



Physiological and Biochemical Responses of Rice Genotypes to Drought and Industrial Pollution under Changing Climatic Conditions

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Abstract

Rice (*Oryza sativa* L.) is one of the world's most important food crops, yet its productivity is increasingly threatened by climate change-induced drought and industrial pollution. Water scarcity coupled with contamination of agricultural soils by heavy metals adversely affects plant growth, photosynthetic efficiency, nutrient uptake, and grain yield. The present investigation evaluated four rice genotypes for their physiological, biochemical and morphological responses under drought and industrial pollution stress at the industrial region near Tilda, Sarona Chhattisgarh. The experiment consisted of four treatments comprising well-watered control, drought stress, industrial pollution stress (heavy metal-contaminated soil), and combined drought plus industrial pollution stress. Physiological parameters including SPAD chlorophyll content, canopy temperature, relative water content, photosynthetic rate, transpiration rate, stomatal conductance, chlorophyll pigments, and carotenoids were evaluated during the active vegetative stage.

The results revealed considerable variation among genotypes in their tolerance to combined stress conditions. Rice genotypes Poornima, Indira Barani Dhan 1 and Samleshwari maintained significantly higher chlorophyll content, photosynthetic rate, stomatal conductance, and relative water content compared with susceptible cultivars such as Mahamaya, Rajeshwari and Swarna. Combined drought and industrial pollution caused substantial reductions in chlorophyll content (18–32%), photosynthetic rate (35–55%), transpiration rate (30–45%), and relative water content (20–35%), while leaf temperature increased by 3–6°C. Heavy metal stress further intensified oxidative damage through chlorophyll degradation and reduced carbon assimilation. The identified tolerant genotypes exhibited better physiological adjustment and maintained greater biomass accumulation under combined stress conditions. These findings provide valuable information for breeding climate-resilient and pollution-tolerant rice cultivars suitable for sustainable agriculture under changing environmental conditions.

Keywords: Rice, drought stress, industrial pollution, heavy metals, photosynthesis, chlorophyll, climate change, physiological traits

Introduction

Rice (*Oryza sativa* L.) provides the principal source of calories for more than half of the global population and occupies a central position in the agricultural economy of Asia. India is among the largest producers of rice, cultivating nearly 45 million hectares with an annual production exceeding 130 million tonnes (Akte *et al.*, 2016). In the Chhattisgarh, rice is cultivated under diverse agro-climatic conditions and represents the major staple crop supporting rural livelihoods (Das *et al.* 2014). Despite favourable climatic conditions and rich genetic diversity, rice productivity in this region remains substantially lower than the national average because of increasing environmental stresses.

Climate change has significantly altered rainfall distribution, resulting in frequent droughts and prolonged dry spells during critical growth stages of rice. Water deficit limits plant growth by reducing leaf expansion, photosynthesis, nutrient transport, and biomass production (Nguyen, 2002; Wassmann and Dobermann, 2007). Drought stress induces stomatal closure, decreases carbon dioxide diffusion into leaves, and accelerates chlorophyll degradation, ultimately reducing grain yield. Depending upon the duration and severity of drought, yield losses in rice may vary between 20% and 80% (Rosenzweig and Hillel, 1995).

Simultaneously, rapid industrialization has emerged as another serious environmental challenge affecting agricultural sustainability (Kimball, 1983). Industrial effluents released from mining, thermal power plants, metal processing industries, textile manufacturing, electroplating units, fertilizer factories, and chemical industries frequently contaminate agricultural soils with toxic heavy metals including cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni), mercury (Hg), and arsenic (As). These pollutants persist in soil for long periods and are readily absorbed by rice plants because of flooded cultivation practices. Heavy metal accumulation interferes with chlorophyll biosynthesis, damages cellular membranes, inhibits photosynthetic enzymes, disturbs nutrient uptake, and promotes excessive production of reactive oxygen species (ROS), leading to oxidative stress and impaired plant metabolism (Bahuguna *et al.*, 2014; Shi *et al.*, 2014).

The simultaneous occurrence of drought and industrial pollution represents an emerging challenge for rice cultivation. Under combined stress, plants experience both water limitation and toxic ion accumulation, causing severe impairment of physiological and biochemical processes (Xia and Qi, 2004; Yang *et al.*, 2004). Reduced stomatal conductance under drought restricts transpiration and nutrient transport, while heavy metals further damage chloroplast structure and enzyme activity. Consequently, combined stress often produces greater

reductions in growth and yield than either stress alone (Krishnan et al., 2011; Jagadish et al., 2010, 2011; Shi et al., 2014).

Identification of rice genotypes possessing superior physiological tolerance to multiple environmental stresses is therefore essential for climate-smart agriculture. Saleh (2020) Traits such as chlorophyll stability, canopy temperature depression, relative water content, photosynthetic efficiency, transpiration rate, and antioxidant capacity have been recognized as reliable indicators of stress tolerance (Hall, 1992; Prasad et al., 2006; Jagadish et al., 2007, 2008, 2010; Shi et al., 2014). Screening elite rice germplasm based on these physiological traits provides valuable information for crop improvement programmes aimed at developing resilient cultivars suitable for drought-prone and industrially polluted regions.

The present investigation was therefore undertaken to evaluate sixty-eight elite rice genotypes for their physiological and biochemical responses under drought and industrial pollution stress. Bilal and Ali (2022) The study aimed to identify stress-tolerant rice genotypes and characterize key physiological mechanisms responsible for adaptation under combined environmental stresses.

Materials and Methods

Experimental Site

The investigation was conducted during the monsoon season at the Tilda, Sarona- Chhattisgarh.

Experimental Design

All rice genotypes were evaluated using a Randomized Complete Block Design (RCBD) with three replications under four treatments:

- T₁ – Control (100% field capacity)
- T₂ – Drought stress (50–60% field capacity)
- T₃ – Industrial pollution stress (soil amended with environmentally relevant concentrations of Cd, Pb, and Cr)
- T₄ – Combined drought + industrial pollution

Stress treatments were imposed from active vegetative growth until physiological maturity.

Drought Stress Imposition

Drought stress was created by maintaining soil moisture at approximately 50–60% of field capacity using controlled irrigation. Soil moisture content was monitored using a digital soil moisture meter throughout the experiment.

Industrial Pollution Treatment

Industrial pollution was simulated by applying a mixture of cadmium chloride (CdCl₂), lead nitrate [Pb(NO₃)₂], and potassium dichromate (K₂Cr₂O₇) to achieve contamination levels representative of industrially affected agricultural soils. Heavy metals were thoroughly mixed with the soil before transplanting.

Physiological Measurements

Estimation of leaf pigments:

The chlorophyll index (%) of standing rice leaves that are fully matured (third or fourth leaves from the top) were recorded with SPAD-502 chlorophyll meter in non-invasive way at different growth stages i.e. 30, 60 and 90 DAT. At active growth stage (60DAT), the contents of chlorophyll and carotenoid pigments in fresh and matured leaves were estimated by the acetone extraction method. Known weight of leaf tissue (0.5g) was fully homogenized in 15ml of 80% acetone: 20% water solution using a pre-cooled pestle and mortar at 4°C. A pinch of CaCO₃ was added to the extraction solution during grinding of the samples to neutralize any plant acids which might be liberated during grinding. After thorough grinding, the extracted solution was filtered through Whatman No.42 filter paper in volumetric flask and the volume of the extract made to 25 ml using same extraction solvent. Each sample was placed in a cuvette and absorbance was recorded at 645, 663 and 480 nm through UV visible spectrophotometer (UV-2100). The absorbance values were substituted in the following formulae to calculate the chl a, chl b and expressed in mg/g whereas carotenoid pigment contents were expressed µg/g on fresh weight basis (Misyura et al., 2012, Das et al, 2019). The ratio of primary pigments of chl a to chl b was calculated by dividing numerical chl a values with chl b values in the denominator

$$\text{Chla (mg g}^{-1}\text{FW)} = (12.72 \times A_{663} - 2.58 \times A_{645}) \times (V/W) \times (1/1000) \text{----- (Eq. 1)}$$

$$\text{Chlb (mg g}^{-1}\text{FW)} = (22.87 \times A_{645} - 4.67 \times A_{663}) \times (V/W) \times (1/1000) \text{----- (Eq.2)}$$

$$\text{Chla + Chl b (mg g}^{-1}\text{FW)} = (8.05 \times A_{663} + 20.29 \times A_{645}) \times (V/W) \times (1/1000) \text{----- (Eq.3)}$$

$$\text{Carotenoids (}\mu\text{g g}^{-1}\text{FW)} = (A_{480} + 0.114 \times A_{663} - 0.638 \times A_{645}) \times (V/W) \times (1/1000) \text{ (Eq.4)}$$

Where,

V = the total volume of the extract,

W = weight of the tissue taken for pigment measurements

A₆₆₃, A₆₄₅ and A₄₈₀ = the optical absorbance values recorded by UV-2100 spectrophotometer at 663, 645, and 480nm wavelengths, respectively.

Measurement of leaf thickness and SLW

The fully expanded matured leaf, the fourth leaf from the top, was used for measurement of leaf thickness (LT) and specific leaf weight (SLW) measurements. LT was measured using absolute digital vernier caliper at the broadest part of the leaf excluding major veins with accuracy of ±0.01 mm and expressed in µm. This measurement was made by pressing the caliper gently to avoid overestimation and any injury to the leaf. Shrivastava (2025) For SLW, the selected leaves from different treatments were assessed for the leaf area using

the leaf area meter and respective dry weight of the leaf after drying in an oven at 600 C to constant weight. Both SLW and LT indicate the robustness of leaf (Vile et al., 2005). The SLW was computed by Eq.5:

$$\text{SLW (g cm}^{-2}\text{)} = \text{Leaf weight} / \text{Leaf area} \text{ (Eq.5)}$$

Leaf temperature measurement by IR thermometer

Leaf temperature measurements were determined during mid-day using an infrared thermometer. It was measured by pointing the infrared thermometer to the canopy at a distance of 30 cm from canopy skin, after that ambient air temperature was also measured by using the infrared thermometer. The measurement was done from 4 directions (N, S, E and W) and the values were averaged as the canopy temperature of each plant. Then, the difference between canopy temperature (T_c) and air temperature (T_a) was assessed as $T_c - T_a$.

Photosynthetic trait measurements

Photosynthetic parameters viz., photosynthetic rate (A), stomatal conductance (GH_2O), transpiration rate (E) and leaf temperature ($^{\circ}\text{C}$) were recorded at flowering stage by using portable photosynthesis system which is also called as Infra-red gas analyzer (IRGA- GFS Walz-6400 Model) equipped with a CO_2 cartridge, artificial light source to adjust and maintain a constant CO_2 level of 400 ppm and light required within the leaf cuvette). The measurements were done in the fully expanded (3rd leaf from top) leaf of randomly selected representative plants. IRGA majorly consists of a main console that harbors separate detectors for two different apparent hetero-atomic molecules CO_2 and H_2O involved during photosynthesis, an internal air supply unit a leaf chamber to clamp the leaves and the necessary software for the computation of gas exchange parameters. Shrivastava (2025) It accommodates a leaf area of 3.00 cm^2 and equipped to measure the light intensity in the PAR range by a point quantum sensor, relative humidity by a thermocouple and temperature of the air by at thermostat. Tasneem (2025) Butyl rubber tubing is used to carry air from the leaf chamber and into the IRGA. In addition leaf level water use efficiency was calculated by using gas exchange data and expressed as instantaneous water use efficiency. Instantaneous water use efficiency was also calculated by using following formula as indicated Eq. 6

Instantaneous WUE = Net photosynthetic rate (A) / Transpiration rate (E) (Eq.6)

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using a randomized complete block design. Mean comparisons among treatments were performed using the Least Significant Difference (LSD) test at the 5% probability level. Correlation analysis was conducted to determine relationships among physiological parameters under stress conditions.

Results

The sixty-eight rice genotypes exhibited significant variability in their physiological and biochemical responses under drought, industrial pollution, and combined stress conditions. The magnitude of reduction in growth and physiological parameters was greater under combined drought and industrial pollution than under individual stress treatments, indicating a synergistic interaction between water deficit and heavy metal toxicity. Analysis of variance revealed significant differences ($P \leq 0.05$) among genotypes, treatments, and genotype $\times$ treatment interactions for most physiological parameters evaluated.

Physiological responses under drought and industrial pollution

SPAD chlorophyll values ranged from 24.8 to 41.6 under drought stress, 23.5 to 40.2 under industrial pollution, and 20.3 to 37.8 under combined stress. Compared with the control, average chlorophyll content has been declined by 11.8% under drought, 15.4% under industrial pollution, and 24.9% under combined stress. However, tolerant genotypes such as Poornima, Indira Barani Dhan 1 and Samleshwari maintained comparatively higher chlorophyll content than susceptible cultivars including Mahamaya, Rajeshwari and Swarna.

Leaf temperature increased progressively under stress treatments due to reduced transpiration and impaired stomatal regulation. Mean canopy temperature increased from 26.4°C under control to 30.2°C under drought, 31.1°C under industrial pollution, and 33.3°C under combined stress. The increase was particularly pronounced in susceptible genotypes, whereas tolerant lines maintained relatively cooler canopies through better stomatal regulation.

Relative water content (RWC) also declined significantly under stress. Mean RWC decreased from 91.2% in control plants to 74.5% under drought, 71.6% under industrial pollution, and 63.4% under combined stress, indicating substantial impairment of plant water relations.

Photosynthetic characteristics

Drought and industrial pollution substantially reduced photosynthetic performance (Table 5). Net photosynthetic rate declined by 28.4% under drought, 34.8% under industrial pollution, and 48.6% under combined stress compared with control plants.

Tolerant rice genotypes maintained significantly higher photosynthetic rates despite stress conditions. Indira Barani Dhan 1 recorded the highest photosynthetic rate under combined stress ($13.4 \mu\text{mol m}^{-2} \text{s}^{-1}$), followed by Poornima ($12.9 \mu\text{mol m}^{-2} \text{s}^{-1}$) and Samleshwari ($11.9 \mu\text{mol m}^{-2} \text{s}^{-1}$). In contrast, susceptible genotype Rajeshwari exhibited only $8.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, representing nearly a 57% reduction compared with control conditions.

Transpiration rate and stomatal conductance showed similar declining trends. Mean transpiration decreased by 18.6%, 26.8%, and 41.2% under drought, industrial pollution, and combined stress, respectively. Stomatal conductance declined by nearly 56% under combined stress, resulting in restricted CO_2 diffusion into leaves and reduced photosynthetic carbon assimilation.

Intercellular CO₂ concentration exhibited contrasting responses. Although stomatal closure reduced CO₂ availability under drought, industrial pollution impaired mesophyll utilization of carbon dioxide, resulting in moderate accumulation of internal CO₂ in susceptible genotypes.

Chlorophyll pigments

The chlorophyll pigments responded markedly to environmental stress. Total chlorophyll content declined from 4.61 mg g⁻¹ under control conditions to 4.02 mg g⁻¹ under drought, 3.82 mg g⁻¹ under industrial pollution, and 3.28 mg g⁻¹ under combined stress.

Chlorophyll-a was more severely affected than chlorophyll-b, indicating preferential degradation of photosystem reaction centres under oxidative stress. Carotenoid concentration decreased moderately but remained comparatively higher in tolerant genotypes, suggesting enhanced antioxidant protection against reactive oxygen species.

Among the evaluated rice lines, Indira Barani Dhan 1 retained the highest chlorophyll content under combined stress (3.29 mg g⁻¹ total chlorophyll), whereas Rajeshwari exhibited the greatest pigment degradation (2.61 mg g⁻¹).

Table.1 Variability selected stress physiological traits of rice lines grown under ambient and elevated Drought conditions

Parameter	SPAD Chlorophyll Meter Reading SCMR- (Leaf chlorophyll)			Leaf Temperature-Infrared (IR) thermometer readings (°C)		
	Elevated D	Ambient D	Average	Elevated D	Ambient D	Average
Gross Mean	29.11	31.05	35.67	28.34	27.79	28.06
Range	29.0-43.4	21.9-49.3	25.2-47.4	23.1-35.9	24.7-33.3	24.5-33.1
CD@0.05	G=3.44 (±1.23)			G=1.6(±0.57)		
	T=0.59(±0.21)			T=0.27(±0.09)		
	G*T=4.86 (±1.74)			G*T=2.26(±0.81)		

Biomass accumulation and yield attributes

Plant growth parameters were significantly influenced by drought and industrial pollution. Combined stress reduced plant height by 17.3%, total dry matter accumulation by 34.6%, and grain yield by 41.5% compared with control plants.

Indira Barani Dhan 1 and Poornima recorded the smallest reduction in biomass accumulation (approximately 20–24%), whereas susceptible cultivars experienced reductions exceeding 40%. The maintenance of higher biomass under stress reflected superior photosynthetic capacity, improved water conservation, and greater chlorophyll stability.

Heavy metal accumulation

Heavy metal analysis indicated significantly greater accumulation of cadmium, lead, and chromium in roots than shoots. Root tissues accumulated approximately 2.5–3.0 times higher concentrations than leaves, suggesting restricted translocation of toxic metals to aerial tissues.

Tolerant rice genotypes exhibited lower translocation factors and higher sequestration of heavy metals within root tissues, thereby minimizing toxicity to photosynthetic organs.

Correlation analysis

Correlation analysis revealed strong positive relationships between chlorophyll content and photosynthetic rate ($r = 0.84$), relative water content and biomass accumulation ($r = 0.79$), and stomatal conductance and transpiration rate ($r = 0.88$).

Leaf temperature exhibited significant negative correlations with photosynthetic rate ($r = -0.81$), chlorophyll content ($r = -0.77$), and grain yield ($r = -0.73$), indicating that cooler canopy temperature is an important physiological indicator of stress tolerance.

Discussion

The present investigation demonstrated that drought and industrial pollution substantially impaired physiological performance and growth of rice genotypes. Water deficit reduced stomatal conductance, thereby restricting carbon dioxide diffusion into mesophyll tissues and limiting photosynthetic carbon fixation. Naaz and Mehrotra (2025) Simultaneously, industrial pollutants, particularly cadmium, lead, and chromium, disrupted chloroplast ultrastructure, inhibited chlorophyll biosynthesis, and induced oxidative damage through excessive generation of reactive oxygen species.

The greater reduction observed under combined stress compared with individual stress treatments indicates a synergistic interaction between drought and heavy metal toxicity. Water deficit decreases transpiration-driven nutrient transport, thereby increasing heavy metal concentration around root surfaces. Heavy metals further impair root membrane integrity, reducing water uptake and aggravating drought injury. Consequently, plants experience severe reductions in chlorophyll content, photosynthesis, and biomass accumulation.

Maintenance of higher chlorophyll concentration by Indira Barani Dhan 1, Poornima and Samleshwari suggests greater stability of the photosynthetic apparatus under stress. Higher chlorophyll retention allows sustained light harvesting and carbon assimilation, resulting in improved biomass production. Similarly, cooler canopy temperatures observed in tolerant genotypes indicate efficient transpirational cooling and better stomatal regulation.

Industrial pollution significantly reduced chlorophyll-a concentration, reflecting degradation of reaction centre pigments responsible for photochemical activity. The comparatively smaller decline in carotenoids suggests activation of antioxidant defense mechanisms, which protect chloroplast membranes from oxidative injury. Similar physiological responses have been reported in rice exposed to cadmium and chromium contamination, where carotenoids function as non-enzymatic antioxidants against photooxidative damage.

The reduction in relative water content under combined stress demonstrates impairment of plant water status. Tolerant genotypes maintained higher leaf hydration through improved osmotic adjustment and efficient root water uptake. Better water status contributed to sustained stomatal opening, higher photosynthetic rate, and improved biomass accumulation.

physiological traits including chlorophyll stability, canopy temperature depression, relative water content, stomatal conductance, and photosynthetic rate proved effective indicators for identifying drought- and pollution-tolerant rice genotypes. These parameters may therefore serve as reliable selection criteria in breeding programmes aimed at developing climate-resilient rice cultivars for environmentally stressed regions.

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