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Coal-based Carbon Quantum Dots as Plant Growth Promoter for Empowering Plant Productivity: A Sustainable Nano-Solution

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1. Fabrication of carbon quantum dots (CQDs) as PGP

Each coal sample (25g) was mixed with 250 mL of 30% hydrogen peroxide under ice-cold conditions. The mixture was then subjected to ultrasonication treatment in a microprocessor-controlled bench-type ultrasonic bath (Model: Rtul), operating at approx. 40 kHz frequency, for 5–6 hours at ambient pressure and temperature. After sonication, the reaction slurry was cooled to room temperature, followed by drop-wise addition of ammonia solution until the pH was adjusted to neutral. The neutralised slurry was filtered through Whatman no. 42 filter paper under vacuum pressure. Then further filtered and purified using a Cross/Tangential flow filtration system using a 1 kDa membrane. The excess water from the purified solutions was removed using a rotary evaporator. Finally, the concentrated purified solutions of carbon quantum dots (CQDs-I, CQD-II, and CQD-III) were preserved for subsequent application as PGP[27].

2. X-ray photoelectron spectroscopic (XPS) analysis

XPS survey spectra of CQD-I, CQD-II, and CQD-III along with high-resolution deconvoluted peaks of C 1s, N 1s, O 1s, and S 2p. The analysis confirms the presence of functional groups including C–C/C=C, C–O, C=O, C–N, pyridinic/graphitic N, organic C=O, C–S, and S=O, evidencing successful heteroatom doping and surface functionalization of the CQDs.

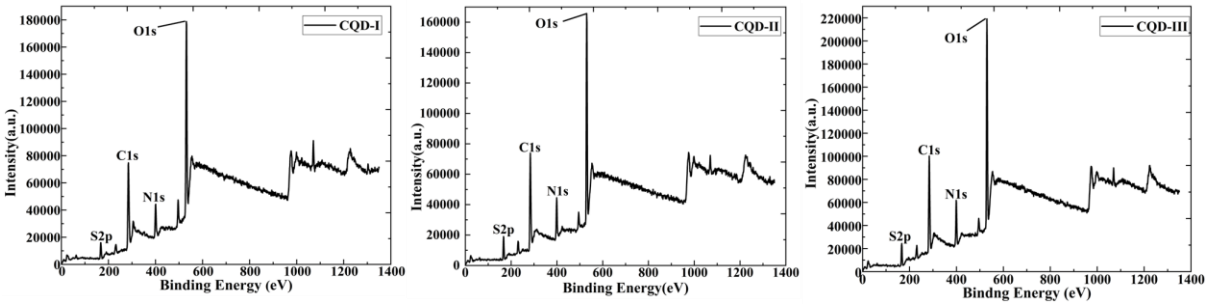

Fig. S1: X-ray Photoelectron Spectroscopy (XPS) survey spectra of CQD-I, CQD-II, and CQD-III showing characteristic binding energy peaks corresponding to C1s, O1s, N1s, and S2p bonding.

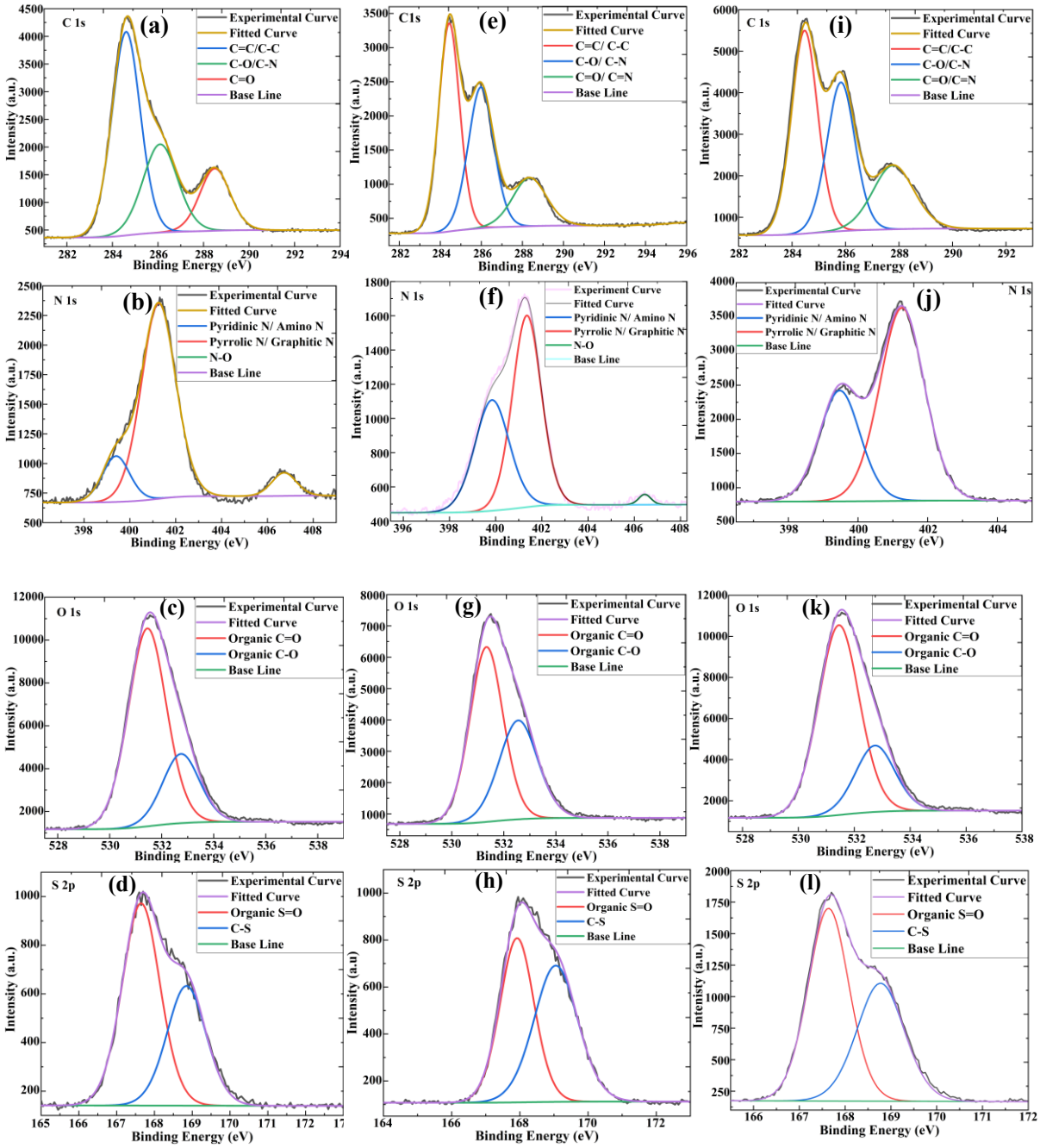

Fig. S2: High-resolution X-ray Photoelectron Spectroscopy (XPS) spectra of the C1s, N1s, O1s, and S2p regions for CQD-I, II, and III samples, labelled as (a–d), (e–h), and (i–l), respectively. For CQD-I (a–d), the C1s spectrum (a) is deconvoluted into peaks corresponding to C–C/C=C, C–O/C–N, C=O, and O–C=O groups, while the N1s spectrum (b) reveals the presence of Pyridinic N, Pyrrolic N/Graphitic N, and N–O species. The O1s spectrum (c) shows contributions from organic C=O and C–O functionalities, and the S2p spectrum (d) indicates the presence of organic S=O and C–S groups. In CQD-II (e–h), the C 1s peak (e) displays C–

C/C=C, C–N/C–S, and C=O bonding, while the N1s peak (f) includes Pyridinic N, Pyrrolic N/Graphitic N, and N–O contributions. The O1s peak (g) is resolved into organic C=O and C–O components, and the S2p peak (h) confirms the presence of organic S=O and C–S bonds. For CQD-III (i–l), the C1s spectrum (i) reveals C–C/C=C, C–N/C–S, and C=O groups, and the N 1s spectrum (j) shows Pyridinic N and Pyrrolic N/Graphitic N features. The O1s peak (k) includes organic C=O and C–O functionalities, while the S2p spectrum (l) again indicates the presence of organic S=O and C–S species.

3. Plant growth promotion activity studies

Table S 1 presents physiological and biochemical data from plants treated with (S,N-self-CQDs) at varying concentrations, both alone (T1–T3) and in combination with herbicide stress (T4–T9), compared to herbicide-only treatments (T10–T11) and an untreated control (C). S,N-CQDs alone improved plant physiological and biochemical health (increased chlorophyll and protein, low stress indicators), showing potential as growth enhancers. When co-applied with herbicides, they alleviated oxidative and metabolic damage to a certain extent, though not completely. Thus, S, N-CQDs demonstrate protective roles under herbicide-induced stress, promoting antioxidant responses and metabolic stability.

Table S 1. Chlorophyll, Protein, SOD, Lipid peroxidase, Proline, NAG, DH, and ALP content in all the treated plants.

S. NO.	Chlorophyll (mg/g FW)	Protein (mg/g)	SOD (unit/g/FW)	LP	Proline	NAG (µgPNP/g)	DH (µgTPF/g)	ALP (µgPNP/g)
C	0.81±0.02	0.127±0.005	0.58±0.06	0.011±0.001	0.082±0.008	182±7	26.1±0.2	196±2
T1	0.89±0.06	0.156±0.006	0.55±0.03	0.012±0.001	0.046±0.008	179±10	32.3±0.6	211±7
T2	0.88±0.03	0.148±0.006	0.52±0.06	0.011±0.001	0.035±0.006	181±13	27.7±0.9	197±5
T3	0.88±0.01	0.136±0.009	0.58±0.06	0.012±0.001	0.074±0.013	168±7	28.1±0.8	195±7
T4	0.56±0.03	0.095±0.005	0.88±0.05	0.018±0.001	0.221±0.018	121±5	17.1±0.8	146±6
T5	0.62±0.04	0.119±0.008	0.82±0.01	0.014±0.001	0.126±0.018	129±9	17.3±1.3	160±7
T6	0.46±0.04	0.090±0.009	0.97±0.03	0.021±0.001	0.196±0.020	123±10	13.7±0.6	152±6
T7	0.56±0.00	0.102±0.006	0.98±0.00	0.018±0.001	0.182±0.019	138±3	13.2±0.7	185±2
T8	0.51±0.00	0.069±0.007	0.96±0.02	0.020±0.001	0.288±0.019	109±19	11.3±0.7	159±8
T9	0.56±0.04	0.083±0.003	0.96±0.04	0.017±0.001	0.252±0.008	121±17	10.6±0.6	176±7
T10	0.23±0.01	0.035±0.007	1.24±0.04	0.026±0.001	0.372±0.014	56±10	6.2±0.3	87±8
T11	0.31±0.04	0.045±0.009	1.10±0.05	0.022±0.001	0.347±0.005	69±4	9.4±0.5	122±5

4. Gene expression studies

In *Table S 2* the six genes selected for qRT-PCR analysis, along with their primer sequences and functional descriptions. Three target genes*, FeSOD, G6PD6,* and *ZAT12*, are involved in oxidative stress response, highlighting antioxidant activity and defence signalling. *bZIP* and *CRF6* are transcription factors associated with stress and hormone signalling. *ACT2* is used as

a housekeeping gene to normalize gene expression data. The primer sequences provided enable specific amplification of each gene for accurate expression analysis.

Table S 2. Genes selected for qRT-PCR analysis and primer sequences

Sl. No	**Gene name**	**Gene Description**	**Primer Sequence: Forward/Reverse**
1	ACT2	Actin-2	F-CCTCACCCTCAAGTACCCCAT R-TTGGCCTTTGGGTTGAGTG
2	bZIP	bZIP transcription factor family protein	F-AGTGGGTCCGTGCTTTGTTC R-GCAACTAGTGGGTCCTACCCAAT
3	CRF6	Cytokinin response factor 6	F-TTGCAGCCTAAGCAGAAGGG R-ACGCCCCTGAACTTCCTAAC
4	SOD	Superoxide dismutase Fe	F-CAGCCGCTCCGATGAAACT R-GAAATGCAGGCGGAAGGATT
5	G6PD6	Glucose-6-phosphate 1-dehydrogenase	F- TCCGACAAGTGGTGTCTTGG R- GGTTCAACTCCCAGCAAAGG
6	*ZNF*	Zinc finger family protein	F-ACTAGCCAATACTCTGCCCC R-TTTGCTCTAAGGCTGCCCAA