

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~~Indica 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~~ransform ~~i~~nfrared ~~s~~pectroscopy (~~FTIR~~), ~~T~~ransmission ~~e~~lectron ~~m~~icroscopy (~~TEM~~), and X-ray ~~d~~iffraction (~~XRD~~). The biosynthesized silver nanoparticles (PHAgNPs), when supplemented in tissue culture medium, 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 optimal ~~upon~~ ~~augmentation of by supplementing the~~ regeneration medium with 5 mg l⁻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).

Commented [MR1]: I have reviewed your manuscript according to your specifications. Please review my changes and comments and return the document to me with any additional questions or revisions.

Commented [MR2]: Note that I have inserted a serial or "Oxford" comma here and throughout the text to improve clarity; check with your target journal to ensure this is in line with their style guide.

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.

Commented [MR5]: Ensure that your target journal does not utilize the capitalized "L" for the unit abbreviation here.

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.

~~Genetic~~Genetic 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).

Commented [MR7]: Check with your target journal to ensure that "T-DNA" is an acronym that is understood well by the target audience and is not expected to be defined at its first mention in the main text.

~~It is in common knowledge that plant~~Plant growth and development is ~~basically~~ modulated by endogenous plant growth regulators (PGRs). ~~Amongst all the identified PGRs, The most prevalent plant hormones are a~~Auxins, ~~c~~Cytokinins, ~~g~~Gibberellins (GA), ~~a~~Abscisic acid (ABA), and ~~c~~Ethylene ~~have been acknowledged as the most prominent natural plant hormones. Auxin is the most~~Auxin 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 cytokinin~~The 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. Gibberellins-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 eytokinins 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 plants~~plant senescence (5, 6). ~~Earlier studies~~Studies have demonstrated that *in 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

Commented [MR8]: Phrases like this to begin a sentence or paragraph are unnecessary and negatively impact the academic tone of the manuscript, so I have removed instances like this.

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

Commented [MR10]: This is a good location in the main text to define this acronym.

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~~Reactive ~~o~~Oxygen ~~s~~Species (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 alleviating ~~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 ~~eo-relate~~correlate the influence of exogenously supplemented AgNPs on endogenous ROS as well as PGR levels ~~in ef~~ 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, requires~~ toxic chemicals and involves complex purification steps; ~~h~~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 ~~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 ~~eeconomical~~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).

Commented [MR11]: Check with your target journal to ensure that the use of first-person language (like "we") is acceptable for the publication.

