Supplementary Information for

Stress response to nanoplastics with different charges in *Brassica napus* L. during seed germination and seedling growth stages

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Text S1. Preparation and characterization of nanoplastics.

Styrene (St, 99.5%, AR, Sinopharm Chemical Reagent Co. Ltd) was purified by distillation under reduced pressure and stored in a refrigerator prior to use. Unmodified polystyrene nanoplastics (PS) were synthesized using soap-free emulsion polymerization method (Xiang et al., 2020). Absolute ethanol (EtOH, 99.7%, AR) and methanol (99.7%, GR) were procured from Sinopharm Chemical Reagent Co. Ltd. Potassium persulfate (KPS, 99.5%, AR, Sinopharm Chemical Reagent Co. Ltd) was used as initiator in this method. Nanoplastics with different charges were synthesized using emulsion polymerization method (Feng et al., 2018). Positively charged polystyrene nanoplastics (PS-NH_2) used dodecylamine hydrochloride (98%, AR, Shanghai Macklin Biochemical Co. Ltd) as emulsifier and 2,2'-azobisisobutyronitrile (97%, AR, Shanghai Aladdin Biochemical Technology Co. Ltd) as initiator; negatively charged polystyrene nanoplastics (PS-SO_3H) used sodium dodecyl sulfate (AR, Sinopharm Chemical Reagent Co. Ltd) as emulsifier and potassium persulfate as initiator. Nitrogen protection and ultrapure water (H_2O) with resistivity higher than 18.2 MΩ cm were used throughout the synthesis. All emulsions were transferred to a dialysis bag (1 kDa) to remove impurities for 3-5 days before the experiment. The concentration of pristine NPs was quantified after freeze-drying (Tan et al., 2021).

The particle size and morphology of NPs were characterized by scanning electron microscopy (SEM). The average particle size and ζ-potential (mV) of the particles were determined by dynamic light scattering (DLS). The hydrodynamic diameter of PS, PS-NH_2 and PS-SO_3H were 203 nm, 38 nm and 39 nm, respectively. The ζ-potential of PS, PS-NH_2 and PS-SO_3H in deionized water were -23.2±1.14 mV, +30.8±1.21 mV and -35.0±1.78 mV, respectively. PS and PS-SO_3H were negatively charged in water, while PS-NH_2 was positive charged in water. Fourier transform infrared spectroscopy (FTIR) was used to characterize the functional groups contained in NPs.

Text S2. Seed germination assay.

Rape seeds (Qingyou 21) in this study were obtained from the Academy of Agriculture and Forestry Sciences (Qinghai University, China), and stored in sealed kraft paper bags for later use. This variety is a spring rape variety commonly grown in Qinghai Province, China.

Rape seeds were rinsed with 75% alcohol for 15 s, disinfected the surface with 5% (v/v) sodium hypochlorite solution for 10 minutes, rinsed with deionized water for 3 times, and then patted dry with absorbent paper. The germination assay was conducted by the reference of Lian et al. (2020) with modification, where the sterilized seeds were soaked in different concentrations of NPs (PS, PS-NH_2 and PS-SO_3H) dispersion (0, 1, 10, 50, 100, 200 mg/L. 200 mg/L was defined as high concentration, since it was close to or higher than IC50, Table S1) for 3 h. Exposure solutions were prepared from stock solutions and then stirred by ultrasonic vibration (100 W, 25 kHz) for 10 min. 50 seeds were placed in a square Petri dish (13 cm × 13 cm) covered with 2 layers of sterile filter paper, added with 20 mL test solution, sealed with parafilm, and incubated for 3 days. The relative seed germination (seeds germinated with NPs were divided by seeds germinated in control, %), relative root elongation (mean root length in NPs were divided by mean root length with control, %), and seed vigour index (germination% × seedling length) were calculated after 3 days of incubation. Another 50 seeds were taken and weighed, placed in a beaker containing 25 ml test solution. At 4h, 8h, 12h, 16h, 20h, and 24h, all seeds were carefully removed, quickly weighed after blotting dry, and the water uptake of the seeds was calculated (Bhardwaj et al., 2012). All tests were carried out in a growth chamber under dark condition at 25°C, three replicates were applied in each treatment.

Water uptake (%) = (Fresh weight of seeds-Dry weight of seeds) / Dry weight of seeds × 100

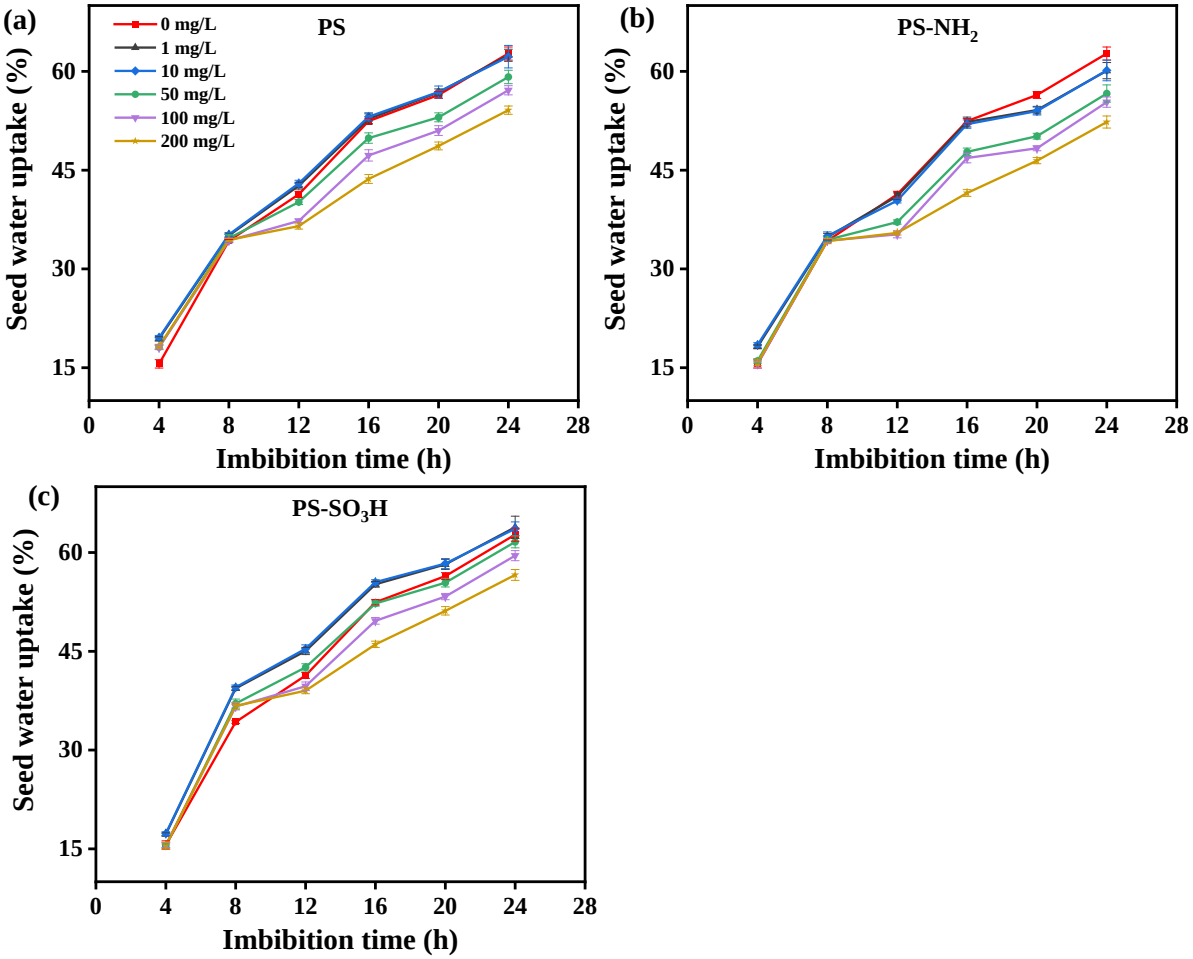

Figure S1. Effects of PS (a), PS-NH_2 (b) and PS-SO_3H (c) on water uptake of rape seeds.

Table S1. Characteristics of PS, PS-NH_2 and PS-SO_3H in 1/4 Hoagland solution.

Treatment	Hydrodynamic diameter (nm)			Zeta potential (mV)		
	PS	PS-NH_2	PS-SO_3H	PS	PS-NH_2	PS-SO_3H
1 mg/L	368.3±10.21	454.1±13.11	478.7±11.24	-18.5±0.69	+19.5±0.56	-24.6±0.89
10 mg/L	250.6±8.64	221.8±10.52	236.9±8.63	-18.7±0.71	+20.2±0.73	-25.4±0.75
25 mg/L	243.5±7.31	186.5±5.24	212.5±6.71	-19.1±0.97	+21.5±1.12	-26.3±1.02
50 mg/L	238.3±3.21	123.3±6.33	146.9±5.33	-19.6±1.21	+23.7±0.86	-28.9±1.13
100 mg/L	235.0±4.32	82.4±3.15	97.4±2.15	-20.3±0.98	+24.8±0.67	-31.1±1.07
200 mg/L	226.2±2.55	62.2±1.16	72.1±1.22	-21.5±1.69	+26.5±1.14	-34.6±1.28

Table S2. IC50 of PS, PS-NH_2 and PS-SO_3H on seed germination.

NPs	Index（%）	Regression equation	R^2	IC50
PS	Germination	y = -0.2668x + 96.388	0.927	173.87 mg·L^{-1}
PS-NH_2	Germination	y = -0.2837x + 98.069	0.966	169.44 mg·L^{-1}
PS-SO_3H	Germination	y = -0.1933x + 99.628	0.955	256.74 mg·L^{-1}

Table S3. Effects of PS, PS-NH_2 and PS-SO_3H on water uptake of rape seeds.

	Treatment	4 h	8 h	12 h	24 h	36 h	48 h
PS	0 mg/L	15.57±0.64	34.26±0.21	41.33±0.45	52.45±0.41	56.42±0.52	62.71±0.99
	1 mg/L	19.39±0.33**	35.07±0.34	42.64±0.52*	52.78±0.76	56.81±0.52	62.44±0.92
	10 mg/L	19.56±0.34**	35.22±0.3*	42.99±0.75**	53.1±0.62	56.87±0.91	62.23±1.71
	50 mg/L	18.12±0.35**	34.66±0.58	40.16±0.38*	49.87±0.79**	53.02±0.68**	59.15±1.02*
	100 mg/L	18.06±0.31**	34.31±0.37	37.29±0.28**	47.24±0.86**	51.01±0.74**	57.13±0.69**
	200 mg/L	18.24±0.3**	34.4±0.25	36.51±0.47**	43.66±0.66**	48.68±0.59**	54.11±0.62**
PS-NH_2	0 mg/L	15.57±0.64	34.26±0.21	41.33±0.45	52.45±0.41	56.42±0.52	62.71±0.99
	1 mg/L	18.19±0.25**	34.83±0.57	41.06±0.48	52.31±0.75	54.12±0.57**	60.11±1.23
	10 mg/L	18.46±0.34**	34.99±0.62	40.38±0.37	51.99±0.64	53.97±0.59**	60.15±1.57
	50 mg/L	16.02±0.35	34.41±0.31	37.11±0.33**	47.77±0.58**	50.15±0.44**	56.64±1.3**
	100 mg/L	15.56±0.61	34.28±0.31	35.24±0.52**	46.84±0.72**	48.32±0.38**	55.33±0.78**
	200 mg/L	15.74±0.45	34.22±0.38	35.46±0.31**	41.53±0.51**	46.44±0.47**	52.31±0.92**
PS-SO_3H	0 mg/L	15.57±0.64	34.26±0.21	41.33±0.45	52.45±0.41	56.42±0.52	62.71±0.99
	1 mg/L	17.22±0.23**	39.37±0.31**	45.04±0.54**	55.17±0.44**	58.2±0.76*	63.83±1.68
	10 mg/L	17.31±0.28**	39.52±0.37**	45.39±0.59**	55.5±0.37**	58.32±0.76*	63.62±1.03
	50 mg/L	15.45±0.28	37.06±0.68**	42.56±0.55	52.26±0.4	55.42±0.65	61.61±0.91
	100 mg/L	15.58±0.4	36.61±0.52**	39.69±0.65*	49.63±0.51**	53.31±0.48**	59.53±0.76*
	200 mg/L	15.49±0.49	36.7±0.51**	39.01±0.45**	46.05±0.48**	51.14±0.64**	56.6±0.82**

Values were represented as mean ± SD (n = 3). Significance of difference between the control and NP treatments: *, p <0.05; **, p<0.01.

Table S4. Correlation analysis between 10 indicators of oxidative stress, antioxidants and quality.

	Chlorophyll a	Chlorophyll b	Carotenoid	SOD	POD	CAT	MDA	Soluble protein	Soluble sugar	Crude fiber
Chlorophyll a	1									
Chlorophyll b	0.992**	1								
Carotenoid	0.914**	0.935**	1							
SOD	-0.390	-0.414	-0.27	1						
POD	-0.192	-0.226	-0.089	0.3	1					
CAT	0.173	0.083	0.013	0.215	0.24	1				
MDA	-0.611*	-0.624*	-0.481	0.666**	0.755**	0.053	1			
Soluble protein	0.635*	0.657**	0.501	-0.781**	-0.703**	-0.057	-0.897**	1		
Soluble sugar	0.868**	0.851**	0.691**	-0.504	-0.435	0.262	-0.758**	0.803**	1	
Crude fiber	0.767**	0.758**	0.605*	-0.661**	-0.561*	0.174	-0.880**	0.906**	0.911**	1

Table S5. Membership function values of each treatment in the experiment.

Treatment		Chlorophyll a	Chlorophyll b	Carotenoid	MDA	Soluble protein	Soluble sugar	Crude fiber	Mean	Rank
L0	PS	0.33	0.38	0.14	0.75	0.92	0.65	0.64	0.54	5
	$PS\text{-}NH_2$	0.33	0.38	0.14	0.75	0.92	0.65	0.64	0.54	5
	$PS\text{-}SO_3H$	0.33	0.38	0.14	0.75	0.92	0.65	0.64	0.54	5
L25	PS	0.83	0.80	0.52	0.97	0.88	0.94	0.70	0.81	2
	$PS\text{-}NH_2$	0.39	0.35	0.12	0.64	0.90	0.69	0.66	0.54	5
	$PS\text{-}SO_3H$	0.90	0.93	0.97	0.70	0.94	0.95	0.75	0.88	1
L50	PS	0.38	0.34	0.09	0.54	0.68	0.95	0.61	0.51	9
	$PS\text{-}NH_2$	0.68	0.70	0.41	0.32	0.57	0.71	0.51	0.56	4
	$PS\text{-}SO_3H$	0.81	0.73	0.49	0.64	0.72	0.97	0.83	0.74	3
L100	PS	0.10	0.11	0.02	0.48	0.57	0.39	0.48	0.31	11
	$PS\text{-}NH_2$	0.19	0.20	0.04	0.10	0.42	0.34	0.15	0.20	12
	$PS\text{-}SO_3H$	0.29	0.27	0.08	0.43	0.55	0.56	0.52	0.39	10
L200	PS	0.05	0.07	0.01	0.32	0.18	0.12	0.17	0.13	14
	$PS\text{-}NH_2$	0.03	0.03	0.00	0.05	0.08	0.04	0.10	0.05	15
	$PS\text{-}SO_3H$	0.12	0.16	0.04	0.21	0.14	0.34	0.19	0.17	13

Rank referred to sorting the mean in descending order. The smaller the mean value, the later the rank, and the stronger the toxicity.