

Article

Integrated Effects of Soil Moisture on Wheat Hydraulic Properties and Stomatal Regulation

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Abstract: The development of water-saving management relies on understanding the physiological response of crops to soil drought. The coordinated regulation of hydraulics and stomatal conductance in plant water relations has steadily received attention. However, research focusing on grain crops, such as winter wheat, remains limited. In this study, three soil water supply treatments, including high (H), moderate (M), and low (L) soil water contents, were conducted with potted winter wheat. Leaf water potential (Ψ_{leaf}), leaf hydraulic conductance (K_{leaf}), and stomatal conductance (g_s), as well as leaf biochemical parameters and stomatal traits were measured. Results showed that, compared to H, predawn leaf water potential (Ψ_{PD}) significantly reduced by 48.10% and 47.91%, midday leaf water potential (Ψ_{MD}) reduced by 40.71% and 43.20%, K_{leaf} reduced by 64.80% and 65.61%, and g_s reduced by 21.20% and 43.41%, respectively, under M and L conditions. Although g_s showed a significant difference between M and L, Ψ_{leaf} and K_{leaf} did not show significant differences between these treatments. The maximum carboxylation rate (V_{cmax}) and maximum electron transfer rate (J_{max}) under L significantly decreased by 23.11% and 28.10%, stomatal density (SD) and stomatal pore area index (SPI) under L on the abaxial side increased by 59.80% and 52.30%, respectively, compared to H. The leaf water potential at 50% hydraulic conduction loss (P_{50}) under L was not significantly reduced. The g_s was positively correlated with Ψ_{MD} and K_{leaf} , but it was negatively correlated with abscisic acid (ABA) and SD. A threshold relationship between g_s and K_{leaf} was observed, with rapid and linear reduction in g_s occurring only when K_{leaf} fell below $8.70 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$. Our findings demonstrate that wheat leaves adapt stomatal regulation strategies from anisohydric to isohydric in response to reduced soil water content. These results enrich the theory of trade-offs between the carbon assimilation and hydraulic safety in crops and also provide a theoretical basis for water management practices based on stomatal regulation strategies under varying soil water conditions.

Keywords: winter wheat; soil water stress; leaf water potential; leaf hydraulic conductance; stomatal conductance; stomatal trait



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1. Introduction

Soil drought stress is a critical constraint to grain crop production, and the increasing frequency and intensity of soil drought are exacerbating crop water demand [1]. To cope with this challenge, plants have evolved a great number of physiological adaptive mechanisms, especially for the regulation of stomatal conductance (g_s) as a water-saving mechanism, which has steadily received attention [2]. However, research focusing on grain crops, such as winter wheat, is still insufficient, including the coordinated regulation of hydraulics and stomatal conductance in plant water relations. Therefore, it is necessary to explore the complex relationship between stomatal behavior, leaf water status, and

morphological adaptations of stomata in response to various drought stresses. This is essential for optimizing water use efficiency in agricultural production.

There have been several studies on the effects of soil drought on hydraulic and stomatal indices. For example, Wang et al. [3] demonstrated that soil water stress significantly decreased g_s in winter wheat, highlighting the plant's vulnerability to drought conditions. The midday leaf water potential (Ψ_{MD}) in field winter wheat [4] and potted rice [5] decreased significantly under soil water stress. Soil water stress was shown to diminish leaf hydraulic conductance (K_{leaf}) in *Arabidopsis* and olive, as documented by Hernandez et al. [6] and Scoffoni et al. [7]. Previous studies showed that the variable K_{leaf} was closely related to the response of g_s to drought stress in two Mediterranean fruit tree species [6]. The sensitivity of the plant to drought can be assessed by fitting a vulnerability curve to the relationship between K_{leaf} and Ψ_{leaf} . Xylem water potential at 50% hydraulic conduction loss (P_{50}) can be derived from this curve, serving as an indicator of the plant's susceptibility to drought. A higher P_{50} value indicates greater vulnerability of the crop to drought [8]. Stiller et al. [9] identified xylem cavitation as the primary factor in the reduction of the K_{leaf} of rice during soil drought. These findings provide valuable insights into the effects of soil drought on key hydraulic and stomatal indices, but it is necessary to consider the integrated effects of soil moisture on plant hydraulic properties and stomatal regulation.

Based on the sensitivity of stomata to hydraulic imbalance induced by drought, strategies for regulating stomata are categorized into isohydric and anisohydric types [10]. There is an ongoing debate among researchers about whether K_{leaf} is the primary factor behind stomatal closure. Studies have shown that the decrease in g_s was not primarily influenced by K_{leaf} . For instance, Corso et al. [11] proposed that during varying degrees of soil water stress, the decline in K_{leaf} did not predominantly control stomatal closure in wheat under soil water stress conditions ranging from 0 to -5 MPa. On the contrary, some studies suggested that K_{leaf} was the main driving force for the decrease in g_s . For instance, Tombesi et al. [12] discovered that in grapes, stomatal closure due to drought was initially caused by a drop in K_{leaf} and was maintained by abscisic acid (ABA). Wang et al. [5] reported that during dehydration, K_{leaf} was the primary driver of stomatal closure and reduction in g_s under soil drought stress in rice. In addition, the decrease in g_s could be attributed to other factors besides K_{leaf} . For instance, Aasamaa et al. [13] found that the reduction in g_s was predominantly governed by Ψ_{leaf} under drought conditions in tomatoes. Abdalla et al. [14] identified that the reduction in root hydraulic conductivity was the primary driver of stomatal closure in tomato under soil drought. Hence, it is necessary to further investigate the relationship between stomatal behavior and leaf water status (e.g., Ψ_{leaf} and K_{leaf}) in winter wheat under soil drought stress.

Crops can adapt to drought-induced environmental changes through both short-term control of stomatal pore size and long-term control of stomatal development and morphology [15]. Stomatal traits are important factors that affect g_s [16]. Numerous studies have focused on the response of stomatal length (SL_{en}) and stomatal density (SD) under soil drought stress. The stomata traits of different crops, or even the same crops, reflect different responses to drought stress. For instance, Li et al. [17] observed that wheat exposed to drought exhibited reduced SL_{en} on both the adaxial and abaxial sides, accompanied by a negative correlation between SD and g_s . Zhao et al. [18] reported that drought notably increased SD and caused a reduction in both stomatal pore sizes in maize. Studies on *Leymus chinensis* showed that moderate soil drought increased SD, while severe drought decreased SD. Xu et al. [19] and Yang et al. [20] discovered that drought induced a gradual increase in adaxial SD in tomato, while adaxial SD initially decreased and then increased. Although the present study has revealed the variability of stomatal responses to drought stress across different species and even within the same species, it remains unclear how these stomatal traits interact with other physiological indices under different intensities and durations of soil drought stress.

At present, there are few studies on the stomatal traits of wheat under various soil drought stress conditions and their quantitative relationships with physiological parame-

ters. Previous studies have primarily focused on the response of crops to water stress in terms of physiological and growth parameters. However, there has been less emphasis on understanding the internal mechanisms of g_s in response to drought stress through leaf water status and stomatal traits. In this study, winter wheat was subjected to high, moderate, and low soil water supply treatments. Various parameters including the g_s , Ψ_{leaf} , K_{leaf} , maximum carboxylation rate (V_{cmax}), maximum electron transfer rate (J_{max}), stomatal morphology traits, relative water content (RWC), and ABA of leaves were measured. The aims of this study were to analyze how these parameters respond to different soil drought stress and how they interrelate, thus revealing the physiological mechanism of winter wheat's drought tolerance under various soil water conditions. It is of great scientific significance to explain the internal causes of g_s changes caused by different drought stress treatments in terms of plant physiology and stomatal characteristics in order to enhance wheat water use efficiency.

2. Results

2.1. Stomatal Conductance, Leaf Hydraulic Conductivity and Leaf Water Potential Response

There was no significant difference in K_{leaf} between the M and L soil water supply. However, compared to H, K_{leaf} significantly reduced by 64.80% in M and 65.61% in L (Figure 1a). Specifically, g_s significantly reduced by 21.20% in M and 43.41% in L, compared to H (Figure 1b). Ψ_{PD} and Ψ_{MD} did not significantly change between M and L. However, when compared to H, Ψ_{PD} significantly reduced by 48.10% in M and 47.91% in L, and Ψ_{MD} significantly reduced by 40.71% in M and 43.20% in L (Figure 1c).

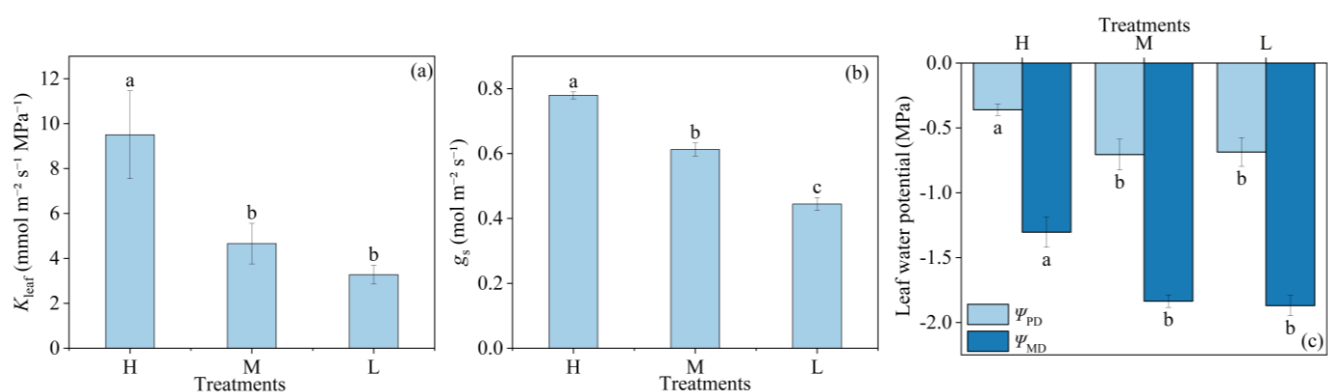


Figure 1. Leaf hydraulic conductivity (K_{leaf}) (a), stomatal conductance (g_s) (b), and leaf water potential at predawn (Ψ_{PD}) and midday (Ψ_{MD}) (c) under high (H), moderate (M), and low (L) soil water supply. All data represent means $\pm$ standard errors of five replicates. Different letters indicate significant differences at the $p < 0.05$ level between treatments according to Duncan's multiple range test.

The relationship between g_s and K_{leaf} was found to be piecewise linear ($R^2 = 0.49$, $p = 0.049$). When K_{leaf} exceeded $8.70 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$, g_s decreased at a slower rate. Conversely, when K_{leaf} was less than $8.70 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$, g_s decreased more rapidly (Figure 2a). Similarly, a piecewise linear relationship was observed between g_s and Ψ_{MD} ($R^2 = 0.67$, $p = 0.004$). When Ψ_{MD} was above -1.48 MPa , g_s decreased slowly; however, when Ψ_{MD} dropped below -1.48 MPa , g_s decreased rapidly (Figure 2b). K_{leaf} and Ψ_{MD} presented a significant positive correlation ($R^2 = 0.46$, $p = 0.004$) (Figure 2c).

The P_{50} was estimated as -1.04 and -1.01 MPa in H and M, respectively. The slope (S) of the vulnerability curve at the inflection point (P_{50}) was estimated as -58.3% and -52.67% in H and M, respectively. The overlapping confidence intervals for the hydraulic vulnerability curves of the leaves suggest that, when assessed at a 95% confidence level, there was no statistically significant difference between H and M treatments (Figure 3).

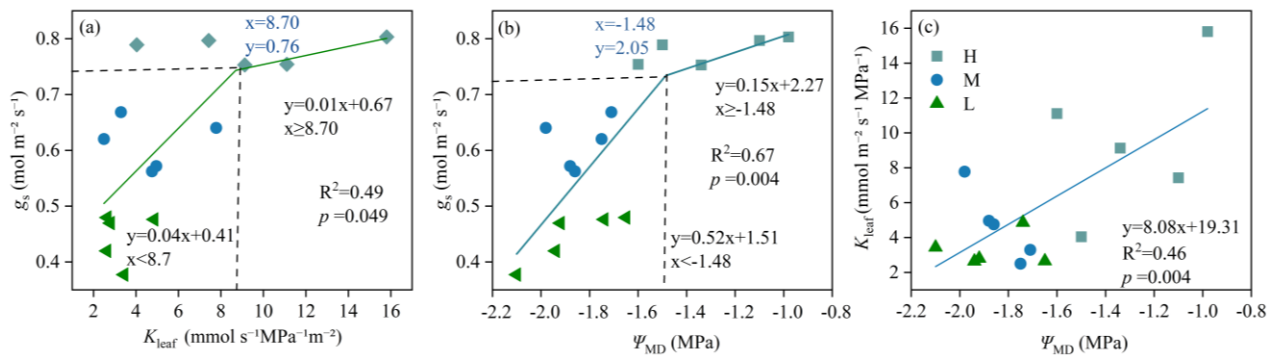


Figure 2. Relationships between stomatal conductance (g_s) and leaf hydraulic conductivity (K_{leaf} , (a)), g_s and midday leaf water potential (Ψ_{MD} , (b)), and K_{leaf} and Ψ_{MD} (c) under high (H), moderate (M), and low (L) soil water supply. Piecewise linear regressions were used to model g_s vs. K_{leaf} and g_s vs. Ψ_{MD} , and linear regression of K_{leaf} vs. Ψ_{MD} was used to fit the data. The dashed lines in (a,b) indicate the horizontal and vertical coordinates of the breakpoints, which are known as segment breakpoints. Before the point, the model shows a clear linear correlation, while, after the breakpoints, the model follows a different linear relationship.

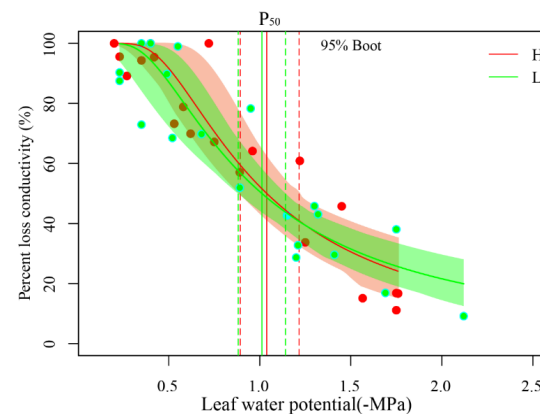


Figure 3. Percentage loss of wheat leaf hydraulic conductance with decreasing leaf water potential under high (H) and low (L) soil water supply treatments. The vertical solid line in each curve represents leaf water potential at 50% hydraulic conduction loss (P_{50}), while the vertical dashed lines indicate its 95% confidence interval. The light green area for the low treatment (L) and the light red area for the high treatment (H) represent the standard error of the estimated parameters.

2.2. Biochemical Indices of Leaves

The V_{cmax} and J_{max} varied from 65.73 to 89.87 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 117.14 to 223.22 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. The V_{cmax} and J_{max} of L significantly reduced by 23.11% and 28.10%, respectively, compared to H. There was no significant difference in V_{cmax} and J_{max} between M and H (Table 1). The RWC of the L treatment significantly reduced by 11.47% compared to H. There was no significant difference in RWC between H and M. ABA significantly increased by 69.35% in M and 89.32% in L.

Table 1. Biochemical indicators of different water treatments.

Treatments	V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	RWC (%)	ABA (ng g^{-1})
H	89.87 a	182.49 a	0.61 a	34.95 c
M	83.05 a	181.05 a	0.54 a	59.19 b
L	65.73 b	131.18 b	0.37 b	66.17 a

Note: All data represent the means of five replicates. Different letters indicate significant differences at the $p < 0.05$ level between treatments according to Duncan's multiple range test. H: high soil water supply; M: moderate soil water supply; L: low soil water supply. V_{cmax} , maximum rate of carboxylation; J_{max} , maximum photosynthetic electron transport; RWC, relative water content; ABA, abscisic acid.

There was a significant positive correlation between g_s and V_{cmax} ($R^2 = 0.64$, $p < 0.001$), and g_s and J_{max} ($R^2 = 0.35$, $p = 0.019$) (Figure 4a,b). A significant negative correlation was found between g_s and ABA ($R^2 = 0.81$, $p < 0.001$) (Figure 4c).

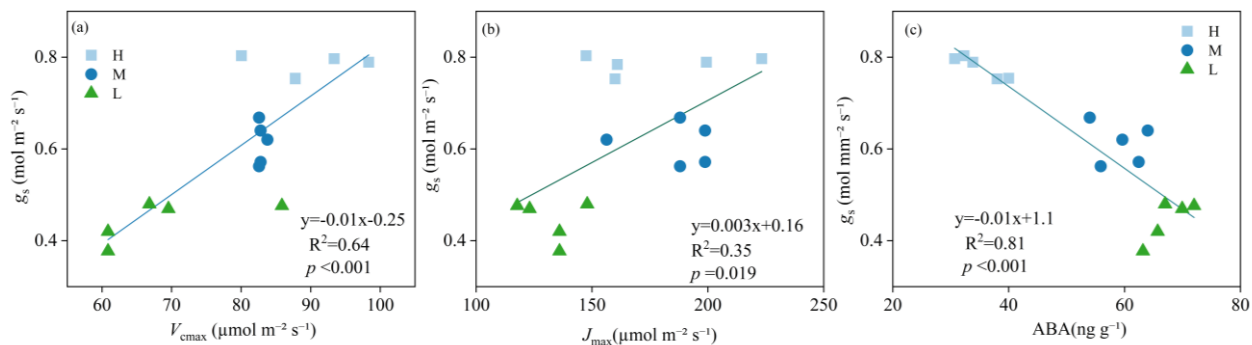


Figure 4. Relationships between stomatal conductance (g_s) and maximum rate of carboxylation (V_{cmax}), (a); g_s and maximum photosynthetic electron transport rates (J_{max}), (b); g_s and abscisic acid content (ABA), (c) under high (H), moderate (M), and low (L) soil water supply. Linear regressions of g_s vs. V_{cmax} , g_s vs. J_{max} , and g_s vs. ABA were used to fit the data.

2.3. Stomatal Traits

There were no significant differences in SL_{en} on both the adaxial and abaxial leaf sides among three soil water levels (Figure 5a). The SD on the adaxial side significantly increased by 81.82% in L compared to H. The SD on the abaxial side significantly increased by 24.71% in M and 59.80% in L compared to H (Figure 5b). The stomatal pore index (SPI) on the abaxial side significantly increased by 52.30% in L compared to H. Conversely, there was no significant difference for SPI on the adaxial side among the three soil water levels (Figure 5c).

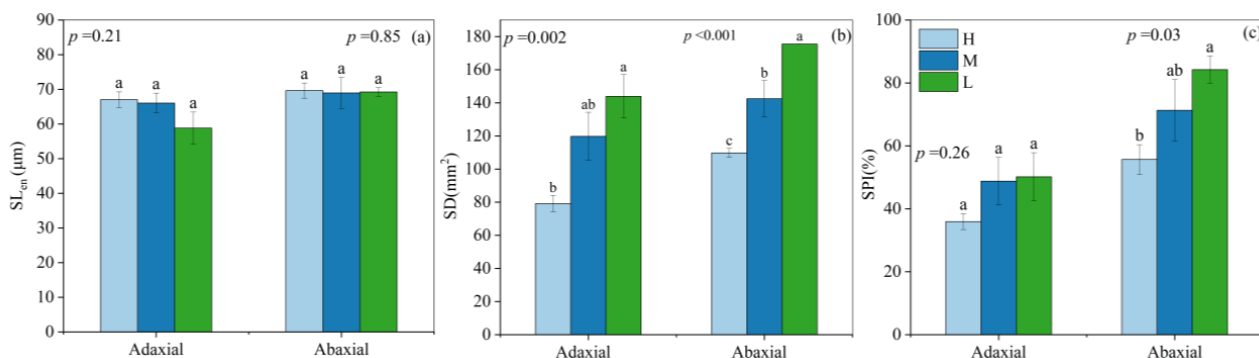


Figure 5. Stomatal traits on the adaxial and abaxial epidermis under high (H), moderate (M), and low (L) soil water supply, including stomatal length (SL_{en} , (a)), stomatal density (SD, (b)), and stomatal pore index (SPI, (c)). All data represent the means $\pm$ standard errors of five replicates. Different letters indicate significant differences at the $p < 0.05$ level between soil water levels according to Duncan's multiple range test.

A strong negative correlation was observed between g_s and SD on the adaxial side ($R^2 = 0.73$) and on the abaxial side ($R^2 = 0.74$). In contrast, a significant correlation between V_{cmax} and SD on the adaxial side ($R^2 = 0.53$) and on the abaxial side ($R^2 = 0.52$) was found in Figure 6.

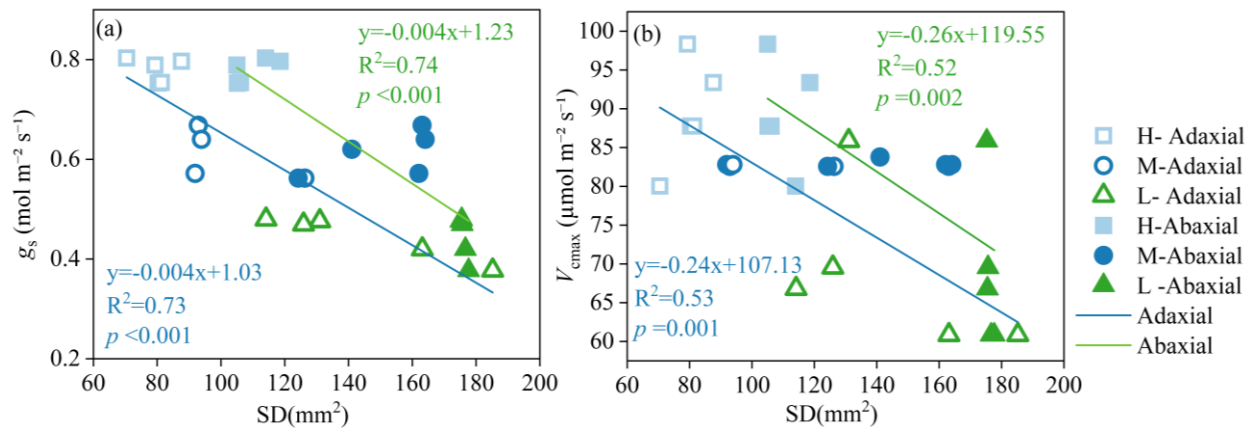


Figure 6. Relationships between stomatal conductance (g_s) and stomatal density (SD) (a), and maximum rate of carboxylation (V_{cmax}) and SD (b), on the adaxial and abaxial epidermis under high (H), moderate (M), and low (L) soil water supply. Linear regressions of g_s vs. SD and V_{cmax} vs. SD were used to fit the data. The lines are colored green for the adaxial side and light blue for the abaxial side.

3. Discussion

It is crucial to understand how plants respond to drought stress, particularly given the ongoing global climate change. The responses of crops to drought are primarily shaped by imbalances in their water transport systems and the regulation of stomatal movements. Our findings further support this view and reveal how crops adapt to drought conditions by balancing g_s and K_{leaf} . As previously documented [21], crops absorb CO_2 through their stomata while releasing water vapor, demonstrating the intricate relationship between g_s and K_{leaf} .

Our study revealed significant physiological adaptations in wheat plants under varying soil drought stress. As soil water content declined to 65–75% of field capacity (FC), plants under M showed significantly lower Ψ_{PD} and Ψ_{MD} , K_{leaf} , and g_s compared to H. Notably, the degree of reduction in Ψ_{PD} and Ψ_{MD} was approximately one-third of the K_{leaf} reduction, while the reduction in g_s was nearly two-thirds of the K_{leaf} reduction. This indicated that in early soil drought stress, plants prioritize limiting water loss by reducing g_s , followed by a decrease in K_{leaf} . Meanwhile, photosynthetic capacity parameters (i.e., V_{cmax} and J_{max}) remained stable, suggesting that photosynthesis is initially preserved. However, as drought intensified to 45–55% FC, g_s , V_{cmax} , and J_{max} declined significantly, indicating a sequential physiological response. The K_{leaf} and Ψ_{leaf} did not continue to decrease significantly with increasing soil drought intensity, while g_s showed consistent reductions, highlighting its sensitivity to soil drought stress. These findings demonstrated a shift in wheat strategies from favoring growth to prioritizing survival under soil drought, reflecting the complexity of plant adaptations to soil drought stress.

The results of our study confirm previous findings that wheat plants undergo a significant decline in leaf water status under soil drought stress. Specifically, Nayyar et al. [22] reported that RWC in wheat leaves decreased by 34.8% when soil moisture dropped by 46.7%. These findings are consistent with our observations, where RWC significantly declined by 11.5% in L compared with H. Akter et al. [4] found a 39.5% reduction in the Ψ_{PD} of wheat under conditions when soil water content is around 18%. We also observed that both Ψ_{PD} and Ψ_{MD} under M and L similarly reduced compared to H (Figure 1c). Previous studies have demonstrated that under drought stress, the reduction in K_{leaf} directly impacts both Ψ_{leaf} and stomatal regulation [23]. In our study, we also observed that g_s showed a significant positive correlation with Ψ_{leaf} (Figure 2b). This finding was consistent with previous research [24], which demonstrated a strong positive correlation between the decrease in Ψ_{leaf} and g_s during soil drought in tomato.

There have been few studies focusing on the driving factors that influence stomatal behavior under soil water stress. Müllers et al. [23] reported the impact of soil water stress on Ψ_{leaf} and noted the role of Ψ_{leaf} in facilitating stomatal closure in maize. This observation was consistent with our own study, as illustrated in Figure 2b, where similar patterns of stomatal response to water stress were observed. The difference in stomatal behavior was observed in our study when K_{leaf} dropped below $8.70 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$. As K_{leaf} decreased, g_s reduction rapidly occurred. When K_{leaf} was higher than $8.70 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$, g_s decreased slowly with K_{leaf} decreasing. The plant's ability to transport water efficiently through the xylem is greatly diminished, leading to a rapid decline in g_s as a protective mechanism to prevent further water loss. This threshold effect suggests that plants have evolved specific physiological mechanisms to respond to varying degrees of soil water stress. When K_{leaf} remains above the threshold, plants can maintain relatively high g_s levels, balancing the need for carbon assimilation through photosynthesis with the risk of excessive water loss. However, as soil water stress intensifies and K_{leaf} decreases below the threshold, plants rapidly close their stomata to minimize water loss, even if it comes at the cost of reduced carbon assimilation. The reasons for this difference in stomatal behavior compared to previous studies that observed a linear correlation between g_s and K_{leaf} in rice [25] are likely multifaceted. One possibility is that different plant species exhibit varying degrees of sensitivity to soil water stress, with some species being more reliant on stomatal closure as a protective mechanism than others. Additionally, environmental factors such as temperature, humidity, and soil texture can influence stomatal behavior and the relationship between g_s and K_{leaf} .

We observed that g_s started to decrease rapidly in correlation with Ψ_{MD} (Figure 2b). It was less than embolism (P_{50} , Figure 3). This result is similar to previous studies. Martin et al. [26], through a meta-analysis of functional traits associated with stomatal response to drought, revealed that in most species, stomatal closure precedes the occurrence of embolism during periods of water scarcity. Similarly, Chen et al. [27] reported that Ginkgo trees closed their stomata before a significant loss of xylem hydraulic conductivity. These observations support the view that stomatal behavior is a primary mechanism for plants to regulate water status and avoid water loss during drought stress.

Notably, the relationship between g_s decline and xylem embolism was not consistent across all conditions. Corso et al. [11] demonstrated that the decrease in K_{leaf} and g_s in wheat under soil drought was not attributed to xylem embolism but might be induced by changes in the water absorption capacity of roots. This suggests that the interaction between stomatal behavior and xylem embolism may vary among plant species and under different soil drought conditions. Our results further indicated that as soil water deficit reached severe levels (M and L treatments), the decline in g_s was primarily driven by K_{leaf} (Figure 2a). This suggested that under severe water stress, plants may regulate water transport in the leaves to further reduce water loss. Such regulation could be associated with the redistribution of water within the plant and its preferential allocation to vital life processes such as photosynthesis.

Understanding the interaction between stomatal closure, which regulates water loss through transpiration, and xylem embolism, which affects water transport in the plant vasculature, is key to comprehending plant adaptation strategies. In our study, we observed that as soil water deficit surpassed a certain threshold (under M and L), the decline of g_s was primarily driven by changes in K_{leaf} (Figure 2a). This finding highlights the intricate interplay between stomatal behavior and xylem embolism in plants experiencing water stress. Notably, no significant difference in P_{50} was observed between H and L (Figure 3). This could be explained by the fact that cavitation, the formation of air embolisms in the xylem, is a common occurrence in crops, including rice, and that rice plants have the ability to quickly reverse cavitation [9]. This resilience to embolism reversal is crucial for plants to maintain water transport efficiency and survive drought stress. Furthermore, studying the interaction between stomatal behavior and xylem embolism under different environmental conditions could provide valuable information for predicting plant responses to climate

change. In summary, our study provides new insights into how plants cope with water stress through stomatal behavior and xylem embolism. Future research should further explore the complexity of this interaction across different plant species and environmental conditions, as well as how these mechanisms influence plant adaptability and survival. This will aid in more precise predictions and responses to the impacts of climate change on global plant communities.

The revelation of diverse stomatal regulation strategies exhibited by winter wheat under varying drought intensities is indeed groundbreaking. Traditionally, wheat grown in pots has been known to adopt an anisohydric stomatal regulation, maintaining a relatively high g_s even under conditions of declining K_{leaf} [10]. Our findings were consistent with this observation, indicating that under mild drought (H or early-stage drought), wheat stomata tend to regulate anisohydrically, with g_s not experiencing a significant drop as K_{leaf} decreases. This allows for a larger stomatal aperture, potentially enabling a higher influx of CO_2 into the leaves. This response represents a radical growth strategy, crucial for plant survival and productivity during periods of moderate water stress. However, our results also demonstrated that wheat stomata tend to regulate in a more isometric strategy with an increased intensity of soil drought stress (Figure 2). This transition in stomatal regulation strategies suggests that wheat exhibits a complex and adaptive synergism between stomatal and hydraulic regulatory mechanisms.

The ability of crops to regulate water loss through the modulation of stomatal pore size has been the focus of numerous investigations [26]. The SL_{en} and SD are key stomatal traits that govern the regulation of g_s [28]. Our results indicated that SL_{en} on both the adaxial and abaxial surfaces remained unaffected by varying soil water levels (Figure 5a). This finding suggests that under the experimental conditions, SL_{en} may not be a primary regulator of g_s in response to soil drought stress. In contrast, SD displayed a distinct pattern, with significantly higher SD on the adaxial surface in L compared to H (Figure 5b). Moreover, SD on the abaxial surface increased significantly with increasing soil moisture of the three soil water levels.

g_s and SD showed a significant negative correlation (Figure 6a), which corresponds to a previous study [20], suggesting that the number of stomata per leaf area plays a crucial role in modulating g_s . Under drought conditions, plants tend to increase SD, likely as a compensatory mechanism to maintain gas exchange while minimizing water loss. This phenomenon was further supported by the observation that the decrease in g_s and V_{cmax} under soil drought was mediated by the increase in SD (Figure 6). These findings highlight the importance of SD as a primary response mechanism to drought stress in plants. By adjusting the number of stomata, plants can optimize water use efficiency and maintain physiological functions during periods of water scarcity. In summary, our research provides insights into the role of stomatal traits, particularly SD, in regulating g_s and plant responses to drought stress. The findings have the potential to contribute to the development of drought-tolerant crop varieties and sustainable agricultural practices.

Our findings revealed that SPI in the L treatment was significantly higher than in H. This observation suggests that SPI may be a contributing factor to the difference in g_s under soil water stress. The elevated SPI in plants under drought conditions likely reflects an adaptive strategy to maximize photosynthetic capacity per unit leaf area, compensating for the reduced water availability [29]. Interestingly, our results also revealed a significant decrease in V_{cmax} and J_{max} under L compared to H (Table 1). This finding was inconsistent with previous reports that showed no significant changes in V_{cmax} and J_{max} in drought-treated trees [30]. The discrepancy in these results indicates that crops may be more sensitive than trees to drought stress, experiencing a decline in their photosynthetic potential. The combined increase in SPI and decrease in V_{cmax} and J_{max} observed in our study could be interpreted as an adaptive response of plants to soil drought, aimed at reducing energy consumption while maintaining photosynthetic efficiency. In addition, g_s was also regulated by ABA in our study, and the relationship between g_s and ABA showed a significant negative correlation (Figure 4c), consistent with the results of previous studies. For instance,

Chen et al. [27] and Hussain et al. [31] found that soil water stress caused a significant increase in ABA, which was consistent with our results (Table 1). These findings further support the role of ABA in mediating g_s responses to drought stress. In conclusion, the present study provides valuable insights into the adaptive responses of plants to drought stress, particularly with regard to SPI, $V_{\max}$, $J_{\max}$, and ABA-mediated regulation of g_s . These findings have important implications for developing effective water management strategies for sustainable agriculture.

4. Materials and Methods

4.1. Experimental Site and Design

The pot trials were conducted in a climate-controlled growth chamber at the Experimental Station of the China Institute of Water Resources and Hydropower Research, located in Daxing District, Beijing. Temperatures were maintained at 25 °C during the day and 18 °C at night, and the relative humidity was controlled at 75% during the day and 80% at night in the growth chamber. The photoperiod was set for 12 h, from 7:00 A.M. to 7:00 P.M., with photosynthetically active radiation surpassing $100 \mu\text{mol m}^{-2} \text{s}^{-1}$, provided by a combination of sunlight and LED lamps (Signify Holding company, Eindhoven, The Netherlands). Dynamic variations of temperature and humidity in the growth chamber are shown in Figure 7a.

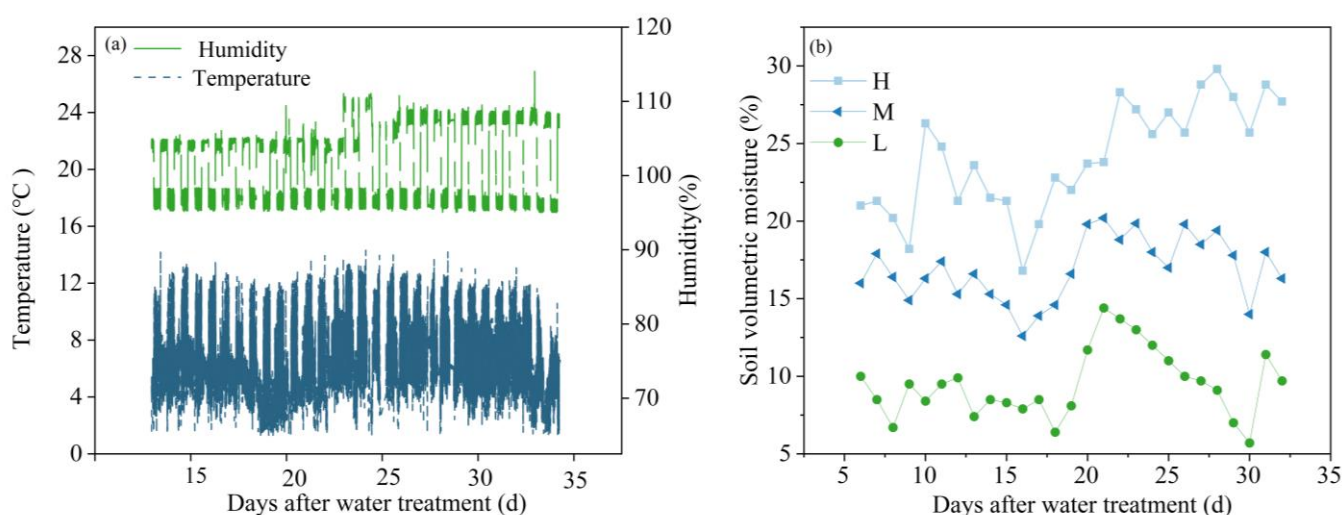


Figure 7. Dynamics of temperature, humidity, and soil moisture in the artificial climate chamber during the experimental treatment (a) and soil moisture dynamics under high (H), moderate (M), and low (L) soil water supply (b).

Twenty seeds of the “Jimai 22” (Qingfeng Seed Industry Company, Cangzhou, China) wheat variety were sown in each plastic pot, each with a capacity of 5.6 L and dimensions of 24 cm in upper diameter, 20 cm in bottom diameter, and 25 cm in height. The pots were filled with 2.5 kg of mixed soil, consisting of a 1:1 mixture of nutrient-rich soil (organic matter >90%, pH 5–6, EC 2–3 mS/cm) and local topsoil from the experimental site (0–30 cm depth). The soil texture was classified as loam according to the international standard for soil texture classification. Field capacity (FC) and bulk density of the mixed soil were 26% and 1.20 g/cm^3 .

The wheat plants were well-watered for a week before the soil drought treatment. Each pot received 1.6 g of urea every two weeks after the drought treatment. To reduce soil surface water evaporation, we added a 2-cm layer of perlite on top of each pot. According to previous studies by Ding et al. [32] and Mu et al. [33], we set three treatments: high soil water supply (H), which was maintained at a moisture content of 85–95% FC; moderate soil water supply (M), which was maintained at a moisture content of 65–75% FC; and low soil water supply (L), which was maintained at a moisture content of 45–55% FC. Soil moisture

in all pots was measured daily using the weighing method, and the soil moisture dynamics at three different soil water supply levels are shown in Figure 7b. The soil volumetric water content during the three treatment periods was 25.61%, 18.01%, and 12.03% in H, M, and L, respectively.

4.2. Measurements

4.2.1. Photosynthetic Parameters

The g_s of the newly fully expanded flag leaf was measured using the Li-6800 portable photosynthetic system (Li-COR, Inc., Lincoln, NE, USA) between 10:00 and 13:00. The Li-6800 was equipped with a 2 cm² leaf chamber. The photosynthetic photon flux density of the leaf chamber was set at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The temperature of the leaf chamber was set at 26 °C. The CO₂ concentration of the leaf chamber was controlled at 400 ppm by a steel cylinder containing CO₂. The flow rate was set at 500 $\mu\text{mol/s}$. The relative humidity was maintained at 55%. Following the g_s measurements, A_n - C_i curves were measured using the method of rapid A_n - C_i response (RACiR) on the same leaves after g_s measurements [34]. The temperature during measurement of the leaf chamber was maintained at 25 °C, and the light intensity of the leaf chamber was set at 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The V_{cmax} and J_{max} were calculated using the “plantecophys” package in R version 4.1.2 (<https://cran.r-project.org> accessed on 1 November 2021) [35]. Each treatment was replicated five times.

4.2.2. Leaf Water Potential (Ψ_{leaf})

The Ψ_{PD} was measured between 6:00 and 7:00, and the Ψ_{MD} was measured between 11:30 and 13:30 from flag leaves and brought to the lab within 10 min of sample collection. Ψ_{leaf} was measured using a pressure chamber (PMS Instrument Company, Albany, OR, USA). The leaf incision was made on the air chamber lid and then the air chamber was tightened. It was slowly pressurized, and the incision was examined with a magnifying glass. Once small droplets of water formed at the incision, the pressure application was immediately stopped, and the pressure value was recorded. Each treatment was replicated five times.

4.2.3. Leaf Hydraulic Conductivity (K_{leaf})

Wheat plants with fully developed flag leaves were selected, and their flag leaves were excised using scissors, leaving approximately 5 cm of the sheath. The selected plants were immediately submerged in water, and a second pruning was done to eliminate any remaining air bubbles from the stem. Subsequently, the cut leaves were placed in a dark environment for rehydration, and then exposed to an irradiation lamp to induce the opening of the leaf stomata. After reaching a steady state for 30 min, the transpiration rate (E) was measured using the LI-COR 6800 photosynthesis measurement system (LI-COR, Inc., USA). The E was recorded once the leaves reached a stable transpiration state. Subsequently, the leaves were quickly placed into a sealed bag with damp paper towels. Then, they were kept in a dark environment for about 30 min to achieve equilibrium before measuring the Ψ_{leaf} . Each treatment was replicated five times.

The K_{leaf} was calculated as follows [36]:

$$K_{\text{leaf}} = E / (0 - \Psi_{\text{leaf}}) \quad (1)$$

4.2.4. Leaf Vulnerability Curves

Twenty tillering stems with three fully developed leaves were chosen and cut before the lights were turned on. Subsequently, the stems were chopped and placed in water to hydrate. Initially, we measured the maximum hydraulic conductance (K_{max}) of the middle leaf of the aforementioned stems using the method described above. Following this, the remaining wheat stems were dehydrated on the laboratory table for varying durations.

Subsequently, the stems with varying degrees of dehydration were wrapped in black plastic bags and left to stabilize for at least 30 min to measure the leaf water potential. The

initial leaf water potential values (Ψ_0) were calculated as the average of the top and bottom Ψ_{leaf} values.

The difference in leaf water potential between the top and bottom leaves was below 0.1 MPa (below 0.3 MPa for severely dehydrated). The percentage loss of hydraulic conductance (PLC) was calculated as follows [37]:

$$\text{PLC} = 100 \times (1 - K_{\text{leaf}}/K_{\text{max}}) \quad (2)$$

The least squares method, based on empirical functions, was used to fit the vulnerability curves for H and L. This was done to determine the xylem pressure (P_{50}) at 50% hydraulic conductance loss and the slope (S) of the vulnerability curve at the inflection point (P_{50}) [11]:

$$\text{PLC} = a/(1 + \exp(S(\Psi_0 - c))) \quad (3)$$

The K_{leaf} loss is expressed as a percentage of change per megapascal (%/MPa). The slope (S) reflects the sensitivity of embolism spread in the xylem, and a steeper S indicates a faster spread of embolism. One vulnerability curve was measured for each treatment. The vulnerability curve is modeled using a Weibull function from the “fitplc” package [38] in R 4.1.2 (<https://cran.r-project.org> accessed on 1 November 2021).

4.2.5. Leaf Relative Water Content

The newly fully expanded flag leaves were weighed, then soaked in distilled water, weighed again, and finally weighed after drying. The relative water content (RWC) of the leaves was calculated using the following formula [39]:

$$\text{RWC} = (W_f - W_d)/(W_t - W_d) \quad (4)$$

W_f represents the fresh weight of leaves; W_t is the saturated weight of the leaves after they have been fully immersed in distilled water for 2 h; and W_d represents the weight of the leaves after they have been fixed at 105 °C for 30 min and then dried in a 70 °C oven for 72 h. Each treatment was measured in three replicates.

4.2.6. Absciscic Acid Content (ABA)

The flag leaves adjacent to those that had been measured for g_s under the same treatment were promptly collected. They were then wrapped in aluminum foil, rapidly frozen in liquid nitrogen, and stored in a refrigerator at −40 °C. ABA was measured using an enzyme-linked immunosorbent assay (ELISA) [40]. The BNTY kit, produced by Beijing Beinong Tianyi Biotechnology (Beijing, China), was used in the experiment. Each treatment was measured in five replicates.

4.2.7. Stomatal Characteristics

Transparent nail polish was applied to the leaf surface in a thin layer with a sample size of 5 mm × 15 mm and left to air dry for 5 min. The dry nail polish was then peeled off using clear tweezers and placed on a microscope slide [41,42]. Stomata on leaf imprints were observed with a Leica light microscope (DM2500, Leica Corp, Wetzlar, Germany) connected to a camera.

Stomatal density (SD) was measured under a 10 × 10 magnification microscope by counting the number of stomata within three randomly selected fields of view. SD was then calculated as the average value divided by the corresponding image area, resulting in the number of stomata per square millimeter. Stomatal length (SL_{en}) was measured under a 10 × 40 magnification microscope using Motic Panthera software version 1.0.23 (Motic China Group Limited, Xiamen, China), and the average length was calculated for three randomly selected stomata. Stomatal Pore Index (SPI) was calculated as follows [43]:

$$\text{SPI} = S_{\text{Len}}^2 \times \text{SD} \times 10^{-4} \quad (5)$$

This index can be interpreted as an approximate measure of the proportion of the stomatal aperture area to the leaf area. SPI is an indicator of the degree of stomatal opening in crop leaves [44]. The SD represents the stomatal density, and SL_{en} represents the stomatal length in Equation (5). Each treatment's SL_{en} , SD, and SPI were measured in five replicates.

4.3. Statistical Analyses

SPSS 23 software (IBM, Inc., Armonk, NY, USA) was used to conduct a one-way ANOVA on the data related to Ψ_{leaf} , K_{leaf} , g_s , stomatal traits, physiology, and biochemistry under various treatments. The differences between the means were analyzed using the least significant difference (LSD) multiple comparison test, with a significance level of 5%. Regression analysis was performed on physiological indexes using Origin 2021. A significance threshold of $p < 0.05$ was applied, and only results with significant differences were presented. The figures and tables were created using Origin 2021 (OriginLab, Northampton, MA, USA) and Microsoft Excel 2021.

5. Conclusions

This study revealed the integrated effects of soil water stress on winter wheat hydraulic properties and stomatal regulation. We found that ABA and stomatal traits including SD and SPI increased, while Ψ_{PD} and Ψ_{MD} , K_{leaf} , g_s , V_{cmax} , J_{max} , and RWC, all considerably decreased under soil water stress. Piecewise linear positive relationships between g_s and Ψ_{MD} and K_{leaf} were found in this study. These results revealed that wheat's stomatal regulation transitioned from anisohydric to isohydric during soil drought. Specifically, during mild soil drought (65–75% FC), wheat adopted anisohydric stomatal regulation, characterized by stomata remaining open to maximize carbon assimilation. Meanwhile, K_{leaf} and Ψ_{leaf} significantly decreased. Under severe drought (45–55% FC), isohydric stomatal regulation was observed, with g_s decreasing to prevent further reduction in K_{leaf} and Ψ_{leaf} , thereby avoiding hydraulic imbalance. Our study also found a negative correlation between g_s and SD, as well as a negative correlation between V_{cmax} and SD, showing that water relations of wheat were also influenced by stomatal traits. These findings enhance our understanding of how crops regulate their stomatal strategy to balance growth and survival and provide theoretical guidance for tailoring water management practices to correspond with crop stomatal regulation strategies under varying soil moisture.

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