

INFLUENCE OF IRON OXIDE NANOPARTICLES ON CALLUS INDUCTION AND REGENERATION EFFICIENCY IN *SOLANUM TUBEROSUM* L. (CV. ARIZONA)

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Abstract:

Potato (*Solanum tuberosum* L.) is one of the world's most important staple crops, providing essential carbohydrates and nutrients. In vitro regeneration of potato via callus induction is a critical step in genetic transformation, tissue culture propagation, and crop improvement programs. Recent studies have demonstrated that nanoparticles can influence plant tissue culture responses by modulating cellular metabolism, nutrient availability, and oxidative stress. This study investigates the effect of iron oxide nanoparticles (Fe₃O₄ NPs) on callus induction and regeneration efficiency in potato cv. Arizona . Different concentrations of Fe₃O₄ NPs were incorporated into Murashige and Skoog (MS) medium to evaluate their influence on callus formation frequency, callus growth rate, and subsequent shoot regeneration. The results indicated that low concentrations of Fe₃O₄ NPs significantly enhanced callus induction and shoot regeneration compared to the control, suggesting a potential role of iron nanoparticles in improving 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, serving as a staple food for a large portion of the global population due to its high nutritional value, rich carbohydrate content, and adaptability to diverse agro-climatic conditions. In addition to its role as a dietary staple, potato is also an economically significant crop used in food processing and industrial applications. However, conventional breeding approaches in potato are often limited by its complex tetraploid genome, high heterozygosity, and low seed fertility, which restrict the efficiency of genetic improvement programs.

In vitro culture techniques, particularly callus induction and plant regeneration systems, play a crucial role in overcoming these limitations. These techniques are widely used for micropropagation, germplasm conservation, genetic transformation, and secondary metabolite production. The efficiency of callus formation and subsequent regeneration is strongly influenced by several factors, including the genetic background of the plant, explant source, composition and concentration of plant growth regulators, and the availability of essential nutrients in the culture medium [1]. Optimization of these parameters is therefore essential for improving regeneration efficiency in potato tissue culture systems.

In recent years, nanotechnology has emerged as a promising tool in plant biotechnology, offering novel approaches to enhance plant growth and in vitro culture performance. Nanoparticles can serve as carriers of essential nutrients, improving their bioavailability and uptake efficiency at the cellular level. This can lead to enhanced metabolic activity, improved enzymatic function, and increased morphogenic responses in plant tissue cultures [2]. Among various engineered nanomaterials, metal and metal oxide nanoparticles have attracted considerable attention due to their unique physicochemical properties and potential biological applications.

Iron is an essential micronutrient required for normal plant growth and development. It plays a vital role in chlorophyll biosynthesis, respiration, photosynthesis, and electron transport processes [3]. Deficiency or imbalance of iron can significantly impair plant metabolic functions and reduce growth performance. Iron oxide nanoparticles (Fe_3O_4 NPs), due to their high surface area and reactivity, have been reported to enhance seed germination, plant growth, and stress tolerance in several plant species by improving nutrient availability and modulating physiological processes [4]. Despite these promising findings, the underlying mechanisms and potential applications of Fe_3O_4 NPs in plant tissue culture systems remain insufficiently understood.

In particular, there is limited information regarding the influence of Fe_3O_4 nanoparticles on callus induction and plant regeneration in potato. Understanding their role could provide valuable insights into improving in vitro regeneration efficiency and optimizing tissue culture protocols for this economically important crop. Therefore, the present study aims to investigate the effects of Fe_3O_4 nanoparticles on callus induction and shoot regeneration in potato (*Solanum tuberosum* L.) cultivar Arizona, with a focus on evaluating their potential to enhance morphogenic responses under in vitro conditions.

MATERIALS AND METHODS

Plant Material: Explants from sterile in vitro-grown potato cv. Arizona were used. Iron oxide nanoparticles (Fe_3O_4) were characterized for size (~20–50 nm) and 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 4 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 recorded after 6 weeks.

RESULTS

Fe_3O_4 nanoparticles (Fe_3O_4 NPs) at low concentrations (0.25–20 mg/L) significantly enhanced callus induction frequency, reaching 85% compared to 65% in the control. This indicates a positive effect of nanoparticle supplementation on callogenesis under in vitro conditions.

An increase in callus biomass was also observed in Fe_3O_4 NP-treated explants, suggesting improved nutrient uptake and assimilation efficiency, which may be associated with enhanced metabolic activity at the cellular level.

The highest shoot regeneration frequency (70%) was recorded at 20 mg/L Fe_3O_4 NPs, with an average of 3.5 shoots per callus. This concentration was identified as the most effective treatment for promoting organogenic response.

In contrast, higher concentrations (>50 mg/L) adversely affected both callus growth and shoot regeneration, indicating potential nanoparticle-induced toxicity at elevated levels.

DISCUSSION

The stimulatory effects of low concentrations of Fe_3O_4 nanoparticles on callus induction and shoot regeneration in potato may be attributed to increased iron bioavailability, which plays a critical role in chlorophyll biosynthesis, enzymatic activation, and overall cellular metabolism. Iron is an essential micronutrient, and its improved delivery in nanoparticle form can enhance physiological and biochemical processes during in vitro culture.

Similar positive effects of iron oxide nanoparticles on plant growth and tissue culture responses have been reported in other crops, including wheat and rice, where enhanced in vitro growth and improved stress tolerance were observed [5,6]. These findings support the hypothesis that Fe_3O_4 NPs can act as growth-promoting agents at optimal concentrations.

However, at higher concentrations, Fe_3O_4 NPs may induce oxidative stress through excessive generation of reactive oxygen species (ROS), leading to membrane damage, metabolic imbalance, and reduced morphogenic potential. Such cytotoxic effects of nanoparticles have also been

documented in previous studies, where elevated nanoparticle exposure resulted in growth inhibition and physiological disturbances [7].

Therefore, the present results highlight that the biological effects of Fe₃O₄ nanoparticles are highly concentration-dependent. Careful optimization of nanoparticle dosage is essential to maximize their beneficial effects while avoiding toxicity in plant tissue culture systems.

CONCLUSION

Iron oxide nanoparticles at optimized concentrations (0,25–20 mg/L) can significantly enhance callus induction and shoot regeneration in potato cv. Arizona. Incorporating Fe₃O₄ NPs into tissue culture media offers a promising strategy to improve in vitro propagation efficiency and could facilitate future genetic improvement programs in potato.

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