**A comprehensive study of the hormetic influence of biosynthesized AgNPs on regenerating rice calli of indica cv. IR64**

Rice is one of the most widely cultivated crops worldwide; however, it is not amenable to genetic manipulations, owing to its poor response to tissue culture and regeneration *in vitro*. To improve its response to tissue culture, we evaluated the influence of biosynthesized silver nanoparticles on callus induction, regeneration, and rhizogenesis of indica rice cv. IR64. Silver nanoparticles were biosynthesized using silver nitrate and *Parthenium hysterophorus* plant extract, and they were characterized by UV-visible spectroscopy, Fourier-transform infrared spectroscopy, Transmission electron microscopy, and X-ray diffraction. The biosynthesized silver nanoparticles (PHAgNPs), when supplemented in tissue culture medium, promoted callus induction frequency, callus regeneration, and rhizogenesis at variable concentrations of 10 mg l-1, 5 mg l-1, and 10 mg l-1, respectively. Further analysis of the endogenous hormonal levels in regenerating calli revealed that AgNPs enhanced regeneration by alleviating abscisic acid and ethylene levels in the plant tissue. The stimulatory influence eliciting a regeneration response was found to be optimal by supplementing the regeneration medium with 5 mgl-1 PHAgNPs; the malondialdehyde, proline, and hydrogen peroxide levels were also lower than those in the control, suggesting an improved antioxidant status. The results indicate that biosynthesized PHAgNPs have an unexplored potential to positively influence the tissue culture of recalcitrant varieties.

**Introduction:**

Indica rice is predominantly cultivated in tropical and subtropical regions of Asia, and it accounts for 80% of the rice cultivated worldwide. As the demand for rice production continues to increase, the germplasm tolerance to biotic and abiotic stress conditions must be improved without compromising yield (1, 2).

Genetic transformation of rice calli using *Agrobacterium tumefaciens* is frequently employed to improve the crop, as it ensures a low copy number and stable integration of T-DNA. The *Agrobacterium*-mediated transformation of indica rice calli has limitations, however, owing to poor regeneration and callogenesis, which are influenced by numerous internal and external factors (3). With the advent of nanobiotechnology, researchers have demonstrated that application of nanotechnology in plant tissue culture has shown promise, positively influencing the germination rate of seeds, plant growth, metabolite production, organogenesis, callus induction frequency, and regeneration frequency as well as eliminating microbial contamination (4).

Plant growth and development is modulated by endogenous plant growth regulators (PGRs). The most prevalent plant hormones are auxins, cytokinins, gibberellins (GA), abscisic acid (ABA), and ethylene. Auxin is the most important modulator found throughout the plant, and its accumulation is imperative for initiation of apical meristem. Cytokinin is involved in germination, meristematic functions, and leaf senescence. The interaction of these hormones is crucial for the development of plants, so they are commonly employed in *in vitro* plant tissue culture to regulate differentiation in explants. GA are primarily involved in organ elongation, seed development, and regulation of flowering time. ABA, however, is regulated by external factors that are involved in stomatal closure, germination, root elongation, and flowering; it is involved in a complex regulatory network with auxins and cytokinins and is imperative for embryogenesis and shoot regeneration. Another important PGR is ethylene, a gaseous hormone that primarily influences the ripening of fruits and plant senescence (5, 6). Studies have demonstrated that *in vitro* tissue cultures result in the accumulation of ethylene, and supplementation with silver nanoparticles (AgNPs) in the plant tissue culture medium renders the explants healthier, improving their growth vigor and regeneration frequency, which can be attributed to the ability of silver ions to inhibit ethylene synthesis (7–11). The influence of silver nanoparticles on other PGRs during regeneration, however, remains unclear.

Another study has reported that, even when propagated under optimal conditions, plant tissues produce reactive oxygen species (ROS) as an unavoidable by-product of general plant metabolism, which is detrimental for plant growth and development. Harmful free radicals are reduced by an internal antioxidant system, but the process consumes vital resources in the cells, hindering growth and development (12). In addition to functioning as ethylene inhibitors, silver ions serve as electron acceptors and donors in redox reactions, supporting the exchange of electrons with CO3+ and Fe2+ (13), in particular, thereby reducing the ROS and alleviating the strain on the plant antioxidant system. Relative to silver ions, AgNPs are more efficient in chemical reactions and interact better with their surrounding environment because of their higher surface-area-to-mass ratio (7). Hence, in the present study, we have attempted to correlate the influence of exogenously supplemented AgNPs on endogenous ROS as well as PGR levels in regenerating calli, to better understand the influence of AgNPs on plant development. Because of their small size (1–100 nm), AgNPs possess unique optical and physiochemical properties, and they are therefore used in various fields for conduction, biological detection, catalysis, wound healing, anti-microbial activity, and phytostimulation (14).

Synthesis of AgNPs using chemical and physical methods requires toxic chemicals and involves complex purification steps; however, AgNPs can be synthesized by utilizing plant extracts, which is a simple, economical, and eco-friendly process. *Parthenium hysterophorus* (PH),used for bio-fabrication of AgNPs in the present study,is one of the most difficult-to-control weeds worldwide. It is toxic to animals, harmful to biodiversity, and responsible for economic losses in agriculture. Control measures such as burning the weeds, spraying chemical herbicides, and introducing pests, mycoherbicides, and competitive crops have their own constraints. Using PH plants for biosynthesis of nanoparticles, however, is a productive use of the weeds (15, 16).