water and then treated with tween-80 for 10 minutes, followed by a rinse with distilled water. Subsequently, the explants were washed with 70% ethanol for 5 minutes and then rinsed with distilled water again. Next, the explants were treated with bavistin at concentrations of 1%, 2%, and 3% for 45 minutes. After 45 minutes, the explants were washed three times with sterilized distilled water. The explants were then transferred to a laminar airflow chamber and treated with 0.1% mercuric chloride for different time intervals (2, 4, 6, 8, and 10 minutes). Following this treatment, the explants were washed 2-3 times with sterilized distilled water. Once surface sterilization was complete, the explants were trimmed and inoculated onto MS media.

Initiation of shoots

The sterilized explants were inoculated on MS media containing 2 mg/l of BAP for shoot initiation.

III. RESULTS AND DISCUSSION

Effect of bavistin on surface sterilization

The explants were subjected to various concentrations of bavistin (1%, 2%, and 3%) for 45 minutes. Among these concentrations, the highest survival rate (90%) was observed with the 3% concentration, followed by a 70% survival rate with the 2% concentration, while the control explants showed no survival.

Table 1: Effect of bavistin on surface sterilization

Sr. no.	Concentration of Bavistin	Number of explants inoculated	Number of explants contaminated (after 10 days)	Number of explants survived (after 10 days)	Percentage of survival after 10 days
1	Control	10	10	0	**0**
2	1%	10	9	1	**10**
3	2%	10	7	3	**70**
4	**3%**	**10**	**1**	**9**	**90***

*indicates concentration of Bavistin with highest survival percentage

Effect of mercuric chloride on surface sterilization

The explants treated with bavistin later exhibited bacterial contamination. Therefore, they were subjected to another treatment with 0.1% mercuric chloride ($HgCl_2$) for various time intervals. Among these intervals, both 10 and 8 minutes of treatment showed the highest survival rate (100%), followed by a 70% survival rate when the explants were treated with 0.1% mercuric chloride for 6 minutes. However, browning was observed in the explants treated for 10 and 8 minutes, leading to the selection of the 6-minute treatment as the optimized protocol. The contamination-free explants were then inoculated onto MS media containing 2 mg/l of BAP for shoot initiation. After 28 days of inoculation, shoots were successfully initiated from the explants, as shown in Figure 1.

Table 2: Effect of $HgCl_2$ on surface sterilization

Sr. No.	0.1% $HgCl_2$ time interval (min)	Number of explants inoculated	Number of explants contaminated (after 10 days)	Number of explants survived (after 10 days)	Percentage of contamination free plants (after 10 days)
1	Control	10	10	0	**0**
2	2	10	7	3	**30**
3	4	10	5	5	**50**
4	6	10	3	7	**70**
5	8	10	1	9	**90***
6	**10**	**10**	**1**	**9**	**90***

*indicates time interval of $HgCl^2$ with highest survival percentage

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 8, May 2024

Fig. 1: Shoot initiation in turmeric

IV. CONCLUSION

In this study, we have established an effective and dependable surface sterilization protocol for the *in-vitro* regeneration of *Curcuma longa* L. rhizome explants. This standardized protocol facilitates large-scale plant propagation through *in-vitro* micropropagation

REFERENCES

[1]. Dosoky, N. S and Setzer W. N (2018) Chemical Composition and Biological Activities of Essential Oils of Curcuma Species; Nutrients. 10 (9): 1–42.

[2]. Rathaur, P., Raja, W., Ramteke, P.W and John, S.A (2012) Turmeric: The golden spice of life; Int. J. pharm. Sci. Res., 3(7):19-87.

[3]. Seran, T.H (2013). In vitro propagation of ginger (Zingiber officinale Rosc.) through direct organogenesis: A review; Pak. J. Biol. Sci., 16:1826-1835.

[4]. Sharifi-Rad, J., Rayess, Y.E., Rizk, A.A., Sadaka, C., Zgheib, R., Zam, W., Sestito, S., Rapposelli, S., Neffe-Skocińska, K., Zieliñska, D., Salehi, B., Setzer, W. N., Dosoky, N. S., Taheri, Y., Beyrouthy, M., Martorell, M., Ostrander, E.A., Suleria, H. A. R., Cho, W. C., Maroyi, A and Martins, N (2020). Turmeric and its major compound curcumin on health: Bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications; Front. Pharmacol., 11:01021.

[5]. Shrishail, D., Ravichandra, H. K. H., Tulsianand, G and Shruthi, S.D (2013). Turmeric: Nature's precious medicine; Asian J. Pharm. Clinical Res., 6:11-16.

ISSN 2581-9429 IJARSCT