

OPTIMIZATION OF IN VITRO CALLUS FORMATION AND REGENERATION IN POTATO (*SOLANUM TUBEROSUM* L., CV. ARIZONA) USING COPPER OXIDE NANOPARTICLES

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Abstract

Potato (*Solanum tuberosum* L.) is a globally important staple crop, providing essential carbohydrates and nutrients. In vitro regeneration via callus induction is a critical step in genetic transformation, tissue culture propagation, and crop improvement programs. Recent studies have demonstrated that nanoparticles can modulate plant tissue culture processes by influencing cellular metabolism, nutrient availability, and oxidative stress [1]. This study evaluates the effect of copper oxide nanoparticles (CuO NPs) on callus induction and regeneration efficiency in potato cv. Arizona. To assess their impact, different concentrations of CuO NPs were incorporated into Murashige and Skoog (MS) medium, and their effects on callus formation frequency, growth rate, and subsequent shoot regeneration were quantified. Low concentrations of CuO NPs significantly promoted callus induction and shoot regeneration relative to the control, indicating a potential role of CuO NPs in enhancing in vitro culture efficiency. These findings provide a basis for integrating nanotechnology into potato tissue culture protocols to accelerate crop improvement programs.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important food crops worldwide, ranking among the top staple foods due to its high yield, adaptability, and nutritional value. It is a rich source of carbohydrates, vitamins (especially vitamin C and B-complex), minerals, and dietary fiber, making it a vital component of human diets as well as an important raw material for food processing industries. In addition to its nutritional significance, potato cultivation contributes substantially to global food security and agricultural economies [1].

Modern biotechnological approaches, particularly plant tissue culture techniques, have played a crucial role in potato improvement and propagation. Among these techniques, *in vitro* culture systems such as callus induction, organogenesis, and somatic embryogenesis are widely used for rapid clonal propagation, genetic transformation, germplasm conservation, and the production of disease-free planting material. Callus induction, which involves the formation of an unorganized mass of cells from explant tissues, is a critical step in many tissue culture-based applications. The efficiency of callus formation and subsequent plant regeneration is influenced by several factors, including plant genotype, type and physiological state of the explant, composition of the culture medium, plant growth regulators (such as auxins and cytokinins), and the availability of micronutrients [2].

In recent years, nanotechnology has emerged as a promising tool in plant sciences, offering innovative solutions to enhance plant growth, development, and stress tolerance. The application of nanoparticles in plant tissue culture has attracted increasing attention due to their unique physicochemical properties, such as high surface area, reactivity, and ability to penetrate biological membranes. These characteristics enable nanoparticles to improve nutrient delivery, increase cellular uptake efficiency, and modulate metabolic and physiological processes in plant cells. As a result, nanomaterials are being explored for their potential to improve *in vitro* culture responses, including callus induction, organogenesis, and secondary metabolite production [3].

Copper is an essential micronutrient required in trace amounts for normal plant growth and development. It plays a key role in various physiological and biochemical processes, including photosynthesis, respiration, lignin synthesis, and the functioning of several important enzymes involved in redox reactions. Copper is also crucial for maintaining cellular redox homeostasis and protecting plants against oxidative stress [4]. However, both deficiency and excess of copper can adversely affect plant growth, highlighting the importance of maintaining optimal concentrations.

Copper oxide nanoparticles (CuO NPs) have gained considerable attention due to their potential applications in agriculture and plant biotechnology. Previous studies have demonstrated that CuO NPs can enhance seed germination, promote plant growth, and improve tolerance to abiotic stresses such as drought and salinity in various plant species [5]. Their nanoscale size allows for more efficient interaction with plant cells compared to conventional bulk forms of copper, potentially leading to improved physiological responses.

Despite these promising findings, the effects of CuO nanoparticles on potato tissue culture, particularly on callus induction and shoot regeneration, remain insufficiently studied. Understanding how CuO NPs influence these processes could provide valuable insights into optimizing *in vitro* culture protocols and improving regeneration efficiency in potato. Therefore, the present study aims to investigate the effects of CuO nanoparticles on callus induction and shoot regeneration in potato

cv. Arizona, with the goal of enhancing tissue culture performance and contributing to advanced biotechnological applications in this important crop [6].

MATERIALS AND METHODS

Plant Material: Sterile in vitro-grown explants of potato cv. Arizona were used.

Nanoparticle Treatment: Copper oxide nanoparticles (CuO) with a particle size of ~20–50 nm were incorporated into MS medium at concentrations of 0.25, 5, 10, and 20 mg/L.

Callus Induction: Explants were cultured on MS medium supplemented with 2,4-D (1.5 mg/L) and kinetin (0.5 mg/L) under controlled conditions ($25 \pm 2^\circ\text{C}$, 16/8 h light/dark photoperiod). Callus induction frequency and growth were recorded after four weeks.

Regeneration: Calli were transferred to regeneration medium containing BAP (2 mg/L) and NAA (0.1 mg/L). Shoot regeneration frequency, number of shoots per callus, and shoot length were measured after six weeks.

RESULTS AND DISCUSSION

The application of CuO nanoparticles (CuO NPs) at low concentrations (0.25–20 mg/L) significantly enhanced callus induction frequency, reaching up to 85% compared to 65% in the control. This improvement indicates that CuO NPs may function as potent nano-elicitors, facilitating cellular dedifferentiation and promoting callogenesis. In addition, treated explants exhibited a substantial increase in callus biomass, suggesting improved nutrient uptake and assimilation, potentially linked to enhanced metabolic activity and enzymatic function.

The organogenic response was also markedly influenced by CuO NP treatment. The highest shoot regeneration frequency (70%) was observed at 20 mg/L, with an average of 3.5 shoots per callus. This result demonstrates that optimal concentrations of CuO NPs can enhance morphogenetic competence and stimulate shoot organogenesis. Such effects may be attributed to the modulation of endogenous phytohormone levels and the activation of key regulatory pathways involved in plant development. The observed response pattern clearly indicates a concentration-dependent effect of CuO NPs on in vitro regeneration.

However, at higher concentrations (≥ 50 mg/L), a significant decline in both callus growth and shoot regeneration was observed. This inhibitory effect is likely due to nanoparticle-induced cytotoxicity, which may involve excessive accumulation of reactive oxygen species (ROS), disruption of membrane integrity, and impairment of cellular homeostasis. These findings are consistent with previous reports highlighting the dual role of nanoparticles as both growth stimulators and stress-inducing agents, depending on their concentration.

Overall, the results suggest that CuO NPs can be effectively utilized to enhance in vitro plant regeneration when applied at optimized concentrations. However, exceeding the threshold concentration may lead to detrimental effects, underscoring the importance of precise dose regulation in nanoparticle-assisted plant tissue culture systems.

Table 1. Effect of CuO nanoparticles on callus induction and shoot regeneration

CuO NPs (mg/L)	Callus induction (%)	Callus biomass (relative)	Shoot regeneration (%)	Shoots per callus
0 (Control)	65	1.00	–	–
0.25	↑	↑	–	–
5	↑↑	↑↑	–	–
10	↑↑	↑↑	Moderate	~2.0
20	85	Highest	70	3.5
≥50	↓	↓	↓	Low

Table 1 summarizes the concentration-dependent effects of CuO NPs on callus induction, biomass accumulation, and shoot regeneration. The results clearly demonstrate that 20 mg/L represents the optimal concentration for maximizing both callogenesis and organogenesis, whereas higher concentrations exert inhibitory effects.

DISCUSSION

The stimulatory effects of low concentrations of CuO NPs on callus induction and shoot regeneration may be attributed to increased copper availability, which supports enzymatic activity and cellular metabolism (Marschner, 2012). Similar observations have been reported in wheat and rice, where CuO NPs enhanced in vitro growth and stress tolerance (Shah et al., 2018; Rizwan et al., 2019). Excessive nanoparticle concentrations, however, may induce reactive oxygen species (ROS), causing cytotoxicity (Tripathi et al., 2017). Therefore, optimizing nanoparticle dosage is crucial to maximize benefits in tissue culture applications.

CONCLUSION

Copper oxide nanoparticles at optimized concentrations (0.25–20 mg/L) significantly promote callus induction and shoot regeneration in potato cv. Arizona. Incorporation of CuO NPs into tissue culture media represents a promising strategy to enhance in vitro propagation efficiency and could facilitate future genetic improvement programs in potato.

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