DROUGHT STRESS RESILIENCE AND ADAPTIVE MECHANISMS IN WHEAT THROUGH GAS EXCHANGE AND BIOCHEMICAL METHODS

BHUTTO, L. A.[1*] – OSBORNE, C. P.[2] – QUICK, W. P.[2] – ALSHAHARNI, M. O.[3] – ALGOPISHI, U. B.[4]

[1]*Agriculture Research Sindh, Tandojam 70060, Pakistan*
(e-mail: l.bhutto@gmail.com)

[2]*Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK*
(e-mail: c.p.oborne@sheffiled.ac.uk; p.quick@sheffiled.ac.uk)

[3]*Biology Department, College of Science, King Khalid University, Abha 61421, Saudi Arabia*
(e-mail: maasalim@kku.edu.sa)

[4]*Department of Biology, College of Science, King Khalid University, 61421 Abha, Saudi Arabia*
(e-mail: ualgopishi@kku.edu.sa)

[*]*Corresponding author*
e-mail: l.bhutto@gmail.com

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Abstract. Drought stress in wheat plants is a crucial aspect affecting various growth stages of the crop. The current research was aimed to investigate the effects of drought stress on leaf gas exchange and biochemical parameters in three wheat (*Triticum aestivum* L.) genotypes: two salt-tolerant varieties (W4909 and W4910) and one drought-sensitive variety (Yecora Rojo). Understanding how plants cope with drought is essential with the escalating global warming concerns. The results show that the salt-tolerant varieties, W4909 and W4910, exhibit higher Rubisco efficiency compared to Yecora Rojo, underscoring the importance of Rubisco content in carboxylation efficiency under drought conditions. Furthermore, these salt-tolerant varieties demonstrate greater RuBP regeneration capacity than Yecora Rojo. Significant variations in Rubisco content were noted among the genotypes, with the tolerant varieties maintaining higher levels during drought stress. Additionally, these varieties exhibited higher chlorophyll and total chlorophyll content under drought, enabling them to sustain photosynthesis even under severe stress. Conversely, the tolerant varieties had lower transpiration rates due to stomatal closure, preserving leaf water potential and enhancing photosynthetic rates. Despite a decrease in overall leaf protein content in the tolerant varieties, an increase in Rubisco degradation was observed in Yecora Rojo. Overall, the salt-tolerant varieties showed superior photosynthetic performance, higher Rubisco content, and lower transpiration rates under drought stress, demonstrating their improved water use efficiency and Rubisco efficiency compared to the drought-sensitive Yecora Rojo.
Keywords: *wheat, drought stress, leaf gas exchange, biochemical adaptations, water use efficiency, Rubisco efficiency, chlorophyll content*

Introduction

Wheat is a staple food used in global nutrition and agriculture, not just for its role as a staple food but for its profound nutritional benefits to human health (Ahmed et al., 2020). Challenges that confront agricultural productivity, abiotic stresses, particularly drought, emerge as formidable adversaries, testing the resilience and adaptability of crop species (Faisal et al., 2017). The quest to understand and mitigate the impacts of drought stress on wheat has unraveled complex mechanisms underpinning the development and physiological attributes contributing to yield, driven by the nuanced interplay of genes

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(Hussain et al., 2019). Wheat revealed to be a rich source of proteins, carbohydrates, minerals, lipids, fibers, vitamins, and essential amino acids, efficient in unparalleled dietary value (Shewry and Hey, 2019; Ahmed et al., 2022). Notably, gluten-a complex protein accounting for a significant portion of wheat's protein content-embodies the elasticity and texture that define many wheat-based foods (Shewry, 2019). However, the productivity in agriculture lies on the efficacy of the crop. Drought is the biggest problem of the world affecting the plant growth and yields through a cascade of agronomic, morphological, physiological, biochemical, and metabolic responses, marking a critical area of study for ensuring food security (Comas et al., 2013; Ahmed et al., 2022). Drought disrupts the water balance, impairs cellular metabolism, and compromises photosynthesis, laying bare the vulnerability of vital physiological processes (Gupta et al., 2020; Upadhyaya et al., 2021). In the crucible of drought stress, chlorophyll—central to photosynthesis—bears the brunt, its degradation leading to the generation of reactive oxygen species and consequent cellular damage (Khalilzadeh et al., 2016; Ahmed et al., 2022). Yet, in the face of these challenges, wheat's drought tolerance emerges as a multifaceted trait, encompassing physiological, morphological, and molecular dimensions. Attributes such as water retention, leaf morphology, and biochemical stability underscore the resilience of certain wheat genotypes, offering a beacon of hope for breeding drought-tolerant varieties (Bala et al., 2018; Ahmed et al., 2020; Merrium et al., 2022). This narrative sets the stage for an in-depth exploration of how drought impacts wheat at the gas exchange and biochemical levels. Through meticulous experiments, this study examines photosynthetic rates, transpiration, stomatal behavior, CO_2 concentrations, and leaf temperatures, alongside protein, Rubisco, and chlorophyll content across different wheat cultivars. The response to drought on wheat plants can help to ensure crop with future improvement towards food security.

Materials and methods

Plant materials

The seeds of six genotypes were obtained from the USDA (United States Department of Agriculture) and CIMMYT (International Maize and Wheat Improvement Centre Mexico). Two bread wheat cultivars W4909 and W4910 (salt tolerant) (*Triticum aestivum* L.) carrying registration numbers Gp 370 and Gp 371, PI 63114 and PI 63115 (Wang et al., 2003b) were used along with cultivar Yecora Rojo, two progeny lines 5757-3 and 5746-20 (R. C. Wang- USDA- ARS-Forage and Range Research Laboratory, Utah State University, Logan, UT USA), and Babax (drought tolerant) (from CIMMYT Mexico).

Drought treatment and experimental design

When the fourth leaf was fully expanded, the drought treatment was applied by withholding water. Sampling of the fully expanded fourth leaf was carried out at 0, 5, 10, 15, and 20 days of the drought treatment and controlled conditions. Only one plant was grown in each pot. Randomized Block Design (RBD) was used in whole the series of experiments i.e., growth, physiological, biochemical and metabolic experiments (also in tillering and de-tillering experiments).

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Sampling for growth analysis

Five replicates were used for each data point. Every 5 days, 10 plants per genotype were harvested to take measurements under the drought stress and control conditions. Fresh weight (g), dry weight (g), leaf number, live and dead eaves, water content (%) and leaf area (cm^2), leaf and soil water potential, and relative water content were measured as described below (*see Measurements sections*).

Sampling for photosynthetic parameter measurements

Photosynthesis (A), transpiration (E), leaf temperature (T_{leaf}) stomatal conductance (g_s) and sub stomatal CO_2 concentration [C_i] were measured in the fourth fully expanded leaf with six replicates for each treatment. Samples were measured on 0, 5, 10, 15 days of the drought treatment and control conditions.

Sampling for biochemical analyses

The fully expanded fourth leaf was harvested on 0, 5, 10, 15, and 20 days under the drought stress and control conditions, frozen immediately in liquid nitrogen and then stored in -80˚C until required for the determination of soluble carbohydrate, insoluble carbohydrate, total amino acids, and total protein analysis, rubisco (enzyme), chlorophyll a + b, and Carotenoids.

Measurements of fresh weight, dry weight, and water content

Samples of the fully expanded fourth leaf (five replicates for each point) were harvested using a razor blade, immediately weighed, and then dried in an oven at 72˚C for 48 hours. Dry weight of samples was recorded to a precision of 0.001 g. Determination of water content was expressed as both a percentage of fresh and dry weights (Michael et al., 1999).

Measurements of leaf area and leaf water potential

Leaf area of the fully expanded fourth leaf was measured using a leaf-area meter (Delta-T, Cambridge, UK) calibrated with a known area. The data were expressed as means of five replicates ±SE on a cm^2 basis. Water potential of the fully expanded fourth leaf was measured using a pressure chamber, as first described by Scholander et al. (1965). The leaf was cut and quickly sealed inside the chamber with the cut end appearing above the rubber seal. The pressure inside the chamber was gradually increased until xylem sap started to appear from the cut end of the leaf (detected by using a magnifying glass). The gas flow was immediately stopped and the pressure on the gauge was noted. The units of pressure used are the Bar (1 Bar = 14.5 pounds per square inch) with negative sign and data expressed as MPa (1 MPa = 10 Bars).

Determination of leaf mortality

The number of live leaves and dead/senesced leaves were recorded on 0, 5, 10, 15, and 20 days of the drought stress and control. All the data were expressed as means of five replicates ± SE. A dead/senesced leaf was recorded if the leaf looked dried i.e., brownish (absence of water and chlorophyll, etc.).

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Determination soil water potential

Soil water potential was measured with a soil psychrometer (Wescor, Logan, UT, PST-55; Briscoe 1984), which was calibrated using standard salt solutions (NaCl) of 0.1, 0.2, 0.5, and 1.0 m (molality) (Brown and Bartos, 1982). The psychrometer executed measurements on an hourly basis with a 30-s cooling time to adjust for the Peltier effect. Psychrometer data were logged and downloaded from a PSYPRO (Model PST-55, Wescor, Logan, UT) water potential system.

Determination of relative water content

Relative water content (RWC) was expressed as the percentage water content at a given time as related to the water content at full turgor: RWC (%) = [(FW-DW)/(TW-DW)] X 100 (Slatyer, 1967). Where: FW= Fresh weight, TW= Turgid weight, and DW= Dry weight, Fresh weight and dry weight measurements are outlined above. The TW was measured by keeping the leaves in ddH_2O over night (12 hours) at room temperature.

Instantaneous gas exchange measurements

Net rates of photosynthesis (A), transpiration (E), stomatal conductance (g_s), leaf internal CO_2 [C_i], and leaf temperature (T_{leaf}) were determined in the fully expanded fourth leaf at 0, 5, 10, and 15 days of the drought stress and 0, 5, 10, and 15 days under fully-watered control conditions. Six replicates from each treatment were measured using a portable gas exchange system (LCA-4 Analytical Development Company Limited, Hertfordshire, England). The middle part of the fourth fully expanded leaf was enclosed in a leaf chamber (PLC4) that was 2.5 cm^2 wide. The leaf area was measured using a ruler by determining length and width. Gas exchange measurements were made in the growth chamber under saturating light conditions. The light source was filtered using a small heat-absorbing filter and adjusted to a flux density of 1000 µmol photons m^{-2} s^{-1}. All the measurements were made on a leaf area basis (cm^2). The data were expressed as means ±SE (standard errors).

A/Ci curve

Another experiment was conducted through the A/Ci curve, in which each entry was determined from various levels ranging from (~1 ppm) to abundant (~1300 ppm). This technology involves compressed air, a meticulous setup of mass flow controllers, and precision of temperature-controlled environments. Various responses of wheat leaves vary carbon dioxide concentrations.

A/C_c data analysis and curve fitting

A/C_c curve analyses were analyzed by using the A/C_i curve fitting utility version 2007.1 (Sharkey et al., 2007), C_i (ppm) data were also converted to C_c (Pa) by using the same utility software. All the curves were analysed in Sharkey model and drawn by using SigmaPlot version 10.0. This analytical crescendo offered profound insights into wheat's photosynthetic efficiency and resilience under of drought stress. Through this odyssey of scientific inquiry, from the genetic selection of robust wheat cultivars to the precise analysis of their physiological and biochemical responses, this can fortify our crops against the ever-growing challenge of drought.

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Measurements of leaf pigments/chlorophyll content

Frozen leaf tissue from the fully expanded fourth leaf was weighed (0.2 g FW) and then placed separately in an Eppendorf tube (1.5 ml) containing 1ml of 80% ethanol, and heated in a water bath at 70°C for 20 minutes. The green solution was transferred to a new Eppendorf tube and a second 1 ml of 80% ethanol was added to the first Eppendorf and heated until all chlorophyll was extracted (the leaf was cleaved). Absorbance was recorded at three wavelengths 470 nm, 649 nm and 665 nm using a spectrophotometer (Lambda 40-UV/vis spectrometer Perkin-Elmer instruments).

The amounts of chlorophyll a, b and carotenoids were calculated using the equations of Lichtenthaler (1983).

$$\text{Chl a} = (13.95 \times A\,\lambda\,665.0) - (6.88 \times A\,\lambda\,649.0) \quad \text{(Eq.1)}$$

$$\text{Chl b} = (24.96 \times A\,\lambda\,649) - (7.32 \times A\,\lambda\,665.0) \quad \text{(Eq.2)}$$

$$\text{Car}_{x+c} = (1000 \times A_{\lambda}\,470) - (2.05 \times \text{Chl a}) - (114.8 \times \text{Chl b}) \quad \text{(Eq.3)}$$

where Chl a = Chlorophyll a, Chl b = Chlorophyll b, Car = carotenoids and A_{λ} = absorbance at wavelength λ nm. The amounts of chlorophyll a, b and carotenoids were expressed on a fresh weight basis.

Water use efficacy

Water use efficiency or productivity is the yield of marketable crop produce. Per unit of water used in evapotranspiration.

$$\text{WUE or CWP} = Y/ET \quad \text{(Eq.4)}$$

where, WUE = water use efficiency or CWP is the crop water productivity (kg/ha-mm), Y = marketable yield (kg/ha) and ET = evapotranspiration (mm).

Measurement of total protein

The fully expanded fourth leaves were sampled, immediately frozen in liquid nitrogen and stored at -80°C. Samples (0.1 g FW) were weighed (± 0.001 g precision) and ground to a fine powder using a pestle and mortar in 750 µl of protein extraction buffer (containing a HEPES 50 mM, pH 7.4, $MgCl_2$ 5 mM, EDTA 1 mM, EGTA 1 mM, Glycerol 10%, TritonX-100 1%, and DTT 5 mM stock solution), Bensamidine 2 mM and PMSF 0.5 mM were prepared fresh on the day and added to the ground leaf tissue. The homogenate was transferred into an Eppendorf tube (1 ml), centrifuged at 4˚C, 14000 g for 3-5 min. The supernatant was decanted in a new Eppendorf and the total protein was determined as described by the method of Bradford (1976). In each cuvette 200 µl of diluted Bradford protein reagent (1 Bradford protein reagent: 4 dH_20, BioRad Laboratories, Hercules, CA, USA) was placed with 5µl of protein extract samples. The absorbance was measured at 595 nm (Perkin- Elmer instruments- lambda 40-UV/vis spectrometer were used) and the amount of protein was determined from a standard curve using bovine serum albumin (BSA), in the range 0-5 mg / ml, as a standard. Four replicates were used for each sample.

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SDS-PAGE of protein

Proteins were resolved as described by Laemlli (1970) using the BioRad Mini Protean 3 (BioRad laboratories, Hercules, CA, USA). The resolving gel contained 11% acrylamide and the stacking gel contained 5% acrylamide. Protein samples of 3.75 µg (according to the Bradford estimation above) were loaded into each lane of the gel. The gels were stained with coomassie brilliant blue R-250 (Bio-Rad) and then de-stained with 20% methanol on a rotatest shaker (Model 100, 220/240 Volts, Luckham, Sussex England). Gels were dried using a Bio-Rad gel drier (Model 583, 220/240 Volts Bio-Rad USA) and scanned (Model F915900/N124OU, Canon Solutions Inc., China).

Quantification of Rubisco from SDS PAGE using LabImage

The gels were stained dried and scanned. The scanned gel images of Rubisco protein were analysed using LabImage V 2.7.2 software to quantify the band amount in arbitrary units. The bands were measured three times from three different gels, then data were analysed on the basis of arbitrary units and data were expressed in percentage means ±SE (standard errors).

Results

Gas exchange results

Photosynthetic rate (µmol m^{-2} s^{-1})

Gas exchange measurements, specifically photosynthetic rate (µmol m^{-2} s^{-1}), were conducted under both drought stress and control conditions across the three wheat genotypes (*Fig. 1*). By the tenth day of drought treatment, a significant ($P<0.001$) decrease in photosynthetic rate was observed in all cultivars compared to day zero, with the largest reduction seen in Yecora Rojo. Notably, Yecora Rojo experienced a substantial decrease from ~30 to 0.2 µmol m^{-2} s^{-1}, while W4909 and W4910 exhibited a smaller but significant ($p<0.001$) decline from ~30 to 8 µmol m^{-2} s^{-1} by the fifteenth day of drought stress. Additionally, by the fifteenth day, W4909 and W4910 displayed a significantly ($p<0.05$) higher leaf photosynthetic rate compared to Yecora Rojo. Under control conditions, leaf photosynthetic rates were similar across all three cultivars.

Transpiration rate (mmol m^{-2} s^{-1})

At the onset of both drought stress and control conditions, Yecora Rojo demonstrated notably higher transpiration rates compared to W4909 and W4910, persisting until the fifth day of the drought treatment. In contrast, W4909 and W4910 experienced a significant reduction in transpiration rates during the initial five days of drought stress ($P<0.001$). By the tenth day, all cultivars exhibited significantly decreased transpiration rates ($P<0.001$), with Yecora Rojo showing a substantial decrease from ~7 to 0.4 mmol m^{-2} s^{-1}, while W4909 and W4910 decreased from ~5.7 to 0.4 mmol m-2 s-1 over the experimental period under drought stress conditions (*Fig. 2*). Under control conditions, Yecora Rojo maintained a significantly higher transpiration rate compared to W4909 and W4910, while W4910 and W4909 displayed significant reductions in transpiration rate by the fifteenth day.

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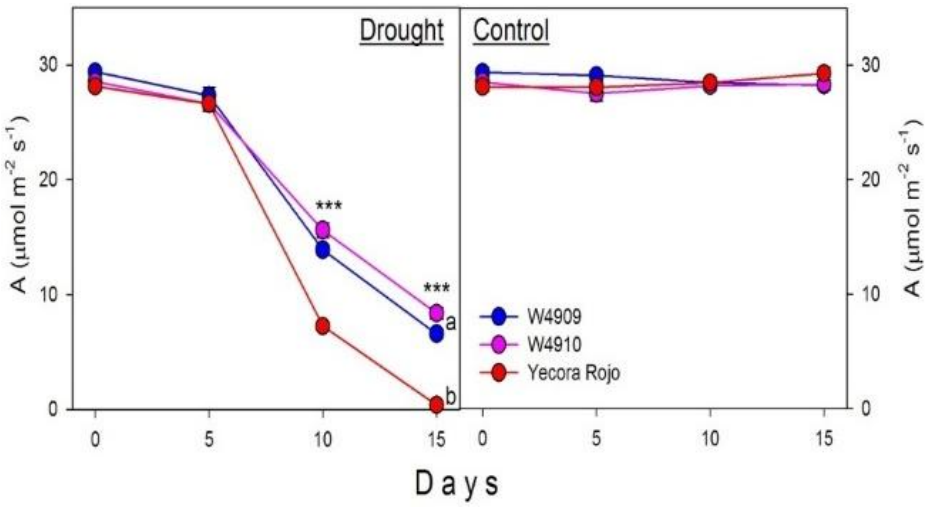

Figure 1. *Gas exchange measurements were conducted to assess the photosynthetic rate (A) ± SE (Standard Error) of three wheat genotypes under both drought stress and control conditions. Significant differences between day zero and subsequent days were determined using ANOVA, with asterisks indicating statistical significance (***, P=0.001). Additionally, significant differences among genotypes on each selected day were identified using letters (P<0.05)*

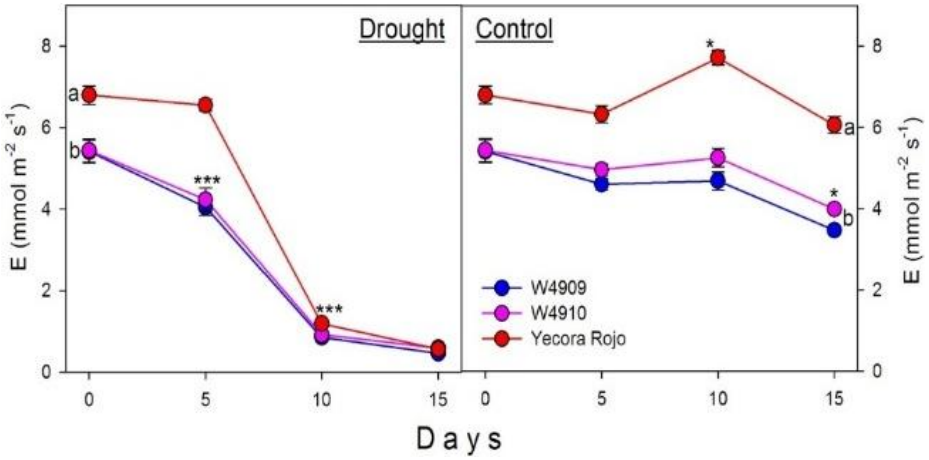

Figure 2. *The transpiration rate (E) ± SE (Standard Error) of three wheat genotypes (n=6) was assessed under drought stress and control conditions. Statistical analysis using ANOVA revealed significant differences between day zero and subsequent days, denoted by asterisks (* for p=0.05, *** for p=0.001). Furthermore, variations among genotypes on each selected day were indicated by letters (p<0.05)*

Stomatal conductance (mol m^{-2} s^{-1})

During the initial five days of the drought treatment, Yecora Rojo displayed notably higher stomatal conductance than W4909 and W4910, with significant differences observed ($p<0.05$). In contrast, W4909 and W4910 exhibited lower stomatal conductance during this period. By the fifth day, W4909 and W4910 had significantly reduced their stomatal conductance ($p<0.01$), while Yecora Rojo did not exhibit such a reduction. However, by the tenth day, all cultivars showed a significant decrease in stomatal conductance ($p<0.001$), with no further significant decline observed between the tenth

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and fifteenth days. Throughout the drought treatment, W4909 and W4910 gradually closed their stomata until the fifteenth day, whereas Yecora Rojo responded to drought by dramatically closing its stomata on the fifth day. The total reduction in stomatal conductance was approximately from ~2 to 0.1 mol m^{-2} s^{-1} in Yecora Rojo, while W4909 and W4910 decreased their stomatal conductance from ~0.8 to 0.1 mol m^{-2} s^{-1} over the same period. Under control conditions, Yecora Rojo maintained significantly higher stomatal conductance, while W4909 and W4910 exhibited significantly reduced stomatal conductance by the tenth day (*Fig. 3*).

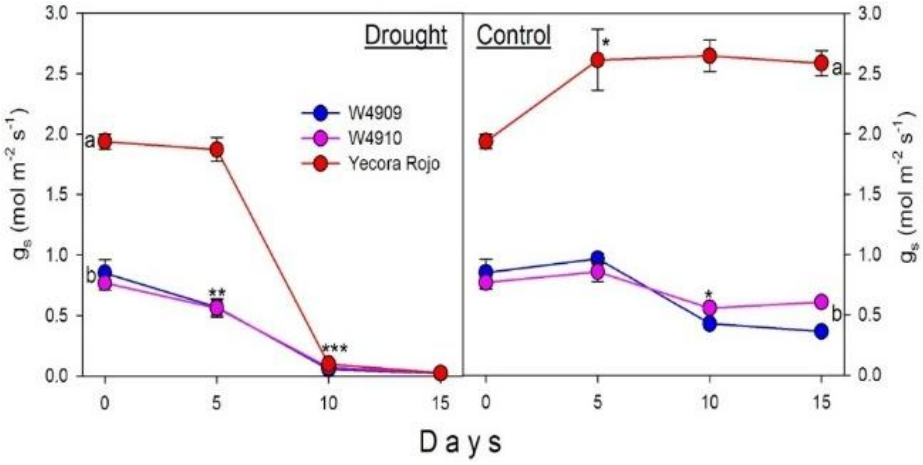

Figure 3. *The stomatal conductance (gs) ± SE (Standard Error) of the three wheat genotypes (n=6) was assessed under both drought stress and control conditions. Any statistically significant differences between day zero and the following days, as determined by ANOVA, are represented by asterisks (* for p=0.05, ** for p=0.01, and *** for p=0.001). Furthermore, significant disparities among genotypes on each selected day were indicated using letters (p<0.05)*

Sub-stomatal CO_2 concentration (µmol mol^{-1})

At the onset of the drought treatment, all cultivars exhibited similar leaf sub-stomatal CO2 concentration (Ci). By the fifth day, Yecora Rojo showed a slight increase in leaf Ci, while W4909 and W4910 remained stable. Notably, Yecora Rojo demonstrated a significant increase in leaf Ci ($p<0.05$) by the tenth day, whereas W4909 and W4910 showed either no increase or a slight decrease. By the fifteenth day, Yecora Rojo had substantially elevated Ci levels ($p<0.001$), whereas W4909 and W4910 maintained lower leaf Ci compared to Yecora Rojo. Throughout the drought treatment, W4909 and W4910 maintained consistent leaf Ci levels, while Yecora Rojo exhibited a significant increase. No significant changes were observed in any cultivar under control conditions (*Fig. 4*).

Leaf temperature (°C)

During the initial five days of the drought treatment, a noteworthy increase in leaf surface temperature was evident across all three cultivars, showing significant differences ($p<0.01$). By the fifth day, Yecora Rojo exhibited a particularly significant rise in leaf surface temperature ($p<0.001$), while W4909 and W4910 also experienced significant increases ($p<0.05$) by the fifteenth day of the drought treatment. Notably, genotypic disparities were observed, with Yecora Rojo displaying significantly higher leaf surface

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temperatures ($p<0.05$) compared to W4909 and W4910 on the fifteenth day of the drought treatment (*Fig. 5*). Conversely, no significant changes were noted in leaf surface temperatures under control conditions for any of the three cultivars, remaining consistently stable throughout the experimental period (*Fig. 5*).

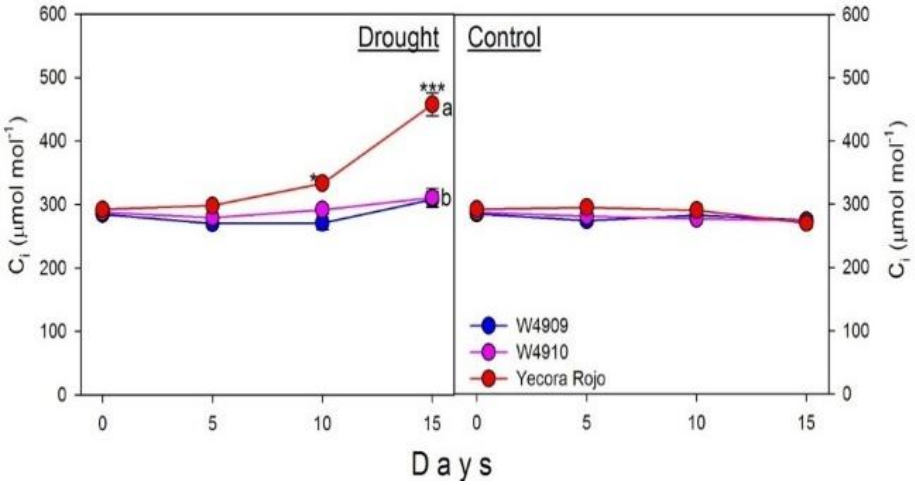

Figure 4. *Sub-stomatal CO_2 concentration (Ci) ± SE (Standard Error) of the three wheat genotypes (n=6) was evaluated under both drought stress and control conditions. Statistically significant differences between day zero and subsequent days, determined by ANOVA, are indicated with asterisks (* for p=0.05 and *** for p=0.001). Additionally, significant disparities among genotypes on each selected day were denoted using letters (p<0.05)*

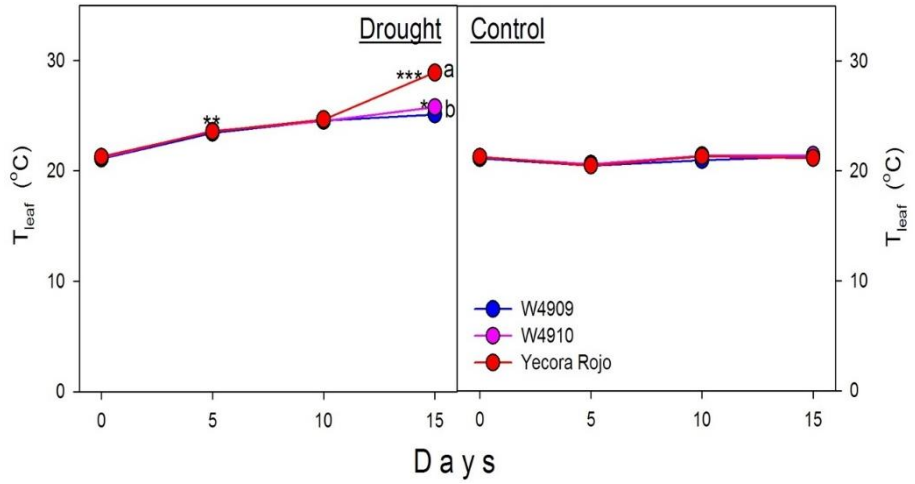

Figure 5. *Leaf temperature (Tleaf) ± SE (Standard Error) of the three wheat genotypes (n=6) was assessed under both drought stress and control conditions. Statistically significant differences between day zero and subsequent days were determined using ANOVA, denoted with an asterisk (* for p=0.01 and *** for p=0.001). Additionally, significant disparities among genotypes on each selected day were indicated using letters (p<0.05)*

Photosynthetic rate v/s relative water content

Highly significant positive relationships of R2= 0.93 were observed between the photosynthetic rate and relative water content in the cultivars W4909, W4910, and Yecora

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Rojo (*Fig. 6)*. Photosynthetic rate was generally dependent on relative water content in all the cultivars, but photosynthetic rate declined faster for a given change of relative water content in the sensitive cultivar Yecora Rojo.

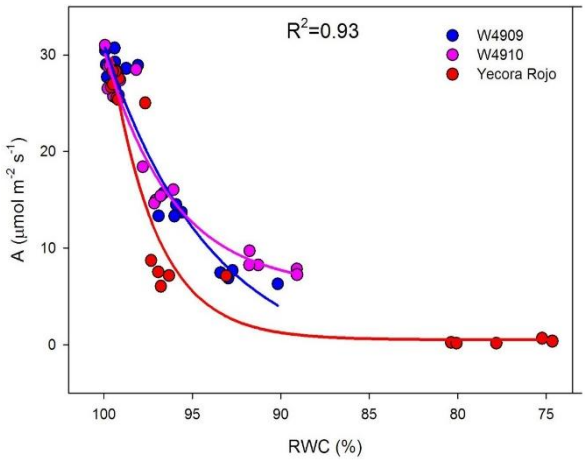

Figure 6. *Relationship between photosynthetic rate (A) and relative water content (RWC) in the tolerant and sensitive cultivars (R^2=0.93) in response to drought stress. Curves $y = a^{exp(-bx)}$, fitted using Sigmaplot. All give statistical significant fits (p<0.0001)*

A/C$_c$ curve results under the drought and control conditions

Under control conditions, all cultivars exhibited similar maximum carboxylation rates allowed by Ribulose 1,5-bisphophate carboxylase/oxygenase (Rubisco), rates of photosynthetic electron transport, mesophyll conductance, and daytime respiration. However, under drought stress, cultivars W4909 and W4910 maintained their photosynthetic performance relative to controls, while Yecora Rojo suffered severe effects. Although no genotypic differences were observed in photosynthetic performance at elevated CO_2 under control conditions, W4909 and W4910 maintained their photosynthetic rate at elevated CO2 under drought stress, unlike Yecora Rojo (*Fig. 7*). In the tolerant cultivars, decreased photosynthetic rates were associated with stomatal closure, limiting CO_2 uptake during drought stress, whereas in Yecora Rojo, the collapse of photosynthetic capacity even at high CO_2 levels suggests a different mechanism. Furthermore, based on A/Ci curves, W4909 and W4910 exhibited greater maximum carboxylation rates allowed by Rubisco and rates of photosynthetic electron transport compared to Yecora Rojo under drought stress.

Biochemical parameters results

Total protein content

By the tenth day of the drought treatment, a gradual but slight decrease in leaf total proteins was evident across all three cultivars (*Fig. 8*). However, on the fifteenth day, distinct differences emerged: W4910 exhibited significantly greater total protein content compared to W4909 and Yecora Rojo (P<0.05), while W4909 also had significantly higher total protein levels compared to Yecora Rojo (P<0.05). Notably, Yecora Rojo, the drought-sensitive cultivar, experienced a substantial 76% reduction in total protein content, followed by W4909 with a 36% decrease compared to day zero.

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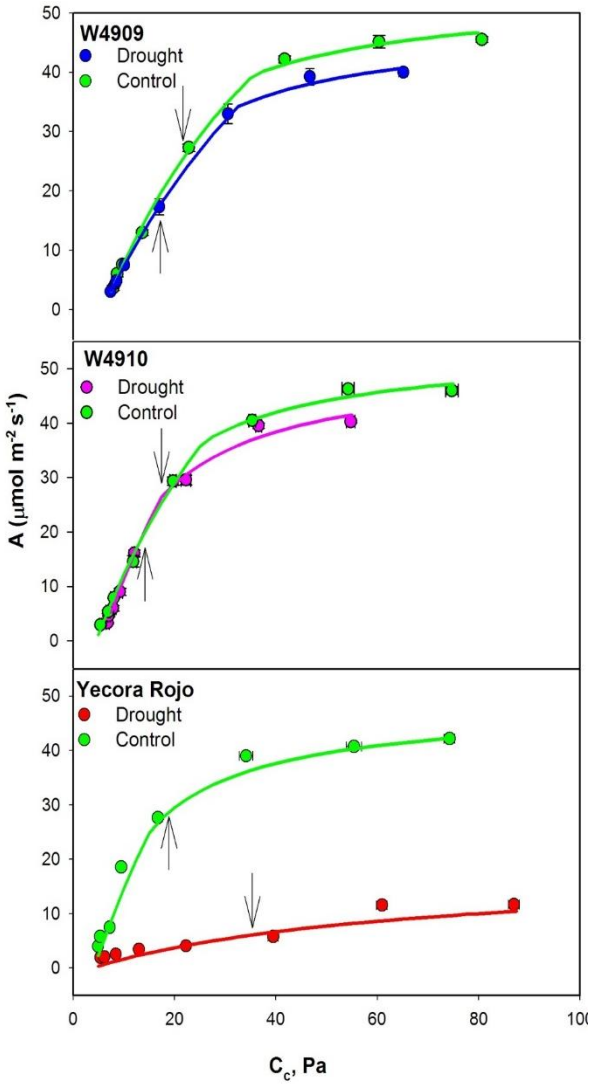

Figure 7. *Visual representation of the relationship between photosynthetic rate (A) and calculated CO_2 partial pressure at the sites of carboxylation (Pa) on the tenth day under both drought and control conditions. The curves were fitted utilizing the model developed by Sharkey et al. 2007 (model: 2007.1). Arrows indicate the operating points of A on the tenth day under drought and control conditions*

Rubisco content

Throughout the drought treatment, cultivars W4909 and W4910 exhibited no reduction in Rubisco content, maintaining consistency in this crucial enzyme (*Fig. 9*). Conversely, the drought-sensitive cultivar Yecora Rojo experienced a notable decline in leaf Rubisco content, with a significant decrease observed by the tenth day of the drought treatment. This decline persisted until the fifteenth day, resulting in a substantial loss of approximately 77% in Rubisco content. In contrast, W4909 and W4910 experienced milder reductions of 17% and 15% in Rubisco content, respectively, over the entire experimental period under drought conditions. By the fifteenth day, a significant genotypic difference emerged, with W4909 and W4910 displaying higher leaf Rubisco content compared to Yecora Rojo.

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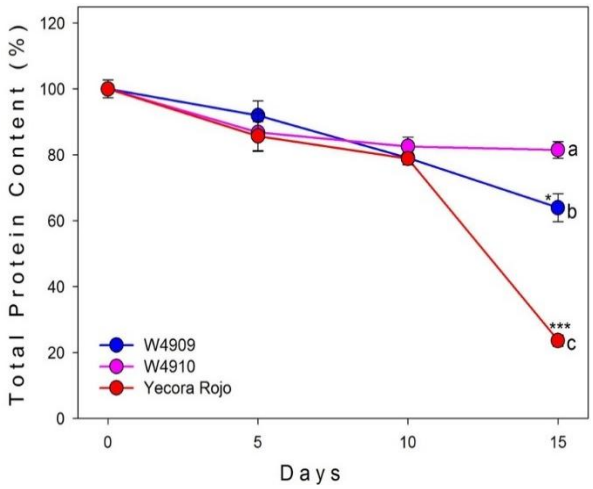

Figure 8. *The leaf total protein content (% of control) ± SE (Standard Error) of the three wheat genotypes (n=4) was evaluated under both drought stress and control conditions. Significant differences between day zero and subsequent days were determined using ANOVA, with asterisks denoting statistical significance levels (* for P=0.05 and *** for P=0.001). Letters indicate significant differences (P<0.05) among genotypes on each selected day*

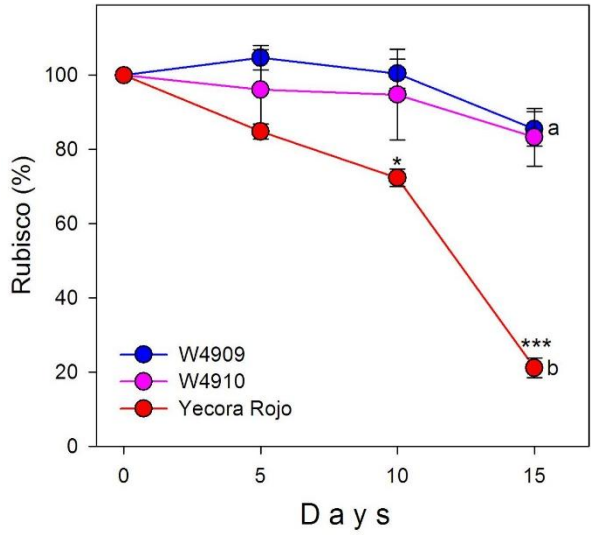

Figure 9. *The graph illustrates the Leaf Rubisco protein content (% of control) ± SE (Standard Error) of three wheat genotypes (n=4) under both drought stress and control conditions. Significant differences resulting from ANOVA between day zero and subsequent days are denoted by asterisks, where ** and *** signify P-values of 0.01 and 0.001, respectively. Furthermore, letters are used to indicate significant differences (P<0.05) among genotypes on each selected day*

Chlorophyll a

By the fifteenth day of the drought treatment, the chlorophyll a content in the cultivar Yecora Rojo had significantly decreased (P<0.05), whereas no significant decrease was observed in the chlorophyll a levels of cultivars W4909 and W4910 under the same conditions (*Fig. 10*). On the fifteenth day of the drought treatment, both cultivars W4909

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and W4910 exhibited significantly higher chlorophyll a levels compared to Yecora Rojo ($P<0.05$). Notably, there were no significant changes in chlorophyll a levels under control conditions across all three cultivars (*Fig. 10*).

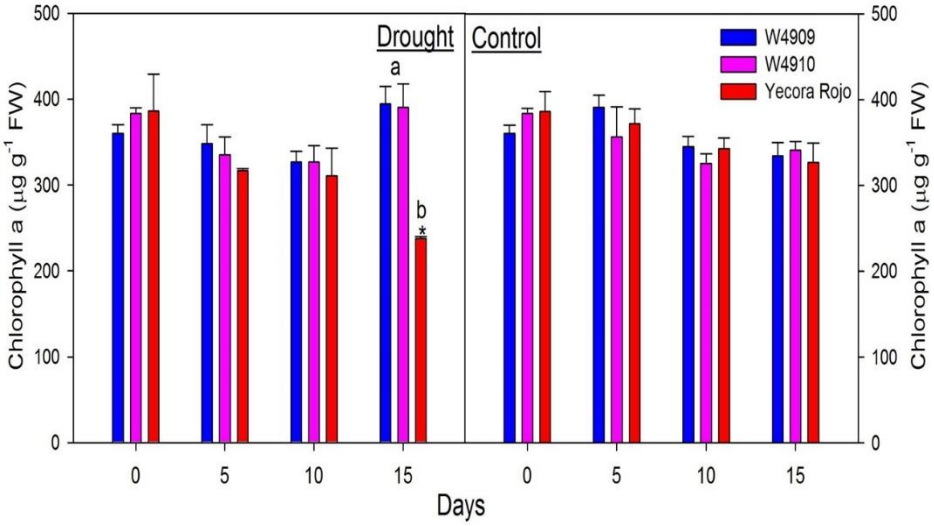

Figure 10. *The graph illustrates the chlorophyll a content ± SE (Standard Error) of the three wheat genotypes (n=4) under both drought stress and control conditions. Statistically significant differences between day zero and subsequent days (ANOVA) are denoted with an asterisk where *, indicating P=0.05. Additionally, letters indicate significant differences (P<0.05) among genotypes on each selected day*

Chlorophyll b

Chlorophyll b content remained relatively stable under both drought stress and control conditions across all three wheat cultivars, as depicted in *Figure 11*. No statistically significant changes were observed in chlorophyll b levels throughout the experimental period, indicating consistent levels of this pigment in the leaves of all cultivars under both conditions.

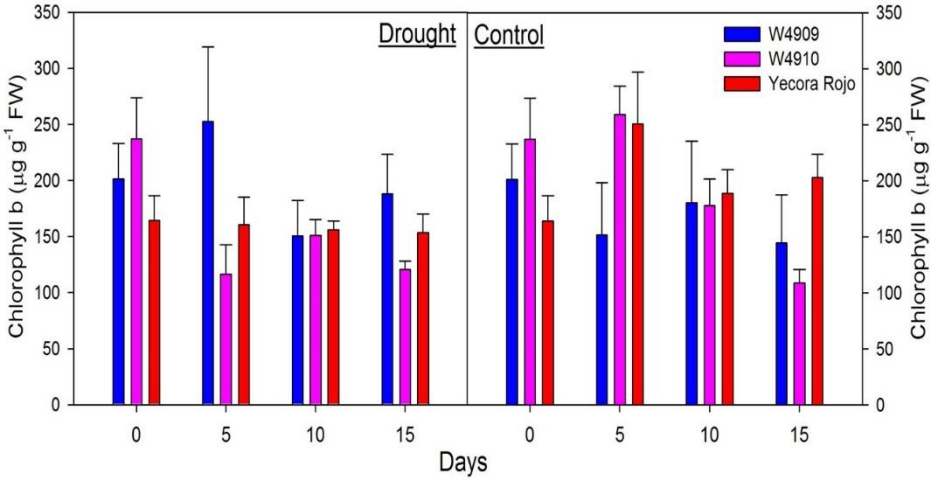

Figure 11. *The chlorophyll b content of the three wheat genotypes (n=4) was measured under both drought stress and control conditions*

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Total chlorophyll content

By the fifteenth day of the drought treatment, the leaf total chlorophyll content of Yecora Rojo significantly decreased ($P<0.05$), whereas no significant changes were observed in cultivars W4909 and W4910 under drought stress conditions. Conversely, both W4909 and W4910 exhibited significantly ($P<0.05$) higher total chlorophyll content compared to Yecora Rojo by the fifteenth day of the drought treatment. No significant alterations were noted in total chlorophyll content under control conditions across all three cultivars (*Fig. 12*).

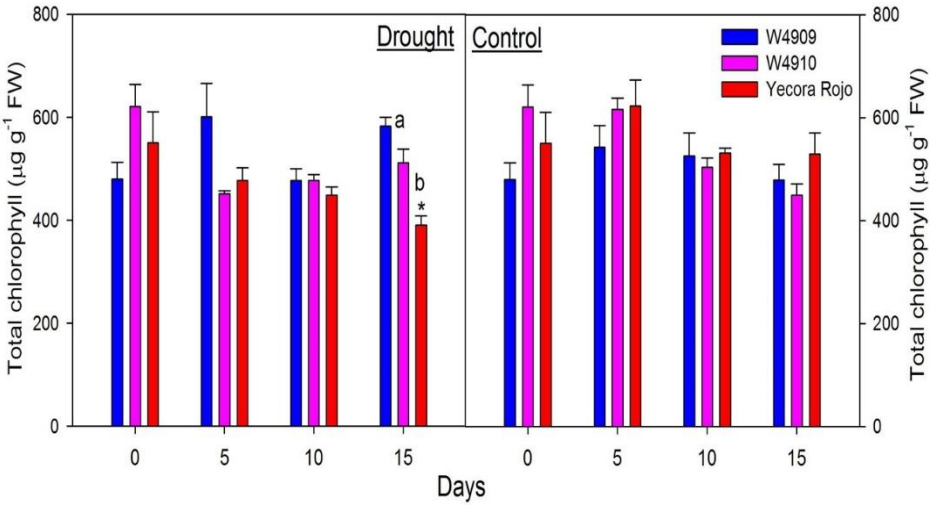

Figure 12. *The graph illustrates the total chlorophyll content ± SE (Standard Error) of the three wheat genotypes (n=4) under both drought stress and control conditions. Statistically significant differences between day zero and subsequent days (ANOVA) are indicated with an asterisk where *, representing P=0.05. Additionally, letters signify significant differences (P<0.05) among genotypes on each selected day*

Carotenoid content

Until the tenth day of the drought treatment, there were no notable changes in carotenoid content across all three wheat cultivars (*Fig. 13*). However, by the fifteenth day, cultivars W4909 and W4910 exhibited a significant ($P<0.05$) increase in carotenoid content. In contrast, there was no significant change observed in the drought-sensitive cultivar Yecora Rojo under drought conditions. By the fifteenth day, both tolerant cultivars, W4909 and W4910, showed significantly ($P<0.05$) higher carotenoid content compared to Yecora Rojo. No significant alterations were observed in carotenoid content under control conditions for any of the three cultivars (*Fig. 13*).

Water use efficiency

By the tenth day of the drought treatment, all cultivars exhibited a noteworthy increase in water use efficiency (WUE), with significant changes observed across the board ($P<0.001$ and $P<0.01$) (*Fig. 14*). However, by the fifteenth day, the drought-sensitive cultivar Yecora Rojo showed a significant decrease in WUE ($P<0.001$), while cultivars W4909 and W4910 continued to experience a sustained increase in WUE throughout the drought treatment. Notably, by the fifteenth day, W4909 and W4910 demonstrated

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significantly higher WUE compared to Yecora Rojo, which, in turn, exhibited significantly lower WUE (P<0.05) (*Fig. 14*).

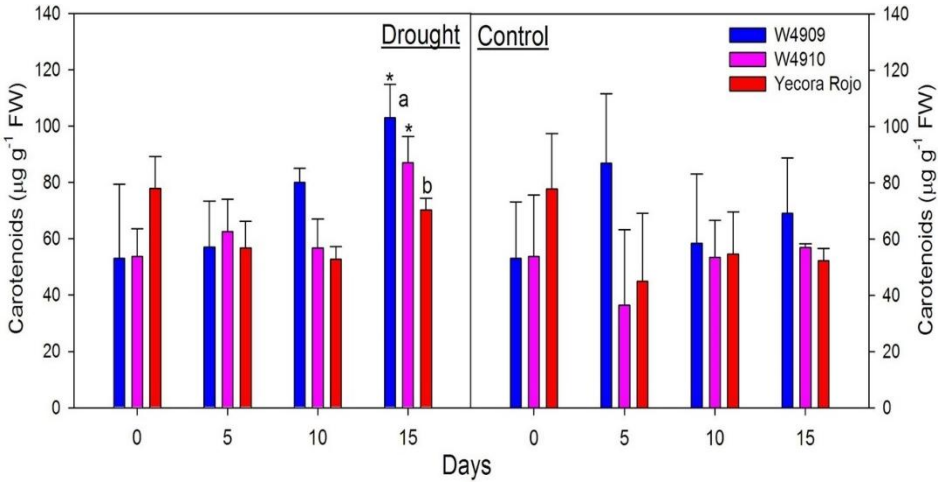

Figure 13. *Carotenoids content ± SE (Standard Error) of the three wheat genotypes (n=6) under drought stress and control conditions. Statistically significant differences between day zero and the other days (ANOVA) are marked with an asterisk where *, equals P=0.05; letters show significant differences (P<0.05) among genotypes on each selected day*

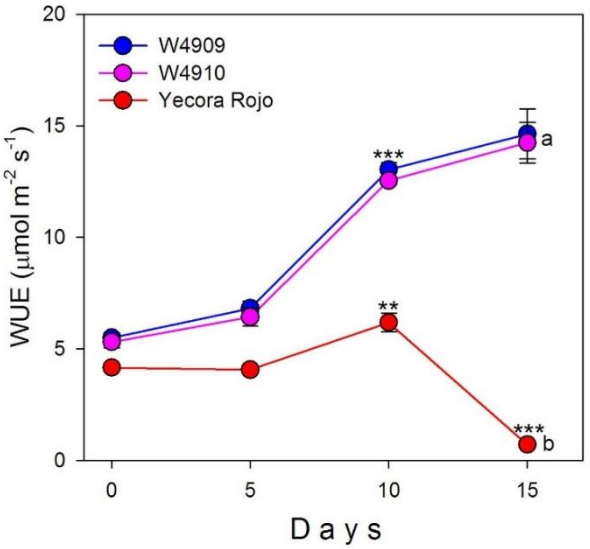

Figure 14. *Water use efficiency (WUE), calculated as the ratio of photosynthetic rate to transpiration rate in drought-stressed plants, was assessed across six replicates of three wheat genotypes under both drought stress and control conditions. Significant changes (P<0.01 and P<0.001) in WUE were evident by the tenth day of drought treatment, indicating an overall increase in all cultivars. However, by the fifteenth day, while cultivars W4909 and W4910 sustained this improvement, the drought-sensitive Yecora Rojo experienced a significant decrease (P<0.001). Notably, on the fifteenth day, W4909 and W4910 demonstrated significantly higher WUE compared to Yecora Rojo (P<0.05)*

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Rubisco efficiency

A consistent trend akin to water use efficiency (WUE) was observed in Rubisco efficiency (RE) across all cultivars (*Fig. 15*). By the tenth day of the drought treatment, all cultivars exhibited a significant increase ($P<0.01$) in RE. However, cultivars W4909 and W4910 continued to display a sustained and statistically significant ($P<0.001$) increase in RE by the fifteenth day of drought treatment. In contrast, Yecora Rojo experienced a notable decrease in RE by the fifteenth day ($P<0.05$), compared to the tenth day of drought treatment. Furthermore, on the fifteenth day, W4909 and W4910 demonstrated significantly higher RE compared to Yecora Rojo ($P<0.05$).

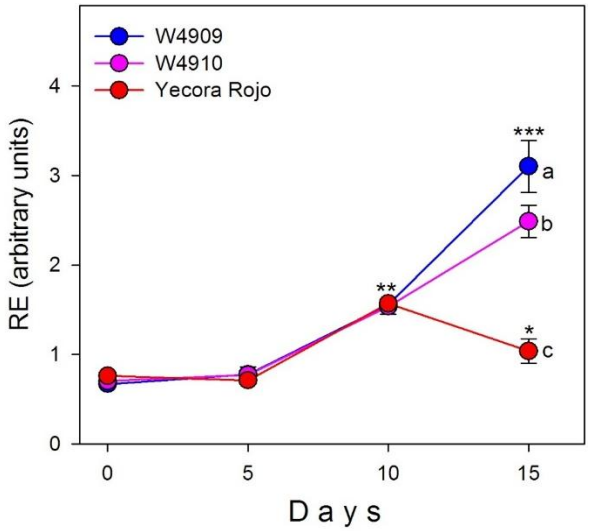

Figure 15. *Rubisco efficiency (RE) ± SE (Standard Error) of the three wheat genotypes (n=6) under drought stress and control conditions is presented. RE was calculated as the photosynthetic rate divided by Rubisco content in the drought-stressed plants. Statistically significant differences between day zero and subsequent days (ANOVA) are indicated with an asterisk where *, **, and ***, represent P=0.05, P=0.01, and P=0.001, respectively. Letters denote significant differences (P<0.05) among genotypes on each selected day*

Discussion

In the vast agricultural tapestry, drought unfurls as a formidable antagonist, orchestrating a complex array of morpho-physiological challenges for wheat. Hussain et al. (2019) unveil the adversity brought upon plant height, leaf area, and the very essence of water content, painting a picture of struggle and adaptation. This narrative delves deeper into the heart of the plant's resilience, exploring how wheat cultivars, through a ballet of stomatal adjustments, endeavor to conserve life's elixir, water, amidst the throes of water scarcity (Meriam et al., 2018; Upadhyaya et al., 2021). Our investigation highlights the strategic maneuvers of salt-tolerant cultivars W4909 and W4910, which exhibit an early reduction in transpiration, a testament to their ingenious adaptation to minimize water loss, thereby sustaining photosynthetic efficiency. This contrast starkly with the drought-sensitive Yecora Rojo, which, in failing to curb early water loss, succumbs to diminished water potential and content (Shewry et al., 2015; Paul et al., 2018; Abro et al., 2021). This pivotal distinction underscores the nuanced survival strategies that distinguish the resilient from the vulnerable. As the narrative unfolds, we

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witness how these early decisions in water management ripple through to stomatal conductance and transpiration rates, setting the stage for a dynamic interplay between water conservation and gas exchange. The saga of leaf temperature escalation, particularly pronounced in the drought-sensitive Yecora Rojo, reveals the intricate linkages between stomatal behavior, transpiration, and thermal regulation under drought stress, inviting further contemplation on the cascading effects on photosynthesis (Abro et al., 2022; Semahegn et al., 2022). The plot thickens with the curious case of internal CO_2 concentration (Ci), where the sensitive Yecora Rojo exhibits an unexpected rise, hinting at a deeper metabolic turmoil or a shift in photosynthetic dynamics under duress (Bala et al., 2018; Ahmed et al., 2022). This anomaly beckons a closer examination of the photosynthetic conundrum, where the intricacies of CO_2 supply and demand under drought are laid bare, weaving a complex tale of survival and adaptation. Amidst this physiological odyssey, the role of chlorophyll and carotenoids emerges as a beacon of hope, illuminating the path to resilience (Abro et al., 2019). The higher chlorophyll content in the salt-tolerant cultivars not only sustains photosynthesis but also embodies a shield against the ravages of drought, a narrative supported by the legacy of research spanning decades (Kraus et al., 1995; Sairam et al., 1997/1998). Similarly, the elevation in carotenoid content underlines a strategic antioxidant defense, safeguarding the cellular sanctuaries from oxidative assailants (Bala et al., 2018). This exploration into the realm of wheat under drought stress unveils a saga of resilience, adaptation, and the relentless pursuit of survival. The nuanced dance of physiological and biochemical responses, from the strategic management of water to the bolstering of antioxidant defenses, sketches a portrait of wheat's indomitable spirit. It's a testament to the plant's evolutionary prowess, a beacon of hope for breeding future generations of wheat capable of thriving in an increasingly thirsty world.

Conclusion

In the scientific odyssey to unveil wheat's resilience against drought, the salt-tolerant cultivars W4909 and W4910 emerge as champions of endurance. Their strategy is elegantly simple yet profoundly effective: modulating transpiration through stomatal closure to safeguard water potential and photosynthesis. This not only preserves leaf vitality but ensures a robust defense against the arid challenge. While W4909 and W4910 flourish, harnessing lower transpiration, enhanced photosynthetic prowess, and a wealth of Rubisco and chlorophyll, the drought-sensitive Yecora Rojo narrates a tale of vulnerability, marked by a decline in leaf proteins and Rubisco, signaling an onset of senescence. Amidst elevated CO_2, a remarkable phenomenon unfolds: W4909 and W4910 resiliently rebound in photosynthetic rate by the tenth day of drought, a feat unachieved by Yecora Rojo, which suffers under the weight of compromised photosynthetic machinery. Moreover, W4909 and W4910 unveil an antioxidant strategy, bolstering carotenoids to counteract oxidative stress, highlighting a sophisticated layer of protection against drought-induced damage. This scientific saga reveals not just the traits that confer drought tolerance but underscores the nuanced genotypic interplay under stress. It offers a lens into the future of crop improvement, where understanding and harnessing these mechanisms can lead to the cultivation of wheat varieties that stand resilient in the face of drought, securing food sustainability in our changing world.

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