Supplementary material file S1

Unravelling mechanisms of CaO nanoparticles-induced drought tolerance in *Brassica napus*: An analysis of metabolite and nutrient profiling

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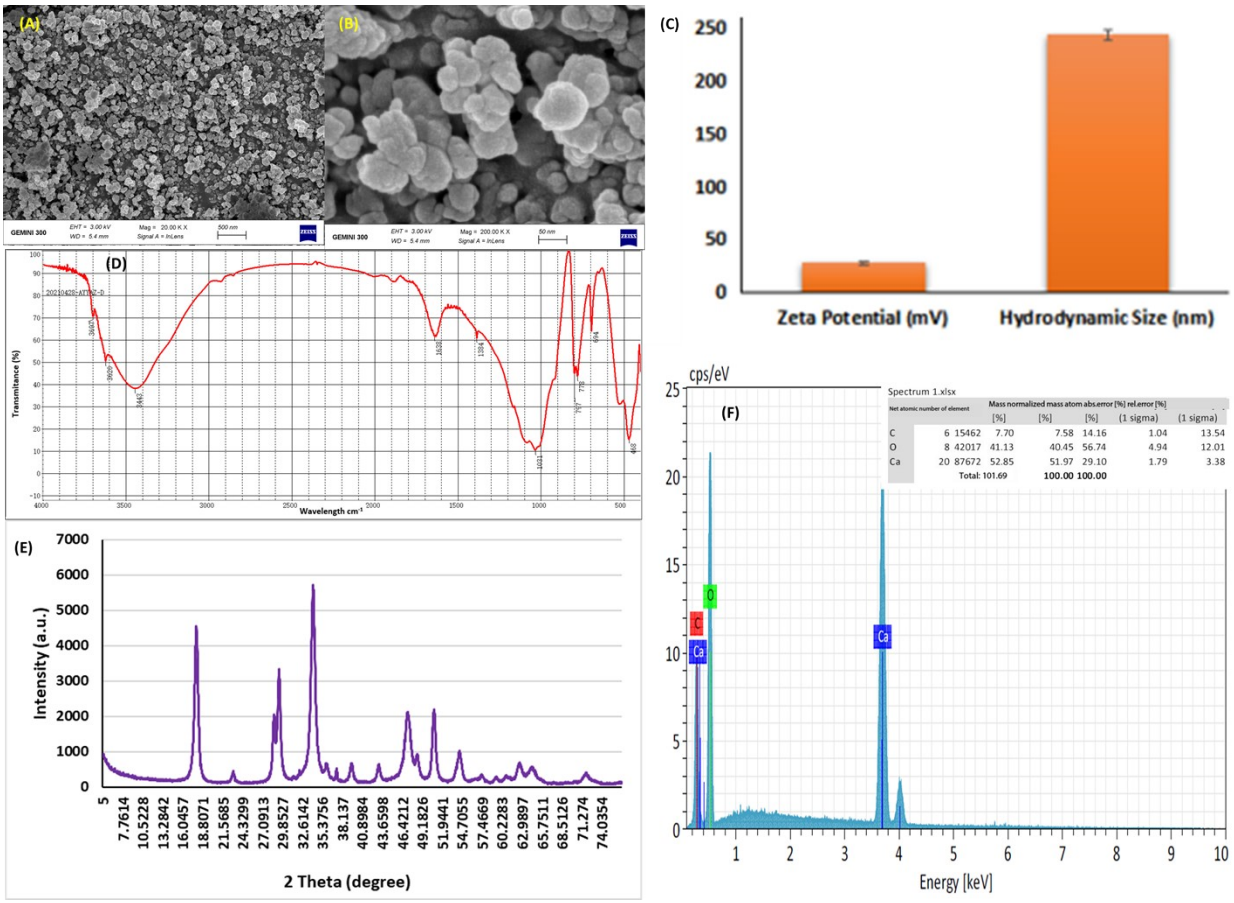
1. Non-modulated method (OJIP test)

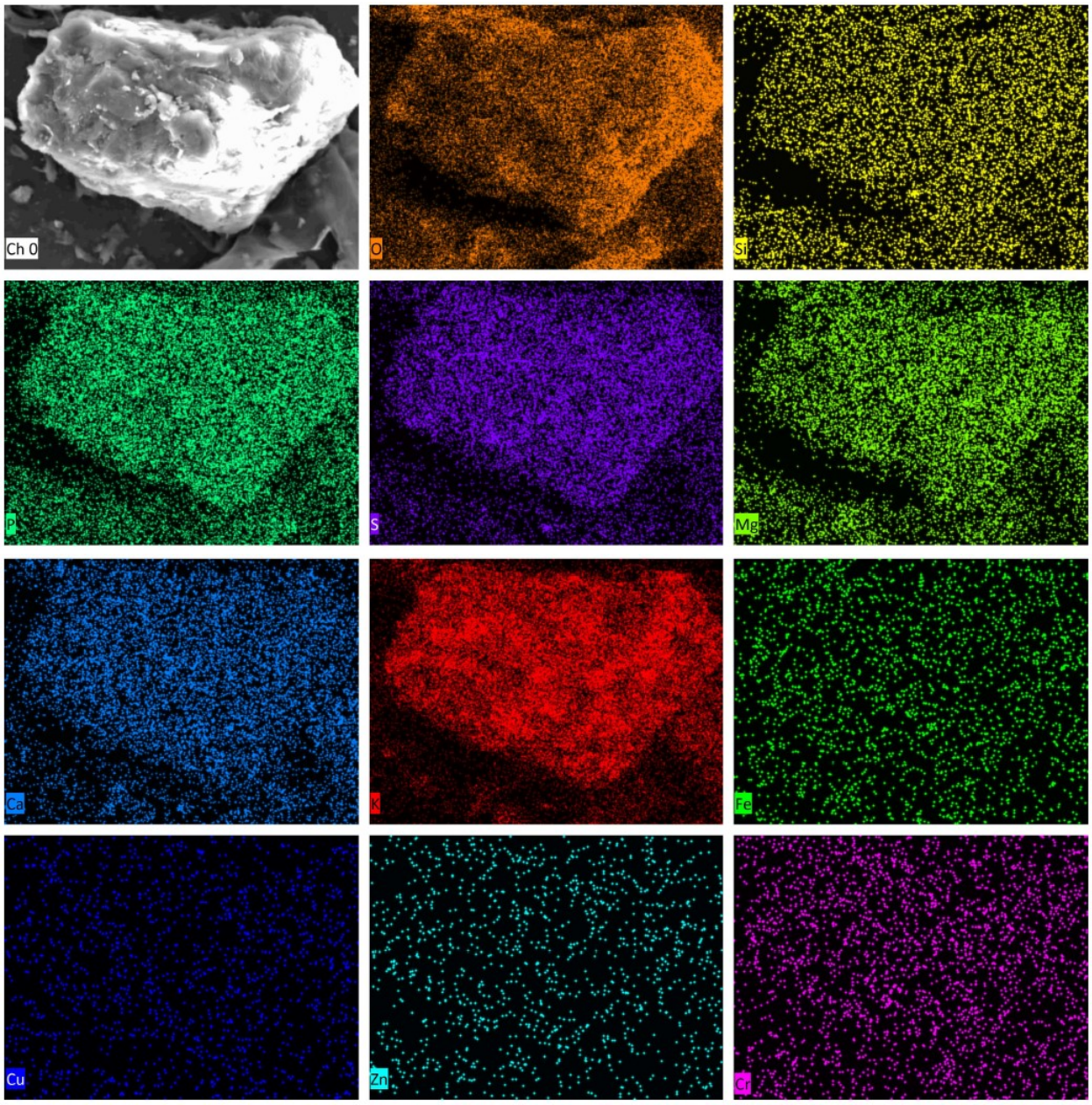
OJIP can also be measured in the absence of ambient light. It is known as the rapid fluorescence transient approach because it requires kinetics that are shorter than 1s. OJIP are the transient phases of fluorescence amplitudes during certain induction stages. The commercially available device for measuring the OJIP curve is a flour pen (FP 100, Photon System Instruments, Czech Republic) [39]. Dark adaptation of leaf is compulsory for the estimation of OJIP [40, 41]. Third mature leaf of each plant was selected, wrapped in aluminum foil for 15-20 min. Flour pen was switched on and default setting of 0 to 100% saturated light (3000 µmol photon m^{-2} s^{-1}) used for this purpose. After the removal of foil cover, dark-adapted leaf was fixed into the clip and reading was recorded. All the data taken by flour pen remained in it and can be taken from device memory out. Statistical methods were used for the analysis and comparison of data. JIP-test parameters including (Fi, Fo, Fj, Fm, Fv/Fm, Vj, Fv/Fo, Vi, TRo/RC, DIo/RC, ETo/ RC, PIABS) were calculated by the method of [42, 43].

2. Quantitative real time PCR analysis

Frozen leaf samples were subjected to total RNA extraction using the TRIzol (Tiangen Biotech, Beijing, China), and RNA reverse transcription using the FastKing RT Kit (Tiangen Biotech). The threshold cycle values were determined using the iCycler IQ Real-Time Detection System Software, and the quantification of mRNA levels was determined using the technique [44]. In order to assist users in creating primers that are particular to their desired PCR target, Primer-BLAST was design at NCBI (detailed provided in Table S1). The $2^{-\Delta\Delta Ct}$ method was used to compute the relative expression levels. There are three biological replicates of each treatment.



Supplementary Figure S1: Calcium oxide nanomaterial morphological and physical characterization as observed through (A-B) scanning electron microscopy (SEM) ZEISS Gemini SEM 300, (C) hydrodynamic size and zeta potential, (D) Fourier Transform Infrared (FTIR), (E-F) energy dispersion X-ray (EDX), respectively.



Supplementary Figure S2: The elemental composition of CaO NPs as revealed by The EDS analysis, X-ray spectroscopy SEM-EDS.