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Enhancement of ginsenoside content and Alleviation of oxidative stress via Glycol chitosan coated selenium nanoparticles (GC-Se NPs)

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Abstract

Background : Chitosan nanoparticles which are considered one of the potential candidate for inducing plant growth, and considering its less toxici ty are conjugated with Glycol and selenium to make (GC-Se NPs) to enhance the ginsenosides content and alleviate the oxidative stress.

Methods and Results : In the current study, glycol chitosan coated selenium nanoparticles (GC-Se NPs) are synthesized and characterized by different techniques. Furthermore, application of (GC-Se NPs) on *P. ginseng* Meyer to alleviate the Oxidative stress and enhancement of ginsenosides. After the application of 20 mg/L Se NPs and GC-Se NPs, the moderate accumulation of ROS ($O2^{\bullet-}$ and H2O2) and upregulation of *PgSOD* and *PgCAT* showed good biocompatibility and less toxicity even at the highest concentration. Furthermore, ginsenoside biosynthetic pathway genes (*PgHMGR*, *PgSS*, *PgSE*, *PgDDS*) also showed significant upregulation upon 20 mg/L GC-Se NPs treatment along with ginsenoside accumulated up to 217.47 mg/mL and 169.86 mg/mL (total ginsenoside content 493.58 mg/mL) mainly due to the increased proportion of Rb1 and Re ginsenosides.

Conclusion : The results suggested that ecofriendly conjugation of GC with Se NPs could be used as a bio fortifier to enhance the ginsenoside profile and to increase the quality of ginseng roots.

Results

Characterization of Se and GC-Se NPs

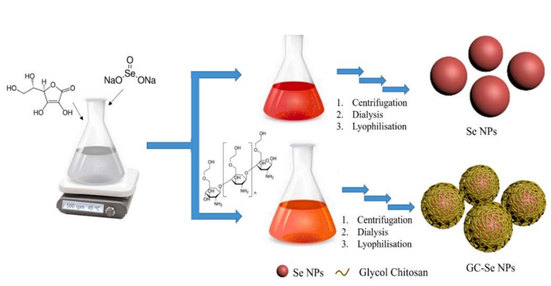

Fig. 1. Schematic representation of synthesis of Se and GC-Se NPs.

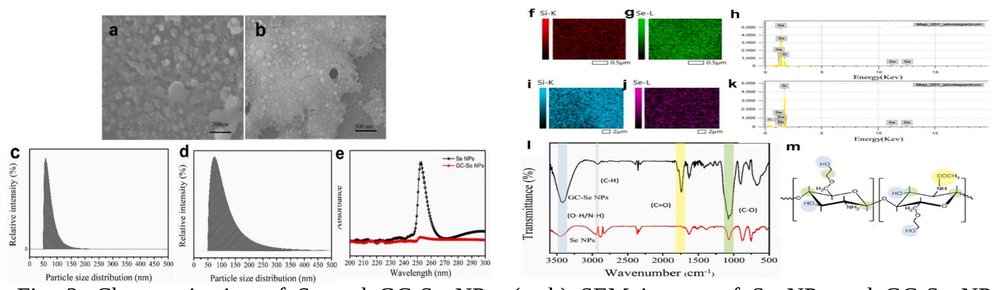

Fig. 2. Characterization of Se and GC-Se NPs. (a, b) SEM images of Se NPs and GC-Se NPs respectively, (c, d) DLS particle size analysis of Se NPs and GC-Se NPs, (e) UV–Vis absorbance spectroscopy of Se NPs and GC-Se NPs, (f-h & i-k) EDS mapping images of Se NPs and GC-Se NPs, respectively, (l) FTIR spectra of Se NPs and GC Se NPs, (m) Chemical structure of Glycol chitosan

ROS production and antioxidant related gene expression studies

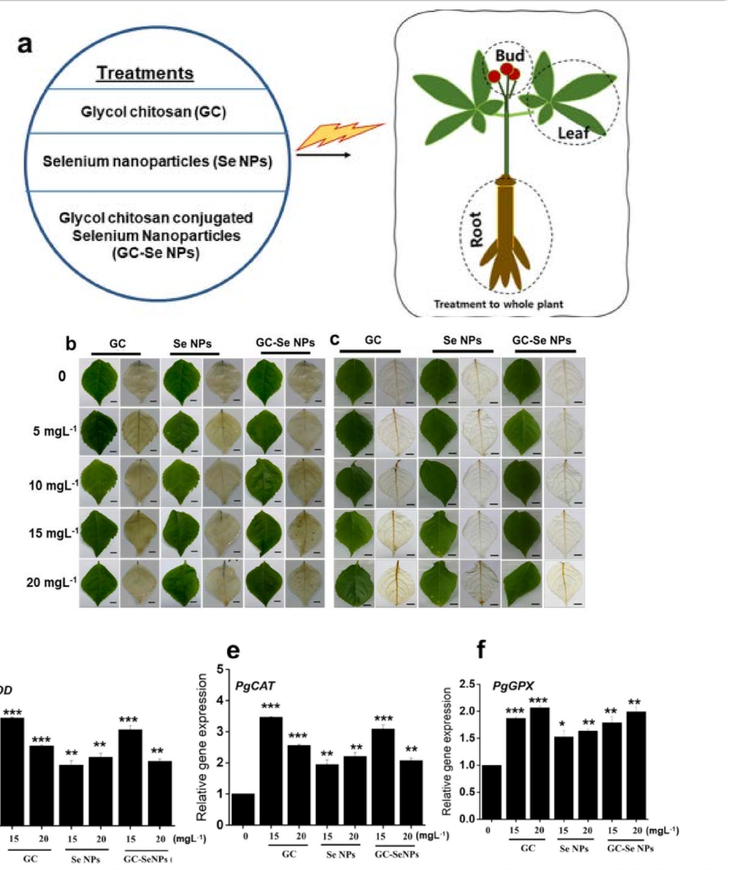

Fig. 3 (a) Two year old *P. ginseng* leaves was treated with GC, Se NPs or GC-Se NPs using foliar spray method.b, c) Reactive oxygen species (ROS) accumulation was measured in leaves using NBT & DAB staining (d–f) Presence of $O2^{\bullet-}$ and H2O2 were detected using brown and blue color formation. The real time PCR analysis of antioxidant genes such as *PgSOD*, *PgCAT*, *PgGPX* were performed using different concentration of GC, Se NPs or GC-Se NPs. Expression was normalized using housekeeping gene *β-actin*. The statistical significance of three biological replicates were determined by student t-test (*p < 0.05, **p < 0.01, p < 0.001).

Gene expression pattern of ginsenoside related pathway

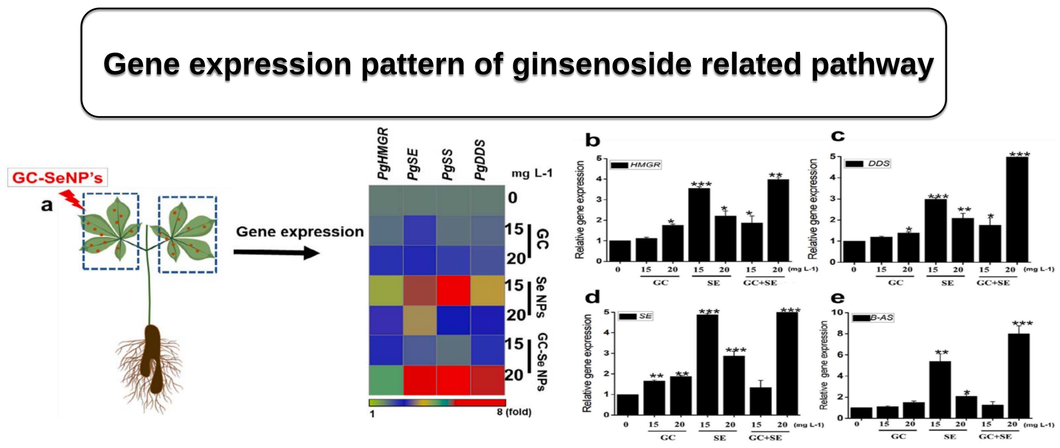

Fig. 5. (a) Heat map showing the differential expression pattern of ginsenoside biosynthetic pathway. (b) Using foliar method, *ginseng* leaves were sprayed with different concentration of GC, Se NPs, GC-Se NPs and samples were collected after 5 days post treatment and subjected to mRNA studies. The relative gene expression of *PgHMGR*, *PgDDS*, *PgSS*, *PgSE* were normalized using internal standard *β-actin*. The statistical significance of three biological replicates were determined by student t-test (*p < 0.05, **p < 0.01, p < 0.001).

Quantification of protopanaxadiol (PPD) and protopanaxatriol (PPT) type ginsenosides.

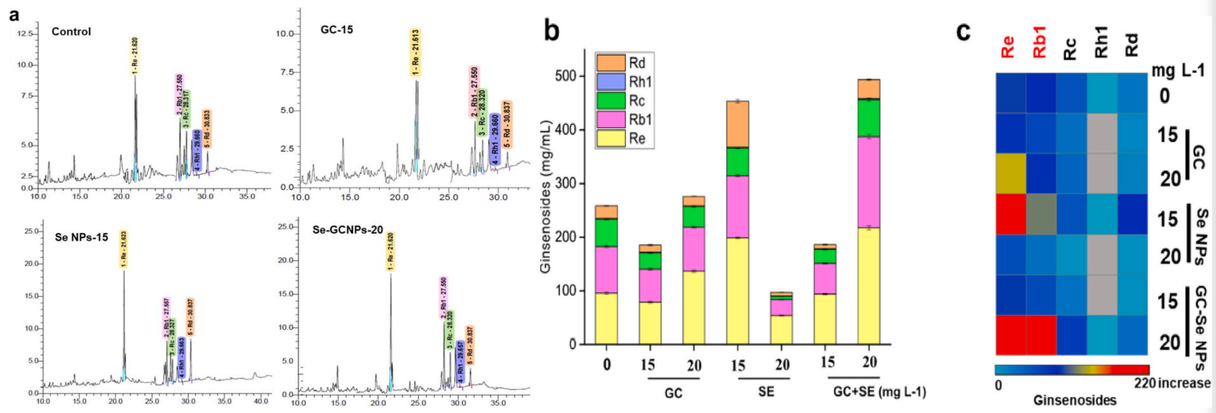

Fig. 6. Quantification of protopanaxadiol (PPD) and protopanaxatriol (PPT) type ginsenosides. (a) With or without treatment of Se, GC and GC-Se NPs, root samples were collected and subjected to HPLC analysis. Representative selective chromatogram showing identified ginsenoside with or without treatments. (b, c) Ginsenoside profile of treated and non-treated 2-year old root samples.

Conclusions & Discussion

1. The crosstalk between antioxidant mechanism and ginsenoside accumulation were studied upon foliar treatment of GC-Se NPs in *P. ginseng*
2. Our present study concludes that 20 mg L^{-1} GC-Se NPs as the safe optimal concentration or ginsenoside accumulation.
3. Moderate GC conjugation at this concentration could also moderate the expression of antioxidative genes including *PgSOD* and *PgCAT* and thus maintaining homeostasis.
4. The concentration of selenium sprayed through the foliar application may not be equivalent to the concentration of selenium uptake by ginseng.
5. We conclude that ecofriendly GC conjugation with Se can be used as a bio fortifier to improve the quality of *P. ginseng.*

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