

Biological Responses of Tomato and Okra to Oil-Contaminated Soils in Southern Iraq

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ABSTRACT

Oil fields, pipelines, refineries, and contaminated drainage networks, have led to increasing levels of petroleum contamination in cultivated soils of Southern Iraq, and are a growing threat to agricultural productivity. The study was conducted in a controlled randomized complete block pot experiment with four contamination levels (control, low, moderate and high) of total petroleum hydrocarbon (TPH) (48, 5,082, 14,819, and 30,050 mg kg⁻¹) each replicated five times for tomato (*Solanum lycopersicum*) and okra (*Abelmoschus esculentus*). After 45 days of growth, germination behaviour, root architecture, leaf anatomy, photosynthetic pigments and chlorophyll-a fluorescence, gas exchange and rhizosphere microbial activity were measured. The germination percentage, seedling vigour, total root length, root biomass, palisade and spongy mesophyll thickness, the chlorophyll content, the maximum photosynthetic efficiency (Fv/Fm), and the net photosynthetic rate (NP) of the two species decreased significantly ($p < 0.001$) when TPH was increased, and stomatal density increased and root-to-shoot ratio shifted under stress. Total culturable bacteria and dehydrogenase activity decreased at high contamination levels, while, the hydrocarbon-degrading bacteria were highly enriched in the rhizosphere of contaminated plants. In almost every character observed, tomato was more sensitive than okra which was the relatively tolerant crop. Principal component analysis and correlation analysis was used to verify the correlation between soil TPH, plant physiological decline and microbial degradation response, and it was found that the three were highly correlated. The results offer a comprehensive phytotoxicity framework suitable for crop safety considerations, monitoring of soils in the vicinity of oil polluted areas and for rhizoremediation design.

1. INTRODUCTION

In southern Iraq, some of the world's largest hydrocarbon reserves overlap with cultivated land, and the oil fields, pipelines, gathering stations and refineries that support the industry are frequently surrounded by agricultural areas. Routine operational discharges, pipeline leaks, accidental spills, produced-water disposal and conflict-related damage have released crude oil and refined products into surface and subsurface soils across the Basrah, Maysan and Dhi Qar governorates [1], [2], where total petroleum hydrocarbon (TPH) concentrations typically range from several thousand to several tens of thousands of mg/kg, well above background levels [3]. Once in soil, petroleum hydrocarbons coat soil particles with hydrophobic films, reducing wettability and oxygen diffusion, increasing electrical conductivity through associated salts, raising apparent organic matter content and immobilizing plant-available N, P and K [3, 4]. These physicochemical changes trigger a cascade of phytotoxic effects: hydrocarbons on the seed coat impede water and gas uptake and delay the hydrolysis of storage reserves, reducing germination percentage and emergence [4, 13]; root initiation and elongation are inhibited [5], [14]; membrane permeability and aquaporin function are compromised, impairing water and nutrient uptake and increasing reactive oxygen species accumulation and oxidative stress [6, 7, 31]; and, downstream, stomatal conductance falls,

chlorophyll degrades, and the photosynthetic apparatus is damaged, ultimately reducing biomass production [8, 9]. Despite this broad understanding, relatively few studies have incorporated the entire cascade of biological effects within a single experimental design; most investigations report only germination or biomass, or describe soil pollution and microbiology without linking them to plant performance, leaving the mechanistic chain from soil TPH through germination, root architecture, leaf anatomy, photosynthetic efficiency and rhizosphere microbial response incomplete, particularly for economically important crops [12], [23], [26]. Tomato (*Solanum lycopersicum*) and okra (*Abelmoschus esculentus*) were chosen as complementary test species because they are among the most widely consumed vegetables in southern Iraq where contamination is most severe [10], [11], are fast-growing and controllable under experimental conditions, are sensitive enough to edaphic stress to serve as bioindicators of soil quality [11, 12], and belong to contrasting families (Solanaceae and Malvaceae) with different root systems and leaf architectures, making them well suited for species-specific tolerance comparisons [12, 26]. The aim of this study was therefore to evaluate the biological responses of tomato and okra grown along an oil-contamination gradient representative of southern Iraqi soils by integrating germination, root development, leaf anatomical traits, photosynthetic performance and rhizosphere microbial interactions. Four hypotheses were tested: (H1) oil-

contaminated soils reduce germination, root growth and photosynthetic efficiency in both crops; (H2) the two species differ in their sensitivity to petroleum-contaminated soil; (H3) rhizosphere microbial abundance, particularly hydrocarbon-degrading groups, increases near contaminated roots; and (H4) root traits and photosynthetic efficiency are strongly associated with soil hydrocarbon concentration and microbial activity.

2. Materials and Methods

2.1 Study area and sampling design

The soil samples were taken in the dry season from an adjacent agricultural area of the oil-production area in Basrah Governorate in Southern Iraq (30.5 N, 47.8 E). It is an arid climate with hot summers (July mean temperature is greater than 38 °C), mild winters, and low annual rainfall (usually less than 150 mm). Three impacted sampling locations were chosen at various distances downwind and downstream from the spill, and one reference location was selected more than 3km upwind and upstream away from the known petroleum source. For each site, a topsoil sample was taken five times and composited (0–20 cm) with the debris and root fragments removed. Table 1: site descriptors are summarised.

Table 1. Description and classification of soil sampling sites in the study area, Southern Iraq. GPS coordinates were recorded at each site (omitted here for site privacy); values are illustrative of the sampling design.

Site code	Description	Approx. distance to source (m)	Use class
REF	Reference cropland, no visible contamination	> 3,000	Control
S-1	Field margin near gathering line	180	Low
S-2	Drainage-affected plot down-gradient of spill	70	Moderate
S-3	Heavily oil-stained soil adjacent to wellpad	25	High

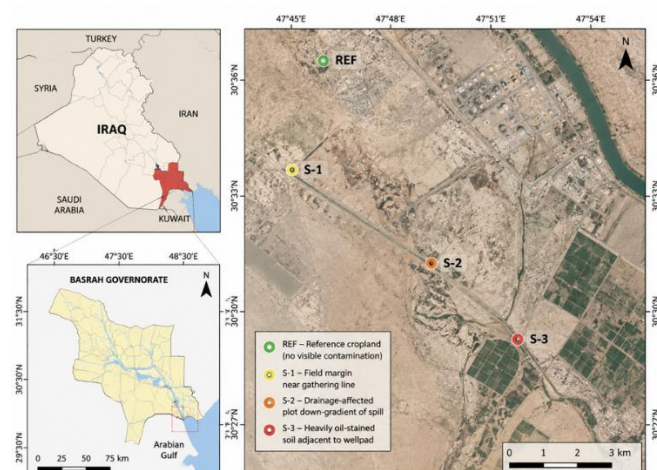


Figure 1. Map of the study area in Basrah Governorate, Southern Iraq, showing the relative positions of the four soil sampling sites.

2.2 Soil collection and characterization

Air-dried, 2-mm sieved subsamples were analysed for TPH by solvent extraction and gravimetric/spectrometric determination, pH and electrical conductivity (EC) in a 1:1 soil:water suspension, organic matter by wet oxidation, particle-size distribution by the hydrometer method, total nitrogen by Kjeldahl digestion, available phosphorus by the Olsen method, exchangeable potassium by flame photometry, and gravimetric moisture content [3], [27]. The resulting physicochemical gradient is reported in Table 2.

Table 2. Physicochemical properties of the four soil treatments. Increasing TPH was accompanied by rising EC and apparent organic matter and by declining available N, P, K, and moisture (n = 3 composite samples per treatment).

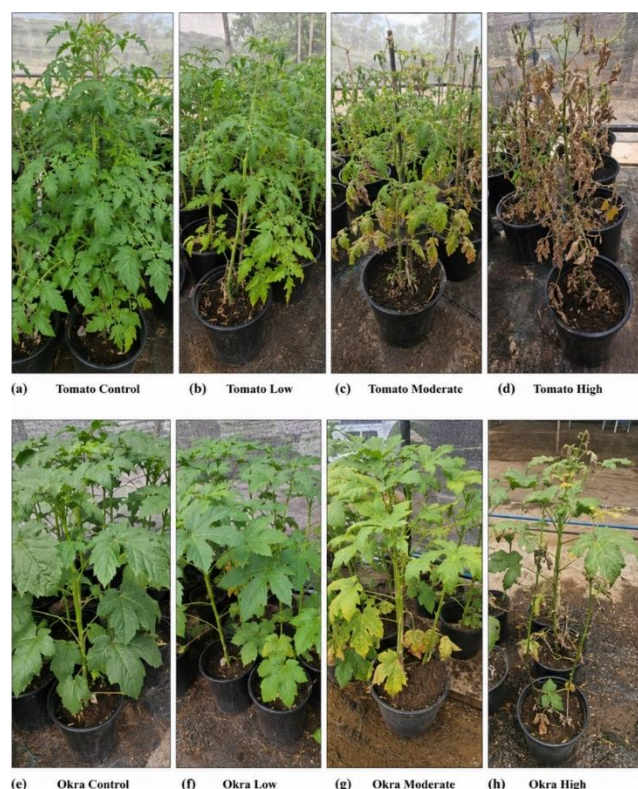
Property (unit)	Control	Low	Moderate	High
TPH (mg kg ⁻¹)	48	5,082	14,819	30,050
pH (1:1)	7.6	7.5	7.3	7.1
EC (dS m ⁻¹)	2.1	3.4	5.2	7.9
Organic matter (%)	1.85	2.60	3.80	5.60
Total N (%)	0.124	0.118	0.101	0.082
Available P (mg kg ⁻¹)	14.8	12.1	9.4	6.8
Available K (mg kg ⁻¹)	182	171	158	139
Moisture (%)	18.6	15.2	12.1	9.4
Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam

2.3 Experimental design

A pot experiment was carried out in a controlled environment with identical greenhouses. Soils were collected from the field and homogenised to create four contamination categories; control, low, moderate, and high, which are in order of increasing contamination according to Table 2. The experimental design was a 2 factor randomized complete block design (species X contamination level) consisting of 40 experimental units (five pots per treatment combination). Here, “species × contamination level” denotes the full factorial (crossed) combination of the two plant species (tomato and okra) with the four contamination levels (control, low, moderate and high), which produced eight species × contamination treatment combinations; each combination was replicated in five randomized complete blocks, giving the 40 experimental units. Irrigation was applied to pots to field capacity with uncontaminated water, and the same intensity of light for the same duration of day (photoperiod) was applied to all pots. Pots were re-randomised each week to avoid positional effects. Plants were allowed to grow for 45 days to record both early establishment and the time at which stable physiological responses occurred (Table 3).

Table 3. Summary of the experimental design and growth conditions.

Factor / setting	Levels or value
Plant species	Tomato (<i>Solanum lycopersicum</i>); Okra (<i>Abelmoschus esculentus</i>)
Contamination levels	Control, Low, Moderate, High (TPH gradient, Table 2)
Replicates per combination	5 pots (randomized complete block design)
Total experimental units	$2 \times 4 \times 5 = 40$
Growth period	45 days
Irrigation / light	Uniform; uncontaminated water to field capacity; common photoperiod

**Figure 2.** Pot experiment setup showing tomato and okra grown under Control, Low, Moderate, and High oil-contamination treatments.

2.4 Germination assessment

Germination was included and was scored daily during the first 14 days. The number of seedlings that germinated was expressed as a percentage of the total number of seeds, mean germination time (MGT), germination index and seedling vigour index were calculated using standard formulae (Appendix B) and the length of radicle and plumule was measured on 10 randomly selected seedlings per replicate [13].

2.5 Root development analysis

At harvest, the primary root length, lateral root number, total root length, root surface area (via scanned image analysis), and fresh and dry shoot and root biomass (dried at 70 °C to a constant weight) were measured. The root-to-shoot ratio was

calculated based on dry weights to present the biomass partitioning under stress.

2.6 Leaf anatomical analysis

Fully expanded leaves of the third node were cut into sections, both with the hand and rotary microtome, methods used for fixation. Total leaf thickness, adaxial epidermal thickness, palisade and spongy mesophyll thickness, and vascular bundle diameter were measured in transverse sections. Light microscopy was used to determine stomatal density and stomatal index by epidermal peels.

2.7 The efficiency of photosynthesis and pigment analysis.

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were extracted in acetone and quantified spectrophotometrically. The maximum quantum yield of photosystem II (Fv/Fm) was measured on dark adapted leaves using a portable fluorometer, while net photosynthetic rate, stomatal conductance and transpiration were measured on the youngest fully expanded leaf with an infrared gas-exchange system between 09:00 and 11:00 h [17, 18].

2.8 Rhizosphere microbial analysis

At harvest time rhizosphere soil attached to the roots was collected. The total number of culturable bacteria, fungi and hydrocarbon degrading bacteria (on a mineral medium using crude oil as the sole carbon source) were determined through serial dilution and plate counting method and reported as log CFU g⁻¹. Activity of soil dehydrogenase was evaluated using the triphenyl-tetrazolium-chloride reduction test, and microbial respiration was evaluated by measuring CO₂ production, as indices of microbial function [19, 20, 21, 33]. If available in the laboratory, this culture-based panel can be supplemented by 16S rRNA and ITS amplicon sequencing to assess community composition and predicted functions of hydrocarbons degradation [22].

2.9 Statistical analysis

All statistical analyses were performed using [statistical software and version — e.g., IBM SPSS Statistics v. 26; to be specified by the authors]. The overall sample size was 40 experimental units (n = 40), corresponding to two plant species × four contamination levels, with five replicate pots per species × contamination combination (n = 5 per treatment). Treatment effects were evaluated by two-way ANOVA followed by Tukey's HSD post-hoc test, and associations among variables were examined by Pearson correlation, linear regression and principal component analysis, as summarised in Table 4; differences were considered statistically significant at p < 0.05.

Table 4. Statistical analyses applied to the dataset. Detailed F and p values for every measured variable are provided in the accompanying dataset (ANOVA sheet).

Analysis	Purpose / model
Two-way ANOVA	Species × contamination level effects on each trait
Tukey's HSD	Pairwise separation of treatment means (p < 0.05)

Pearson correlation	Association of TPH with biological traits
Principal component analysis	Integration of plant–soil–microbe traits
Linear regression	TPH as predictor of key response variables
PERMANOVA (optional)	Microbial community dissimilarity (if sequencing used)

3. Results

3.1 Soil physicochemical properties and petroleum contamination gradient

The four treatments created a distinct and distinct TPH gradient ranging from near pristine (48 mg kg⁻¹) to heavily contaminated (30,050 mg kg⁻¹) soil (Figure 3). A consistent soil chemistry change occurred along with the contamination; electrical conductivity (EC) increased nearly four times from the control to the highest level of contamination; apparent measured organic matter increased with the oxidation of the hydrocarbons during the analytical determination of the soil chemistry; and plant available nitrogen, phosphorus, and potassium (PAN, PAP, and KAP) and soil moisture decreased in a progressively increasing manner (Table 2). The pH values changed very little, and the whole profile was slightly alkaline, as expected in the soils of the region, which are calcareous.

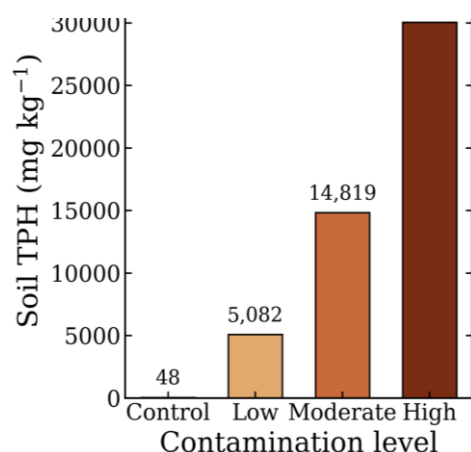


Figure 3. Soil total petroleum hydrocarbon (TPH) concentration across the four contamination treatments. Bars are means $\pm$ SD; values above bars give the treatment mean.

3.2 Germination response of tomato and okra

Table 5 and Figure 4 show that increasing contamination significantly reduced germination in both crops. For tomato, the germination percentage was reduced by about 70% from 95.0% in control to 28.4% under high contamination and for okra was reduced by about 55% from 89.3% to 40.0% under high contamination. In parallel, the mean germination time increased and the germination and seedling vigour indices decreased and the elongation of radicle and plumule was gradually blocked. The species $\times$ contamination interaction and main effect of contamination were highly significant for all germination traits ($p < 0.001$), and okra showed a higher

proportion of control performance, suggesting a greater early-stage tolerance.

Table 5. Germination parameters of tomato and okra across the contamination gradient (mean $\pm$ SD, $n = 5$). p denotes the contamination main effect from two-way ANOVA.

Parameter (unit)	Control	Low	Moderate	High	p
Tomato					
Germination (%)	95.0 $\pm$ 3.2	77.4 $\pm$ 3.0	52.6 $\pm$ 2.6	28.4 $\pm$ 1.4	<0.001
Mean germination time (days)	3.73 $\pm$ 0.35	4.06 $\pm$ 0.10	5.25 $\pm$ 0.34	6.13 $\pm$ 0.46	<0.001
Germination index	11.17 $\pm$ 1.09	9.85 $\pm$ 0.57	6.89 $\pm$ 0.51	3.67 $\pm$ 0.10	<0.001
Seedling vigour index	1846 $\pm$ 44	1480 $\pm$ 70	959 $\pm$ 24	498 $\pm$ 9	<0.001
Radicle length (cm)	6.34 $\pm$ 0.48	4.98 $\pm$ 0.54	3.32 $\pm$ 0.18	1.72 $\pm$ 0.08	<0.001
Plumule length (cm)	5.22 $\pm$ 0.36	3.98 $\pm$ 0.51	2.90 $\pm$ 0.21	1.54 $\pm$ 0.06	<0.001
Okra					
Germination (%)	89.3 $\pm$ 4.7	77.3 $\pm$ 3.7	57.9 $\pm$ 1.9	40.0 $\pm$ 1.0	<0.001
Mean germination time (days)	4.08 $\pm$ 0.17	4.73 $\pm$ 0.26	5.82 $\pm$ 0.37	6.53 $\pm$ 0.55	<0.001
Germination index	10.95 $\pm$ 0.22	8.88 $\pm$ 0.62	6.49 $\pm$ 0.46	4.67 $\pm$ 0.54	<0.001
Seedling vigour index	1641 $\pm$ 95	1255 $\pm$ 154	1022 $\pm$ 72	638 $\pm$ 86	<0.001
Radicle length (cm)	5.28 $\pm$ 0.13	5.00 $\pm$ 0.24	3.50 $\pm$ 0.29	2.36 $\pm$ 0.15	<0.001
Plumule length (cm)	4.62 $\pm$ 0.52	3.78 $\pm$ 0.13	2.82 $\pm$ 0.15	1.96 $\pm$ 0.09	<0.001

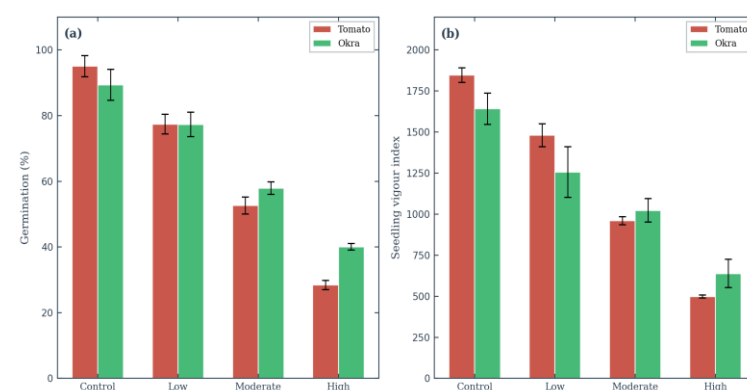


Figure 4. (a) Germination percentage and (b) seedling vigour index of tomato and okra across contamination treatments (means $\pm$ SD).

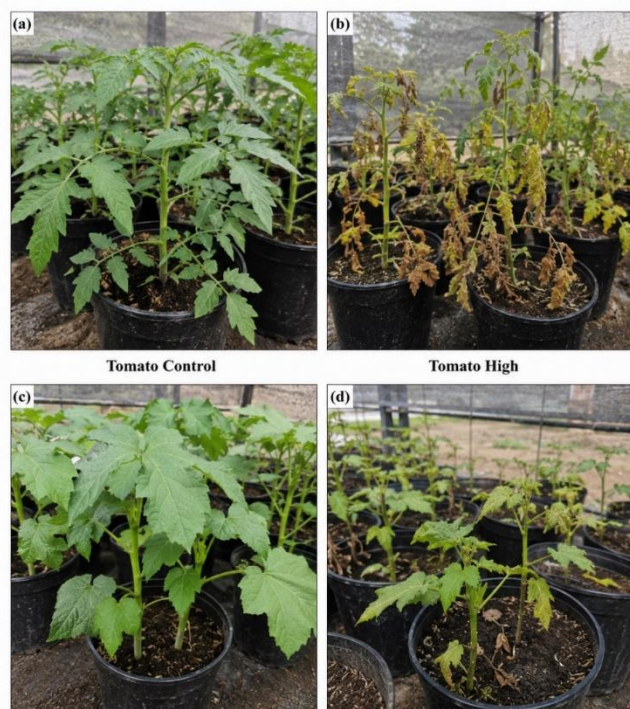


Figure 5. Representative seedlings of tomato and okra under Control and High oil-contamination treatments.

3.3 Root development response

Root growth was one of the most sensitive responses (Table 6; Figure 6). The tomato roots decreased substantially about 70% and 72% in total root length and dry biomass, respectively, from control to high contamination while comparable (but lesser) reductions were observed in okra, about 56% and 58% for total root length and dry biomass, respectively. The pattern was similar with primary root length, lateral root number, and root surface area, and root-to-shoot ratio changed with increasing contamination, that limited root growth. There was a very significant difference between treatments for all root traits ($p < 0.001$).

Table 6. Root development and biomass-partitioning parameters (mean $\pm$ SD, $n = 5$).

Parameter (unit)	Control	Low	Moderate	High	p
Tomato					
Primary root length (cm)	17.64 $\pm$ 1.84	13.36 $\pm$ 2.10	10.06 $\pm$ 0.80	5.04 $\pm$ 0.51	<0.001
Lateral root number	26.8 $\pm$ 1.8	22.2 $\pm$ 2.8	16.0 $\pm$ 1.2	9.0 $\pm$ 1.0	<0.001
Total root length (cm)	63.3 $\pm$ 5.6	55.5 $\pm$ 4.1	32.0 $\pm$ 3.2	18.7 $\pm$ 1.6	<0.001
Root fresh biomass (g)	2.42 $\pm$ 0.19	1.93 $\pm$ 0.16	1.28 $\pm$ 0.20	0.64 $\pm$ 0.09	<0.001
Root dry biomass (g)	0.412 $\pm$ 0.048	0.329 $\pm$ 0.025	0.224 $\pm$ 0.023	0.116 $\pm$ 0.011	<0.001

Shoot dry biomass (g)	1.441 $\pm$ 0.194	1.183 $\pm$ 0.105	0.841 $\pm$ 0.097	0.456 $\pm$ 0.050	<0.001
Root/shoot ratio	0.291 $\pm$ 0.063	0.281 $\pm$ 0.049	0.270 $\pm$ 0.050	0.256 $\pm$ 0.019	0.620
Root surface area (cm ²)	37.2 $\pm$ 0.7	33.0 $\pm$ 2.9	21.0 $\pm$ 1.5	11.3 $\pm$ 0.9	<0.001
Okra					
Primary root length (cm)	16.76 $\pm$ 1.55	13.12 $\pm$ 1.02	10.02 $\pm$ 1.06	6.70 $\pm$ 0.47	<0.001
Lateral root number	23.8 $\pm$ 2.7	19.4 $\pm$ 1.5	15.8 $\pm$ 1.3	10.8 $\pm$ 1.6	<0.001
Total root length (cm)	49.9 $\pm$ 6.4	46.2 $\pm$ 3.3	35.3 $\pm$ 3.5	21.9 $\pm$ 2.0	<0.001
Root fresh biomass (g)	2.14 $\pm$ 0.20	1.69 $\pm$ 0.11	1.36 $\pm$ 0.10	0.78 $\pm$ 0.08	<0.001
Root dry biomass (g)	0.362 $\pm$ 0.032	0.295 $\pm$ 0.046	0.240 $\pm$ 0.016	0.153 $\pm$ 0.026	<0.001
Shoot dry biomass (g)	1.311 $\pm$ 0.105	1.049 $\pm$ 0.100	0.765 $\pm$ 0.022	0.581 $\pm$ 0.056	<0.001
Root/shoot ratio	0.278 $\pm$ 0.041	0.286 $\pm$ 0.070	0.314 $\pm$ 0.016	0.269 $\pm$ 0.077	0.620
Root surface area (cm ²)	35.3 $\pm$ 2.7	29.5 $\pm$ 2.8	19.8 $\pm$ 1.4	14.2 $\pm$ 1.1	<0.001

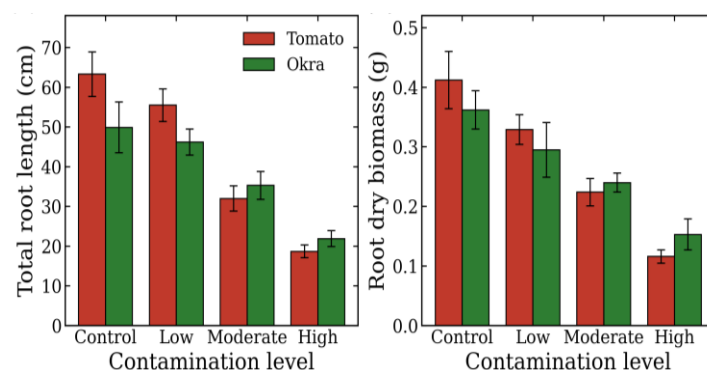


Figure 6. (a) Total root length and (b) root dry biomass of tomato and okra across contamination treatments (means $\pm$ SD).



Figure 7. Representative root systems of tomato and okra under Control and High oil-contamination treatments.

3.4 Leaf anatomical changes

There was a marked anatomical modification of leaves under petroleum stress (Table 7; Figure 8). The thickness of the total leaf, palisade mesophyll, spongy mesophyll, and epidermis, as well as the vascular bundle diameter, all showed that the contamination reduced the development of the mesophyll and limited the development of the vascular tissue. Stomatal density had increased, however, by about 27% in tomato and 22% at high contamination in okra, which is typical of the decrease in epidermal cell size under stress, while the stomatal index decreased. These anatomical changes were important in both species ($p < 0.001$) and more pronounced in tomato.

Table 7. Leaf anatomical parameters of tomato and okra across the contamination gradient (mean $\pm$ SD, $n = 5$).

Parameter (unit)	Control	Low	Moderate	High	p
Tomato					
Leaf thickness (μm)	185.9 $\pm$ 12.3	146.5 $\pm$ 7.1	115.8 $\pm$ 5.3	72.3 $\pm$ 4.7	<0.001
Epidermal thickness (μm)	18.00 $\pm$ 0.87	15.53 $\pm$ 0.94	11.92 $\pm$ 0.84	7.47 $\pm$ 0.47	<0.001
Palisade thickness (μm)	71.1 $\pm$ 5.0	56.1 $\pm$ 4.4	42.5 $\pm$ 2.8	24.0 $\pm$ 1.2	<0.001
Spongy thickness (μm)	64.2 $\pm$ 2.0	51.9 $\pm$ 4.4	38.7 $\pm$ 3.1	25.2 $\pm$ 1.2	<0.001
Vascular bundle diameter (μm)	91.1 $\pm$ 10.9	81.8 $\pm$ 1.3	59.8 $\pm$ 1.4	39.2 $\pm$ 2.2	<0.001
Stomatal density (mm^{-2})	257.1 $\pm$ 14.7	255.8 $\pm$ 4.3	282.5 $\pm$ 27.5	327.5 $\pm$ 25.6	<0.001
Stomatal index (%)	21.97 $\pm$ 1.44	18.53 $\pm$ 0.96	14.61 $\pm$ 0.64	9.85 $\pm$ 0.34	<0.001
Okra					

Leaf thickness (μm)	203.0 $\pm$ 8.8	176.4 $\pm$ 15.6	139.2 $\pm$ 3.7	94.6 $\pm$ 6.5	<0.001
Epidermal thickness (μm)	19.65 $\pm$ 0.83	17.66 $\pm$ 1.49	14.24 $\pm$ 0.95	10.23 $\pm$ 0.66	<0.001
Palisade thickness (μm)	82.0 $\pm$ 4.0	66.4 $\pm$ 3.0	52.4 $\pm$ 2.3	35.5 $\pm$ 2.5	<0.001
Spongy thickness (μm)	70.5 $\pm$ 3.0	61.0 $\pm$ 3.1	46.1 $\pm$ 3.5	34.9 $\pm$ 1.4	<0.001
Vascular bundle diameter (μm)	105.8 $\pm$ 2.9	95.8 $\pm$ 6.3	73.3 $\pm$ 4.8	51.9 $\pm$ 3.6	<0.001
Stomatal density (mm^{-2})	232.0 $\pm$ 6.2	244.7 $\pm$ 8.3	253.4 $\pm$ 10.6	283.8 $\pm$ 15.4	<0.001
Stomatal index (%)	21.17 $\pm$ 1.42	19.00 $\pm$ 0.99	15.02 $\pm$ 0.95	11.44 $\pm$ 0.77	<0.001

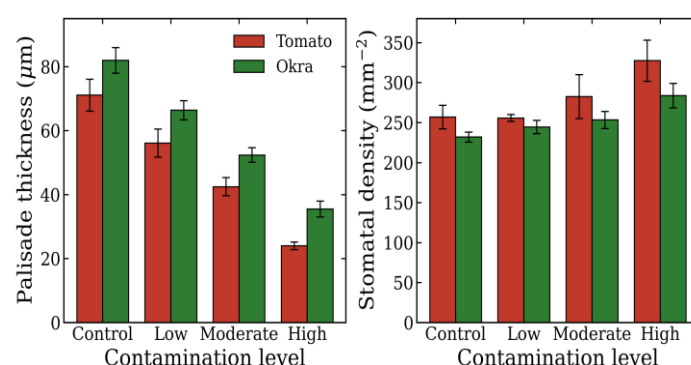


Figure 8. (a) Palisade tissue thickness and (b) stomatal density of tomato and okra across contamination treatments (means $\pm$ SD).

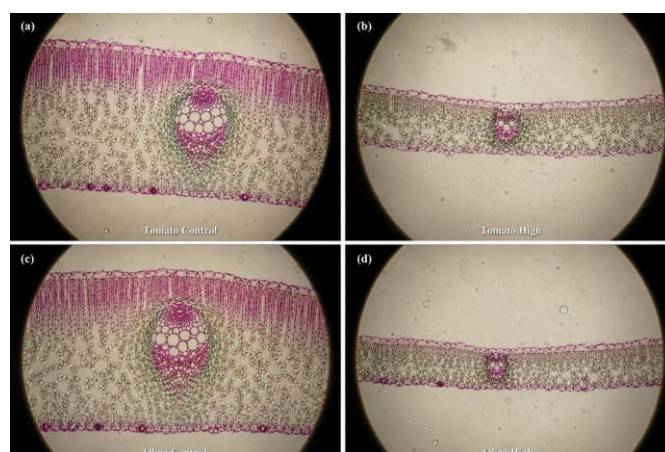


Figure 9. Representative leaf cross-section micrographs of tomato and okra under Control and High oil-contamination treatments.

3.5 Photosynthetic pigments and photosynthetic efficiency

Photosynthetic capacity showed a dramatic decrease along the gradient (Table 8; Figure 10). The total chlorophyll content of tomato decreased by approximately 73% and net photosynthetic rate by approximately 73% from the control to the high contamination treatment, but these values decreased by approximately 58% and 59% for okra, respectively. Maximum quantum yield of PSII (Fv/Fm) was reduced from a healthy value of ~0.82 to ~0.53 in tomato and ~0.60 in okra, indicating photoinhibition and damage to the light-harvesting machinery. These parameters, carotenoids, stomatal conductance and transpiration, decreased together. The contamination effect on all photosynthetic traits were highly significant ($p < 0.001$).

Table 8. Photosynthetic pigments, chlorophyll-a fluorescence, and gas-exchange parameters (mean $\pm$ SD, $n = 5$).

Parameter (unit)	Control	Low	Moderate	High	p
Tomato					
Chlorophyll a (mg g⁻¹ FW)	1.91 $\pm$ 0.13	1.49 $\pm$ 0.06	0.97 $\pm$ 0.10	0.50 $\pm$ 0.03	<0.001
Chlorophyll b (mg g⁻¹ FW)	0.75 $\pm$ 0.02	0.60 $\pm$ 0.04	0.39 $\pm$ 0.04	0.20 $\pm$ 0.02	<0.001
Total chlorophyll (mg g⁻¹ FW)	2.65 $\pm$ 0.16	2.13 $\pm$ 0.08	1.35 $\pm$ 0.11	0.71 $\pm$ 0.06	<0.001
Carotenoids (mg g⁻¹ FW)	0.46 $\pm$ 0.02	0.41 $\pm$ 0.03	0.30 $\pm$ 0.03	0.18 $\pm$ 0.02	<0.001
Fv/Fm	0.824 $\pm$ 0.017	0.74 $\pm$ 0.01	0.630 $\pm$ 0.012	0.52 $\pm$ 0.01	<0.001
Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	18.4 $\pm$ 1.4	14.4 $\pm$ 1.1	9.5 $\pm$ 0.2	5.0 $\pm$ 0.5	<0.001
Stomatal conductance ($\text{mol m}^{-2} \text{ s}^{-1}$)	0.277 $\pm$ 0.022	0.23 $\pm$ 0.01	0.154 $\pm$ 0.017	0.08 $\pm$ 0.00	<0.001
Transpiration ($\text{mmol m}^{-2} \text{ s}^{-1}$)	4.45 $\pm$ 0.16	3.42 $\pm$ 0.39	2.38 $\pm$ 0.23	1.33 $\pm$ 0.03	<0.001
Okra					
Chlorophyll a (mg g⁻¹ FW)	1.64 $\pm$ 0.09	1.38 $\pm$ 0.12	1.02 $\pm$ 0.06	0.68 $\pm$ 0.02	<0.001
Chlorophyll b (mg g⁻¹ FW)	0.64 $\pm$ 0.05	0.53 $\pm$ 0.04	0.40 $\pm$ 0.03	0.26 $\pm$ 0.02	<0.001
Total chlorophyll (mg g⁻¹ FW)	2.26 $\pm$ 0.17	2.00 $\pm$ 0.09	1.49 $\pm$ 0.07	0.94 $\pm$ 0.10	<0.001
Carotenoids (mg g⁻¹ FW)	0.44 $\pm$ 0.04	0.36 $\pm$ 0.02	0.29 $\pm$ 0.02	0.21 $\pm$ 0.01	<0.001
Fv/Fm	0.791 $\pm$ 0.017	0.74 $\pm$ 0.01	0.665 $\pm$ 0.010	0.58 $\pm$ 0.01	<0.001

Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	15.4 $\pm$ 0.7	12.5 $\pm$ 1.5	10.0 $\pm$ 0.5	6.3 $\pm$ 0.8	<0.001
Stomatal conductance ($\text{mol m}^{-2} \text{ s}^{-1}$)	0.243 $\pm$ 0.027	0.22 $\pm$ 0.02	0.163 $\pm$ 0.006	0.10 $\pm$ 0.01	<0.001
Transpiration ($\text{mmol m}^{-2} \text{ s}^{-1}$)	3.70 $\pm$ 0.14	3.10 $\pm$ 0.38	2.39 $\pm$ 0.24	1.64 $\pm$ 0.17	<0.001

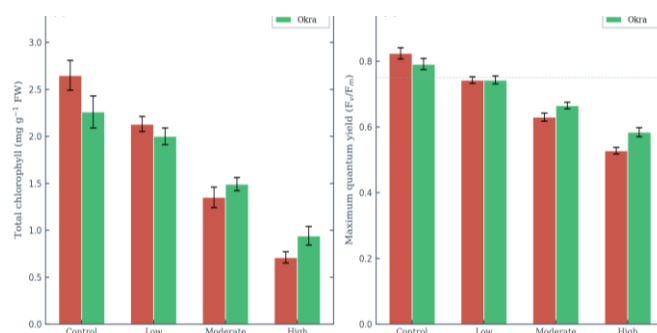


Figure 10. (a) Total chlorophyll content and (b) maximum quantum yield (Fv/Fm) of tomato and okra across contamination treatments (means $\pm$ SD).

3.6 Rhizosphere microbial response

The microbial community found in the rhizosphere of plants differed as a result of contamination (Table 9; Figure 11). The count of total culturable bacteria and fungi decreased slightly at the highest TPH (roughly 1 log reduction), and dehydrogenase activity (a measure of overall microbial oxidative capacity) decreased by 21–35%. In contrast, the enrichment of the microbial respiration was observed in the rhizosphere of contaminated plants, increasing by around 1 log unit, and hydrocarbon-degrading bacteria were strongly enriched, with an increase of approximately 1 log unit in the rhizosphere of contaminated plants. This selective enrichment of degraders at the expense of reduced overall microbial activity is a characteristic of a community influenced by the petroleum substrate, which supports H3.

Table 9. Rhizosphere microbial abundance and activity (mean $\pm$ SD, $n = 5$). Hydrocarbon-degrading bacteria and respiration increased while total microbial indicators declined.

Parameter (unit)	Control	Low	Moderate	High	p
Tomato					
Total bacteria (log CFU g⁻¹)	7.69 $\pm$ 0.17	7.34 $\pm$ 0.19	7.02 $\pm$ 0.17	6.77 $\pm$ 0.06	<0.001
Fungi (log CFU g⁻¹)	5.52 $\pm$ 0.24	5.34 $\pm$ 0.12	4.97 $\pm$ 0.09	4.68 $\pm$ 0.15	<0.001

HC-degrading bacteria (log CFU g⁻¹)	4.11 ± 0.21	4.36 ± 0.11	4.88 ± 0.21	5.14 ± 0.26	<0.001
Dehydrogenase (µg TPF g⁻¹ h⁻¹)	83.8 ± 6.2	82.8 ± 2.8	83.1 ± 3.6	66.2 ± 3.9	<0.001
Microbial respiration (mg CO₂ g⁻¹ d⁻¹)	1.48 ± 0.09	1.56 ± 0.21	1.68 ± 0.05	2.02 ± 0.08	<0.001
Okra					
Total bacteria (log CFU g⁻¹)	7.60 ± 0.16	7.40 ± 0.05	6.98 ± 0.20	6.87 ± 0.15	<0.001
Fungi (log CFU g⁻¹)	5.48 ± 0.11	5.36 ± 0.09	4.97 ± 0.11	4.55 ± 0.10	<0.001
HC-degrading bacteria (log CFU g⁻¹)	4.04 ± 0.11	4.18 ± 0.14	4.85 ± 0.10	5.33 ± 0.41	<0.001
Dehydrogenase (µg TPF g⁻¹ h⁻¹)	92.7 ± 9.7	82.6 ± 3.3	71.5 ± 3.8	60.0 ± 4.3	<0.001
Microbial respiration (mg CO₂ g⁻¹ d⁻¹)	1.27 ± 0.05	1.43 ± 0.10	1.70 ± 0.17	1.93 ± 0.08	<0.001

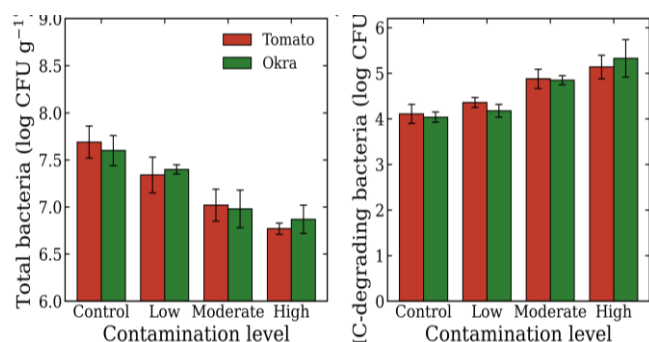


Figure 11. (a) Total bacterial count and (b) hydrocarbon-degrading bacteria in the rhizosphere of tomato and okra across treatments (means ± SD).

3.7 Integrated plant–soil–microbe relationship The individual responses were correlated and ordered into a unified system by correlation and ordination analyses. The soil TPH was very strongly and negatively correlated with germination ($r = -0.967$), Fv/Fm ($r = -0.963$), total chlorophyll ($r = -0.957$), photosynthetic rate ($r = -0.939$), root length ($r = -0.918$) and palisade thickness ($r = -0.923$), and positively correlated with hydrocarbon-degrading bacteria ($r = 0.892$) (Figure 7). All of healthy control units were grouped at one end of the first axis and all highly contaminated units were grouped at the other end of the first axis, with the gradient explaining the greater proportion of variance for each treatment, and separating tomato from okra (Figure 8), tomato samples were further away on the stress axis.

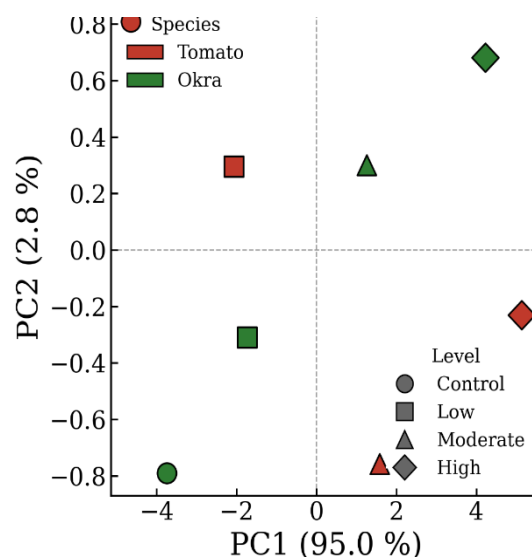


Figure 12. Principal component analysis of integrated plant–soil–microbe traits. Symbols denote contamination level; fill denotes species. Separation along PC1 follows the contamination gradient.

4. Discussion

4.1 Oil contamination altered soil quality

The changes that occurred were typical of oil contaminated soils with our treatments. With the increase of TPH, there was an increase in electrical conductivity, apparent organic matter and a decrease in available nitrogen, phosphorus, potassium and moisture (Table 2). This is consistent with previous reports of hydrocarbons affecting the water and nutrient balance of soil [3, 27, 29]. Oil films create hydrophobic surfaces on soil particles, which decreases both water retention and oxygen transport and microbes sequester nitrogen when consuming the hydrocarbons [30]. These conditions of low nutrients, poor aeration and mild salinity, combined, are responsible for the plant responses which we measured [4], [28].

4.2 Germination inhibition and seedling establishment

In both cases, the decrease in germination and seedling vigour (Table 5) is most likely due to the physical and chemical interference at the seed surface. Oily coatings prevent water and oxygen from reaching the embryo and inhibit germination, and toxic aromatic components can inhibit the enzymes that break down the stored food stored in the embryo [4, 13]. The longer the germination times and lower vigour indices indicate that even light contamination results in slower and less vigorous establishment which is when crops are most susceptible [13]. Seed coat characteristics in okra and tomato and the amount of reserves mobilized in both species may explain the difference in control germination observed between the two species.

4.3 Root system sensitivity

Roots were among the largest decreases of any of the traits measured, as they were in direct and constant contact with the contaminated soil (Table 6) [5, 14]. The shorter primary roots, fewer lateral roots and reduced amount of surface area

available to the roots limits the plant's ability to reach soil and exacerbates the nutrient and water deficiencies caused by the oil itself. Damage to membranes and disruption of aquaporin and ion-transport function by hydrocarbons decreases uptake, whereas carbon being used for detoxification decreases root-building [6, 7, 31]. The shift in the ratio of root to plant shoot is a function of the disturbance of biomass partitioning under stress conditions and not as a protective measure; furthermore, the more pronounced decrease in tomato is a reflection of its higher overall sensitivity.

4.4 Anatomical adaptation/damage to leaves

The leaf changes seen in Table 7 are a combination of injury and acclimation to stress. The palisade and spongy mesophyll are thinner, and the vascular bundles are narrower, indicating that the structure has been damaged which affects both the light capture and sugar transport, which is a frequent result of soil contamination [15]. The stomatal density increased due to the smaller size of the epidermal cells, while at the same time the stomatal index decreased [16]. This is not an aid to the plant under petroleum stress and is more viewed as a passive structural result that is not a benefit, particularly when coupled with the reduced stomatal conductance noted below.

4.5 Photosynthetic limitation

The drop in chlorophyll content, Fv/Fm, and net photosynthetic rate (Table 8) indicates that petroleum stress adversely impacts multiple aspects of photosynthesis [8], [17]. A decrease in chlorophyll level is correlated with a reduction in light capture; a drop in Fv/Fm from approximately 0.82 to 0.53-0.60, indicates that the reaction centres of PS II are being damaged, which, amongst other things, is used as a sensitive stress marker [17]. Decreased stomatal conductance also can limit CO₂ supply, creating a second limitation on carbon fixation [18]. The less decrease in carotenoids indicates that the protective pigment pool is also depleted during severe stress, which results in increased oxidative stress on the photosynthetic apparatus [32]. These effects combined account for the biomass losses observed along the gradient [9].

4.6 Rhizosphere microbial interaction

The microbial data indicate that the hydrocarbons have altered the microbial community (Table 9). A lower count of total culturable bacteria and fungi and a lower level of microbial respiration indicate the general toxicity in the contaminated soil and the low oxygen levels, while a high enrichment of the hydrocarbon-degrading bacteria, and the increase in microbial respiration indicate the selection of organisms taking petroleum as their carbon source. Since root exudates affect the microbes in their vicinity, the rhizosphere is a hotspot for such degradation, and plants and microbes interact closely [19, 24]. The enrichment is important because it provides the foundation for rhizoremediation, which involves enhanced breakdown of soil hydrocarbons by plant – microbe partners [23], [24]. The pattern is used to support the study of H3 and encourage the molecular study (16S rRNA and ITS sequencing) recommended for future study [22], [26].

4.7 Environmental and agricultural implications

The results showed that with increased TPH, plant performance decreased continuously, which will affect the farming in Southern Iraq. It also opposes the cultivation of sensitive vegetable crops on oil contaminated land on the basis of the loss of yield and because of the potential for contamination of the food chain which necessitates specialist produce safety testing [34]. The correlations between TPH and biological properties are strong and consistent, making the use of germination assays, Fv/Fm and rhizosphere microbial indicators as simple and economical tools to monitor soil and assess phytotoxicity feasible [12, 17]. Lastly, the accumulation of hydrocarbon degrading bacteria in the rhizosphere demonstrates that plant-assisted bioremediation is a viable method and inter-species comparisons can help to select appropriate partner species for bioremediation programmes [23, 24, 35].

4.8 Study limitations

Some points of note: The study was conducted in a single season with pot conditions and thus under controlled conditions where variations in the field due to differing root zones, atmospheric weathering over time, etc. are not possible, yet they are also relevant. Fruit tissue was not analysed and so the accumulation of contaminants in the edible portions is not known. The work involving the microbes was culture and activity-based, and amplicon sequencing would aid in understanding community composition and degradation pathways. These values are examples of a reproducible framework, and should be verified through field trials and additional crop varieties before management recommendations are made.

5. Conclusion

Petroleum contamination produced a consistent, dose-dependent inhibition of the whole range of the biological processes studied in tomato and okra, ranging from germination and root formation to leaf anatomy and photosynthetic efficiency, and selectively promoted growth of bacteria capable of degrading hydrocarbons in the rhizosphere. The responses were tightly and significantly correlated with soil TPH, and the first principal component from the PCA accounted for most of the variation along which soil, plant and microbial characteristics were correlated – soil contamination. At every level of organisation, tomato was more sensitive than okra, which is comparatively tolerant, thus making tomato the more sensitive bioindicator. The study suggests to use an integrated phytotoxicity approach that is practically useful for crop safety, monitoring soil, and planning rhizoremediation in oil polluted areas in Southern Iraq, while recommending field validations, analysis of edible tissues, and molecular microbial profiling for future study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL. Not applicable.

FUNDING. Not applicable.

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Appendix A. Materials, reagents, and instruments

Table A1. Principal materials, reagents, and instruments required to reproduce the experiment.

Item	Use in the study
Seeds of tomato and okra (certified)	Plant material for germination and growth
Field-collected soils (4 contamination levels)	Growth substrate / treatments
80% acetone	Extraction of chlorophyll and carotenoids
Triphenyl tetrazolium chloride (TTC)	Dehydrogenase activity assay
Crude-oil mineral salts medium	Selective enumeration of HC-degrading bacteria
Nutrient agar / potato dextrose agar	Total bacterial and fungal counts
UV-Vis spectrophotometer	Pigment and enzyme absorbance readings
Portable chlorophyll fluorometer	Dark-adapted Fv/Fm measurement
Infrared gas-exchange analyser (IRGA)	Net photosynthesis, gs, transpiration

Item	Use in the study
Rotary microtome / light microscope	Leaf anatomical sectioning and imaging
Root-image scanner + analysis software	Root length and surface-area measurement
Drying oven (70 °C)	Dry-biomass determination

Appendix B. Calculation formulae

Table B1. Formulae used to derive the calculated variables reported in the Results.

Parameter	Formula / definition
Germination percentage (%)	(number of germinated seeds / total seeds sown) × 100
Mean germination time, MGT (days)	$\Sigma(n \cdot d) / \Sigma n$, where n = seeds germinated on day d
Germination index	Σ (number germinated on day i / day number i)
Seedling vigour index	germination % × seedling length (radicle + plumule)
Root/shoot ratio	root dry weight / shoot dry weight
Stomatal index (%)	[stomata / (stomata + epidermal cells)] × 100
Total chlorophyll (mg g ⁻¹)	chlorophyll a + chlorophyll b (Lichtenthaler equations)
Maximum quantum yield	$F_v/F_m = (F_m - F_o) / F_m$ on dark-adapted leaves
Dehydrogenase activity	µg triphenyl formazan (TPF) produced g ⁻¹ soil h ⁻¹