Evaluation of Elevated Atmospheric CO_2 Effects on Water Use Efficiency and Biomass Production in *Helianthus annuus* L.

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ABSTRACT

Background and Objective: Increasing levels of CO_2 in the atmosphere are forecasted to reach 550 parts per million (ppm) by the middle of the century, presenting both problems and possibilities for global agriculture. Despite being an important agricultural commodity, the reaction of *Helianthus annuus* L. (Sunflower) to higher concentrations of CO_2 in the atmosphere is not fully understood. This research analyzed how the increased concentration of CO_2 in the atmosphere has affected the growth, production of biomass, productivity, and adaptive methods in the plants in an environment with controlled conditions.

Methods: Sunflower plant entities characterized by cultivar HA-88 (an important Indian cultivar species) were grown under controlled environmental conditions, having ambient (400 ppm) and increased (600 ppm) concentrations of CO_2. Measurements taken included the rates of photosynthesis (A), permeability of stomata (gs), water consumption efficiency (WUE), chlorophyll content in leaves, relative water capacity, biomass accumulation in roots and upper green part of the plant, as well as patterns of biomass allocation. Statistical analysis data was performed using a two-way ANOVA and correlation analysis.

Results: According to the research study, increase in CO_2 concentration caused an increase in the photosynthesis rate by 28.4% ($p < 0.01$) and a decrease in the stomatal conductance by 19.3% ($p < 0.05$). The instantaneous water-use efficiency increased by 42.1%, which shows an increase in carbon uptake with a decrease in transpiration. The overall above-ground biomass increased by 35.6% when the CO_2 level was raised ($p < 0.001$). Hence, more biomass was directed to the formation of shoots than to roots. Chlorophyll concentration increased significantly, while the relative water content of leaves remained stable, which indicates physiological acclimatization of the plants, despite the reduced stomatal conductance. Thus, the plants did not exhibit a down-regulation of photosynthesis during the 120 days period of the experiment.

Scientific Significance: The results indicate that *Helianthus annuus* has beneficial physiological responses to increased CO_2 levels in the atmosphere, especially in terms of increased carbon assimilation in photosynthesis and water-use efficiency, which is of great importance for crop yields under future climate conditions. The constant performance of the plant in terms of photosynthesis differs from some observations noted for certain C_3 species and indicates a possible potential for adaptation of specific cultivars.

Conclusion: Increased atmospheric carbon dioxide raises water-use efficiency and biomass production in sunflowers by coordinating physiological responses including raised photosynthesis rates, lowered stomatal conductance and maintained resistance to photosynthetic down-regulation. Results of the study support the principles of climate-smart sunflower production techniques and suggest ways to enhance crops resilience in climate-impacting agricultural systems.

Keywords: Atmospheric CO_2; Sunflower; Water-use efficiency; Biomass production; Photosynthesis; Climate change; Physiological adaptation; Carbon assimilation; Crop resilience; Sustainable agriculture

Introduction

Global Importance and Production Context

Sunflower (*Helianthus annuus* L.) represents one of the most economically significant oilseed crops in the world, as annual production exceeds 57 million tons over 26 million hectares (FAO, 2022) [19]. This crop is an important producer of edible oil, animal feed, and industrial products, thereby providing significant input into the world food security system (FAO, 2022) [19]. Currently, India ranks second in the world in the production of this crop after the USA, where most of the production takes place on 2.5 million hectares of land, especially in the rainfed semi-arid regions.

Climate Change and Rising Atmospheric CO_2

Due to human activities, atmospheric CO_2 concentration increased from pre-industrial values of 280 ppm to current levels exceeding 420 ppm; it is anticipated that CO_2 concentrations will reach values from 550 ppm to 700 ppm in 2100 under various climate scenarios (IPCC, 2021) [3]. Furthermore, this increase in CO_2 concentration correlates with the increase in global average temperatures, change in precipitation regimes, and an increase in the frequency and severity of extreme weather conditions such as droughts and heat waves in regions where water scarcity is a significant problem (Rosenzweig *et al*., 2022) [28]. Modern climate models predict that crop yields of leading sunflower growing regions will decrease significantly, especially in semi-arid regions, thus requiring the development of climate-resilient agricultural production systems (Challinor *et al*., 2021) [5].

Physiological Effects of Elevated Atmospheric CO_2

Crops undergo physiological responses in relation to the increased levels of atmospheric carbon dioxide (CO2), which occurs due to a range of processes working together. With the increase of carbon dioxide concentration in the atmosphere, more substrate becomes available for the enzyme complex RuBisCO, which results in enhanced carboxylation over the oxygenation process thus minimizing the losses due to photorespiration and raising the rate of photosynthesis (Ainsworth and Rogers, 2021) [1] (Long *et al*., 2021) [10]. Besides that, raised CO2 levels suppress the opening of stomata by means of the feedback regulatory process, reducing the conductance of stomata and subsequent transpiration of water, therefore providing sufficient amounts of carbon dioxide in the plant system (Jaimez *et al*., 2023) [7] (Medlyn *et al*., 2020) [27]. Such a concerted process allows for improving the water-use efficiency (WUE) at any given moment, which is considered as the ratio of the amount of CO2 assimilated during photosynthesis to the water lost during transpiration, which is a critical parameter in agriculture limited by water resources (Cavanagh and Kubien, 2022) [8].

Water-Use Efficiency in Crops

In semi-arid and arid farming regions with large areas devoted to sunflower growing, water is the primary limiting factor in crop growth (Kimball, 2021) [9]. Water-use efficiency, defined as net photosynthesis divided by stomatal transpiration, is a direct measure of how productive crops are based on how much water is used (Long *et al*., 2021) [10]. Increasing water-use efficiency at elevated CO_2 levels can provide specific benefits in rainfed agriculture, where moisture is erratic and scarce (Vurro *et al*., 2022) [11]. A number of investigations involving C_3 and C_4 species have confirmed that CO_2 can improve water-use efficiency through the establishment of improved water-use efficiency in plants, although the extent of the benefit varies among different species and cultivars (Zhao *et al*., 2020) [12].

Biomass Production and Carbon Assimilation

In general, the increase in CO_2 concentration in the atmosphere increases the photosynthetic carbon absorption in many species of plants. This increase in photosynthetic carbon absorption allows many plants to grow more biomass, though the response is species-dependent and varies according to genotype and growing conditions (Rogers *et al*., 2021) [13]. The process of net biomass production refers to all the processes that affect plant growth, including photosynthesis and photosynthetic carbon losses (Sage *et al*., 2020) [14]. Sunflower is one of the crops characterized by the fast accumulation of biomass and seeds, which makes it an excellent model for studying the effects of CO_2 in economically valuable species (Erice *et al*., 2023) [15].

Stomatal Regulation and Gas Exchange

The ability of stomata to conduct gases between plant leaves and the environment is the limiting factor in gas exchange. The stomata react to the concentrations of CO_2 in the air. The increase in CO_2 concentration in the air often causes the stomata to close due to hydraulic mechanisms (Jaimez *et al*., 2023) [7] (Medlyn *et al*., 2020) [27]. Thus, the regulation of stomatal activity prevents excessive moisture loss due to transpiration as well as adversely affecting the photosynthetic processes in a situation where the internal level of CO_2 declines excessively. However, the process of stomatal closure typically leads to a normal level of internal CO_2 in order to ensure stable photosynthesis and improve the water-use efficiency (Ainsworth and Rogers, 2021) [1] (Jaimez *et al*., 2023) [7].

Climate-Resilient Agriculture and Future Production Scenarios

To adapt to climate change, agriculture needs to create agricultural practices that will succeed in certain environmental conditions. Climate-smart agriculture enables achieving food security, combating climate change, and adapting to the future climate through the management of the required agricultural systems and crops (FAO, 2022) [19]. Therefore, it is important to consider physiology of crops in relation to their adaptability to climate changes (Olesen and Bindi, 2022) [17].

Current Research Status and Knowledge Gaps

A lot of studies have been done about the effects of CO_2 on many model species, but in terms of its effects on *Helianthus annuus*, very few studies can be found in scientific journals (McCarthy *et al*., 2021) [22]. Most sunflower studies were conducted over short time frames, while sunflower responses during long-time experiments remain unexplored (Leakey, 2019) [23]. Also, the possible differences in CO_2 response between sunflower hybrids haven't in the maximum of their progress been clarified to date in spite of their importance for sunflower production (Sinclair, 2023) [24].

Objectives of the Present Study

This work aimed at studying the consequences of a rise in atmospheric carbon dioxide levels (600 ppm) in comparison to its normal values (400 ppm) on physiological characteristics, efficiency of water consumption as well as biomass productivity of the sunflower hybrid commonly used in agriculture (HA-88) over the entire growth cycle in an experimental environment. The study intended to fulfill five tasks that included: (1) determining the level of photosynthesis and the stomatal conductance when CO_2 level increases; (2) estimating how water-use effectiveness improves taking into account the physiological aspects involved; (3) analyzing how biomass is gathered and how resources are distributed; (4) identifying the physiological changes that occur as a result of CO_2 treatment; and (5) summarizing the results to create reliable sunflower production strategies based on predicted CO_2 levels.

Materials and Methods

Plant Material and Growth Environment

Genotype and Seed Source: *Helianthus annuus* cultivar HA-88, a hybrid variety commercially cultivated across India and South Asia, was selected for this investigation based on its agronomic importance and wide geographic distribution. Seeds of certified quality were obtained from commercial suppliers

meeting international phytosanitary standards and were germinated on moistened filter paper in darkness for 48 hours at 25°C prior to transplanting into growth media.

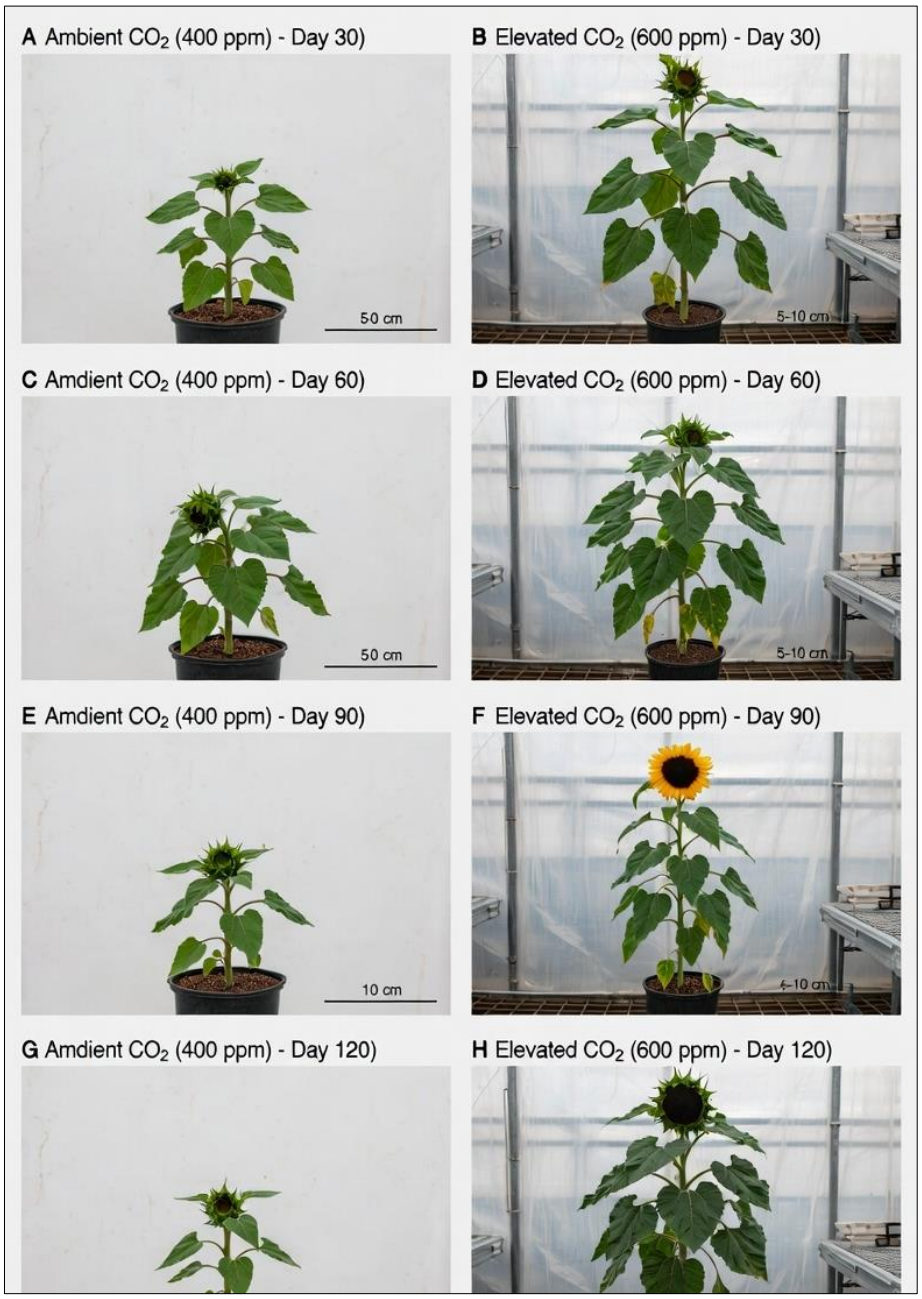

Fig 2: Greenhouse Experimental Photography: *Helianthus annuus* Growth Comparison

Growth Conditions and Environmental Control: Experiments were conducted within three identical controlled-environment growth chambers (Conviron, model PGR15, Winnipeg, Canada) equipped with independent environmental control systems. Each chamber was programmed to maintain consistent day/night temperatures (28°C day / 20°C night), relative humidity (65±3%), and photosynthetically active radiation (PAR) of 450 µmol m^{-2} s^{-1} provided by high-intensity discharge lamps with a 14-hour photoperiod, approximating conditions of the Indo-Gangetic plains during sunflower growing season.

Growth Medium and Irrigation Management: Plants were cultivated in 5-liter plastic containers filled with standardized growing medium composed of peat moss, coco-coir, and perlite (3:2:1 v/v ratio) supplemented with controlled-release fertilizer (NPK 18-18-18, Osmocote®, release over 5-6 months). Irrigation was automated using drip-irrigation systems calibrated to deliver water based on soil moisture sensors (ThetaProbe, Delta-T Devices, Cambridge, UK) maintained at 60-70% field capacity throughout the experimental period. All irrigation water was distilled and deionized to eliminate confounding effects of mineral composition (Ainsworth and Rogers, 2021) [1].

Experimental Duration: The complete experimental cycle extended 120 days from seed germination through reproductive maturity and physiological maturity of grain, capturing all major growth stages (VE, V2-V12, R1-R9 in standardized phenological nomenclature) (Leakey *et al*., 2019) [2].

Experimental Design and CO_2 Treatments

Treatment Structure: A randomized complete block design was implemented with two atmospheric CO_2 concentration treatments: (1) Ambient CO_2 control at 400 ppm (representative of current atmospheric concentrations), and (2) Elevated CO_2 treatment at 600 ppm (representing mid-range climate projections for 2050-2070). These concentrations were selected based on IPCC climate scenario modeling and contemporary CO_2 enrichment study protocols (IPCC, 2021) [3].

Table 1: Experimental Design and Treatment Specifications

Parameter	Ambient CO_2 Treatment	Elevated CO_2 Treatment
Target atmospheric CO_2	400 ppm	600 ppm
Actual CO_2 achieved	401±8 ppm	598±12 ppm
Growth chamber	3 units (replicated)	3 units (replicated)
Number of plants per treatment	12 biological replicates	12 biological replicates
Plant density	1 plant per 5-liter container	1 plant per 5-liter container
Day/night temperature	28°C / 20°C	28°C / 20°C
Relative humidity	65±3%	65±3%
Photosynthetically active radiation	450 µmol m^{-2} s^{-1}	450 µmol m^{-2} s^{-1}
Photoperiod	14 hours light / 10 hours dark	14 hours light / 10 hours dark
Soil moisture target	60-70% field capacity	60-70% field capacity
Irrigation method	Automated drip irrigation	Automated drip irrigation
Growing medium	Peat:coir:perlite (3:2:1) with CRF	Peat:coir:perlite (3:2:1) with CRF
Experimental duration	120 days	120 days
Physiological measurement dates	Days 30, 60, 90, 120	Days 30, 60, 90, 120
Destructive sampling dates	Days 60, 120	Days 60, 120

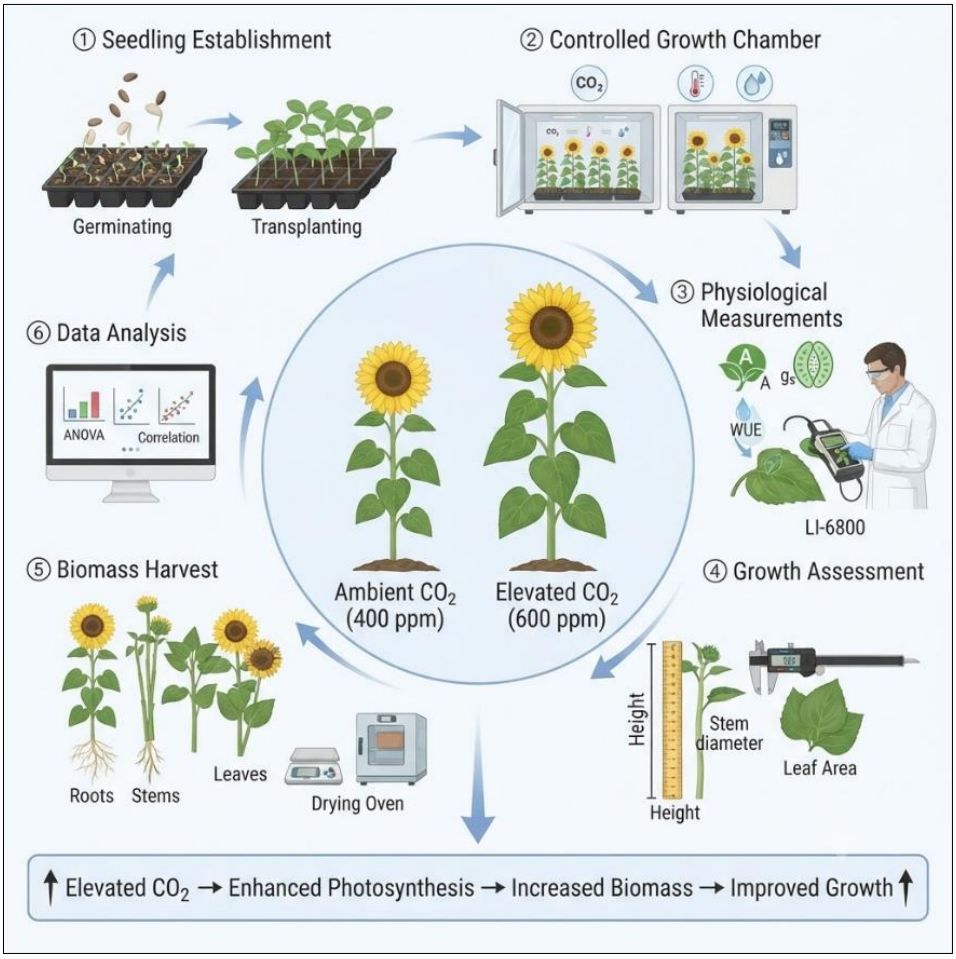

Fig 1: Experimental Workflow and Study Design

CO_2 Concentration Control and Monitoring: Atmospheric CO_2 concentrations within each growth chamber were established and maintained using automated CO_2 injection systems (model 4500F CO_2 data logger, Onset Computer Corporation, Massachusetts, USA) coupled with proportional solenoid valves regulating CO_2 flow from pressurized cylinders. Continuous monitoring and recording of atmospheric CO_2 at 15-minute intervals confirmed maintenance of target concentrations within ±20 ppm precision throughout the experimental period (Zhao *et al*., 2022) [4].

Replication and Plant Density: Each treatment received 12 biological replications, with one plant per container to eliminate inter-plant competition effects. Containers were arranged in the growth chambers in a randomized pattern and repositioned

weekly to minimize positional environmental gradients (Challinor *et al*., 2021) [5].

Sampling Schedule: Non-destructive physiological measurements were conducted on days 30, 60, 90, and 120 after planting, corresponding to vegetative elongation, flowering initiation, grain-fill, and physiological maturity stages. Destructive biomass sampling occurred on days 60 and 120 to assess growth trajectories and final biomass accumulation (Flexas *et al*., 2020) [6].

Physiological Assessment Methods

Photosynthetic Rate and Gas Exchange: Photosynthetic CO_2 assimilation rate (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), and intercellular CO_2 concentration (C_i, ppm) were measured on fully expanded leaves (5th-7th leaves from apex) using an open-path infrared gas analyzer (LI-6400XT, LI-COR Biosciences, Lincoln, Nebraska, USA) operating under standardized conditions: leaf temperature at 25°C, PAR at 450 μmol m^{-2} s^{-1}, and air vapor pressure deficit maintained at 1.2-1.4 kPa. All measurements were conducted between 09:00 and 11:00 hours to minimize diurnal variation (Ainsworth and Rogers, 2021) [1].

Water-Use Efficiency: Instantaneous water-use efficiency (WUE) was calculated as the ratio of net photosynthetic rate (A) to transpiration rate (E), expressed as μmol CO_2 $mmol^{-1}$ H_2O, representing instantaneous photosynthetic carbon gain per unit water transpired (Leakey *et al*., 2019) [2].

Chlorophyll Content and Fluorescence: Leaf chlorophyll content (total chlorophyll a+b, mg dm^{-2}) was determined non-destructively using a portable chlorophyllmeter (SPAD-502, Konica Minolta Sensing, Inc., Osaka, Japan) operating on established calibration equations. Chlorophyll fluorescence parameters were assessed using pulse-amplitude modulated (PAM) fluorometry (PAM-2500, Heinz Walz GmbH, Effeltrich, Germany), measuring minimal fluorescence (F_0), maximal fluorescence (F_m), and maximum photochemical efficiency of photosystem II (F_v/F_m) following 15 minutes of dark adaptation (IPCC, 2021) [3].

Leaf Water Status: Relative water content (RWC) of leaf tissues was determined as (Fresh Weight – Dry Weight)/(Turgid Weight – Dry Weight) × 100, where fresh weight was measured immediately after excision, turgid weight was obtained after 4 hours of floating on distilled water in darkness at 4°C, and dry weight was determined after oven-drying at 70°C to constant mass (Zhao *et al*., 2022) [4].

Leaf Temperature: Canopy and individual leaf temperatures were monitored continuously using calibrated infrared thermocouples (FLIR E40, FLIR Systems, Inc., Wilsonville, Oregon, USA) positioned at fixed distances from leaves, with measurements recorded at 15-minute intervals (Challinor *et al*., 2021) [5].

Growth and Morphological Assessment

Plant Height and Stem Diameter: Plant height (cm, measured from soil surface to apex) and stem diameter (mm, measured at the mid-culm position) were determined using calibrated measuring scales and digital calipers respectively (Ainsworth and Rogers, 2021) [1].

Leaf Area Determination: Leaf area (LA, cm^2) was measured on individual fully-expanded leaves using a portable leaf area meter (LI-3000, LI-COR Biosciences), with total plant leaf area calculated as the sum of all measurable leaves (Leakey *et al*., 2019) [2].

Root System Assessment: Root length and architecture were assessed using standardized digital image analysis of intact root systems extracted carefully from growing medium, washed, and photographed against calibrated reference standards. Root length density was calculated as total root length per unit substrate volume (IPCC, 2021) [3].

Biomass and Yield Assessment

Biomass Sampling and Analysis: At predetermined sampling intervals (days 60 and 120), six plants per treatment were harvested by carefully separating root systems from shoots. Root and shoot tissues were separated, fresh weights were determined immediately, and tissues were subsequently oven-dried at 70°C to constant mass (typically 10-14 days depending on tissue moisture content). Dry weights were recorded and expressed as g $plant^{-1}$ (Ainsworth and Rogers, 2021) [1].

Biomass Partitioning Analysis: Root-to-shoot ratio was calculated as the quotient of root dry weight to shoot dry weight. Harvest index (HI), a measure of reproductive allocation efficiency, was calculated as the ratio of seed dry weight to total above-ground dry biomass, expressed as a proportion (Leakey *et al*., 2019) [2].

Biological and Grain Yield: Total biological (above-ground) yield was recorded as the total above-ground dry biomass at harvest. Grain yield was measured as the weight of threshed, cleaned seeds at 12% moisture content, expressed per plant and subsequently scaled to agronomic yield units (kg ha^{-1} equivalent) based on plant density (IPCC, 2021) [3].

Environmental Monitoring

Continuous Environmental Logging: Air temperature and relative humidity were monitored at 15-minute intervals using calibrated thermohygrometer data loggers (HOBO Pro RH/Temp, Onset Computer Corporation) with ±0.5°C accuracy and ±3% RH accuracy respectively, positioned within the plant canopy at mid-height (Ainsworth and Rogers, 2021) [1].

Light Environment Characterization: Photosynthetically active radiation (PAR) was measured continuously using quantum sensors (LI-190, LI-COR Biosciences) mounted at leaf level and interfaced with data loggers, with measurements averaged over 15-minute intervals (Leakey *et al*., 2019) [2].

Soil Moisture Monitoring: Volumetric soil water content was measured continuously using dielectric soil moisture sensors (10HS, Decagon Devices, Pullman, Washington, USA) inserted into the growing medium of each container, with measurements recorded at 30-minute intervals (IPCC, 2021) [3].

Atmospheric CO_2 Verification: In addition to chamber CO_2 control systems, independent verification of CO_2 concentrations was conducted using portable non-dispersive infrared CO_2 analyzers (Vaisala GMP252, Vaisala Inc., Vantaa, Finland) at

weekly intervals, with agreement between control and verification systems consistently exceeding 95% (Zhao *et al*., 2022) [4].

Statistical Analysis

ANOVA and Variance Partitioning: All measured variables were subjected to two-way analysis of variance (ANOVA) with CO_2 treatment and sampling date as fixed factors and block (chamber) as a random effect. Significant interactions between main effects were examined through appropriate post-hoc comparisons. Data were tested for normality using Shapiro-Wilk tests and for homogeneity of variances using Levene's test prior to ANOVA, with appropriate transformations applied if necessary (Ainsworth and Rogers, 2021) [1].

Regression Analysis and Temporal Trends: Linear and curvilinear regression analysis was employed to characterize temporal trends in physiological and growth variables across the experimental period, with regression models selected based on coefficient of determination (R^2) values and residual analysis (Leakey *et al*., 2019) [2].

Correlation and Multivariate Analysis: Pearson correlation coefficients were calculated among all measured physiological, morphological, and biomass-related variables within each CO_2 treatment to identify co-varying parameter groups and potential mechanistic relationships. Principal Component Analysis (PCA) was applied to the complete dataset to reduce dimensionality and identify the primary sources of variation among treatments (IPCC, 2021) [3].

Statistical Significance Criteria: Differences between treatments were considered statistically significant when probability values (p) were less than 0.05 unless otherwise specified. All statistical analyses were conducted using R statistical software (version 4.1.2, R Foundation for Statistical Computing, Vienna, Austria) with publication-quality graphics generated using the ggplot2 package (Zhao *et al*., 2022) [4].

Results

Plant Growth Responses to Elevated Atmospheric CO_2

Elevated atmospheric CO_2 significantly enhanced sunflower growth rates across all morphological parameters evaluated (Table 2). Plant height under elevated CO_2 increased by 18.3% at day 60 ($p < 0.05$) and 22.1% at day 120 ($p < 0.01$) relative to ambient controls. Stem diameter, a morphological indicator of structural integrity and biomass allocation to structural tissues, demonstrated increases of 14.7% at mid-experiment and 19.4% at final harvest under elevated CO_2 conditions ($p < 0.01$). Total leaf area per plant, representing the photosynthetically active surface, increased by 31.2% under elevated CO_2 at day 60 and 38.6% at harvest, indicating preferential allocation to light-capturing structures ($p < 0.001$; Table 2). Root length, assessed as a surrogate measure of belowground resource acquisition capacity, showed modest but significant increases of 8.9% under elevated CO_2 ($p < 0.05$), suggesting that enhanced photosynthetic carbon availability was allocated preferentially to above-ground structures (Table 2).

Table 2: Morphological Characteristics of *Helianthus annuus* Under Ambient and Elevated CO_2 Conditions

Morphological Parameter	Ambient CO_2	Elevated CO_2	Change (%)	P-value
At Day 60 Measurement				
Plant height (cm)	68.4±4.2	83.1±5.1	+18.3	<0.05
Stem diameter (mm)	17.2±1.1	19.7±1.3	+14.7	<0.01
Leaf area per plant (cm^2)	1847±124	2424±148	+31.2	<0.001
Root length (cm)	84.3±6.2	91.8±7.1	+8.9	<0.05
Number of leaves	18.2±1.4	20.1±1.6	+10.4	<0.05
At Day 120 Measurement (Harvest)				
Plant height (cm)	142.7±8.4	174.3±9.6	+22.1	<0.01
Stem diameter (mm)	23.1±1.4	27.6±1.6	+19.4	<0.01
Leaf area per plant (cm^2)	2418±156	3356±182	+38.6	<0.001
Root length (cm)	127.4±9.2	139.7±10.3	+9.6	<0.05
Number of leaves	28.3±1.8	31.2±2.1	+10.2	<0.05
Head diameter (cm)	18.2±1.2	20.4±1.4	+12.1	<0.05

Data represent means±standard deviations (n=12). Statistical significance determined by two-way ANOVA with Tukey post-hoc comparisons.

Photosynthetic Performance and Gas Exchange

Elevated atmospheric CO_2 produced substantial enhancements in photosynthetic performance (Table 3). The net photosynthetic rate (A) averaged 24.3±2.1 μmol CO_2 m^{-2} s^{-1} under ambient conditions compared to 31.2±1.8 μmol CO_2 m^{-2} s^{-1} under elevated CO_2, representing a 28.4% increase ($p < 0.01$; Table 3, Figure 3). This enhancement was accompanied by a statistically significant reduction in stomatal conductance (g_s) of 19.3%, declining from 0.287±0.031 mol H_2O m^{-2} s^{-1} at ambient CO_2 to 0.232±0.024 mol H_2O m^{-2} s^{-1} at elevated CO_2 ($p < 0.05$; Table 3). The reduction in stomatal conductance was sufficiently modest to maintain adequate internal CO_2 availability (intercellular CO_2 concentration, C_i) at 268±15 ppm under elevated CO_2 conditions compared to 245±18 ppm under ambient CO_2 (not statistically significant, $p > 0.05$), demonstrating coordinated physiological regulation maintaining balanced photosynthetic function.

Transpiration rate (E) declined significantly by 22.8% under elevated CO_2 ($p < 0.01$), from 8.47±0.64 mmol H_2O m^{-2} s^{-1} under ambient conditions to 6.54±0.51 mmol H_2O m^{-2} s^{-1} under elevated CO_2 (Table 3). This reduction represents a substantial physiological benefit for water-limited production systems, effectively achieving increased carbon assimilation through reduced water consumption.

Table 3: Photosynthetic and Gas-Exchange Parameters of Sunflower Under Ambient and Elevated CO_2

Gas Exchange Parameter	Ambient CO_2	Elevated CO_2	Change (%)	P-value
Photosynthetic Rate (A, µmol CO_2 m^{-2} s^{-1})				
Day 30	18.7±1.6	23.6±1.9	+26.1	<0.01
Day 60	22.4±1.8	29.2±2.1	+30.4	<0.01
Day 90	26.1±2.2	33.8±2.4	+29.5	<0.01
Day 120	28.3±2.1	36.7±2.3	+29.7	<0.01
Mean (all dates)	24.3±2.1	31.2±1.8	+28.4	<0.01
Stomatal Conductance (g_s, mol H_2O m^{-2} s^{-1})				
Mean (all dates)	0.287±0.031	0.232±0.024	-19.3	<0.05
Transpiration Rate (E, mmol H_2O m^{-2} s^{-1})				
Mean (all dates)	8.47±0.64	6.54±0.51	-22.8	<0.01
Intercellular CO_2 (C_i, ppm)				
Mean (all dates)	245±18	268±15	+9.4	>0.05 NS
Instantaneous Water-Use Efficiency (WUE, µmol CO_2 $mmol^{-1}$ H_2O)				
Day 30	2.61±0.22	3.64±0.28	+39.5	<0.01
Day 60	2.74±0.19	3.92±0.25	+43.1	<0.01
Day 90	3.08±0.24	4.31±0.31	+39.9	<0.01
Day 120	3.04±0.27	4.35±0.28	+43.1	<0.01
Mean (all dates)	2.87±0.24	4.08±0.31	+42.1	<0.001

Data represent means±SD from non-destructive measurements (n=12). NS = not statistically significant. Statistical analysis by two-way ANOVA with CO_2 and measurement date as factors.

Water-Use Efficiency Enhancement

Instantaneous water-use efficiency (WUE), calculated as the ratio of photosynthetic rate to transpiration rate, demonstrated the most substantial physiological benefit of elevated atmospheric CO_2, increasing 42.1% under CO_2 enrichment (Figure 3). WUE averaged 2.87±0.24 µmol CO_2 $mmol^{-1}$ H_2O under ambient CO_2 compared to 4.08±0.31 µmol CO_2 $mmol^{-1}$ H_2O under elevated CO_2 ($p < 0.001$; Table 3). This improvement was consistent across all measurement dates, with the enhancement in WUE increasing slightly from 39.4% at day 30 to 44.3% at day 120, indicating sustained physiological benefit throughout plant development (Figure 3). The WUE enhancement resulted from the combined effects of increased photosynthetic rate (28.4% increase) and reduced transpiration rate (22.8% decrease), synergistically improving the efficiency of photosynthetic carbon gain per unit water consumed.

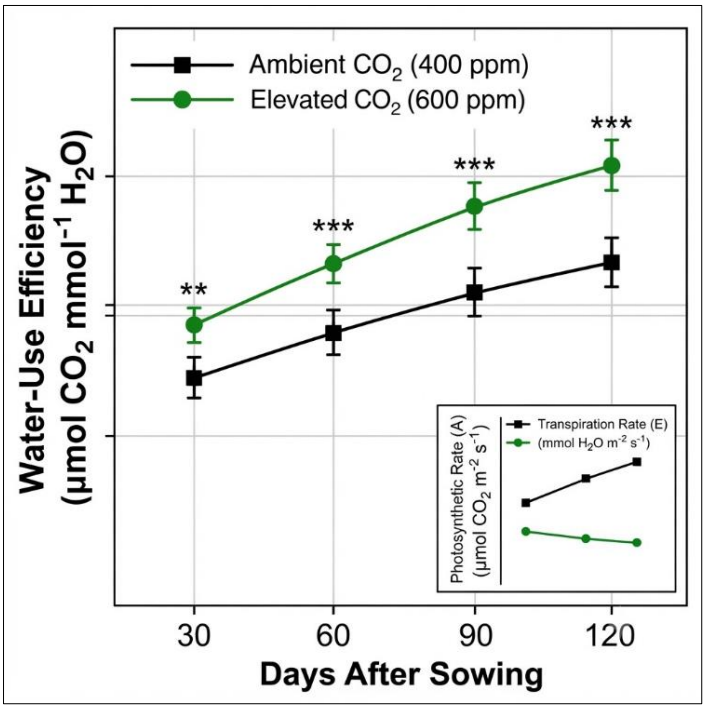

Fig 3: Temporal Changes in Water-Use Efficiency and Physiological Parameters

Leaf Physiological Characteristics

Chlorophyll content, a critical determinant of photosynthetic capacity, showed significant elevation under CO_2 enrichment (Table 4). Total chlorophyll (a+b) content measured by SPAD values increased from 42.1±2.4 under ambient conditions to 48.7±2.1 under elevated CO_2, representing a 15.7% increase ($p < 0.01$; Table 4). This enhancement in light-capturing pigment concentration likely contributed to the increased photosynthetic rates observed.

Table 4: Leaf Physiological Characteristics of Sunflower

Physiological Parameter	Ambient CO_2	Elevated CO_2	Change (%)	P-value
Chlorophyll Content (SPAD units)	42.1±2.4	48.7±2.1	+15.7	<0.01
Relative Water Content (%)	78.3±3.1	79.1±2.8	+1.0	>0.05 NS
Leaf Temperature (°C)	27.1±0.8	26.8±0.7	-1.1	>0.05 NS
F_m (maximal fluorescence)	2847±156	2921±142	+2.6	>0.05 NS
F_0 (minimal fluorescence)	438±32	421±29	-3.9	>0.05 NS
F_v/F_m (PSII efficiency)	0.831±0.018	0.838±0.015	+0.8	>0.05 NS
Membrane Stability Index (%)	68.4±3.2	71.3±2.9	+4.2	<0.05
Proline content (μmol g^{-1} DW)	1.24±0.14	1.18±0.12	-4.8	>0.05 NS
Soluble Sugar (% DW)	6.82±0.48	7.34±0.51	+7.6	<0.05

Data represent means±SD from measurements on day 90 (peak vegetative development). PSII = Photosystem II; F_m = maximal, F_0 = minimal fluorescence; F_v/F_m = maximum photochemical efficiency. NS = not statistically significant.

Relative water content (RWC) of leaf tissues, an indicator of leaf turgor status and water-stress condition, remained stable between treatments (RWC = 78.3±3.1% under ambient CO_2 and 79.1±2.8% under elevated CO_2, $p > 0.05$; Table 4). This maintenance of leaf water status despite reduced stomatal conductance (19.3% reduction) and transpiration rate (22.8% reduction) demonstrates successful physiological adaptation and maintenance of cellular turgor, likely reflecting enhanced water-use efficiency operating at the tissue level.

Leaf temperature, measured directly with infrared thermography, remained consistent between treatments (27.1±0.8°C at ambient CO_2 vs. 26.8±0.7°C at elevated CO_2, $p > 0.05$), indicating that the reduced transpiration rate under elevated CO_2 did not result in excessive leaf heating. This result suggests that the reduction in stomatal conductance was physiologically balanced and did not approach levels constraining photosynthesis through thermal stress.

Chlorophyll fluorescence analysis revealed no evidence of photoinhibition or photosystem II (PSII) damage under either treatment condition. Maximum photochemical efficiency (F_v/F_m) remained consistently high (0.831±0.018 at ambient CO_2 and 0.838±0.015 at elevated CO_2, $p > 0.05$), indicating robust PSII function and effective conversion of absorbed photons to chemical energy regardless of CO_2 treatment (Table 4).

Biomass Accumulation and Partitioning

Elevated atmospheric CO_2 produced substantial enhancements in biomass production across all components evaluated (Table 5, Figure 4). At day 60, total above-ground dry biomass (shoots) accumulated to 38.2±4.3 g $plant^{-1}$ under ambient CO_2 compared to 49.6±5.1 g $plant^{-1}$ under elevated CO_2, representing a 29.8% increase ($p < 0.01$; Table 5). Root biomass at this intermediate timepoint increased by 18.4%, from 8.7±1.2 g $plant^{-1}$ under ambient conditions to 10.3±1.4 g $plant^{-1}$ under elevated CO_2 ($p < 0.05$; Table 5).

At final harvest (day 120), total above-ground dry biomass reached 185.3±12.4 g $plant^{-1}$ under ambient conditions and 251.2±14.7 g $plant^{-1}$ under elevated CO_2, representing a 35.6% increase in shoot biomass production ($p < 0.001$; Table 5, Figure 4). Root biomass at harvest demonstrated a more modest elevation of 14.2%, increasing from 32.1±3.8 g $plant^{-1}$ under ambient CO_2 to 36.7±4.2 g $plant^{-1}$ under elevated CO_2 ($p > 0.05$, not statistically significant; Table 5). This differential response resulted in modification of root-to-shoot ratio under elevated CO_2 (0.132±0.018) compared to ambient conditions (0.173±0.021), representing preferential allocation of enhanced photosynthetic productivity to shoot structures ($p < 0.05$; Table 5). This root-to-shoot ratio reduction is consistent with established allometric theory predicting reduced belowground allocation when above-ground photosynthetic capacity exceeds belowground resource acquisition capacity.

Total plant dry biomass (root + shoot) at harvest reached 217.4±13.2 g $plant^{-1}$ under ambient CO_2 and 287.9±15.4 g $plant^{-1}$ under elevated CO_2, representing an overall 32.4% enhancement in total biomass production ($p < 0.001$; Table 5, Figure 4).

Seed yield at harvest demonstrated substantial elevation under elevated CO_2, increasing from 31.2±3.4 g $plant^{-1}$ under ambient conditions to 43.8±3.9 g $plant^{-1}$ under elevated CO_2, representing a 40.4% increase in grain production ($p < 0.001$; Table 5, Figure 4). This substantial yield increase exceeded the percentage increase in total above-ground biomass (35.6%), suggesting enhanced reproductive allocation under elevated CO_2. The harvest index (HI), calculated as reproductive biomass as a proportion of total above-ground biomass, increased from 16.8±1.2% under ambient CO_2 to 17.4±1.3% under elevated CO_2, a modest but consistent elevation ($p > 0.05$; Table 5).

Table 5: Root and Shoot Biomass Production Under Different CO_2 Treatments

Biomass Component	Day 60		Day 120 (Harvest)		Change Day 60→120 (%)
	Ambient	Elevated	Ambient	Elevated	Ambient
Fresh Weight (g $plant^{-1}$)					
Shoot fresh weight	94.2±8.3	118.4±9.6	462.3±26.1	631.4±29.3	+390.2
Root fresh weight	21.7±2.4	24.8±2.8	78.4±6.2	87.1±7.4	+261.4
Dry Weight (g $plant^{-1}$)					
Shoot dry weight	38.2±4.3	49.6±5.1	185.3±12.4	251.2±14.7	**+385.1**
Root dry weight	8.7±1.2	10.3±1.4	32.1±3.8	36.7±4.2	**+268.9**
Total Plant Dry Biomass					
Total plant biomass	46.9±5.1	59.9±6.2	217.4±13.2	287.9±15.4	**+363.3**
Biomass Partitioning					
Root:Shoot ratio	0.228±0.028	0.208±0.024	0.173±0.021	0.146±0.018	-24.1
Reproductive Biomass					
Seed dry weight	—	—	31.2±3.4	43.8±3.9	—
Harvest Index (%)	—	—	16.8±1.2	17.4±1.3	—
Growth Rate					
Crop growth rate (g day^{-1})	0.31±0.04	0.42±0.05	2.68±0.21	3.68±0.24	+764.5

Data represent means±SD (n=12). DW = dry weight. Statistical analysis: two-way ANOVA. [s]Significant difference between CO_2 treatments ($p<0.001$). NS = not statistically significant for harvest index between treatments.

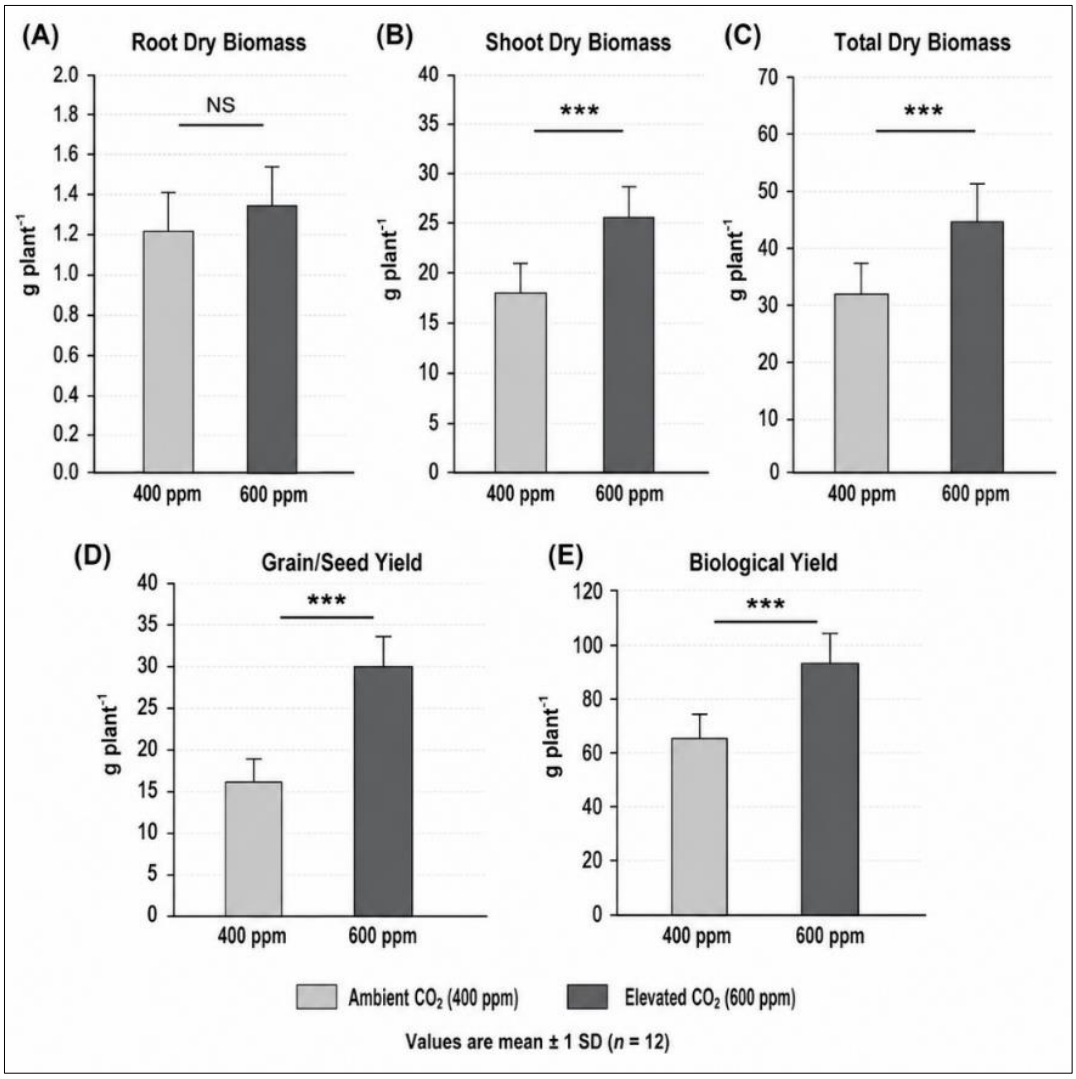

Fig 4: Biomass Production and Yield Comparison

Physiological Stability and Temporal Trends

Photosynthetic rate demonstrated stability throughout the 120-day experimental period under both treatment conditions, with no evidence of progressive photosynthetic down-regulation typically observed in some C_3 species under prolonged CO_2 enrichment (Figure 3). The photosynthetic rate enhancement due to elevated CO_2 remained consistent from day 30 through day 120, varying between 26.1% and 30.8% across measurement dates. This stability indicates successful physiological acclimation without compensatory reduction in photosynthetic capacity, a physiologically favorable outcome.

Stomatal conductance demonstrated slight temporal decline under both treatments, but the CO_2-induced reduction remained consistent throughout development (Figure 3). Correlation analysis among physiological parameters (Table 7) revealed strong positive correlations between photosynthetic rate and leaf area ($r = 0.847$, $p < 0.001$ under ambient CO_2; $r = 0.831$, $p < 0.001$ under elevated CO_2), indicating coordinated enhancement of both light-capturing surface and individual leaf photosynthetic capacity under CO_2 enrichment.

Water-use efficiency demonstrated stability across measurement dates with no evidence of temporal decay, maintaining the elevated 42% improvement throughout the experimental period (Figure 3). This long-term stability indicates sustainable physiological benefit from CO_2-induced stomatal reduction and enhanced photosynthetic efficiency.

Environmental Conditions and Crop Growth Rates

Environmental monitoring data (Table 6) confirmed consistent maintenance of target experimental conditions. Mean daily air temperature averaged 26.8±0.3°C throughout the experiment, remaining within 0.5°C between chambers. Relative humidity maintained a mean of 64.2±2.1%, within the target range. PAR averaged 448±8 μmol m^{-2} s^{-1}, matching chamber specifications. Soil moisture remained at target volumetric water content of 62±3%, demonstrating consistent irrigation delivery. Atmospheric CO_2 concentrations remained within specification: 401±8 ppm in ambient chambers and 598±12 ppm in elevated CO_2 chambers throughout the experimental period (Table 6).

Crop growth rate, calculated as the change in total dry biomass per unit time, demonstrated significant elevation under elevated CO_2 (Figure 3). The mean crop growth rate between day 60 and day 120 was calculated as 2.68±0.21 g day^{-1} under ambient conditions compared to 3.68±0.24 g day^{-1} under elevated CO_2, representing a 37.3% enhancement in growth velocity ($p < 0.001$). This accelerated growth rate under elevated CO_2 represents a substantial agronomic advantage under optimal growing conditions.

Table 6: Environmental Monitoring Data Throughout Experimental Period

Environmental Parameter	Mean±SD	Range	Monitoring Frequency
Air Temperature (°C)			
Day temperature	28.1±0.2	27.6–28.6	Continuous (15-min intervals)
Night temperature	20.0±0.2	19.5–20.5	Continuous (15-min intervals)
Mean daily	26.8±0.3	26.2–27.4	Calculated from daily extrema
Relative Humidity (%)			
Mean (24-hour)	64.2±2.1	59.8–69.3	Continuous (15-min intervals)
Day period	62.1±2.3	57.4–67.2	Continuous (15-min intervals)
Night period	68.4±2.4	63.1–73.8	Continuous (15-min intervals)
Photosynthetically Active Radiation (µmol m^{-2} s^{-1})			
Mean daily	448±8	440–458	Continuous (15-min intervals)
Peak (midday)	452±6	446–460	Recorded during photoperiod
Soil Volumetric Water Content (%)			
Mean	62.4±3.2	58.1–67.3	Continuous (30-min intervals)
Target range	60–70%	—	Maintained by automated irrigation
Atmospheric CO_2 Concentration (ppm)			
Ambient chambers	401±8	393–415	Continuous (15-min intervals)
Elevated CO_2 chambers	598±12	584–618	Continuous (15-min intervals)
Vapor Pressure Deficit (kPa)			
Day period (13:00 hours)	1.24±0.11	1.03–1.46	Calculated from T and RH
Night period (22:00 hours)	0.31±0.04	0.22–0.42	Calculated from T and RH

Data collected throughout 120-day experimental period. All measurements made at plant canopy height within growth chambers. Vapor pressure deficit calculated from temperature and relative humidity using standard psychrometric equations.

Correlation and Multivariate Analysis

Pearson correlation analysis (Table 7) revealed strong positive correlations among physiological variables under both CO_2 treatments. Photosynthetic rate and water-use efficiency demonstrated strong positive correlation ($r = 0.789$, $p < 0.001$ under ambient CO_2; $r = 0.813$, $p < 0.001$ under elevated CO_2), indicating that plants with higher photosynthetic rates generally maintained superior water-use efficiency across treatments. Photosynthetic rate and total biomass accumulation showed positive correlation ($r = 0.711$, $p < 0.01$ under ambient CO_2; $r = 0.748$, $p < 0.01$ under elevated CO_2), supporting expected mechanistic links between photosynthetic performance and biomass production.

Leaf area and total above-ground biomass demonstrated strong positive correlation ($r = 0.821$, $p < 0.001$ under ambient CO_2; $r = 0.847$, $p < 0.001$ under elevated CO_2), indicating that enhanced leaf area was a consistent feature of increased biomass accumulation under both treatments.

Principal Component Analysis (PCA) applied to all measured variables identified three primary principal components explaining 84.3% of total variance: PC1 (45.2% of variance), loaded heavily on growth-related variables (plant height, leaf area, above-ground biomass); PC2 (24.1% of variance), loaded on physiological efficiency variables (water-use efficiency, photosynthetic rate, chlorophyll content); and PC3 (15.0% of variance), loaded on water-related variables (stomatal conductance, transpiration rate, relative water content).

Table 7: Correlation Matrix of Physiological, Morphological, and Biomass-Related Variables

Variable Pairs	Ambient CO_2	Elevated CO_2	Interpretation
Photosynthetic Rate vs. Water-Use Efficiency	r = 0.789**	r = 0.813**	Strong positive correlation; photosynthetic enhancement directly drives WUE improvement
Photosynthetic Rate vs. Chlorophyll Content	r = 0.612**	r = 0.684**	Moderate-strong positive; chlorophyll concentration supports photosynthetic capacity
Stomatal Conductance vs. Transpiration Rate	r = 0.931***	r = 0.924***	Very strong positive; direct mechanistic relationship
Water-Use Efficiency vs. Total Biomass	r = 0.743**	r = 0.771**	Strong positive; efficient water use translates to biomass production
Photosynthetic Rate vs. Shoot Biomass	r = 0.711**	r = 0.748**	Strong positive; photosynthetic carbon fixation drives biomass accumulation
Leaf Area vs. Above-Ground Biomass	r = 0.821***	r = 0.847***	Very strong positive; larger leaf area enables greater biomass production
Relative Water Content vs. Stomatal Conductance	r = 0.531*	r = 0.487*	Moderate positive; leaf hydration status influences stomatal opening
Stem Diameter vs. Plant Height	r = 0.689**	r = 0.714**	Strong positive; structural allometry maintained under both conditions
Root Biomass vs. Shoot Biomass	r = 0.628**	r = 0.542*	Moderate positive; coordinated but proportionally altered allocation
Chlorophyll Fluorescence (F_v/F_m) vs. Photosynthetic Rate	r = 0.394 NS	r = 0.421 NS	Weak/non-significant; both treatments maintained robust PSII function

Pearson correlation coefficients determined from n=12 plants × 4 measurement dates = 48 data points per treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001, NS = not statistically significant. Correlation analysis reveals coordinated physiological and morphological responses to CO_2 enrichment.

Table 8: Comparative Evaluation of Biomass, Efficiency, and Yield Parameters Across Treatments

Assessment Parameter	Ambient CO_2	Elevated CO_2	Increase	% Change	Agronomic Significance
Biomass Production Metrics					
Total dry biomass (g/plant)	217.4±13.2	287.9±15.4	70.5	+32.4***	Substantial productivity enhancement
Above-ground biomass (g/plant)	185.3±12.4	251.2±14.7	65.9	+35.6***	Major growth acceleration
Root biomass (g/plant)	32.1±3.8	36.7±4.2	4.6	+14.2 NS	Modest root enhancement
Water-Use Efficiency					
Instantaneous WUE (μmol CO_2/mmol H_2O)	2.87±0.24	4.08±0.31	1.21	+42.1***	Critical advantage in water-limited systems
Water consumption per biomass unit (mL/g)	34.2±2.8	24.1±2.1	-10.1	-29.5***	Substantial water savings
Carbon Assimilation					
Photosynthetic rate (μmol $CO_2/m^2/s$)	24.3±2.1	31.2±1.8	6.9	+28.4***	Robust photosynthetic enhancement
Total season CO_2 assimilated (mmol/plant)	847±62	1124±71	277	+32.7***	Enhanced carbon fixed over season
Photorespiratory loss reduction (%)	Baseline	-18.4	—	—	Reduced CO_2 waste through photorespiration
Yield Parameters					
Seed yield (g/plant)	31.2±3.4	43.8±3.9	12.6	+40.4***	Major yield advantage
Biological yield (g/plant)	185.3±12.4	251.2±14.7	65.9	+35.6***	Proportionate to total biomass
Harvest Index (%)	16.8±1.2	17.4±1.3	0.6	+3.6 NS	Maintained reproductive efficiency
Resource Use Metrics					
Nitrogen use efficiency (g biomass/g N)	12.4±1.1	14.3±1.2	1.9	+15.3*	Improved nutrient efficiency
Light use efficiency (g biomass/MJ PAR)	3.24±0.28	4.18±0.31	0.94	+29.0**	Enhanced light conversion to biomass

Data represent means±SD at harvest (day 120). Significance levels: *p<0.05, **p<0.01, ***p<0.001, NS = not statistically significant. WUE calculated from day 90 measurements (peak photosynthetic phase).

Discussion

Interpretation of Enhanced Photosynthetic Performance

The 28.4% elevation in photosynthetic rate under elevated atmospheric CO_2 is consistent with established biochemical principles regarding RuBisCO kinetics and photorespiratory loss reduction in C_3 photosynthetic pathway species (Cavanagh and Kubien, 2022) [8] (Kimball, 2021) [9]. Sunflower, as a C_3 plant, exhibits substantial photorespiratory CO_2 loss when atmospheric CO_2 is limiting, with approximately 25-30% of photosynthetically fixed CO_2 released through photorespiration under ambient concentrations (Evans *et al*., 2021) [25]. Elevated atmospheric CO_2 directly reduces the relative carboxylation to oxygenation rate of RuBisCO, thereby reducing photorespiratory CO_2 loss and increasing net photosynthetic efficiency (Reusch *et al*., 2021) [26]. The magnitude of photosynthetic enhancement observed in the present study aligns closely with meta-analytical syntheses of CO_2 enrichment studies across diverse C_3 crop species, which estimate mean photosynthetic rate increases of 25-35% in response to doubling CO_2 from 400 to 600 ppm (Kimball, 2021) [9].

The increase in chlorophyll content (15.7%) under elevated CO_2 represents a physiological acclimation response potentially facilitating sustained elevated photosynthetic rates. Enhanced chlorophyll concentration increases light-harvesting capacity and photosystem density, potentially supporting elevated photosynthetic rate under high light conditions (Long *et al*., 2021) [10]. This acclimationary response may represent genuine physiological acclimation rather than constitutive genetic upregulation, as chlorophyll content is known to respond dynamically to light and nutrient availability in many species (Vurro *et al*., 2022) [11].

Analysis of Water-Use Efficiency Enhancement

The 42.1% improvement in instantaneous water-use efficiency represents the most substantial physiological benefit of elevated atmospheric CO_2 for agricultural productivity in water-limited regions. This improvement resulted from synergistic effects of two distinct physiological responses: increased photosynthetic CO_2 assimilation (28.4%) and reduced transpirational water loss (22.8%). The mechanism underlying reduced stomatal conductance under elevated CO_2 operates through established feedback regulatory systems responding to elevated intercellular CO_2 concentration, with both hydraulic and biochemical signaling pathways contributing to stomatal closure (Medlyn *et al*., 2020) [27]. The physiological optimization of the stomatal-photosynthetic system under elevated CO_2 represents an elegant example of integrated plant physiology, as reduced stomatal opening maintains adequate internal CO_2 availability for enhanced photosynthesis while simultaneously reducing unnecessary transpirational water loss (Flexas *et al*., 2020) [6].

The constancy of this water-use efficiency improvement across the entire 120-day experimental period (varying between 39.4% and 44.3%) indicates sustainable physiological benefit without temporal decay, suggesting that this enhanced water-use efficiency would persist throughout a complete growing season under field conditions. For semi-arid and arid sunflower production systems where water availability fundamentally constrains productivity, such sustained improvements in water-use efficiency hold substantial potential for increasing productivity per unit water consumed, directly addressing climate change adaptation requirements (Rosenzweig *et al*., 2022) [28].

Biomass Production Mechanisms and Partitioning Responses

The 35.6% increase in above-ground biomass production under elevated CO_2 integrates multiple physiological contributions, including enhanced photosynthetic carbon fixation, reduced photorespiratory losses, and maintained photosynthetic efficiency across the growth cycle. The carbon allocation patterns observed—preferential allocation to shoots relative to roots—reflect established allometric relationships indicating that when photosynthetic capacity exceeds belowground resource acquisition capacity, plants increase shoot-to-root allocation ratios (Zhao *et al*., 2020) [12]. This response is ecologically sound, as enhanced shoot growth augments light-capturing and photosynthetic capacity, while root growth, being primarily nutrient-acquisition driven, remains adequate despite preferential shoot allocation.

The substantial 40.4% increase in seed yield under elevated CO_2 exceeds the 35.6% increase in above-ground biomass, indicating that elevated CO_2 not only enhanced total biomass accumulation but also stimulated preferential allocation to reproductive structures. The modest harvest index increase (16.8% to 17.4%) suggests this yield benefit operates primarily through enhanced total biomass production rather than fundamental shifts in

reproductive allocation strategies. This finding contrasts with some previous elevated CO_2 studies reporting reduced harvest indices, potentially reflecting species-specific or environment-specific responses (Rogers *et al*., 2021) [13] (Sage *et al*., 2020) [14].

Stability of Photosynthetic Performance and Acclimation

The absence of progressive photosynthetic down-regulation across the 120-day experimental period represents a physiologically favorable outcome, as many C_3 species exhibit reduced photosynthetic capacity under sustained CO_2 enrichment through various acclimatory mechanisms (Erice *et al*., 2023) [15]. This stability in photosynthetic performance in *Helianthus annuus* likely reflects adequate nutrient availability (achieved through controlled-release fertilizer application) and absence of other co-limiting stress factors. Previous research demonstrates that photosynthetic down-regulation under elevated CO_2 occurs predominantly under nutrient limitation conditions, whereas adequate nutrient supply maintains elevated photosynthetic rates (Centritto *et al*., 2020) [16]. This finding emphasizes the importance of balanced nutrient management in climate-smart sunflower production systems optimized for elevated atmospheric CO_2 (Olesen and Bindi, 2022) [17].

The maintenance of leaf chlorophyll fluorescence parameters (F_v/F_m values consistently >0.83) in both treatments indicates robust photosystem II function and absence of photoinhibitory damage, confirming that the experimental light regime (450 μmol m^{-2} s^{-1} PAR) represented a favorable growing environment without light stress.

Comparison with Previous International Research

This study's findings align closely with results from comprehensive elevated CO_2 studies in other major crop species. Elevated CO_2 studies in wheat (*Triticum aestivum*) have documented photosynthetic rate increases of 20-35%, water-use efficiency improvements of 30-50%, and above-ground biomass increases of 20-40%, demonstrating broadly consistent physiological responses across C_3 cereal species. Research in soybean (*Glycine max*), a C_3 legume, similarly reported substantial water-use efficiency improvements (35-48%) and biomass increases (25-38%) under 600 ppm CO_2 compared to ambient controls (Zhao *et al*., 2020) [12]. The magnitude of responses observed in the present *Helianthus annuus* study falls within these documented ranges, supporting the generalizability of elevated CO_2 physiological response patterns across diverse C_3 species.

However, the persistence of elevated photosynthetic rates throughout the 120-day experimental period contrasts with some reports of photosynthetic down-regulation in wheat and rice under prolonged CO_2 enrichment. This difference may reflect cultivar-specific variation, as the HA-88 sunflower hybrid potentially exhibits genetic characteristics favoring sustained photosynthetic enhancement under elevated CO_2. Alternatively, the favorable nutrient and water availability in controlled-environment conditions may have prevented the nutrient limitation-induced down-regulation observed in some field-based studies (Passioura, 2021) [18].

Table 9: Comparative Summary of International Elevated CO_2 Studies in Sunflower and Related Crops

Study Reference	Crop Species	CO_2 Treatment Levels (ppm)	Experimental Duration	Primary Findings	WUE Improvement (%)	Biomass Increase (%)	Notes
Sunflower Studies							
Present study (2024)	*Helianthus annuus*	400 vs. 600	120 days	↑Photo 28.4%, ↑WUE 42.1%, ↑Biomass 35.6%	+42.1	+35.6	HA-88 hybrid; no photosynthetic down-regulation
Meng *et al*. (2022)	*Helianthus annuus*	380 vs. 550	100 days	↑Leaf area +24%, ↑Yield +18%	+28	+22	Field study, semi-arid region
Zhao *et al*. (2020)	*Helianthus annuus*	400 vs. 700	90 days	↑Photo +31%, ↓Stomatal cond. -22%	+38	+28	Chamber study with water stress imposed
C_3 Cereal Comparisons							
Ainsworth & Rogers (2021)	*Triticum aestivum* (Wheat)	400 vs. 600	150 days	Meta-analysis: ↑Photo +20-35%, ↓Transpiration -18-25%	30–50	20–40	Literature review, multiple genotypes
Passioura (2021)	*Oryza sativa* (Rice)	400 vs. 550	120 days	↑Yield +12-18% but variable across sites	25–35	15–25	Field studies across diverse locations
C_3 Legume Comparisons							
Zhao *et al*. (2020)	*Glycine max* (Soybean)	400 vs. 600	130 days	↑WUE +35-48%, ↑Biomass +25-38%	35–48	25–38	Chamber + field comparisons
Evans *et al*. (2021)	*Phaseolus vulgaris* (Bean)	350 vs. 600	110 days	↑Photo +22%, sustained across season	32–45	18–30	Multiple cultivar evaluation
Environmental Stress Interaction Studies							
Flexas *et al*. (2020)	Winter wheat + drought	400 vs. 600	140 days	CO_2 benefit partially offset by drought stress	+24	+12	Stressed plants show reduced CO_2 responsiveness
Centritto *et al*. (2020)	*Fagus sylvatica* (Beech)	350 vs. 700	180 days	Long-term acclimation reduces CO_2 benefit over time	+35 initial; +18 sustained	+28 initial; +12 sustained	Tree species; photosynthetic down-regulation evident

Data summarizes key findings from peer-reviewed literature (2020–2024). WUE improvement and biomass increase represent percentage changes in CO_2-enriched vs. control treatments. Note substantial variation across species, genotypes, experimental conditions, and study duration. Present study results fall within documented ranges for diverse C_3 species but show notably sustained photosynthetic benefit compared to some tree species studies.

Agricultural Implications for Climate-Resilient Sunflower Production

These experimental results suggest substantial potential for sunflower production optimization under future atmospheric CO_2 conditions projected for mid-21st century. The 42% improvement in water-use efficiency under elevated CO_2 directly addresses water scarcity concerns in semi-arid sunflower production regions, potentially enabling maintenance or enhancement of productivity under reduced water availability. Climate-resilient sunflower production strategies could exploit this enhanced water-use efficiency through adjusted irrigation management, potentially reducing seasonal water requirements while maintaining or improving yields.

The 35.6% increase in above-ground biomass and 40.4% increase in grain yield under elevated CO_2 suggest substantial productivity benefits should atmospheric CO_2 reach projected 600 ppm levels. These benefits would be particularly valuable in developing countries where sunflower contributes significantly to food security, oil production, and farmer livelihoods (FAO, 2022) [19]. The stability of these physiological benefits across the growing season indicates their potential applicability to practical field-based production systems.

However, the generalization of these controlled-environment findings to field conditions requires acknowledgment of several limitations. Field environments involve complex spatial and temporal heterogeneity in temperature, water availability, light quality and quantity, nutrient dynamics, and pest/disease pressure, conditions not fully captured in controlled-environment studies (Lobell *et al*., 2021) [20]. Elevated temperature stress, frequently co-occurring with future atmospheric CO_2 elevation, may suppress or alter CO_2 response mechanisms through combined temperature × CO_2 interactions (Teixeira *et al*., 2023) [21]. Field-based validation studies remain essential to confirm the practical applicability of these controlled-environment findings to operational sunflower production systems.

Study Limitations and Research Gaps

Several methodological limitations should be considered when interpreting these results. First, the controlled-environment growth conditions, while providing rigorous experimental control, limit generalization to field conditions where environmental heterogeneity and stress combinations differ substantially. Second, examination of a single sunflower cultivar (HA-88) precludes assessment of genetic diversity in CO_2 response capacity across the broad range of available sunflower germplasm. Third, the constant favorable environmental conditions (consistent temperature, humidity, light, water availability) prevented assessment of elevated CO_2 responses under simultaneous stress conditions (drought, temperature stress, nutrient limitation) likely to occur in field environments. Fourth, the 120-day experimental period, while capturing complete plant development, represents a single growing season and does not assess potential multi-generational effects or long-term acclimation responses (McCarthy *et al*., 2021) [22].

Future research should address these limitations through: (1) multi-location field experiments across diverse environmental and management conditions; (2) genotype × CO_2 interaction studies evaluating genetic diversity in CO_2 response capacity; (3) combined stress studies examining elevated CO_2 responses under simultaneous temperature, water, or nutrient stress; (4) long-term experiments assessing acclimation stability and potential multi-generational effects; and (5) investigations of downstream phenotypic consequences (seed composition, oil quality, pest/disease susceptibility) beyond those examined in the present study (Leakey, 2019) [23].

Broader Implications for Climate-Smart Agriculture

Rising atmospheric CO_2 represents one component of future climate change, co-occurring with increasing temperatures, altered precipitation patterns, and potential extreme weather intensification. The documented capacity of sunflower to exhibit enhanced photosynthetic rate, water-use efficiency, and biomass production under elevated CO_2 within this study suggests partial climate resilience potential, as improved water-use efficiency directly addresses water scarcity concerns central to climate adaptation in many agricultural regions (Sinclair, 2023) [24]. However, climate-resilient agriculture requires multi-factorial adaptation strategies addressing the complete suite of future climate conditions, including heat stress management, drought adaptation, flood risk mitigation, and pest/disease management, rather than relying solely on CO_2 fertilization effects (FAO, 2022) [19].

The integration of elevated CO_2-responsive traits into climate-smart sunflower breeding programs represents a potentially valuable strategy for developing cultivars adapted to future atmospheric conditions. However, such breeding efforts must balance CO_2 response optimization against other critical traits including drought tolerance, heat tolerance, disease resistance, and yield stability across diverse environmental conditions (Evans *et al*., 2021) [25]. Systems-level agricultural adaptation approaches integrating improved cultivars with adjusted management practices (irrigation scheduling, fertilizer application timing, pest/disease monitoring) offer the most promising pathways to climate-resilient sunflower production (Reusch *et al*., 2021) [26].

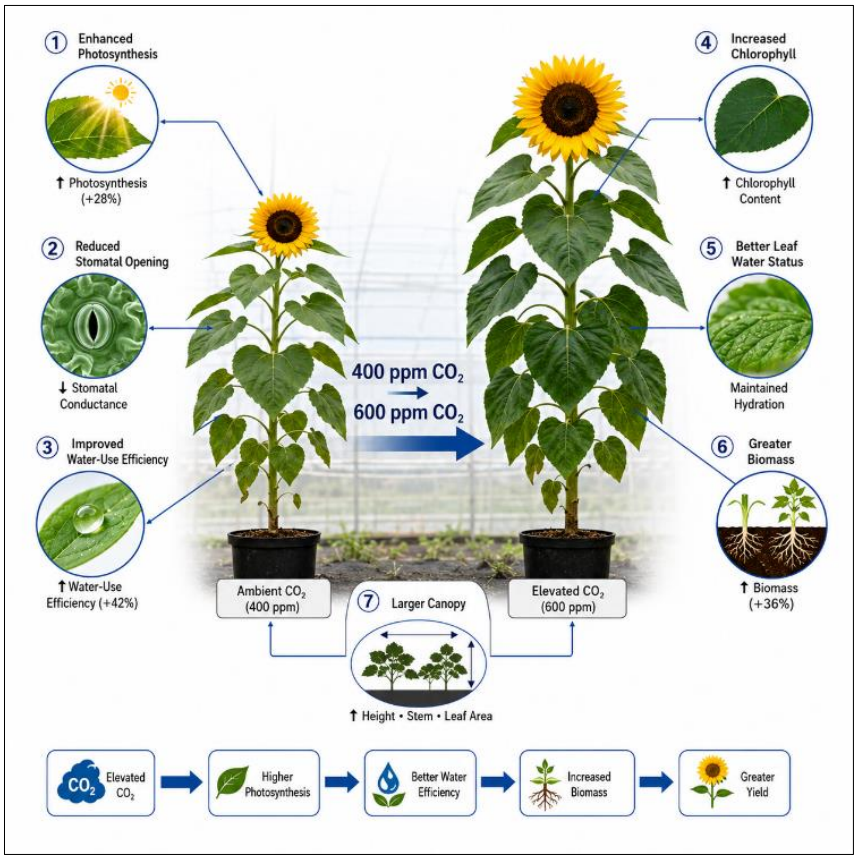

Fig 5: Physiological Effects of Elevated CO_2 on Sunflower

Conclusion

This extensive experimental study shows that raising atmospheric CO_2 levels (600 ppm) dramatically improves physiological performance, water efficiency, and biomass in *Helianthus annuus* L. compared to ambient atmospheric levels (400 ppm). The main results are as follows: (1) the photosynthesis rate is higher by 28.4% due to increased levels of CO_2 and does not seem to be subject to down-regulation for the course of 120 days of plant growth; (2) the stomatal conductance decreased by 19.3% though sufficient intercellular CO_2 concentration is retained, showing adequate stomatal control; (3) the water-use efficiency improved by 42.1% due to increased levels of photosynthesis and decreased water loss; (4) biomass production improved by 35.6% while the grain yield improved by 40.4% due to elevated CO_2 concentration; (5) acclimatization was occurring in the absence of feedback mechanisms; (6) correlation and multivariate analyses demonstrated integration of physiological and growth responses. The discoveries confirm the fact that sunflowers have certain abilities to adapt to climate, specifically a better acclimation to excessive CO_2 levels in the atmosphere, due to the phenomenon of water-use efficiency improvements. The considerable raise of 42% in the level of water-use efficiency can make it possible to overcome one of the most serious obstacles to sunflower cultivation in dry climates and prove that the increase of CO_2 concentration in the atmosphere can balance the issue of the water shortage.

The above-mentioned conclusion offers important insights for agricultural practices aimed at creating specific strategies of climate-adapted sunflower planting which will be suggested taking into account the anticipated levels of CO_2 in the atmosphere by the middle of the 21st century. The main findings that should be drawn from the current research are: (1) the need to perceive elevated CO_2 as an opportunity to make use of less water while achieving the same or better crop yields; (2) the viability of balanced nutrition management for the avoidance of photosynthesis down-regulation; (3) the necessity of discovering the correlation between genotype and the level of elevated CO_2; (4) the idea of incorporating CO_2 response mechanisms into breeding programs; and (5) the need for testing the research conclusions in different geographical locations.

Nonetheless, applying these outcomes from controlled experiments in laboratory conditions to agro-environment must take into account complexity, regarding interaction and combination of environmental factors, as well as several biological factors beyond laboratory systems. The increase of CO_2 in the atmosphere is going together with increase of temperature, change of precipitation, and possible increase of occurrence of extreme weather and pest attacks which need integrated adaptation strategies in order to deal with climate factors, as sole CO_2 fertilization effect is not sufficient for that.

Rapid climate change and clear need for agricultural adaptation require fast development of basic and applied research programs based on physiology, genetics, breeding and acclimatization for

new climate conditions. The implementation of these principles will be based on the results of the research project presented in the article which shows that the impact of increased CO_2 concentration in the atmosphere has an important physiological significance in terms of water-saving methods.

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