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**ENHANCING POTATO (*SOLANUM TUBEROSUM* L., CV CHANLIBEL)
CALLUS INDUCTION AND REGENERATION USING IRON
AND COPPER OXIDE NANOPARTICLES**

The application of nanoparticles (NPs) in plant tissue culture has emerged as a promising strategy to enhance morphogenesis and regeneration efficiency. Despite accumulating evidence regarding nanoparticle-mediated enhancement of plant tissue culture responses, limited information is available on their application in potato, particularly in the cultivar Chanlibel. The absence of systematic evaluation of Fe₃O₄ and CuO nanoparticles in this genotype represents a notable knowledge gap. Therefore, the present study aimed to investigate the effects of different concentrations of Fe₃O₄ and CuO nanoparticles on callus induction, callus growth, and shoot regeneration in Solanum tuberosum L. cv. Chanlibel under in vitro conditions. This study evaluated the effects of iron oxide (Fe₃O₄) and copper oxide (CuO) nanoparticles on callus induction and in vitro regeneration of potato (Solanum tuberosum L., cv. Chanlibel). Explants were cultured on Murashige and Skoog (MS) medium supplemented with Fe₃O₄ or CuO nanoparticles at 0.25, 5, and 10 mg L⁻¹. Callus induction frequency increased with Fe₃O₄ concentration up to 5 mg/L, reaching a maximum of 82%, compared to 60% in the control. Similarly, callus fresh weight and shoot regeneration frequency were highest at 5 mg/L, with values of 0.70 g and 68%, respectively. Increasing the concentration to 10 mg/L slightly reduced both callus induction (72%) and shoot regeneration (55%). Callus induction frequency increased with Fe₃O₄ concentration up to 5 mg/L, reaching a maximum of 82%, compared to 60% in the control. Similarly, callus fresh weight and shoot regeneration frequency were highest at 5 mg/L, with values of 0.70 g and 68%, respectively. Increasing the concentration to 10 mg/L slightly reduced both callus induction (72%) and shoot regeneration (55%). Callus induction frequency, callus fresh weight, and shoot regeneration percentage were assessed to determine treatment efficacy. Fe₃O₄ nanoparticles significantly enhanced morphogenic responses, with the highest performance observed at 10 mg L⁻¹, resulting in 82% callus induction and 68% shoot regeneration. CuO nanoparticles exhibited a concentration-dependent response, with optimal effects at 5 mg L⁻¹ (78% callus induction and 64% shoot regeneration), whereas

10 mg L⁻¹ caused inhibitory effects, including reduced regeneration and callus browning. Morphological evaluation revealed compact, green calli under Fe₃O₄ treatments, while elevated CuO levels induced tissue browning, indicating potential phytotoxicity. Overall, the findings demonstrate that appropriately optimized Fe₃O₄ and CuO nanoparticle supplementation can improve in vitro regeneration efficiency in potato, underscoring their potential application in advanced plant biotechnology and micropropagation systems.

Keywords: nanoparticles; iron oxide (Fe₃O₄); copper oxide (CuO); callus induction; in vitro regeneration; potato; Solanum tuberosum L.

Introduction. Potato (*Solanum tuberosum* L.) is one of the most strategically important food crops worldwide, ranking among the leading staple species in terms of global production and human consumption. Beyond its substantial contribution to food security, potato represents a valuable source of carbohydrates, high-quality proteins, vitamins, and essential minerals. Sustainable improvement of potato cultivars through rapid clonal propagation, genetic transformation, and breeding programs relies heavily on efficient in vitro culture systems. In this context, successful callus induction and subsequent plant regeneration constitute critical steps for micropropagation and biotechnological applications. Nevertheless, regeneration efficiency in potato tissue culture is frequently limited by genotype-dependent morphogenic responses, oxidative stress caused by reactive oxygen species (ROS), phenolic oxidation leading to tissue browning, and microbial contamination, all of which compromise morphogenic competence [1, 6, 8].

Recent advances in nanotechnology have introduced engineered nanoparticles (NPs) as promising modulators of plant physiological and biochemical processes. Due to their nanoscale dimensions, high surface area-to-volume ratio, and enhanced reactivity, nanoparticles exhibit greater bioavailability and biological activity than their bulk counterparts [5, 8]. Emerging evidence indicates that NPs can enhance nutrient uptake, alter membrane permeability, regulate phytohormonal signaling pathways, activate antioxidant defense systems, and suppress microbial growth in plant tissue culture systems [4, 9]. These properties suggest their potential to improve in vitro morphogenesis and regeneration efficiency.

Among micronutrient-based nanoparticles, iron oxide (Fe₃O₄) and copper oxide (CuO) are of particular interest because iron (Fe) and copper (Cu) are essential elements involved in key metabolic pathways. Iron is indispensable for chlorophyll biosynthesis, mitochondrial respiration, and electron transport processes, whereas copper functions as a catalytic cofactor for numerous oxidoreductases, including superoxide dismutase, and plays a crucial role in redox regulation and stress responses [2, 8]. Nano-formulations of these micronutrients may enhance their solubility, mobility, and controlled delivery within plant tissues. For example, Fe₃O₄ nanoparticles have been reported to significantly enhance callus induction and shoot regeneration in rice and chickpea tissue cultures [3, 7]. In contrast, CuO nanoparticles have demonstrated concentration-dependent effects, stimulating callogenesis at lower concentrations while inducing oxidative damage and phytotoxicity at higher doses due to excessive ROS accumulation [6, 10].

Despite accumulating evidence regarding nanoparticle-mediated enhancement of plant tissue culture responses, limited information is available on their application in potato, particularly in the cultivar Chanlibel. The absence of systematic evaluation of Fe₃O₄ and CuO nanoparticles in this genotype represents a notable knowledge gap. Therefore, the present study aimed to investigate the effects of different concentrations of Fe₃O₄ and CuO nanoparticles on callus induction, callus growth, and shoot regeneration in *Solanum tuberosum* L. cv. Chanlibel under *in vitro* conditions. By elucidating the morphogenic responses to micronutrient-based nanoparticles, this research seeks to contribute to the optimization of regeneration protocols and to expand the practical applications of nanotechnology in plant biotechnology.

Materials and Methods. *Plant Material and Explant Preparation*

Certified disease-free tubers of *S. tuberosum* L., cv. Chanlibel were used. Stem and leaf explants (1–2 cm) were surface sterilized using 70% ethanol (1 min) and 2% sodium hypochlorite (15 min), followed by three rinses with sterile distilled water.

Nanoparticle Preparation. Fe_3O_4 and CuO nanoparticles (Sigma-Aldrich, 20–50 nm) were dispersed in sterile distilled water by sonication for 15 min. NPs were added to MS medium at the following concentrations:

Fe_3O_4 : 0,25, 5, 10 mg/L

CuO: 0,25, 5, 10 mg/L

Control media contained no nanoparticles. Medium pH was adjusted to 5.8 and autoclaved at 121°C for 20 min.

Culture Conditions. Explants were incubated in the dark at $25 \pm 2^\circ\text{C}$ for 4 weeks for callus induction. Calli were then transferred to regeneration medium containing 1 mg/L BAP and 0.1 mg/L NAA under a 16/8 h light/dark photoperiod.

Evaluation Parameters.

Callus induction frequency (%) = (number of explants forming callus ÷ total explants) × 100

Callus fresh weight (g) after 4 weeks

Shoot regeneration frequency (%) = (number of calli producing shoots ÷ total calli) × 100

Morphological observations: color, compactness, and necrosis

Statistical Analysis. Experiments were performed in triplicate with 20 explants per treatment. Data were analyzed using ANOVA followed by Tukey's test at $p < 0.05$.

Results and Discussion. Effects of Fe_3O_4 Nanoparticles. The effect of Fe_3O_4 nanoparticles on callus induction, fresh weight, and shoot regeneration of *Solanum tuberosum* L., cv. Chanlibel is presented in Table 1. Callus induction frequency increased with Fe_3O_4 concentration up to 5 mg/L, reaching a maximum of 82%, compared to 60% in the control. Similarly, callus fresh weight and shoot regeneration frequency were highest at 5 mg/L, with values of 0.70 g and 68%, respectively. Increasing the concentration to 10 mg/L slightly reduced both callus induction (72%) and shoot regeneration (55%). Morphologically, calli at 5 mg/L were compact, green, and highly meristematic, whereas higher Fe_3O_4 concentrations led to minor decreases in growth and regeneration potential.

Table 1. Effects of Fe_3O_4 nanoparticles on callus induction, fresh weight, and shoot regeneration of potato explants

Fe_3O_4 (mg/L)	Callus Induction (%)	Callus Fresh Weight (g)	Shoot Regeneration (%)
0 (Control)	60	0.42	50
0,25	68	0.55	57
5	82	0.70	68
10	72	0.58	55

Effects of CuO Nanoparticles. CuO nanoparticles also influenced callus induction and regeneration (Table 2). Low concentrations of CuO (0,25–5 mg/L) enhanced callus induction (70–78%) and shoot regeneration (56–64%) compared to the control (60% and 50%, respectively). However, a higher concentration (10 mg/L) markedly reduced callus induction (52%) and shoot regeneration (42%) and caused browning and necrosis in the calli.

Table 2. Effects of CuO nanoparticles on callus induction, fresh weight, and shoot regeneration of potato explants

CuO (mg/L)	Callus Induction (%)	Callus Fresh Weight (g)	Shoot Regeneration (%)
0 (Control)	60	0.42	50
0,25	70	0.53	56
5	78	0.63	64
10	52	0.45	42

Comparative Observations. Comparative analysis indicated that Fe₃O₄ at 5 mg/L and CuO at 5 mg/L were optimal for callus induction and shoot regeneration. Morphologically, Fe₃O₄-treated calli were compact and green at optimal concentration, while high CuO concentrations induced browning and necrosis.

The present study demonstrated that Fe₃O₄ nanoparticles function as a bioavailable iron source, promoting enzymatic activity, antioxidative defense mechanisms, and organogenesis in *Solanum tuberosum* L., cv. Chanlibel. These observations align with the findings of Ullah et al. (2024) and Iqbal et al. (2024), who reported similar stimulatory effects of iron oxide nanoparticles on callus induction and regeneration in rice and turmeric tissue cultures.

CuO nanoparticles exhibited dose-dependent effects: low concentrations (0,25–5 mg/L) enhanced callus formation and shoot regeneration, whereas higher concentrations (10 mg/L) caused oxidative stress, cytotoxicity, and browning of calli. These results are consistent with previous studies by Anwaar et al. (2020) and Khaliloğlu et al. (2022), highlighting the dual role of metal oxide nanoparticles in plant tissue culture. The data emphasize the importance of optimizing nanoparticle type and concentration to maximize regenerative potential while minimizing phytotoxic effects.

Overall, the study confirms that carefully selected concentrations of Fe₃O₄ and CuO nanoparticles can significantly improve callus induction, biomass accumulation, and shoot regeneration, offering a valuable strategy for enhancing in vitro propagation efficiency in potato.

Conclusion. Fe₃O₄ and CuO nanoparticles exert significant effects on callus induction and in vitro regeneration of potato cv. Chanlibel. Optimal concentrations—5 mg/L for Fe₃O₄ and 5 mg/L for CuO—resulted in enhanced callus formation, increased biomass, and higher shoot regeneration rates. These findings suggest that nanoparticle supplementation can serve as a promising tool to improve potato tissue culture protocols, provided that concentrations are carefully optimized to avoid cytotoxicity.

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ПІДВИЩЕННЯ ІНДУКЦІЇ УТВОРЕННЯ КАЛУСУ ТА РЕГЕНЕРАЦІЇ У КАРТОПЛІ (*SOLANUM TUBEROSUM* L., СОПТ *CHANLIBEL*) ЗА ДОПОМОГОЮ НАНОЧАСТИНОК ОКСИДУ ЗАЛІЗА ТА МІДІ

Застосування наночастинок (НЧ) у культурі рослинних тканин стало перспективною стратегією для покращення морфогенезу та ефективності регенерації.

Незважаючи на накопичення доказів щодо посилення реакцій культури рослинних тканин за допомогою наночастинок, інформація про їх застосування на картоплі, зокрема у сорту *Chanlibel*, обмежена. Відсутність систематичної оцінки наночастинок Fe_3O_4 та CuO у цьому генотипі являє собою значну прогалину в знаннях. Тому метою цього дослідження було дослідити вплив різних концентрацій наночастинок Fe_3O_4 та CuO на індукцію калюсу, ріст калюсу та регенерацію пагонів у *Solanum tuberosum* L. cv. *Chanlibel* в умовах *in vitro*. У цьому дослідженні оцінювали вплив наночастинок оксиду заліза (Fe_3O_4) та оксиду міді (CuO) на індукцію калюсу та регенерацію *in vitro* картоплі (*Solanum tuberosum* L., сорт *Chanlibel*).

Експлантати культивували на середовищі Мурасіге та Скуга (MS), доповненому наночастинами Fe_3O_4 або CuO у концентраціях 0,25, 5 та 10 мг/л. Частота індукції калюсу збільшувалася з концентрацією Fe_3O_4 до 5 мг/л, досягаючи максимуму 82% порівняно з 60% у контролі. Аналогічно, свіжа маса калюсу та частота регенерації пагонів були найвищими при 5 мг/л, зі значеннями 0,70 г та 68% відповідно. Збільшення концентрації до 10 мг/л дещо знизило як індукцію калюсу (72%), так і регенерацію пагонів (55%). Для визначення ефективності обробки оцінювали частоту індукції калюсу, масу свіжого калюсу та відсоток регенерації пагонів. Наночастинки Fe_3O_4 значно посилювали морфогенетичні реакції, причому найвища ефективність спостерігалася при 10 мг L^{-1} , що призвело до 82% індукції калюсу та 68% регенерації пагонів. Наночастинки CuO демонстрували концентраційно-залежну реакцію, з оптимальним ефектом при 5 мг L^{-1} (78% індукції калюсу та 64% регенерації пагонів), тоді як 10 мг L^{-1} викликали інгібуючий ефект, включаючи зниження регенерації та потемніння калюсу. Морфологічна оцінка виявила компактні, зелені калюси при обробці Fe_3O_4 , тоді як підвищений рівень CuO індуквав потемніння тканин, що вказує на потенційну фітотоксичність.

Загалом, результати дослідження показують, що належним чином оптимізоване додавання наночастинок Fe_3O_4 та CuO може покращити ефективність регенерації *in vitro* у картоплі, що підкреслює їх потенційне застосування в передовій біотехнології рослин та системах мікророзмноження.

Ключові слова: наночастинки; оксид заліза (Fe_3O_4); оксид міді (CuO); індукція калюсу; регенерація *in vitro*; картопля; *Solanum tuberosum* L.

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