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

Rice ~~crop~~ is one of the most widely cultivated crops ~~in the world~~worldwide~~;~~ however, it is not amenable to genetic manipulations, owing to its poor response to tissue culture and regeneration *in vitro*. ~~With an aim to~~To improve its response to tissue culture, we ~~have~~ evaluated the influence of biosynthesized silver nanoparticles on callus induction, regeneration, and rhizogenesis of i~~I~~ndica rice cv. IR64 ~~in the present study~~. ~~Biosynthesis of silver nanoparticles has been achieved~~Silver nanoparticles were biosynthesized using silver nitrate and *Parthenium hysterophorus* plant extract, and they were characterized by UV-visible spectroscopy, Fourier-t~~T~~ransform i~~I~~nfrared s~~S~~pectroscopy ~~(FTIR)~~, T~~T~~ransmission e~~E~~lectron m~~M~~icroscopy ~~(TEM)~~, and X-ray d~~D~~iffraction ~~(XRD)~~. The biosynthesized silver nanoparticles (PHAgNPs), when supplemented in tissue culture medium~~a~~, promoted callus induction frequency, callus~~,~~ regeneration, ~~of callus~~ and rhizogenesis at variable concentrations of 10 mg $l^{-1}$, 5 mg $l^{-1}$~~,~~ and 10 mg $l^{-1}$, respectively. Further ~~introspection into~~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 optim~~um upon augmentation of~~ by supplementing the regeneration medium with 5 $mgl^{-1}$ PHAgNPs~~;~~ ~~wherein~~ the~~,~~ malondialdehyde, proline, and hydrogen peroxide levels ~~also reduced as compared to~~were also lower than those in the control, suggesting an improved antioxidant status. ~~From the results it is apparent that the~~ The results indicate that biosynthesized PHAgNPs have an unexplored potential to positively influence the tissue culture of recalcitrant varieties.

**Introduction:**

Indica ~~Rice variety prominently~~rice is predominantly cultivated in ~~the~~ tropical and~~,~~ subtropical regions of Asia, ~~is accounted for~~and it accounts for 80% of the ~~the rice cultivated in the world~~rice cultivated worldwide. ~~With an ever increasing demand for produce,~~As the demand for rice production continues to increase, ~~there is a need to improve the tolerance of germplasm~~the germplasm tolerance to biotic and abiotic stress conditions must be improved without compromising ~~on the~~ yield (1, 2).

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**Commented [MR3]:** Internal references to the current study are not necessary in the abstract.

**Commented [MR4]:** If you do not use these acronyms again in the abstract, they do not need to be defined until the main text.

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**Commented [MR6]:** Your meaning here was a bit unclear; you may be referring to general demand for "produce," but I adjusted this to refer more specifically to the production of rice. Please review this carefully and get back to me with any clarifications.

GeneticGenetic transformation of rice calli calli, mediated by using *Agrobacterium tumefaciens* is frequently employed to improve the crop, is a popular choice to improve the crop, as it ensures a low copy number and stable integration of T-DNA. However,The *Agrobacterium*-mediated transformation using rice indica cv. callus,of indica rice calli has its 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, increase in callus induction frequency, and induction frequency, regeneration frequency as well as eliminating as well as elimination of microbial contamination (4).

It is in common knowledge that plantPlant growth and development is basically modulated by endogenous plant growth regulators (PGRs). Amongst all the identified PGRs,The most prevalent plant hormones are aAuxins, cCytokinins, gGibberellins (GA), aAbscisic acid (ABA), and eEthylene have been acknowledged as the most prominent natural plant hormones. Auxin is the mostAuxin is the most important modulator important modulator found throughout the plant, and its accumulation is imperative for initiation of apical meristem, and then there is cytokinin which. Cytokinin is involved in germination, meristematic functions, and leaf senescence of leaves. Interaction of auxin and cytokininThe interaction of these hormones is crucial for the development of plants, hence are beingso they are commonly employed in *in vitro* plant tissue tissue culture to regulate differentiation in explants. Gibberelins GA also are involved in growth, however, are prominently primarily involved in organ elongation, seed development, and regulating the time of flowering.regulation of flowering time. On the other hand, ABA, however, is regulated by external factors which that are involved in stomatal closure, germination, root elongation, and flowering, and; it is involved in a complex regulatory network along with auxins and cytokinins which with auxins and cytokinins and is imperative for embryogenesis and shoot regeneration. Another important PGR is ethylene, a gaseous hormone, primarily influencing that primarily influences the ripening of fruits and senescence in plantsplant senescence (5, 6). Earlier studiesStudies have demonstrated that *iIn vitro* tissue cultures results in the accumulation of ethylene, and supplementation withof silver nanoparticles (AgNPs) in the plant tissue culture medium rendersed the explants healthier, improvinged their growth vigor as well asand regeneration frequency, which can be attributed to the ability of silver ions to

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**Commented [MR9]:** In American English, "which" must be preceded by a comma. In this context, "that" is the more appropriate term.

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inhibit ethylene synthesis (7–11). ~~However, influence of silver nanoparticles on other PGRs during regeneration is yet not lucid.~~The influence of silver nanoparticles on other PGRs during regeneration, however, remains unclear.

Another study has reported that ~~plant tissues ,~~ even when propagated under optimal conditions, plant tissues produce r~~R~~eactive o~~O~~xygen s~~S~~pecies (ROS) as an unavoidable by-product of general plant metabolism, which is detrimental for plant growth and development. ~~Even though the harmful~~ Harmful free radicals are reduced by an internal antioxidant system, but the process ~~uses up~~consumes vital resources in the cells, hindering growth and development (12). ~~Silver ions apart from~~In addition to functioning as ethylene inhibitors, silver ions ~~are accredited to function~~ serve as electron acceptors and donors in red-ox reactions, ~~especially~~ supporting the exchange of electrons with $CO_3^+$ and $Fe_2^+$ (13), in particular, ~~thereby reducing~~thereby reducing the ROS and alleviat~~ing~~ing the strain on the plant antioxidant system ~~of the plant~~. ~~However, compared~~Relative to silver ions, ~~silver nanoparticles~~ AgNPs are ~~extra~~ more efficient in chemical reactions and interact better with ~~its~~ their surrounding environment because of their higher surface–area–to–mass ratio (7). Hence, in the present study, we have attempted to ~~co-relate~~correlate the influence of exogenously supplemented AgNPs on endogenous ROS as well as PGR levels in~~of~~ regenerating calli, to better understand the influence of AgNPs on plant development. ~~The AgNPs, due to~~Because of their small size (1–100 nm), AgNPs possess unique optical and physiochemical properties,~~,~~ and ~~thereby~~ 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~~,~~ require toxic chemicals and involves complex purification steps;~~.~~ h~~H~~owever, 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 ~~world's most difficult to control weed~~most difficult-to-control weeds worldwide. It is toxic to animals, harmful to biodiversity, and ~~is~~ responsible for ~~economical~~economic losses in agriculture. Control measures such as~~like~~ burning the weeds, spraying chemical herbicides, and introducing pests, mycoherbicides, and competitive crops have their own constraints. ~~However, utilizing~~Using ~~the~~ PH plants for biosynthesis of nanoparticles, however, is a ~~way to put the weeds to good use~~productive use of the weeds (15, 16).

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