

Supplementary Information Data

Biogenic Chitosan–Selenium Nanoparticles for Improved Stress Resilience in Rice Seedlings

Iqra Irshad^{1,2,†}, Saira Zaheer^{1,†}, Muhammad Faheem Iqbal^{1,2}, Muhammad Uzair^{2*}, Sunbal Khalil Chaudhari³, Muhammad Ramzan Khan^{1,2}, Murtaza Hasan^{4*}, Ghazala Mustafa^{1*}

¹Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan

²National Institute for Genomics and Advanced Biotechnology (NIGAB), NARC, Park Road-45500, Islamabad, Pakistan.

³Institute of Molecular Biology and Biotechnology, The University of Lahore, Sargodha Campus, Sargodha 42100, Pakistan

⁴Department of Biotechnology, Faculty of Chemical and Biological Sciences, The Islamia University of Bahawalpur, Bahawalpur, 63100, Pakistan

*Corresponding Author

Murtaza Hasan

Department of Biotechnology,
Faculty of Chemical and Biological Sciences,
The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan.

Email: murtaza@iub.edu.pk

[†] These authors contributed equally.

Ghazala Mustafa

Department of Plant Sciences, Faculty of Biological Sciences,
Quaid-i-Azam University, Islamabad 45320, Pakistan.

Email: mghazala@qau.edu.pk

Tel: +92-51-90643187

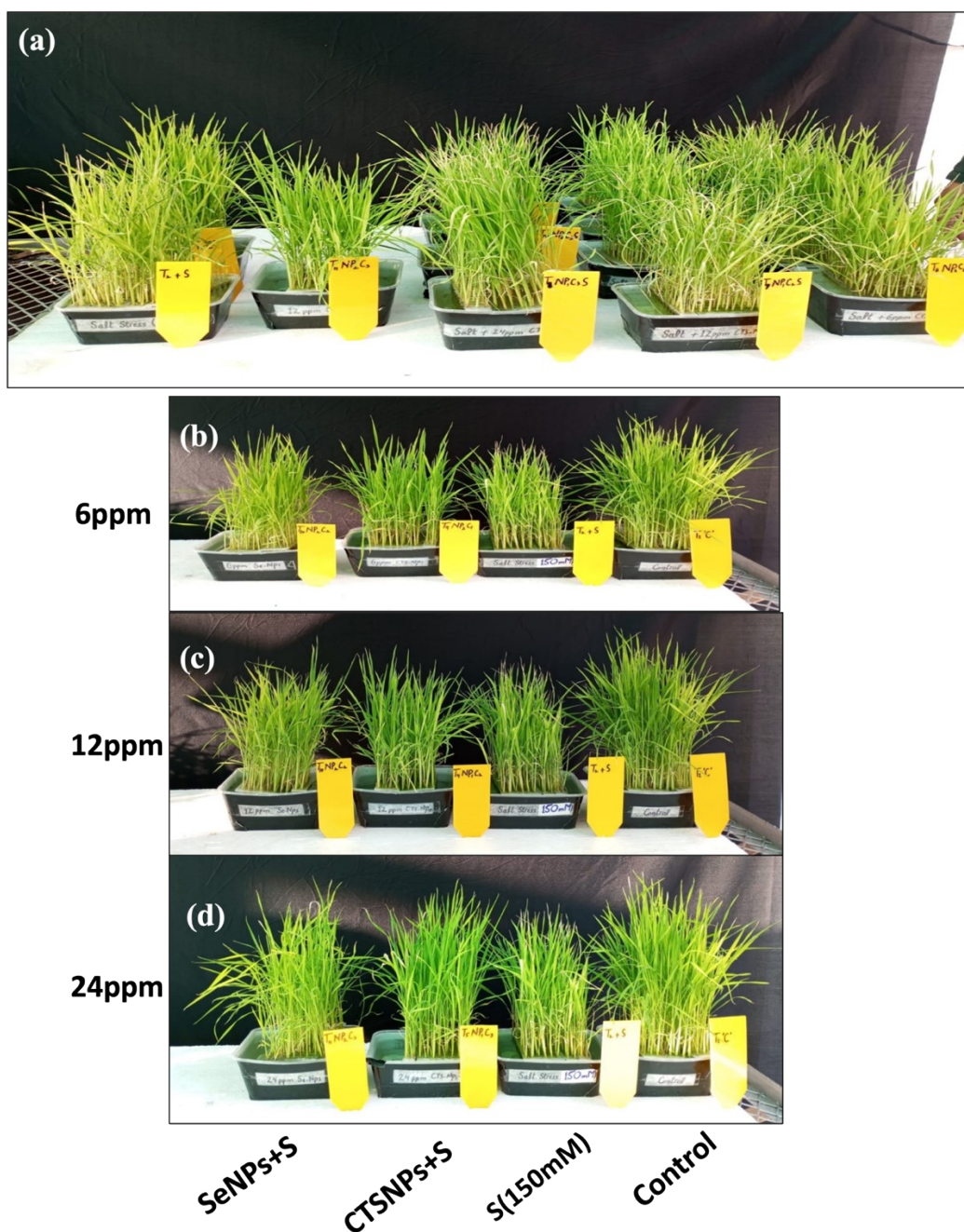


Fig.S1. Comparison of different concentrations of SeNPs & CTSNPs with 150mM salt on *Oryza sativa* before harvesting (a). Complete experiment (b). 6ppm (SeNPs & CTSNPs) (c). 12ppm (SeNPs & CTSNPs) (d). 24ppm (SeNPs & CTSNPs).

Table S1

| Parameters | Se-NPs | CTS-NPs |
|--|---|--|
| (a) UV-Vis Spectra | | |
| Peak range | 260-280 nm | 280-300 nm |
| Absorption Peak | Sharp and well-defined (plasmon resonance) | Broad peaks (less pronounced plasmon band) |
| Interpretation | Indicates nanoparticle formation and a smaller size | Suggests a polymeric structure with embedded or coated particles |
| (b) FTIR analysis (Functional groups) | | |
| -OH, -NH | Present from plant extract as a reducing/ capping agent | Strong signals from the chitosan backbone |
| C=O, C=C | From phytochemicals (phenolics, Flavonoids) | May arise due to minor modifications or additives |
| Se-O or Se=O (if oxidized) | Weak peak may appear ($\sim 600\text{-}700\text{ cm}^{-1}$) | Absent |
| C-N, C-O | Possible with capping agents | Strong, from the Chitosan structure |
| (c) XRD Diffraction Pattern | | |
| Crystallinity | Highly distinct peaks indicate a crystalline nature | Low-broad halo pattern indicating amorphous structure |
| 2 θ Peaks | Characteristics peaks of elemental Se (e.g., 23°, 30°, 43°) | Broad peak around 20° (typical of chitosan) |
| (d) DLS (Dynamic Light Scattering) | | |
| Size range | Typically, 30-100 nm | Typically, 100-200 nm |
| Polydispersity Index | Low to moderate (0.2-0.4) | Higher (0.3-0.6), due to polymer aggregation |
| Zeta potential | -20 to -30 mV (moderate stability) | +30 to +40 mV (cationic chitosan provides high stability) |
| (e) SEM Imaging (Morphology) | | |

| | | |
|--|--------------------------------------|--|
| Shape | Spherical or slightly irregular | Rough, semi-spherical, or irregular |
| Surface Texture | Smooth and slightly agglomerated | Dense and compact polymeric surface |
| Particle Size | 30-80 nm (individual), some clusters | 100-200 nm (due to chitosan matrix) |
| (f) EDS (Energy Dispersive X-ray Spectroscopy) (Key element Detected) | | |
| Selenium (Se) | Strong, confirming elemental Se | Absent |
| C, O | Present due to the organic matrix | Present in Higher amounts (from Chitosan) |
| N | Trace or absent | Prominent due to chitosan |
| Other elements | Possible residual (Na, Cl) | Possibly Na, P, or crosslinking ions (e.g., TPP) |
| Summary of Difference | | |
| Structure | Metallic, Crystalline | Polymeric, mostly amorphous |
| Stability | Moderate (negatively charged) | High (positively charged due to chitosan) |
| Size | Smaller (30-100 nm) | Larger (100-200 nm) |
| Elemental profile | Rich in Se | Rich in C, N, O (from Chitosan) |
| Application Stability | Redox activity, stress mitigation | Controlled release, biocompatibility |