# Electronic Supplementary Information

# for

# Sequestration of Pyridinium Herbicides in Plants by Carboxylated Pillararenes Possessing Different Alkyl Chains

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## Experimental section

### Materials.

All chemicals were commercially available and used without further purification, unless otherwise described. The water used in all the plants experiments were ordinary water except additional noted. Chemical compounds as 2C-WP5A,[1,2] 4C-WP5A[3] and DQ[4] were synthesized according to the literature procedure. All of the experiments were performed at the room-temperature unless noted otherwise.

### General Instrumentation.

$^1$H NMR spectra were recorded on Bruker 400 MHz instrument in $D_2O$, 25℃, and all the chemical shifts were recorded in parts per million (ppm). The cyclic voltammetric curves were recorded on LANLIKE2010. The scanner we used is Epson Perfection V370 Photo. The bright field pictures of protoplast were photographed using an Olympus BX63 microscope. We get the absorption spectrum through the spectrophotometer of Thermo Fisher Scientific OY Ratastie 2, FI-01620 Vantaa, Finland. We centrifuged the solutions of the sample through the centrifuge (Hamburg 5430R). The leaves were lyophilized through the Freeze Dryer (Thermo Scientific Heto). We observed the protoplast by advanced upright fluorescence microscopy (OLYMPUS BX3-CBH).

### Plant Materials and Plant Growth Conditions

The seeds of soybean and maize were obtained from the market. The plants were all grown at approximately 25°C in the greenhouse under the sun for 8 days before they were treated with different chemicals.

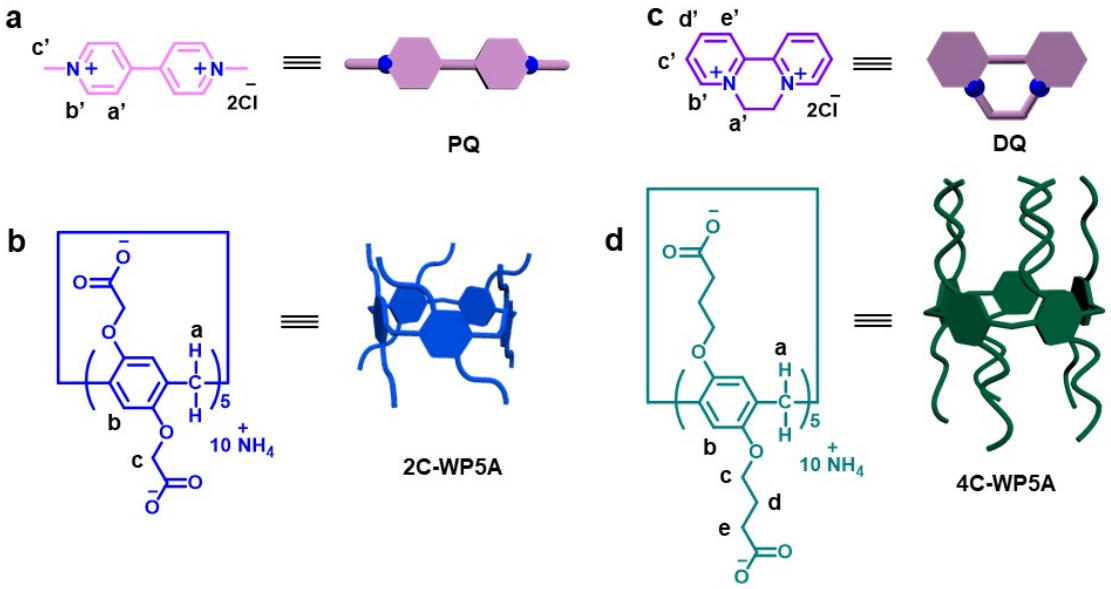



**Fig. S1** Structures and proton designations of PQ, DQ, and carboxylated pillararenes.

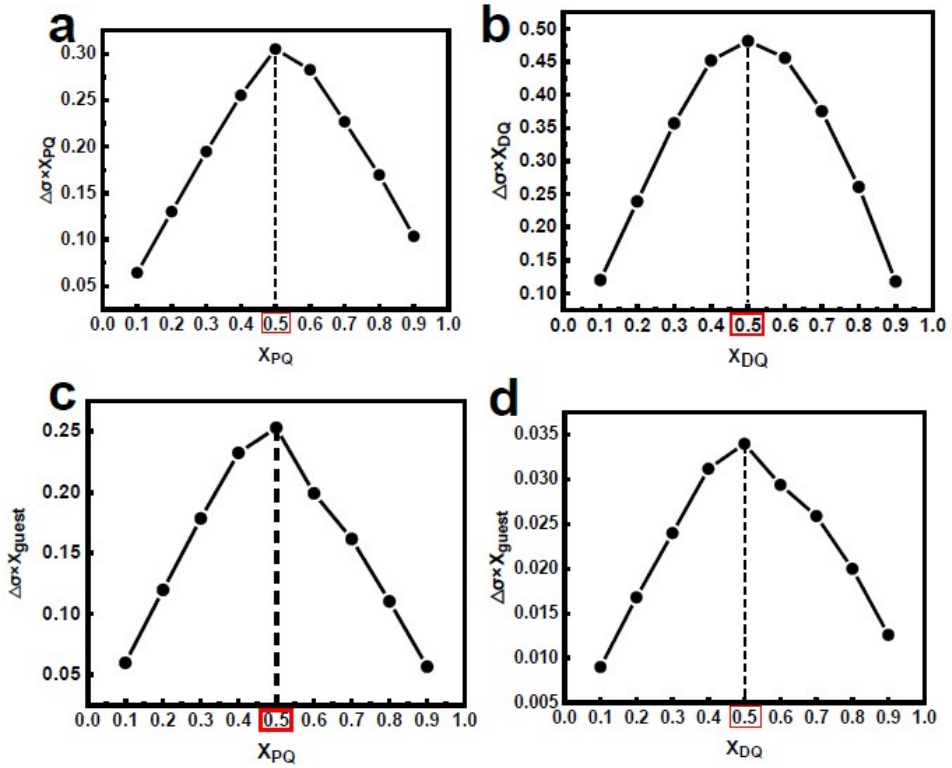



**Fig. S2** Job's plot of (a) PQ⊂2C-WP5A, (b) DQ⊂2C-WP5A, (c) PQ⊂4C-WP5A, (d) DQ⊂4C-WP5A complexation, respectively ([guest] + [host] = 1 mM).

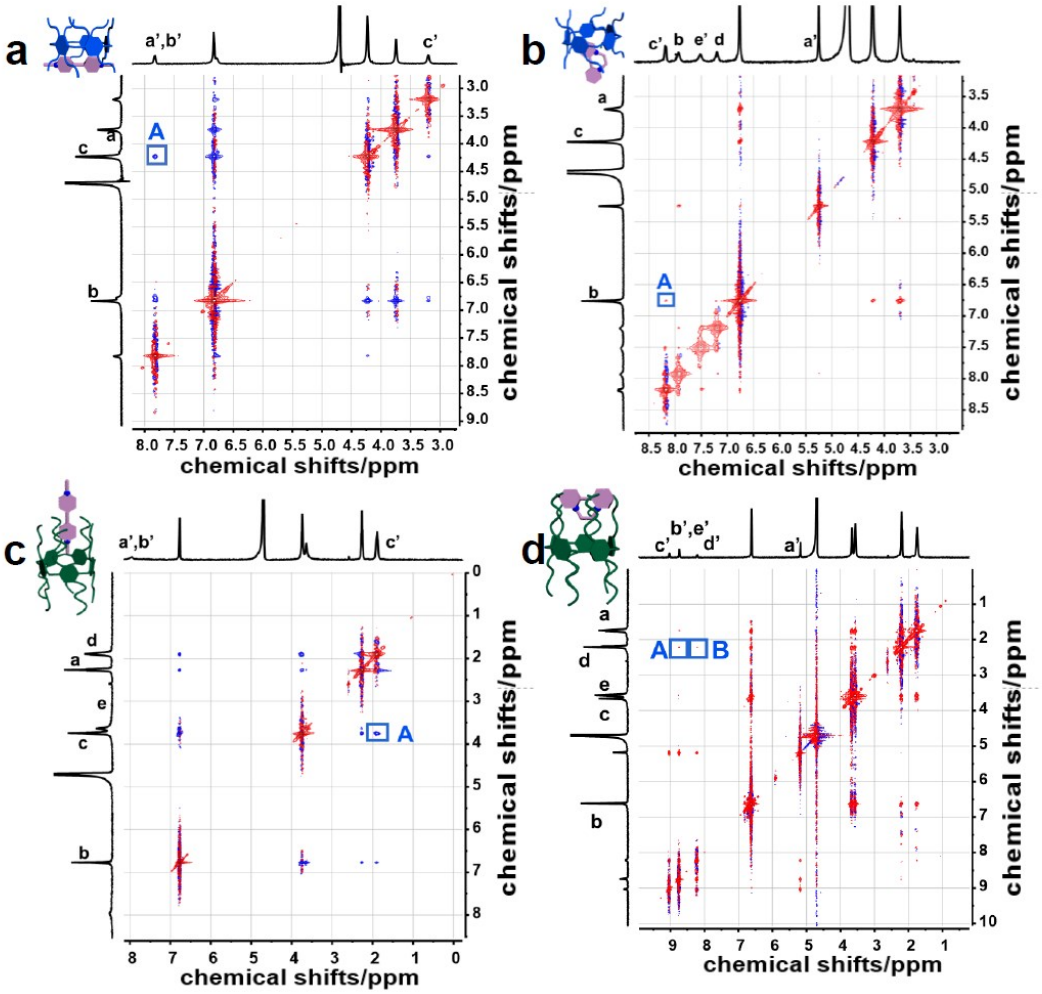



**Fig. S3** 2D ROESY ($D_2O$, 298 K,400 MHz) analysis of a mixture of (a) [2C-WP5A] = [PQ] = 5.0 mM, (b) [2C-WP5A] = [DQ] = 5.0 mM, (c) [4C-WP5A] = [PQ] = 5.0 mM, (d) [4C-WP5A] = [DQ] = 5.0 mM.

We can see that PQ and DQ could be tightly included into the cavities formed by the alkyl chains of WP5A (as shown in Fig.S3). Therefore, PQ and DQ would have less opportunity to occur the oxidation reductions in the cell.

**Cyclic voltammetric curves**

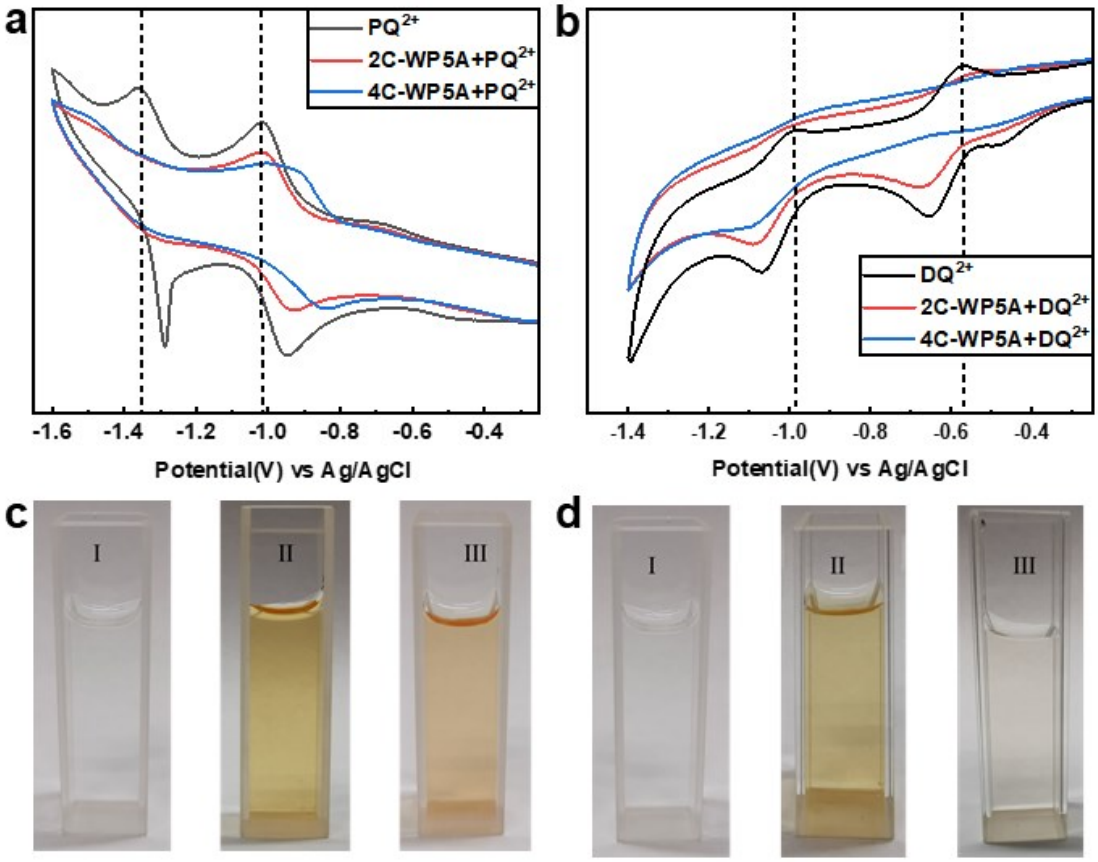
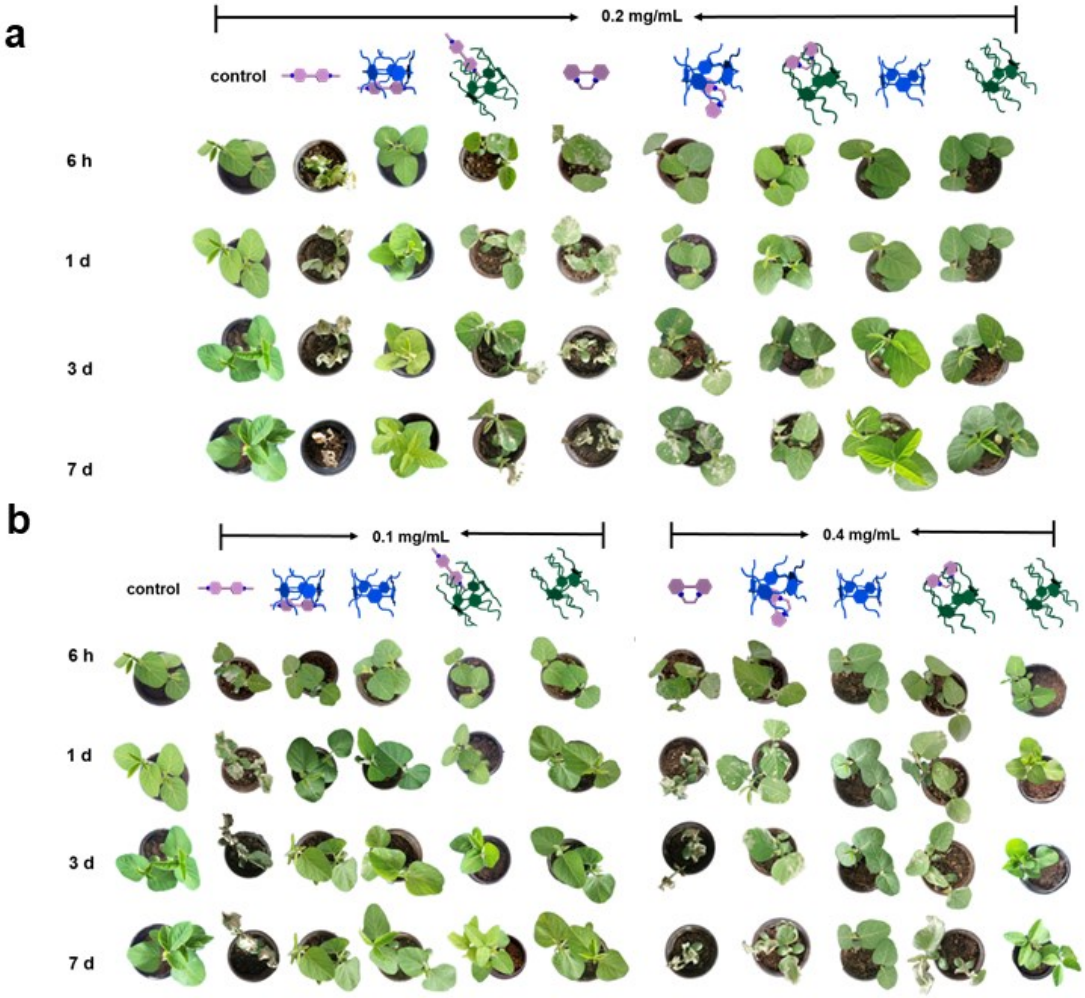



**Fig. S4** Cyclic voltammetric curves of $PQ^{2+}$ (a) and $DQ^{2+}$ (b) (1.0mM in pH=7.2 phosphate buffer solution) in the absence and presence of 1equiv of 2C-WP5A and 4C-WP5A. The scan rate is 100 mV/s. Pictures showing the color changes of $PQ^{2+}$ (c) and $DQ^{2+}$ (d) upon complexation with 1 equiv of 2C-WP5A (1.0 mM in pH = 7.2 phosphate buffer solution), (I) free viologens, (II) + 2C-WP5A and (III) + 4C-WP5A.[5]

**Senescence phenotypes of the plants after different treatments**



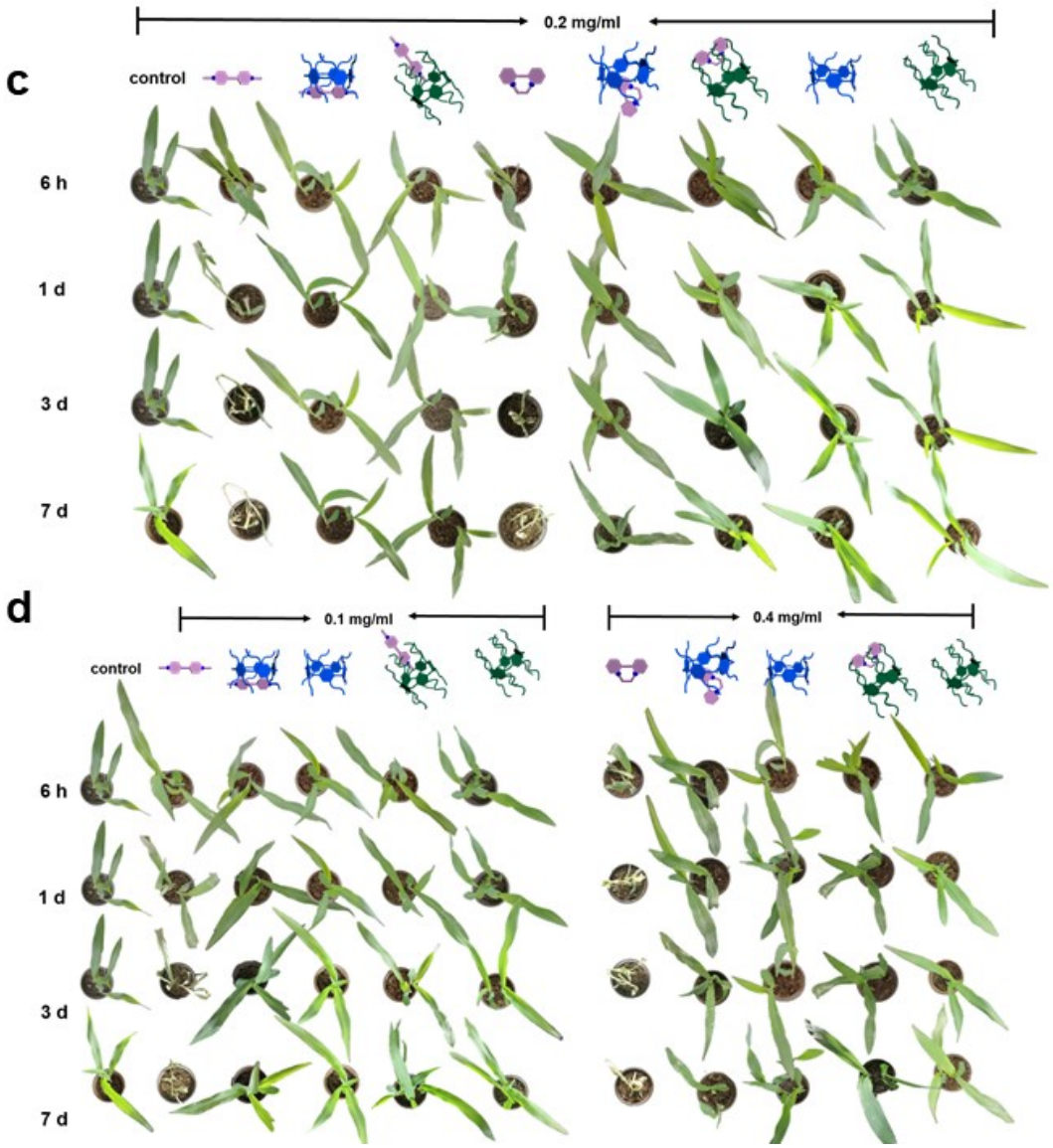



**Fig. S5** Senescence phenotype of the plants treated with control, PQ (0.2 mg/mL), PQ⊂2C-WP5A (1.1 mg/mL 2C-WP5A and 0.2 mg/mL PQ), PQ⊂4C-WP5A (1.3 mg/mL 4C-WP5A and 0.2 mg/mL PQ), DQ (0.2 mg/mL), DQ⊂2C-WP5A (1.1 mg/mL 2C-WP5A and 0.2 mg/mL DQ), DQ⊂4C-WP5A (1.3mg/mL 4C-WP5A and 0.2 mg/mL DQ) , 2C-WP5A (1.1 mg/mL), 4C-WP5A (1.3 mg/mL) for 6 h, 1 d, 3 d, 7 d. (a) soybean (c) maize. Senescence phenotype of the plants treated with control, PQ (0.1 mg/mL), PQ⊂2C-WP5A (0.55 mg/mL 2C-WP5A and 0.1 mg/mL PQ), 2C-WP5A (0.55 mg/mL), PQ⊂4C-WP5A (0.65 mg/mL 4C-WP5A and 0.1mg/mL PQ), 4C-WP5A (0.65 mg/mL), DQ (0.4 mg/mL), DQ⊂2C-WP5A (2.2 mg/mL 2C-WP5A and 0.4 mg/mL DQ), 2C-WP5A (2.2 mg/mL), DQ⊂4C-WP5A (5.2 mg/mL 4C-WP5A and 0.4 mg/mL DQ), 4C-WP5A (5.2 mg/mL) for 6 h, 1 d, 3 d, 7 d. (b) soybean

(d) maize. Note that through a calculation based on the complexation ratios, more than 87% of pyridinium herbicides were converted to the binary inclusion complexes with pillararenes in these experiments.

**DAB staining for analysis of $H_2O_2$ accumulation**

The seeds were planted in pots and grown in the greenhouse for 8 days, then they were treated with different chemicals for 6 h before being taken off for the DAB staining. The detached leaves were all immersed into DAB solution (1 mg/mL, pH = 3.8) and left to stand overnight in the dark at room temperature for 24 h. After the dyeing was completed, we transferred the leaves into absolute ethanol for three hours to fix them and then boiled them in 95% ethanol for 10 min to remove chlorophyll until the leaves all became white (if the green is not completely removed, we can extend the boiling time appropriately). Afterwards, we used the scanner to take photos. [6,7,8] Afterwards, we used the Image J to quantify the gray values of the staining.

**Chlorophyll Assays**

The leaves were triturated in liquid nitrogen and lyophilized, then we weighed 5 mg of every sample. We added 500 μl 80% acetone to a 2 mL centrifuge tube containing the sample and mixed them thoroughly in the dark. After that, we centrifuged the samples for 1 min (12,000 rpm, 4℃), then transferred the supernatant to a new centrifuge tube and let it stand in the dark. Repeated the above steps several times and combined supernatant together, mixed thoroughly. Then, we measured the absorption spectrum at 665 nm and 649 nm of the samples (Herein after referred to as A) using the spectrophotometer to calculate the chlorophyll content according to the following

formulae (using 80% acetone as a blank control). Owing to that higher concentrations of chl a and b can absorb even more light, this method can be calibrated to get the exact concentration of chlorophyll content in the solution.

$Ca + Cb\ (\mu g/g) = [8.02 \times A_{665} + 20.20 \times A_{649}] \times V/W$

Where V = volume of the extract (mL); W = Weight of leaves (g).

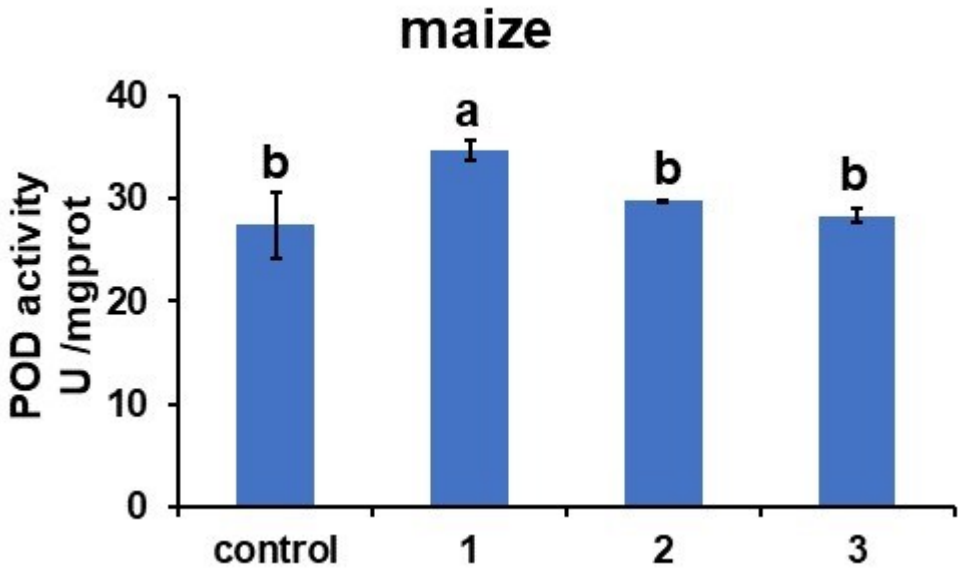



**Fig. S6** The POD activity of maize after treatment of different samples for 6h. The experimental groups were treated with 1: [PQ]= 0.2 mg/mL, 2: [2C-WP5A] = 1.1 mg/mL 3: [PQ]= 0.2 mg/mL, [2C-WP5A] = 1.1 mg/mL.

**Protoplast Assays**

After the protoplasts were isolated from 3-week-old *Arabidopsis* plants, we dropped 13.5 μL of them and 1.5 μL of the chemical solutions (the concentration of them are ten times as used in the plant experiments) onto the slides, then we covered it with the coverslips and began to observe them under the microscope (use the oil mirror of 60×) for 30 minutes.

**POD activity**

For physiological parameter measurements, the leaves of the plants were harvested. We used the POD Kit to measure the POD activity of the plants according to the manufacturer's instruction http://www.njjcbio.com/uploadfile/product/big/20140218094425886.pdf, JianCheng Bioengineering Institute, China. About 0.1 g of the soybean and corn leaves were used in the assays. We recorded the absorbance values at 420 nm through the spectrophotometer. The POD activity was calculated by the following formula: POD activity (U/mgprot) = $\frac{(OD\ value)\ \ measurements-\ \ (OD\ value)\ \ control}{12\times colorimetric\ scene(1cm)}\times \frac{total\ volume\ \ (mL)}{sample(mL)}\ \div\ reaction\ time\ (30\ minutes)$ ÷ concentration of homogenized protein (mgprot/mL) × 1000

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