

***Supplementary material***

**Micronutrient nanoprotectants curtail arsenic-induced physio-oxidative damages by differentially regulating antioxidant and metabolic mechanisms across *Brassica napus* genotypes**

Muhammad Arslan Yousaf <sup>a</sup>, Muhammad Noman <sup>b,c</sup>, Kangni Zhang <sup>a</sup>, Basharat Ali <sup>d</sup>, Muhammad Shahbaz Naeem <sup>e</sup>, Wenqiang Lan <sup>a</sup>, Mengting Lyu <sup>a</sup>, Yiwa Hu <sup>a</sup>, Skhawat Ali <sup>a,\*</sup>, Weijun Zhou <sup>a</sup>,  
\*

<sup>a</sup> Institute of Crop Science, Ministry of Agriculture and Rural Affairs Key Laboratory of Spectroscopy Sensing, Zhejiang University, Hangzhou 310058, China

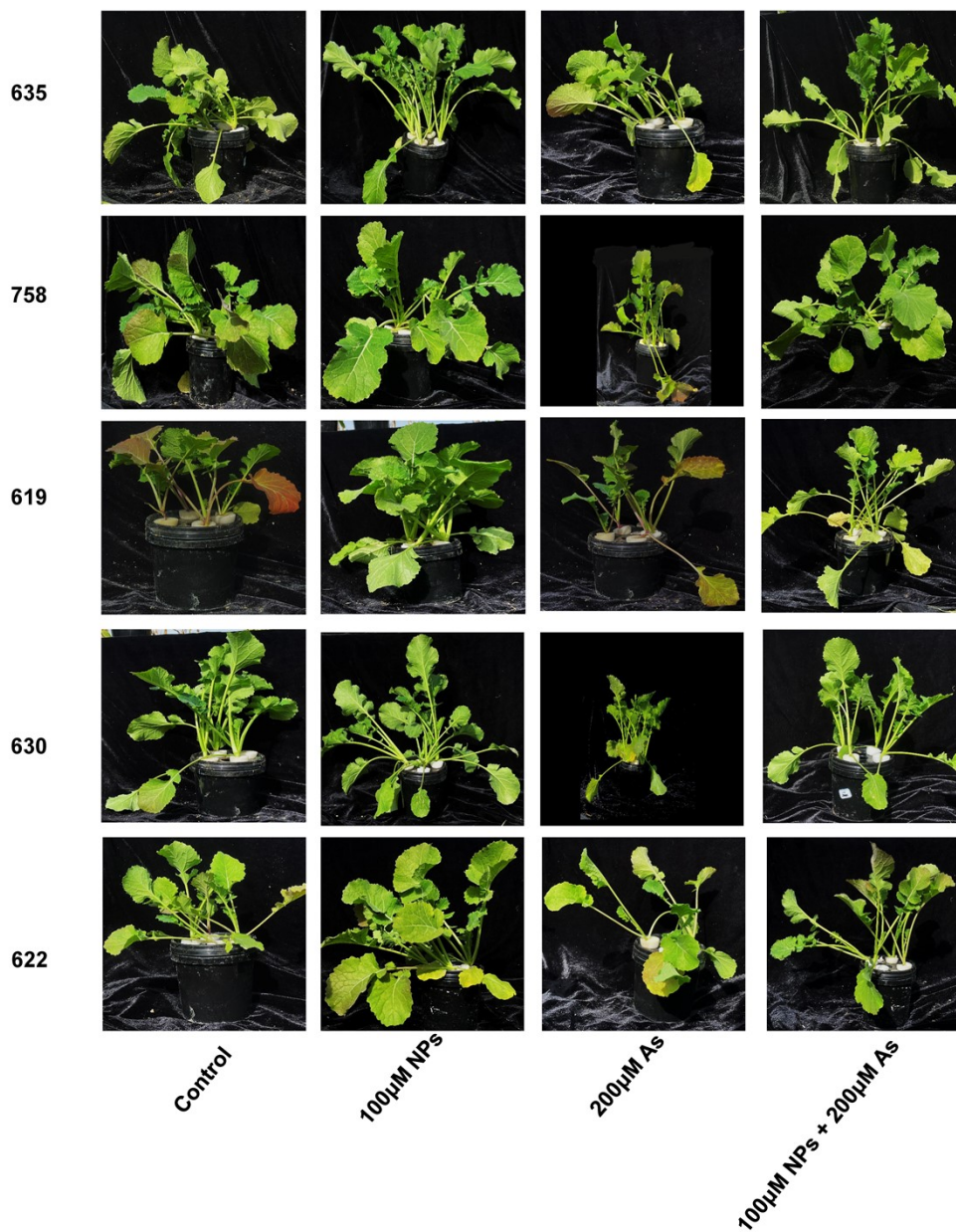
<sup>b</sup> Institute of Plant Protection and Microbiology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China

<sup>c</sup> Department of Plant Biotechnology, Korea University, Seoul 02481, South Korea

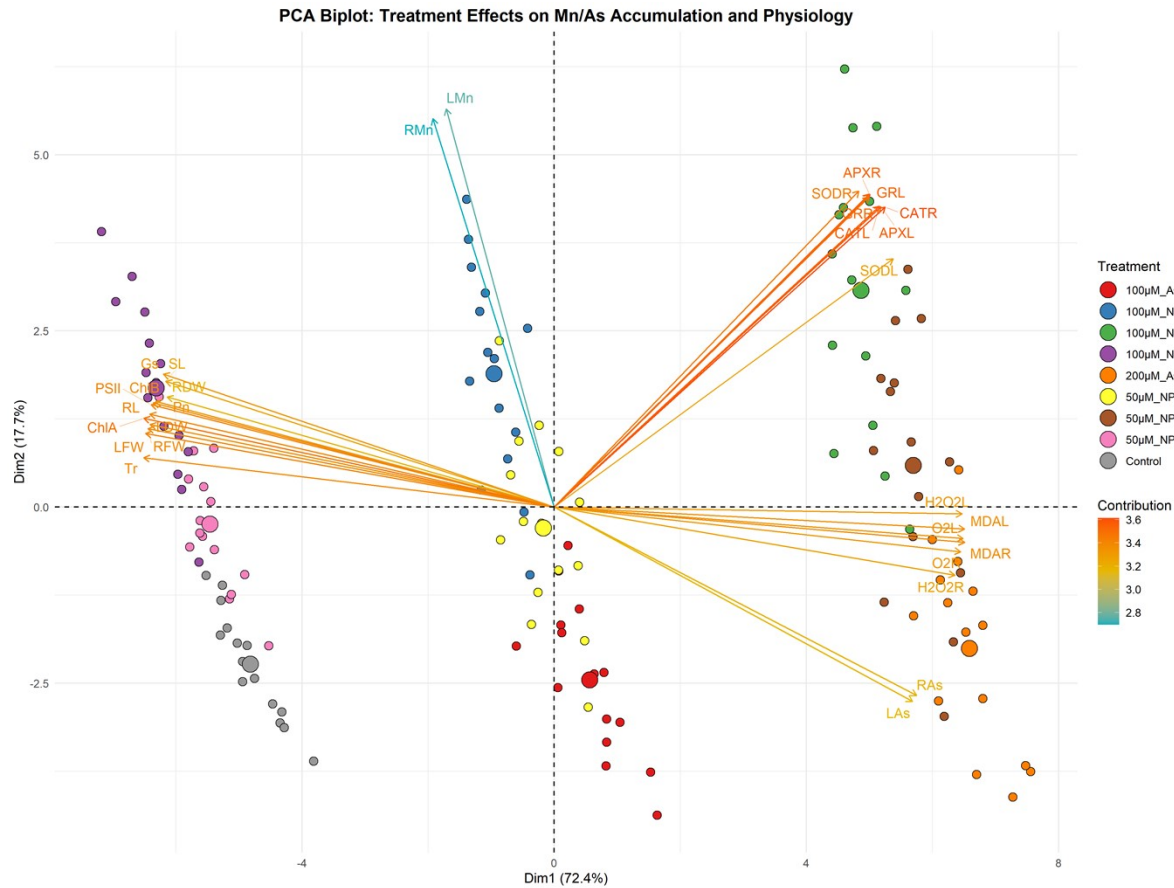
<sup>d</sup> Department of Agricultural Engineering, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan 64200, Pakistan

<sup>e</sup> Department of Agronomy, University of Agriculture, Faisalabad 38000, Pakistan.

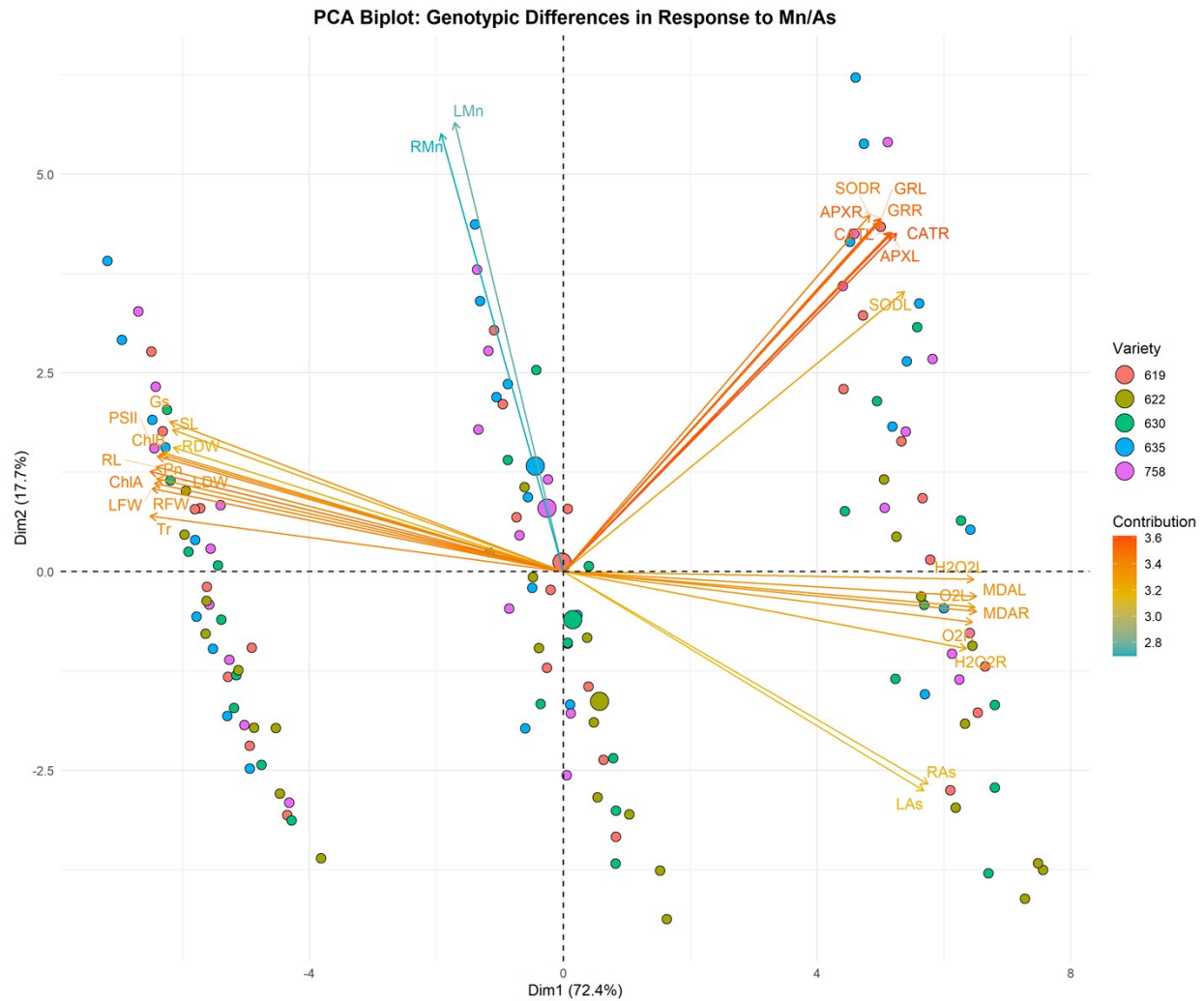
\*Corresponding author email: [skhawataligill@yahoo.com](mailto:skhawataligill@yahoo.com) (SA), [wjzhou@zju.edu.cn](mailto:wjzhou@zju.edu.cn) (WZ)



**Fig. S1.** Visual representation of the effects of different treatments on the growth of *Brassica napus* cultivars (635, 758, 619, 630, and 622). The images show the plant responses to four treatment conditions: Control, 100µM MnNPs, 200µM As, and 100µM MnNPs + 200µM As. The photographs illustrate the varying degrees of As-induced toxicity and the protective effects of MnNPs across different cultivars, highlighting the genotypic differences in stress tolerance.

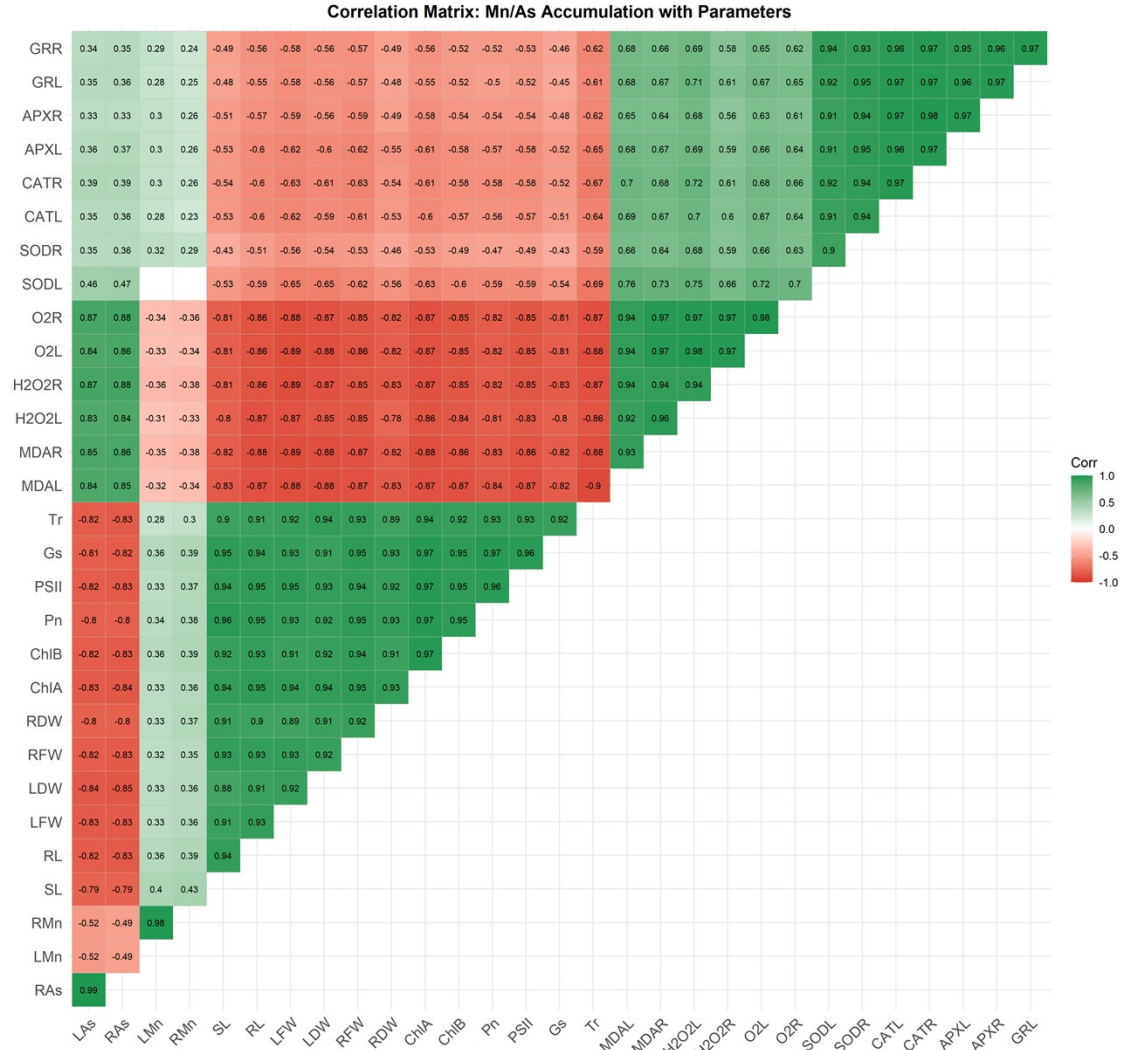


**Fig. S2.** PCA Biplot showing treatment effects on Mn/As accumulation and physiological responses. The biplot displays the relationship between Mn/As accumulation and various physiological and biochemical traits, such as growth, photosynthesis, and antioxidant enzyme activity. Treatments are color-coded as follows: 100μM As (red), 100μM NP + 100μM As (green), 100μM NP + 200μM As (yellow), 200μM As (blue), 50μM NP + 200μM As (purple), 50μM NP + 100μM As (cyan), 50μM NP + Control (pink), and Control (grey). The arrows represent the contribution of various traits (e.g., SOD, GRL, APX) to the principal components (PC1 and PC2).



**Fig. S3.** PCA Biplot showing genotypic differences in response to Mn/As accumulation and physiological traits. The plot illustrates how five *Brassica napus* cultivars (619, 622, 630, 635, and 758) respond to varying treatments, with each cultivar represented by differently colored points. The arrows indicate the contribution of physiological and biochemical traits (e.g., growth, photosynthesis, antioxidant enzyme activity) to the principal components (PC1 and PC2). The percentage of variance explained by each component is indicated, with PC1 explaining 72.4% and PC2 explaining 17.7% of the variance. The color gradient represents the contribution of each trait to the principal components, with darker shades indicating higher contributions.





**Fig. S5.** Correlation matrix showing the relationship between Mn/As accumulation and various physiological and biochemical parameters in *Brassica napus*. The matrix presents Pearson correlation coefficients ( $r$ ) between Mn (RMn, LMn), As (RAs, LAs) accumulation, and various plant traits, including growth parameters, photosynthesis, antioxidant enzymes, and oxidative stress markers. Positive correlations are indicated by shades of green, while negative correlations are shown in red. High positive correlations ( $r > 0.9$ ) are observed between Mn/As accumulation and physiological traits like growth (RDW, RFW, SL) and photosynthetic efficiency (Pn, PSII), while antioxidant markers such as SOD, CAT, and GR show varying degrees of correlation with both Mn and As accumulation.

**Table S1.** Physicochemical characteristics of MnNPs used in this study.

| Parameter                | Description / Value               | Analytical Method |
|--------------------------|-----------------------------------|-------------------|
| Particle size (diameter) | ~33 nm (average)                  | SEM, TEM          |
| Particle morphology      | Predominantly spherical           | SEM, TEM          |
| Aggregation state        | Low agglomeration; well-dispersed | SEM               |
| Elemental composition    | Mn: 75.02%, O: 24.98%             | EDX               |

**Table S2.** Oligonucleotide primer sequences used for qRT-PCR analysis

| Gene name                  | Forward              | Reverse               |
|----------------------------|----------------------|-----------------------|
| Super oxide dismutase(SOD) | ACGGTGTGACCACTGTGACT | GCACCGTGTTGTTTACCATC  |
| Catalase (CAT)             | TCGCCATGCTGAGAAGTATC | TCTCCAGGCTCCTTGAAGTT  |
| Ascorbate peroxidases(APX) | ATGAGGTTTGACGGTGAGC  | CAGCATGGGAGATGGTAGG   |
| Glutathione reductase (GR) | AAGCTGGAGCTGTGAAGGTT | AGACAGTGTTTCGCAAAGCAG |
| <i>Actin</i>               | TTGGGATGGACCAGAAGG   | TCAGGAGCAATACGGAGC    |