Precise evaluation of transpiration patterns in relation to grain yield under drought stress in faba bean

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Abstract

Background

Faba bean (*Vicia faba* L.) is a key crop for sustainable agriculture in temperate cropping systems due to its nitrogen-fixing ability and high protein content, but its productivity is increasingly threatened by drought stress driven by climate change. Precise phenotyping under semi-controlled conditions is crucial for understanding drought responses. High-throughput precision phenotyping enables efficient evaluation of many genotypes, revealing detailed water-use patterns as a basis for breeding productive, drought-resilient cultivars. In this study, faba bean genotypes were grown in a precision phenotyping facility comprising 120-liter containers filled with mineral soil to simulate field like growth conditions. Each container was placed on a high-precision gravimetric scale to record water use in real-time in relation to 3-dimensional spectral image information.

Results

Precise measurement of genotype-specific transpiration behavior using gravimetric methods enabled detailed insights into the transpiration patterns of different genotypes in response to ambient temperature and humidity fluctuations throughout the day, night and across the whole life cycle. The results showed that total water use, water use efficiency, and consequently yield were particularly influenced by specific transpiration parameters, such as the maximum transpiration rate and the vapor pressure deficit threshold at which stomatal conductance was interrupted.

Conclusion

The results revealed genetically determined variation for transpiration responses to drought stress. Genotypes that reduced water loss earlier tended to achieve higher grain yields and use water more efficiently. The findings show that precise automated phenotyping can identify previously undiscovered genetic variation for breeding of drought-tolerant faba bean varieties, which are crucial for ensuring productivity under increasingly water-limited conditions.

Introduction

Drought stress is a major environmental limitation that significantly impacts agricultural productivity worldwide, especially under the growing challenges of climate change (Pequeno et al. 2021) To cope with drought, plants employ various physiological, morphological, and biochemical mechanisms to conserve water and sustain growth under suboptimal conditions (Deligoz and Gur 2015). These adaptive responses are particularly important in crop species like faba bean (*Vicia faba* L.), a legume valued for its high protein content and ability to fix atmospheric nitrogen – both critical factors for global food security (Erbersdobler et al. 2017). Understanding how faba bean responds to drought stress is key to

enhancing drought resilience and ensuring stable yields in water-limited environments. Like many other crops, faba bean experiences reduced growth and yield under drought conditions(Buragohain et al. 2024), primarily due to impaired water uptake and disruptions in key physiological processes such as photosynthesis and transpiration (Farooq et al. 2009). Two critical factors influencing plant water status are vapor pressure deficit (VPD) and soil water content (Xue 2004). VPD represents the difference between saturation vapor pressure and actual vapor pressure at a given temperature (Anderson 1936), while soil water content determines how much water is available for plant roots to absorb (Lobet et al. 2014). These factors interact dynamically: low soil water content restricts a plant's ability to replenish water lost through transpiration, while high VPD accelerates water loss. A combination of low soil water and high VPD creates a major challenge for maintaining plant hydration and physiological function, with serious potential consequences for crop yields. As VPD rises, transpiration rates increase due to greater evaporative demand, leading to rapid water loss unless adaptive mechanisms, such as stomatal closure, are triggered (Jones 2014; Damour et al. 2010).

Stomatal closure is a well-documented response to drought stress and high VPD (Kholová et al. 2010); (Medina and Gilbert 2015), acting as a primary defense mechanism to reduce water loss through transpiration (Agurla et al. 2018). As VPD increases, indicating warmer and/or drier air, plants close their stomata to limit water loss. However, this also restricts CO_2 uptake, reducing photosynthetic efficiency and overall growth (Jones 2014; Damour et al. 2010). In faba bean, stomatal regulation plays a crucial role in maintaining water-use efficiency, as excessive water loss can rapidly lead to dehydration, especially under prolonged drought conditions (Khan et al. 2007). Balancing gas exchange for photosynthesis while minimizing water loss through stomatal closure is therefore a key factor in determining drought tolerance in this species (Ripley et al. 2010).

The transpiration rate, largely driven by VPD, is another critical factor influencing plant drought responses (Grossiord et al. 2020). As VPD increases, the plant's demand for water rises. Under drought conditions, where soil moisture is limited, this can be particularly detrimental, forcing the plant to rely on physiological drought tolerance mechanisms (Haghpanah et al. 2024). Research has shown that managing VPD and monitoring transpiration rates are effective strategies for assessing drought impact and improving water-use efficiency in crops (Lobell et al. 2013). Additionally, nighttime transpiration, which occurs in the absence of photosynthesis, represents water loss without carbon gain, making it an additional key parameter for identifying water-saving genotypes better adapted to drought (Coupel-Ledru et al. 2016). Given the importance of VPD and transpiration in modulating drought stress responses, studying how faba bean genotypes differ in their response to varying VPD levels and soil moisture availability is essential for identifying drought-tolerant varieties. However, all of these factors are notoriously difficult to accurately and consistently measure in genetically diverse populations under field-relevant conditions. Hence, little is known about useful genetic diversity and until now it has been virtually impossible to effectively screen for such traits as potential targets for breeding novel approaches to systematically investigate the relationship between stomatal conductance, VPD, and transpiration rate can thus provide valuable new insights for optimizing faba bean cultivation under drought stress conditions.

In this study, we assessed drought stress responses of diverse faba bean genotypes under controlled drought conditions using a fully automated drought simulation system (Stahl et al. 2020) The experimental design includes real-time monitoring of VPD and soil moisture levels under simulated, field-like drought conditions (Hohmann et al. 2016) and quantify plant responses. This will contribute to a deeper understanding of the physiological mechanisms underlying drought tolerance in faba bean, with the goal of improving resilience and water-use efficiency in this important legume.

Material and Methods

Experimental Setup

The "Droughtspotter XXL" experimental facility (Phenospex, Heerlen) at Rauischholzhausen field station of Justus Liebig University Giessen (50° 45' 40.05" N, 8° 52' 45.96" E) (Fig. 1), consists of 240 large containers arranged in 6 rows of 40 containers each. Each container has a volume of 120 liters and is filled with approximately 168 kg of a clay-sand soil mixture, simulating field-like growth conditions and providing a rooting depth of up to 90 cm. Each container is placed on an individual, high-capacity gravimetric scale which records the individual container weight every 5 minutes throughout the entire plant growth period, with a measurement precision of ± 20 grams (equivalent to approximately 0.13% of the total quantity of water per container at 60% water capacity in this soil). Each container is equipped with an automated irrigation system, enabling precise water management based on real-time weight data. The water-holding capacity of each container is calculated from these weight measurements, allowing for the simulation of drought stress and well-watered conditions. Irrigation was performed daily at 00:05 hours, with the water volume adjusted automatically based on the individual container weight, ensuring that each container starts the day at its predefined water-holding capacity. The amount of irrigation applied for each water-holding capacity was determined through gravimetric measurements (Fig. 2).

This approach ensured precise water management by continuously weighing the containers and adjusting irrigation volumes to maintain the target field capacity accurately.

Experimental Design

The experiment was conducted over one growing season. Each genotype was tested with three replications following a randomized complete block design (RCBD). Containers were sown with 18 seeds, later thinned to 9 plants per container, arranged in a 3x3 grid over an area of 0.16 m², reflecting a typical field sowing density of 56 plants/m². At the start of the experiment, a water-holding capacity of 60% was maintained to support healthy plant growth. At the onset of flowering, the water-holding capacity was reduced to 40% for the remainder of the reproductive phase, simulating severe drought stress conditions for faba bean. This approach ensured consistent drought stress conditions across the whole experiment.

Prior to sowing, all genotypes were inoculated with *Rhizobium fabae* (Rhizofix RF-20, Feldsaaten Freudenberger, Krefeld). Soil nutrient levels were analyzed before the experiment, and fertilization was carried out according to the recommendations of the Hessen State Agency. The fertilization was divided into two applications. The first fertilization, applied 19 days after sowing, included ammonium molybdate, manganese sulfate, zinc sulfate, copper sulfate, boric acid, potassium dihydrogen sulfate, and magnesium sulfate. The second fertilization, applied 35 days after sowing, consisted of potassium dihydrogen sulfate and potassium chloride. No nitrogen fertilization was used (**Supplementary Table 2**). Pest management for aphids (Pyrethroids, Karate Zeon, Syngenta) was applied as needed at the standard application rate.

Genotype Set

The experiment described here was conducted in 2022. A total of 56 genetically diverse faba bean genotypes (**Supplementary Table 1**) representing a collection of breeding accessions and official breeding lines were used, provided by Norddeutsche Pflanzenzucht Hans Georg-Lembke KG (Holtsee). Twenty of the entries are spring faba bean varieties and two are winter varieties, while 34 had no indication of summer or winter annuality (EU database of registered plant varieties 2024).

Environmental Monitoring

Nine evenly distributed data loggers were installed throughout the greenhouse to record temperature and humidity at 10-minute intervals throughout the entire growing period. Based on these measurements, the VPD was calculated, assuming that leaf temperature matched ambient temperature and that relative humidity inside the leaf was 100%. VPD values were interpolated across all six rows and 240 columns, providing corresponding VPD data for each container position throughout the growth period. Additionally, solar radiation was recorded as photosynthetically active radiation (PAR) and integrated into environmental monitoring and analyses. This comprehensive setup, combining precise environmental monitoring, automated irrigation, and real-time weight tracking, enabled a detailed analysis of plant responses to drought stress under controlled, field-like conditions.

Transpiration Response to VPD

As described in detail by (Moritz et al. 2024), VPD was calculated from the difference (**Formula 3**) between VP_{Sat} (**Formula 1**) and VP_{Air} (**Formula 2**). This represents the VPD of the air relative to saturated air, rather than the VPD between leaf and air, which can nonetheless serve as an indicator for transpiration behavior. To calculate VP_{Sat}, it is assumed that the leaf surface temperature equals the ambient temperature and that the transpiration stream is saturated (Day 2000).

The calculation of VPD was conducted as follows:

$$\mathrm{VP}_{Sat}\,[\mathrm{kPA}] = 0.61078 * e^{\left(\frac{17.27*T}{T+237.3}\right)}$$

1

$$\mathrm{VP}_{Air}\,[\mathrm{kPA}] = 0.61078 * e^{\left(\frac{17.27*T}{T+237.3}\right)} * RH\%$$

2

$$\mathrm{VPD}\,[\mathrm{kPA}] = VP_{Sat}\,[kPA] - VP_{Air}\,[kPA]$$

3

where T is the temperature, $RH\%$ is the relative humidity, the constants are part of the Tetens equation used to calculate vapor pressure (Tetens 1930).

The transpiration breakpoint, representing the VPD [kPa] level at which stomatal closure is assumed to have occurred in a given genotype, was calculated using a two-segment regression analysis. (**Formula 4**). For each scale in the data, we fit a linear model to estimate a breakpoint ψ in the relationship between transpiration rate and VPD. The segmented regression model is given by:

$$TR = \begin{cases} \beta_0 + \beta_1 \times VPD & for\, VPD \leq \psi \\ \beta_0 + (\beta_1 + \delta) \times VPD & for\, VPD > \psi \end{cases}$$

4

where β_0 is the intercept, β_1 is the slope before the breakpoint, δ is the change in slope after the breakpoint and ψ is the estimated breakpoint for VPD. The breakpoint ψ is estimated by fitting the segmented regression model, where:

$$\psi = median\,(VPD)$$

The slopes are defined as:

Slope before the vpd breakpoint (Slope1): β_1

Slope after the vpd breakpoint (Slope2): $\beta_1 + \delta$

Separate R^2 values for each segment can be computed by fitting individual linear models before and after the vpd breakpoint:

R^2Slope1: Coefficient of determination for the segment where VPD ≤ ψ

R^2Slope2: Coefficient of determination for the segment where VPD > ψ

This model splits the relationship between VPD and TR into two parts. Below a certain VPD threshold (ψ), TR increases with VPD at one rate. Above this threshold, TR increases (or possibly decreases) at a different rate. This model is useful for capturing changes in stomatal behavior or transpiration under different environmental conditions.

The classification of genotypes into the transpiration-specific groups was based on the threshold of the vpd breakpoint and the change in transpiration rate after the breakpoint was reached (slope2). To achieve a more refined differentiation between transpiration-specific groups, only days with VPD ≥ 3.5 kPa were considered. Genotypic assessments were conducted through daily group comparisons, accounting for both the day-specific variability in breakpoints and the extent of transpiration restriction following the breakpoint. The classification was based on the daily vpd breakpoint magnitude and the subsequent transpiration response once the breakpoint was exceeded. For each day, group comparisons were performed, and a daily classification was established based on the median values of these parameters.

Estimation of Repeatability (h²)

To ensure robust estimates of genotype performance, adjusted means, genetic variance, and repeatability were calculated using a linear mixed-effects model approach (**Formula 5**). The analysis accounted for random effects associated with the experimental design, including spatial variability and replication effects. Adjusted means for each genotype were calculated using a linear mixed-effects model where genotype was treated as a fixed effect, and other experimental factors (columns, rows, blocks, and replications) were treated as random effects. The model was formulated as:

$$P_{ijklm} = \mu + Genotype_i + S_j + Z_k + B_l + W_m + \epsilon_{ijklm}$$

5

where P_{ijklm}represents the measured value of a specific trait for an individual phenotype, indexed by the factors i, j, k, l and m. μ stands for the overall mean, $Genotype_i$ is the fixed effect of the genotype i, S_j is the random effect of the column j, Z_k is the random effect of the row k, B_l is the random effect of the block l, W_m is the random effect of the replication m and ϵ_{ijklm} is the residual error.

To estimate the genetic variance (σ_g^2) a linear mixed-effects model (**Formula 6**) was fitted in which genotype was treated as a random effect:

$$P_{ijklm} = \mu + G_i + S_j + Z_k + B_l + W_m + \epsilon_{ijklm}$$

6

where P_{ijklm}represents the measured value of a specific trait for an individual phenotype, indexed by the factors i, j, k, l and m. μ stands for the overall mean, $G_i \sim N(0, \sigma_g^2)$ is the random effect of the genotype i with σ_g^2 representing the genetic variance component, S_j is the random effect of the column j, Z_k is the random effect of the row k, B_l is the random effect of the block l, W_m is the random effect of the replication m and ϵ_{ijklm} is the residual error. The calculation of repeatability (h²) (**Formula 7**) for transpiration response traits was performed after determining the genetic variance and the standard error of the difference between means using:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \left(\frac{SE\square}{2}\right)}$$

7

where σ_g^2 stands for the genotypic variance component and $SE\square$ is the squared standard error of pairwise genotype comparisons (Piepho and Möhring 2007).

This approach accounts for the random effects in the experimental design, ensuring an estimation of repeatability by partitioning the total variance into genetic and non-genetic components.

Night Transpiration

Transpiration rates during nighttime hours were conducted the same way as during daytime hours. Due to system limitations, weight measurements could not be recorded during irrigation periods. Therefore, nighttime transpiration analysis was restricted to the time period after PAR dropped to ≤ 10 µmol m^{-2} s^{-1} until midnight. To assess the nighttime transpiration dynamics for each genotype, linear regression was performed on transpiration rate as a function of time (in minutes) for each genotype and each day. Repeatability (h^2) for the regression of transpiration rate was calculated after determining the genetic variance and the standard error of the difference between means using the given formula h^2 (**Formula 7**) for repeatability. Tukey's Honestly Significant Difference test was applied post-hoc to group genotypes into statistically distinct categories (compact letter display) based on a significance level of $p < 0.05$. The regression slopes quantify the rate at which transpiration decreases as light diminishes, indicating how efficiently genotypes adapt to nighttime conditions.

Statistical Analysis

All data analysis was performed using the programming language R (Version 4.1.2). For each target trait, a linear mixed-effects model was applied to estimate the genotypic variance components and to calculate adjusted means. The lmer function from the lme4 package was used for modeling, and the emmeans package was employed to derive adjusted genotype means. Repeatability (h^2) was estimated based on variance components. Pairwise comparisons between genotypes were conducted to estimate masking variance, and compact letter displays were generated using the multcomp package. The anonymization of the genotype number was performed based on the nighttime transpiration behavior.

Results

Transpiration Rate in Response to VPD

Using the temperature and relative humidity measurements recorded throughout the phenotyping facility, the specific daily VPD was calculated for each container. By linking the weather data (Fig. 3B) with

transpiration data (Fig. 3A), which were derived from the reduction in container weights, the breakpoints were determined using a two-segment regression analysis. Until the vpd breakpoint is reached, transpiration follows a linear relationship with a coefficient of determination (R^2) of 0.99 (Slope 1). After reaching the vpd breakpoint, transpiration becomes restricted, preventing any further increase in the transpiration rate (Slope 2) (Fig. 3C). This analysis was conducted for each container throughout the entire period of drought stress. It has been observed that both the magnitude of the breakpoint and the degree of transpiration restriction exhibit day-specific values, making it impractical to define a universally high or low vpd breakpoint and slope. Day-specific variations in transpiration-related traits result in a relatively high variation when comparing (Table 1).

Tab. 1 Characteristics of the segmented regression analyses for the 2022 drought stress experiment on faba bean, with the determined values corresponding to the measured transpiration rates per m^2 based on adjusted mean values.

Year	Trait	Min	Max	Mean	CoV	h^2
2022	Breakpoint [kPa]	1.77	2.41	2.17	0.06	0.46
	Slope 1 [g min^{-1} m^{-2} kPa^{-1}]	5.07	9.09	7.28	0.11	0.44
	Slope 2 [g min^{-1} m^{-2} kPa^{-1}]	-1,47	0.38	-0.44	0.96	0.21

An analysis of the continuously recorded transpiration behavior in relation to the prevailing VPD indicates that the threshold for reaching the breakpoint depends on more than just the VPD. Additional factors, particularly light intensity and light duration quantified as the daily PAR sum (Fig. 4A) play a significant role in influencing the rate of transpiration change. Specifically, the magnitude of the changes in transpiration breakpoints follows a linear relationship with PAR up to a critical threshold. This threshold was identified at a PAR sum of 29.417 µmol/m²s in year 2022 (r^2 = 0,39). Beyond this point, the magnitude of the changes in transpiration breakpoint appears to lose its structured relationship with PAR, becoming seemingly arbitrary. The same behavior can be seen by analyzing the temperature sum until the transpiration breakpoint is reached. The relationship between cumulative temperature and the height of the transpiration breakpoints also follows a linear trend up to a temperature sum of approximately 1614°C. Beyond this point, the magnitude of the breakpoint for transpiration changes appears to lose its structured relationship with temperature sum, becoming seemingly arbitrary (Fig. 4B). These influences on the breakpoint magnitude become apparent when analyzing all genotypes collectively over the entire growth period. A continuous change in the magnitude of the breakpoints (BP) is observed across time, though the trend of this change remains consistent among all genotypes (Fig. 4C). Differences among genotypes are primarily reflected in the magnitude of breakpoint changes, rather than in their direction. This underscores the complexity of the breakpoint dynamics: no single, universal classification of breakpoint magnitudes can be established. Instead, meaningful interpretation requires group comparisons for each measurement day, as these daily comparisons allow for a more precise framing of the observed breakpoints. A similar complexity emerges when examining the change in transpiration rate after the vpd breakpoint (Slope 2). The data reveals no clear generalization for how transpiration changes after the vpd breakpoint. This reinforces the need for group comparisons for each

specific day to properly characterize the dynamics of transpiration adjustments following the breakpoint. The adjusted genotype averages (Fig. 5) for the vpd breakpoint varied between 1.77 kPa to 2.41 kPa. Before reaching the breakpoint, the adjusted mean values for Slope 1 varied between 5.07 g min^{-1} m^{-2} kPa^{-1} and 9.09 g min^{-1} m^{-2} kPa^{-1}. After the vpd breakpoint, the adjusted mean values for Slope 2 ranged from – 1.47 g min^{-1} m^{-2} kPa^{-1} to 0.38 g min^{-1} m^{-2} kPa^{-1}, indicating an overall strong restriction in transpiration beyond the breakpoint.

Impact of Transpiration Dynamics on Yield-Related Traits

A clear pattern emerges when grouping the investigated genotypes based on both the magnitude of the transpiration breakpoint and the nature of the transpiration changes beyond the breakpoint. These analyses reveal four distinct response types, allowing the genotypes to be classified into the following categories:

1. “Early Breakpoint + Strong Restriction” (EB + SR): Genotypes that exhibit an early vpd breakpoint with a pronounced reduction in transpiration after the breakpoint.
2. “Early Breakpoint + Weak Restriction” (EB + WR): Genotypes that demonstrate an early vpd breakpoint but with a milder reduction in transpiration.
3. “Late Breakpoint + Strong Restriction” (LB + SR): Genotypes with a delayed vpd breakpoint and a significant decrease in transpiration beyond the breakpoint.
4. “Late Breakpoint + Weak Restriction” (LB + WR): Genotypes with a late vpd breakpoint and only a slight restriction in transpiration.

This classification highlights the complex interplay of environmental factors, genotype-specific responses, and temporal dynamics in determining transpiration behavior. Figure 6 illustrates the cumulative water uptake (kg), grain yield (kg) and water use efficiency (g/l) for four distinct groups during a specific developmental stage. The data reveal significant differences between the groups ($p \leq 0,05$) (Fig. 6). Considering the flowering period, which is the most sensitive stage for faba bean during drought stress (Plies-Balzer et al. 1995; Khan et al. 2007), a groupwise comparison reveals significant differences in cumulative water uptake, water use efficiency, and grain yield. Genotypes that exhibit a strong restriction of transpiration after reaching the vpd breakpoint during the flowering period—whether they have an early (EB + SR) or late (LB + SR) vpd breakpoint—consistently take up significantly less water over their entire life cycle. When considering the complete growth cycle, genotypes with a strong transpiration restriction show lower cumulative water uptake, while those with an early vpd breakpoint maintain a high grain yield. The highest overall water use efficiency was observed in genotypes combining an early vpd breakpoint with a strong transpiration restriction, followed by those with an early vpd breakpoint and a weak restriction. These results indicate that an early interruption of transpiration under critical VPD is a critical trait for identifying genotypes capable of securing high yield under drought stress. Hence, prioritizing an early vpd breakpoint in combination with effective transpiration control emerges as a promising strategy in faba bean for enhancing water use efficiency and yield stability in drought-prone environments.

Genotypes with a late transpiration breakpoint showed correspondingly higher cumulative water uptake, however, the increased water consumption did not translate into higher grain yield, resulting in lower water use efficiency. Nevertheless, strong restriction of transpiration after reaching the vpd breakpoint had positive effects on yield and yield-related traits compared to weak restriction.

Senescence Behavior in Transpiration-Specific Groups

Temporal changes in plant-specific spectral indices from May 22, 2022, to July 5, 2022 (Fig. 7) revealed notable differences, particularly in the senescence behavior of the different transpiration-specific groups. Although no significant differences were observed between groups for the overall digital biomass recorded across the entire growth period, the plant indices revealed physiological differences in senescence behavior between the different groups. For instance, the group characterized by a late transpiration breakpoint and strong restriction exhibited a higher Plant Senescence Reflectance Index (PSRI) at the final measurement compared to the other transpiration-specific groups, indicates more advanced leaf senescence. This group also showed a significantly greater reduction in the average hue value during senescence, reflecting more pronounced yellowing of the plants and suggesting an earlier decline in photosynthetic capacity.

A similar trend was observed in the Normalized Pigment Chlorophyll Index (NPCI), where the late-breakpoint group exhibited stronger changes during senescence. Additionally, the Normalized Difference Vegetation Index (NDVI) showed a more pronounced decline during senescence in groups with strong restriction of transpiration following the vpd breakpoint. These findings highlight the physiological and phenological differences in the senescence process across the transpiration-specific groups.

Genotypic Variation in Nighttime Transpiration

Analysis of nighttime transpiration (Fig. 8) revealed that faba bean genotypes do not achieve complete stomatal closure after sunset, even in the complete absence of photosynthetic active radiation. This results in continued water loss through transpiration during the night. The transpiration rates of all investigated genotypes exhibit a negative regression, indicating a steady reduction in transpiration after sunset and after reaching a measured PAR value of 0 µmol m^{-2} s^{-1}. The magnitude of the regression slope shows significant differences among the genotypes. Overall, the genotypes can be categorized into 13 statistically distinct groups. A particularly steep negative regression slope, observed in Genotype 1 (-0.0008; Fig. 8A), suggests a faster adjustment of transpiration and consequently a faster stomatal closure after sunset. By quickly reducing the transpiration rate, this genotype effectively minimizes water loss during the night when no additional energy gain through photosynthesis occurs. Although differences in the rate of adjustment among genotypes are evident (Fig. 8B), the total amount of water transpired during the night remains minimal compared to daytime water loss. On average, water transpired during the night accounts for only 3.13% of the total transpired water. Analysis of the linear relationships between daytime and nighttime transpiration and the adjusted mean values revealed a strong correlation for daytime transpiration ($r = 0.65$), whereas no linear correlation was observed between nighttime transpiration and yield ($r = -0.02$) (Fig. 8C). These findings indicate a genotype-

specific adaptation response, although nighttime transpiration does not appear to contribute significantly to yield.

Discussion

Transpiration Dynamics and VPD Breakpoint

By simulating drought stress and monitoring transpiration in real time, we gained valuable insights into the relationships between VPD and genotype-specific transpiration behavior in faba bean. The two-segment regression analysis of transpiration rates under varying VPD conditions revealed distinct breakpoint dynamics that define clear phases of stomatal regulation. Our findings demonstrate that the magnitude of the transpiration breakpoint and the degree of transpiration restriction are highly dynamic and vary on a day-to-day basis. These variations are influenced not only by VPD but also by additional environmental factors, particularly PAR. The response of stomata influenced by environmental factors beyond just VPD has already been demonstrated by (Driesen et al. 2020). The linear relationship between vpd breakpoint magnitude, PAR and cumulative temperature underscores the multifactorial nature of drought response. Beyond these thresholds, the relationship becomes inconsistent, suggesting a complex interplay between environmental saturation and plant physiological limits.

Analyzing transpiration breakpoint magnitudes and changes enabled identification of four distinct response types. This classification system offers a robust framework for understanding genotype-specific drought adaptation strategies. The early breakpoint genotypes (EB) demonstrated an ability to regulate water loss early in the drought period, likely conferring a survival advantage by conserving soil moisture for critical reproductive stages (Zia et al. 2021). This suggests that early stomatal closure reduces water loss during periods of high evaporative demand, thereby improving drought resilience and ensuring water availability for pod filling. Various plant genera are known to possess mechanisms to conserve water under drought stress (Skelton et al. 2015). In our experiments with faba bean, the highest water use efficiency was observed in genotypes classified as Early Breakpoint + Strong Restriction (EB + SR). These genotypes effectively minimized water loss without compromising grain yield, indicating a favorable balance between water conservation and productivity. This finding is consistent with previous research indicating that genotypes exhibiting early stomatal regulation under drought conditions can enhance water-use efficiency (Lawson and Vialet-Chabrand 2019) while sustaining yield potential. A study on maize demonstrated that early stomatal closure effectively reduces transpiration, thereby conserving soil moisture and improving drought tolerance (Benešová et al. 2012). Similarly, rice plants with reduced stomatal density were found to conserve water and maintain yield under drought stress (Caine et al. 2019). Identification of genotypes with early stomatal closure and efficient water use has direct implications for agricultural practices in drought-prone regions (Chaves et al. 2016). Integrating these genotypes into breeding programs can enhance the resilience of cropping systems against the increasing frequency of extreme weather events driven by climate change (Rivero et al. 2022). This balance between water conservation and productivity was also previously found in durum wheat, where genotypes exhibiting segmented transpiration responses to increasing VPD performed

better under drought stress, while linear-response genotypes excelled under favorable conditions (Medina et al. 2018). Our study extends this methodology to faba bean (*Vicia faba* L.), employing precision phenotyping in a field-like environment classify genotypes based on transpiration responses to VPD. Such classifications facilitate breeding for drought-resilient crops by identifying genotypes that effectively balance water conservation with productivity. The differential transpiration strategies observed among genotypes highlight their potential impact on soil water balance throughout the growing season. Genotypes with early stomatal closure may reduce water depletion in the upper soil layers, potentially allowing for better water availability (Schoppach and Sadok 2012).

Moreover, our observations that light intensity (PAR) and cumulative temperature significantly influence transpiration breakpoints complement existing studies emphasizing the role of environmental factors in plant hydraulic responses (Cai et al. 2024). These insights facilitate the identification of genotypes with favorable transpiration characteristics, contributing to the development of crops that are better adapted to fluctuating environmental conditions. Although this study provides valuable insights into genotype-specific responses to VPD, the controlled experimental setup may not fully reflect field conditions, where additional factors such as soil heterogeneity, biotic interactions and fluctuating weather patterns play a significant role. Nevertheless, the findings are important to identify useful genetically determined variation as a starting-point for breeding towards yield and yield stability under drought stress.

Genotype-Specific Regulation of Nighttime Transpiration

One potential mechanism that plants might use to conserve water during drought periods is the reduction of transpiration during nighttime (Howard et al. 2009). Since photosynthesis does not occur at night, water loss through night transpiration is not associated with additional carbon dioxide fixation (Caird et al. 2007). Our findings indicate genotype-specific variation in the ability of different faba bean genotypes to regulate stomatal conductance during the day-night transition. Genotypes that more effectively close their stomata after sunset lose less water. Previous studies have shown that nighttime transpiration can contribute significantly to overall plant water loss. The extent of this process varies depending on plant species and environmental conditions and should be considered when assessing plant water budgets (Dios et al. 2015). However, although we identified genotypic differences in nighttime stomatal regulation, it should be noted that overall nighttime water loss remains marginal and did not correlate with faba bean grain yield in this study ($r = -0.02$).

Impact of Daily Irrigation on Soil Moisture and Root Trait Selection

The irrigation protocol used in this study, which involved daily irrigation to reach a predefined target weight, may favor genotypes that utilize water less efficiently. Genotypes that are more wasteful in their water use are complemented with larger water volumes the following day to achieve the target weight, potentially masking the advantages of genotypes with more conservative water use strategies. Furthermore, the daily application of small amounts of water primarily increases field capacity in the upper soil layers. This scenario tends to induce drought stress in deeper soil layers, thereby favoring

shallow-rooted genotypes (Xue et al. 2003). When drought occurs in short, intermittent periods, water is often retained in the upper soil layers after light rainfall or irrigation (A et al. 2018). Shallow-rooted plants are well-positioned to rapidly absorb this water before it evaporates, whereas deep-rooted genotypes may not access this surface moisture as efficiently (Manschadi et al. 2006). In environments where the water table or the soil profile is shallow or has restrictive layers that inhibit deeper rooting, most of the available water is located near the surface (Rossato et al. 2017). Under both scenarios, shallow-rooted genotypes are well-positioned to rapidly absorb surface water before it evaporates, whereas deep-rooted genotypes may not access these brief moisture pulses as efficiently (Manschadi et al. 2006). Hence, as in all cases where controlled-environment phenotyping is implemented to identify genotypes with potentially useful traits for breeding towards drought tolerance, the context specificity of the results in terms of specific drought scenarios must be considered before implementation in breeding (Snowdon et al. 2021).

Expanding the Scope

A logical extension of our findings is to integrate the observed physiological traits such as vpd breakpoint dynamics and stomatal behavior with genetic and molecular data. Detailed phenotyping, as conducted in this study, provides a robust basis for undertaking genome-wide association studies (GWAS) or quantitative trait locus (QTL) mapping (Xiao et al. 2022). Such approaches can help identify specific genomic regions or candidate genes underlying early stomatal closure and effective water-use strategies under drought conditions. Nevertheless, it remains very challenging to accurately assess transpiration dynamics in the large populations required for quantitative genetic analyses. Our DroughtSpotter XXL facility enables simultaneous screening of 80 genotypes with three independent replicates or 60 genotypes with four replicates, which is insufficient for GWAS or biparental QTL mapping. Alternatively, if genotype-specific responses to VPD are also expressed during juvenile development, related facilities which enable screening of larger numbers of genotypes in smaller pots might represent a useful alternative.

Conclusion

Drought periods caused by climate change pose a significant threat to faba bean cultivation worldwide. Therefore, it is crucial to identify genotypes that not only conserve the available water but also use it as efficiently as possible to maintain stable seed yields under water-limiting conditions. The regulation of stomatal conductance, and consequently of transpiration rates, is primarily influenced by VPD under drought stress conditions. Our findings indicate that genotypes capable of rapid and complete stomatal closure in response to VPD exhibit higher water-use efficiency under drought stress, allowing them to utilize available water more sparingly and efficiently. The breakpoint for transpiration regulation was found to be day-specific, indicating that factors other than VPD (for example light intensity) may also play a role, however the overall trend of stomatal response characteristics was consistent across most genotypes. The consideration of VPD in conjunction with pre- and post-breakpoint transpiration rates provides valuable insights into the water management strategies of plants. These findings have practical

implications for breeding programs aiming to enhance drought resilience in crops. Identifying genotypes with efficient stomatal regulation can contribute to the development of faba bean varieties that are better adapted to water-limited environments. Although nighttime transpiration rates reveal differences in the magnitude of the regression after sunset, the total amount of water lost during the night is minimal and the resulting water savings can be considered negligible in comparison to the savings achieved via prompt and efficient response to VPD.

Declarations

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Authors' contributions

RS conceived the study, AM and AS developed the method, LS performed the experiment, data curation, analysis and interpretation of the results, LS wrote the manuscript with contribution from RS & BW. General guidance was provided by BW. Guidance regarding faba bean cultivation was provided by OS and GW. Project coordination on the NPZ side was carried out by HT. All authors revised the manuscript and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author LS.

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Competing Interests

The authors declare that they have no competing interests

Ethics approval and consent to participate

Not applicable

Consent for Publication

Not applicable

Clinical trial number

Not applicable

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Figures

Figure 1

High-throughput phenotyping system "Droughtspotter XXL". The Droughtspotter XXL consisting of 240 containers with a rooting volume of 120 liters each. Gravimetric weight measurements at 5-minute intervals allow for high-resolution monitoring of the transpiration rate. The containers are situated in a foil greenhouse which excludes natural precipitation, however the walls remain open to maintain temperature and humidity at more or less ambient levels.

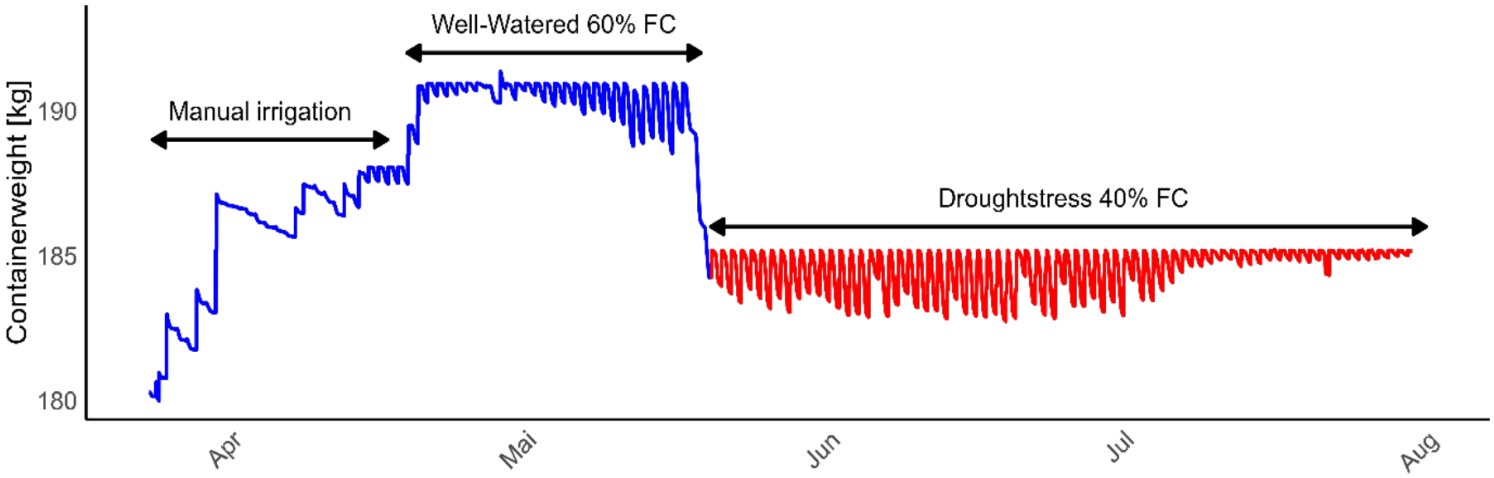

Figure 2

Example showing the irrigation plan applied to each container throughout the vegetation period. Specifically calculated target weights correspond to the desired field capacities of 60% (well-watered) and 40% (drought stress). Irrigation to reach the respective target weights was performed daily at 00:05 hours, when transpiration was no longer occurring for any genotype. The weight changes of the containers served as the basis for container-specific irrigation and gravimetric transpiration measurements. The experiment was initiated with manual irrigation for around 3 weeks after sowing to achieve uniform soil moisture until the target weight was reached.

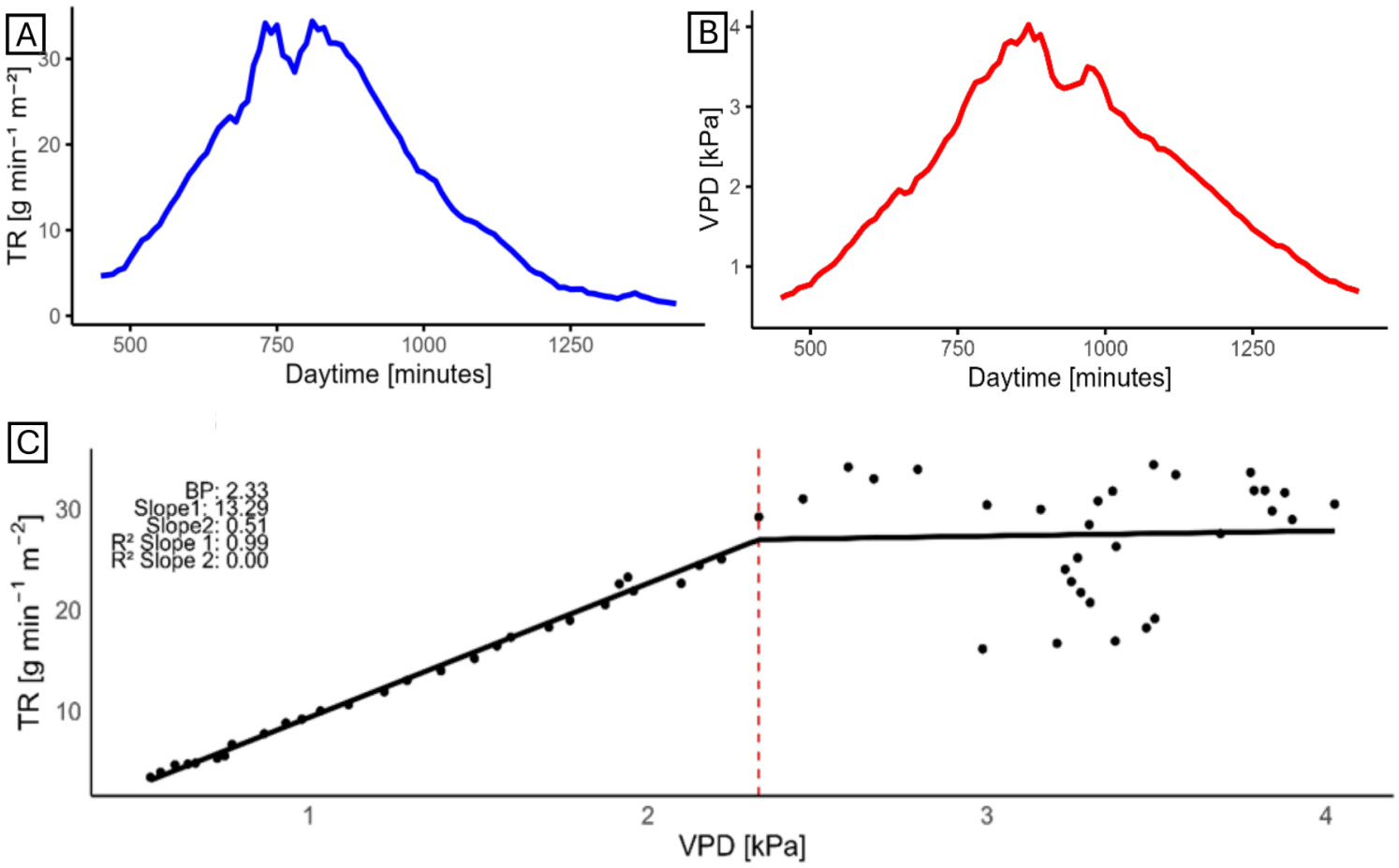

Figure 3

Collected environmental data for VPD and transpiration rate exemplified for one container on June 16. The relationship between transpiration rate (A) and VPD (B) is illustrated using segmented regression (C) over a time window from 8:00 to 18:00 hours. The red dashed line indicates the vpd breakpoint (BP).

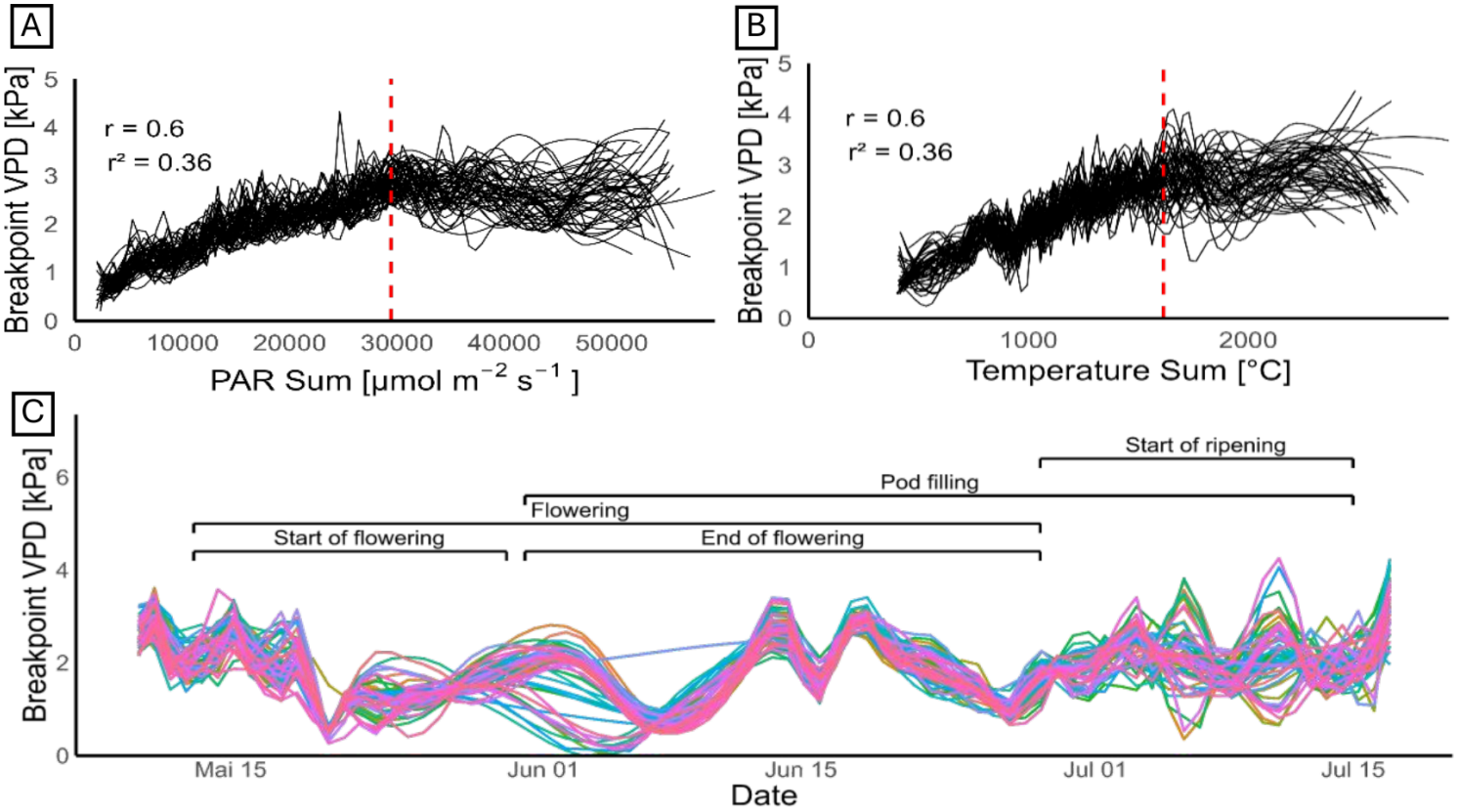

Figure 4

Breakpoints as a function of the cumulative photosynthetically active radiation (A) and temperature (B). Height of vpd breakpoints as a function of the cumulative sum of photosynthetically active radiation (A) and cumulative temperature (B) for each container and day. The progression of vpd breakpoints throughout the growing season is for each container and each day, illustrating their changes during the growth cycle, subdivided into the different developmental stages of the plants (C). The data illustrate how environmental factors influence the breakpoint dynamics during the vegetation period. Correlation coefficients (r) and coefficients of determination (r^2) indicate the strength of the relationships.

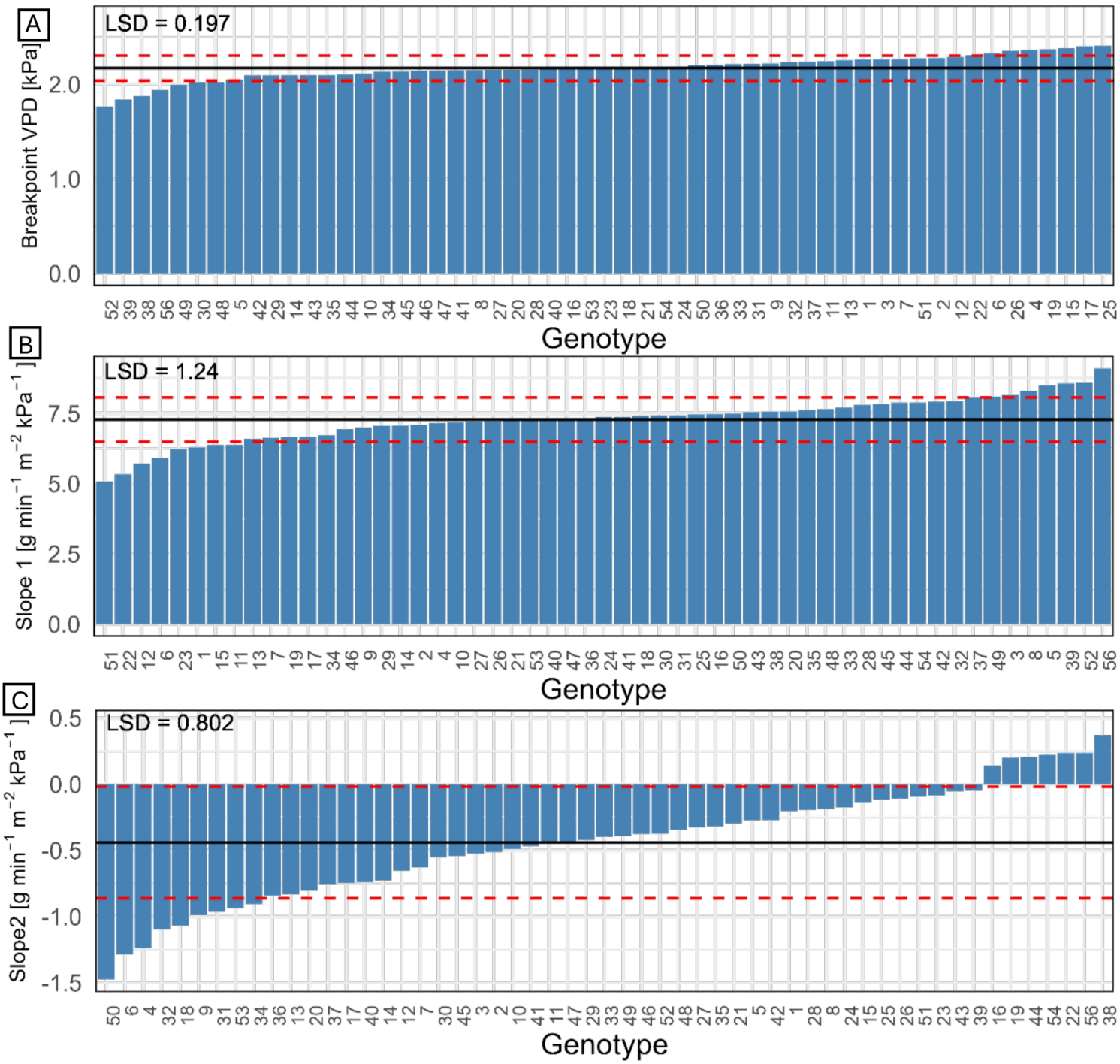

Figure 5

The transpiration response to VPD was assessed in 56 genotypes in 2022. The blue bars represent the adjusted mean values for the breakpoint (A), Slope 1 (B), and Slope 2 (C). The solid black line indicates the overall mean for each variable, while the red dashed lines marks standard deviation. LSD thresholds are calculated at a 10% significance level.

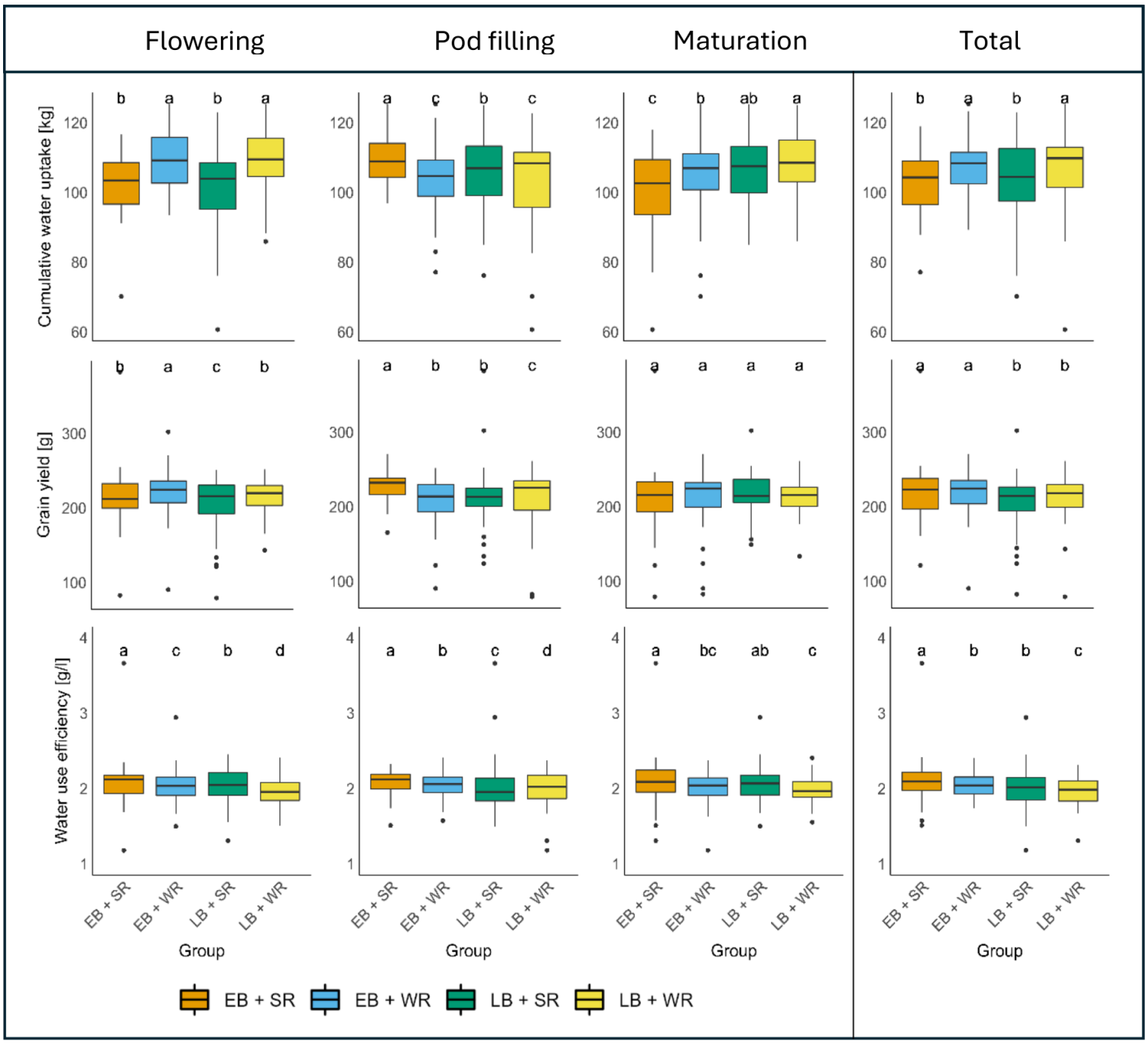

Figure 6

Water uptake (kg), grain yield (g), and water use efficiency (g l^{-1}) for genotype groups with distinct VPD responses: Early breakpoint + strong restriction (EB + SR), early breakpoint + weak restriction (EB + WR), late breakpoint + strong restriction (LB + SR), and late breakpoint + weak restriction (LB + WR), during the flowering, pod-filling, and maturation stages, as well as over the complete growth cycle (total). The data reveal that groups differ significantly in these traits, and variation in transpiration patterns impacted different traits during specific developmental stages.

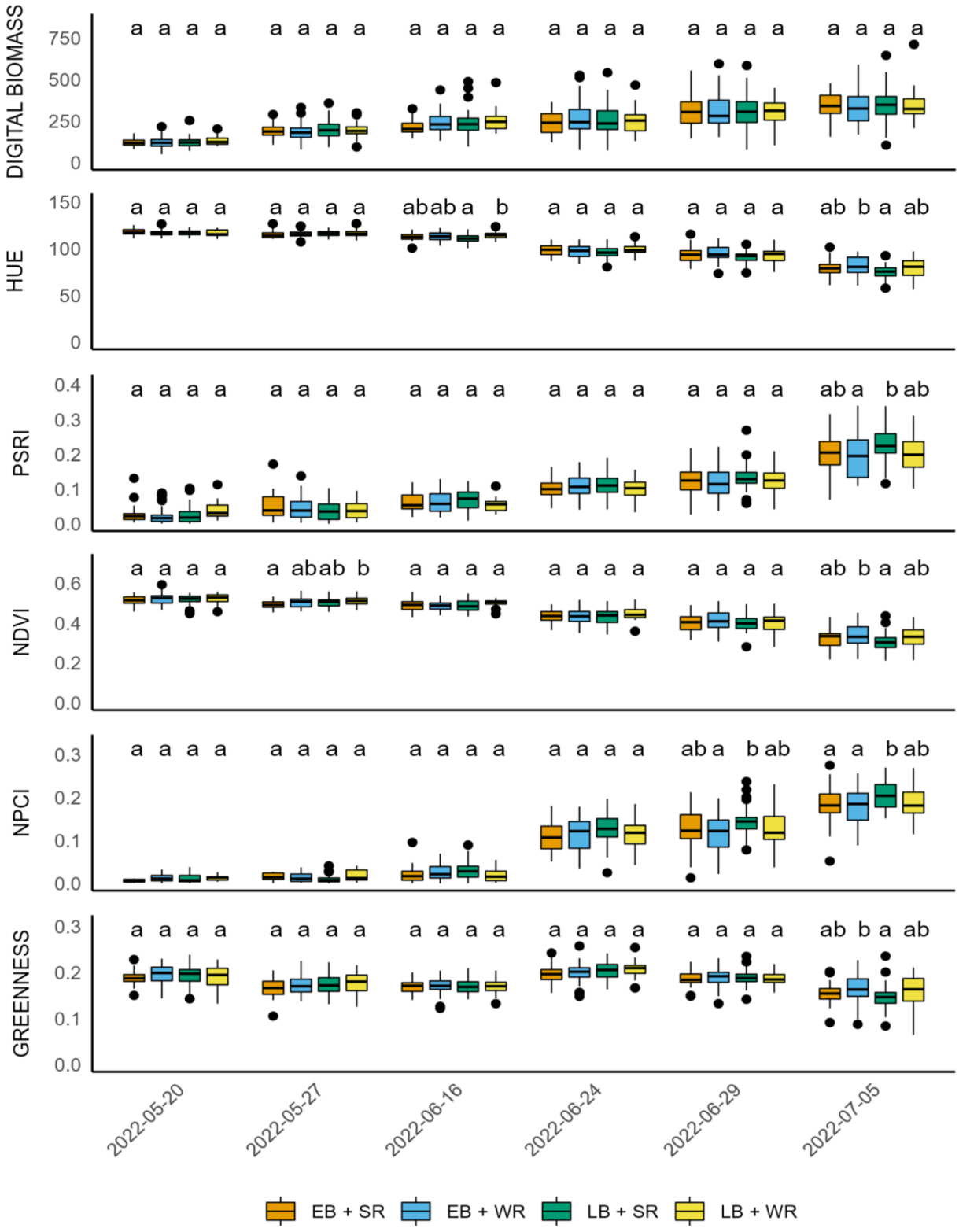

Figure 7

Plant-specific indices in relation to transpiration response groups. Changes in plant-specific indices - Greenness, NPCI, NDVI, HUE, PSRI, and Digital Biomass -were captured using two 3D multispectral scanner heads (F500, Phenospex). Changes were analyzed in relation to four distinct transpiration response groups (EB + SR, EB + WR, LB + SR, LB + WR), revealing significant differences among groups ($p \leq 0.05$).

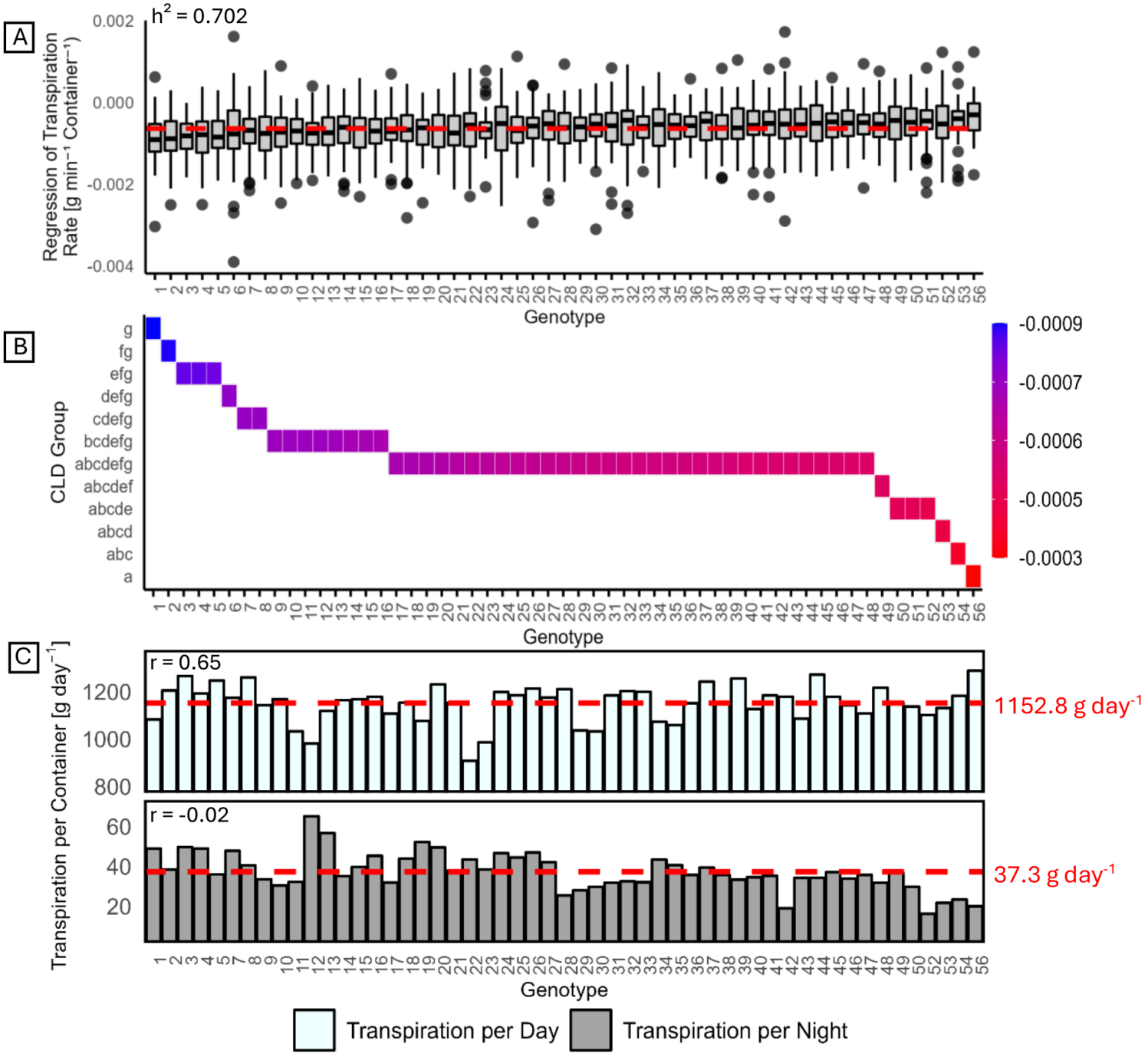

Figure 8

Nighttime transpiration dynamics of faba bean genotypes. Regression analysis of nighttime transpiration rate begins when PAR (photosynthetically active radiation) drops to ≤10 µmol m^{-2} s^{-1} and continues until midnight. Boxplots display the regression slopes for all genotypes, with a red dashed line representing the overall mean slope (A). More negative regression slopes indicate a faster adjustment of plant transpiration in response to the absence of light. The heatmap (B) visualizes the mean regression slope for each genotype and highlights statistically significant groupings ($p \leq 0.05$). Bar plots show the adjusted transpiration means per container for each genotype during both daytime and nighttime. Correlation coefficients (r) linking transpiration and adjusted yield means are displayed (C).

Supplementary Files

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