

## Article

# Sugar Beet Rooting Pattern Mediates Stomatal and Transpiration Responses to Progressive Water Stress

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**Abstract:** Water stress is the main risk facing sugar beet production in Europe and is expected to worsen with climate change. Therefore, future production essentially depends on the traits that sustain growth during water shortages. In this study, we investigated the interplay of stomatal conductance and transpiration rate as well as the root characteristics of six sugar beet cultivars in a climate chamber experiment under environmental conditions progressing from a non-stressed initial state toward high atmospheric water demand, followed by reduced soil water supply and then by a combination of high demand and low supply. Stomatal conductance quickly responded to changing conditions, dropping from 406.4 to 42.5 mmol m<sup>-2</sup> s<sup>-1</sup>, one order of magnitude, with the transition to reduced soil water availability. The transpiration rate showed a slightly delayed response compared with stomatal conductance, while we observed an inverse influence on the subsequent stomatal behavior exerted by the depletion/conservation of available soil water. The rooting pattern substantially differed among genotypes, predominantly at depths between 60 and 80 cm, where 50.5% of the root length was allocated. Longer roots buffered the effects of the reduction in stomatal conductance at the onset of water supply limitation, with 5.4 mmol m<sup>-2</sup> s<sup>-1</sup> higher conductance per 100 cm of root length. Therefore, breeding and/or management measures targeting root system vigor are the key to the growth maintenance of sugar beet during dry periods.

**Keywords:** sugar beet; stress resistance; root system; drought; stomatal conductance; transpiration



**Citation:** Bodner, G.; Alsalem, M. Sugar Beet Rooting Pattern Mediates Stomatal and Transpiration Responses to Progressive Water Stress. *Agronomy* **2023**, *13*, 2519. <https://doi.org/10.3390/agronomy13102519>

Academic Editors: Tihana Marček and Marija Viljevac Vuletić

Received: 4 September 2023

Revised: 14 September 2023

Accepted: 15 September 2023

Published: 29 September 2023



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## 1. Introduction

Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is an important economic crop in Europe for the production of sugar, biogas, and animal fodder. Currently (2019–2021), sugar beet covers on average approximately 1.8% of the European cropland (up to 7.8% in the Netherlands), with production regions featuring humid oceanic to semiarid continental and Mediterranean climates. Yield levels (10-year average) range from 38 t ha<sup>-1</sup> (Portugal) to 86 t ha<sup>-1</sup> (France), with an average yield variability (coefficient of variation) of 14% [1].

The growth and development of sugar beet under central European conditions extend from March to the end of October [2]. The phenological stages with high sensitivity to abiotic stresses occur around row closure (BBCH 31–39: beginning to complete crop cover), coinciding with periods of potentially high temperatures and limited water supply [3]. However, early growth stages might also be negatively affected by spring season water limitations, which have repeatedly occurred in Europe during the last decade [4], leading to delayed emergence and juvenile development [5].

Modern sugar beet originates from the wild ancestor *Beta vulgaris* spp. *maritima* which is found in coastal regions of southern Europe and Africa and is resistant to severe drought and salinity stress [6]. Therefore, sugar beet is also generally considered a crop that is well-adapted to water-limited environments [7]. However, water stress is still the single most important, single yield-limiting factor in many sugar-beet-producing countries and

particularly challenges production in semiarid regions within an increasingly competitive open global sugar market [2,8,9].

The risks facing sustainable sugar beet production are expected to increase with climate change. Although high concentrations of atmospheric CO<sub>2</sub> might positively affect photosynthetic rates and yield via CO<sub>2</sub> fertilization, increasing temperatures lead to higher atmospheric water demand, increased canopy transpiration, as well as (night-time) respiration losses [9]. The total water requirement of sugar beet strongly depends on the environmental conditions and ranges from 385 mm in cool and humid environments with low evaporative demand to 1043 mm in high-temperature environments with high atmospheric water demand [10]. With changes in the rainfall amount, distribution, and frequency, the probability of the soil water supply being lower than the crop water demand increases. In a simulation study, it was shown that drought-induced losses and the annual variability in sugar beet yield were found to rise from 7% and 10% (1961–1990) to 18% and 15% (2021–2050), respectively [11]. Therefore, we need to understand the key mechanisms through which sugar beet responds to water limitation and, thereby, identify traits that might confer increased drought resistance.

Generally, plants can adapt to water-limited environments via drought escape (e.g., early maturity), tolerance of tissue dehydration (e.g., proline accumulation), and/or avoidance of dehydration [12]. Particularly, the traits leading to dehydration avoidance via the effective use of soil moisture are also compatible with high yields, allowing plants to withstand extended periods with limited rainfall supply [13]. The potential target traits for selecting cultivars with superior drought resistance, thus enabling the stronger climate change adaptation of agricultural crops, are highly specific to the drought environment [14]. In Europe, sugar beet is frequently grown on fertile soils, such as chernozems, which have a high water-holding and deep rooting capacity [2]. Under such pedological conditions, the usage of available subsoil water resources stored over winter is particularly important to buffer the intermittent drought spells that are increasingly expected during May and June in central Europe [15]. Therefore, the root system qualifies as a relevant trait for sugar beet breeding.

Root system characteristics are essential for the dehydration avoidance behavior in crops, mediating an effective usage of stored soil moisture (“water spenders”) [16]. In soils with a high water-retention capacity, deep root allocation is considered a key pattern distinguishing water-efficient root systems [17]. Sugar beet is considered a deep rooting crop, capable of also exploring deep-water resources in case of drought [18–21]. Deep rooting, together with the accumulation of osmolytes in the leaf and root tissues upon drought [22], leads to anisohydric behavior, maintaining open stomata and transpiration even during extended periods of water shortage, in sugar beet [23].

Similar to other crops, sugar beet cultivars that perform better under stress might do so at the expense of yield penalty under non-stressed conditions [24]. Therefore, particularly for environments with variable weather conditions where both years with high potential yield as well as intense growth limitation due to abiotic stresses can occur, cultivars are required where stress avoidance is based on an efficient uptake of soil resources. Thus, for sugar beet cultivation and breeding in water-limited environments, we must better understand whether cultivars differ in their root system characteristics and, thereby, control sustained stomatal conductance and transpiration during drought [25,26].

The objectives of this study were to (1) characterize the stomatal and transpiration responses of sugar beet to increasing water limitation severity and (2) to identify the role of the differences in multiple sugar beet varieties regarding root system characteristics in mitigating the stress-induced downregulation of transpiration and stomatal closure. The outcomes of this study provide an improved understanding of the interplay between root system characteristics and drought-sensitive shoot traits, further defining the potential contribution of the root system as a stress-resilience target in sugar beet improvement under climate change conditions.

## 2. Materials and Methods

### 2.1. Sugar Beet Cultivars

Six sugar beet (*Beta vulgaris* subsp. *vulgaris altissima* group) varieties were used to investigate differences in root development and drought response. The selected cultivars (Table 1) are commonly used in sugar beet production under Pannonian climate conditions in central and eastern Europe, where drought is the key yield-limiting factor. Cultivars differ in the region of breeding origin and nematode resistance score, with nematode resistance potentially being related to distinct root system characteristics [27].

**Table 1.** Sugar beet varieties used for the rhizobox investigations of drought response with their country of origin and nematode resistance score (1 = very low, 9 = very high resistance) according to the Austrian Variety list [28].

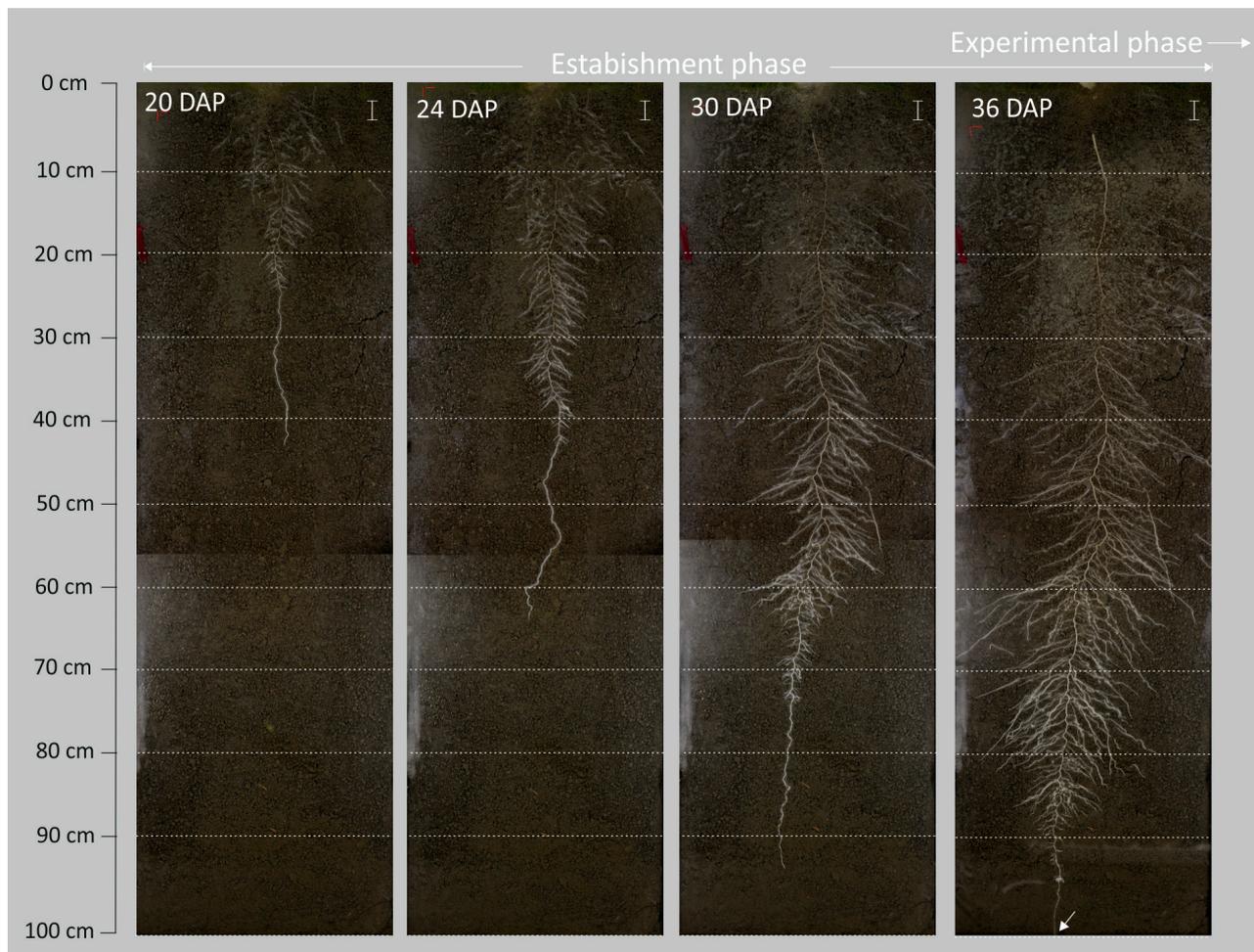
Genotype	Origin	Nematode Resistance
Ferrara	Germany	-
Gladiator	Belgium	2
Inge	Netherlands	6
Marino	Denmark	2
Nauta	Denmark	-
Strauss	Germany	8

### 2.2. Experimental Setup

The sugar beet drought response and its relationships to rooting patterns were studied in soil-filled rhizoboxes (for details of the rhizobox system, cf. [29]). The rhizoboxes (30 × 100 × 1 cm) were filled with sieved (3 mm) silt loam topsoil (sand 21.9%, silt 61.2%, and clay 16.9%), which is representative of the eastern Austrian soils where sugar beet is grown (chernozem), and mounted into a metal holder at an angle of 45° to maximize root visibility from the glass observation window. The effect of different substrates (texture and hydraulic properties) on the root morphology in rhizobox experiments was pretested, demonstrating vigorous root growth when using field soil where the water retention characteristics are similar to field conditions ( $\theta_{\text{field capacity}} = 0.36 \text{ cm}^3 \text{ cm}^{-3}$ ,  $\theta_{\text{wilting point}} = 0.12 \text{ cm}^3 \text{ cm}^{-3}$ ; [30]).

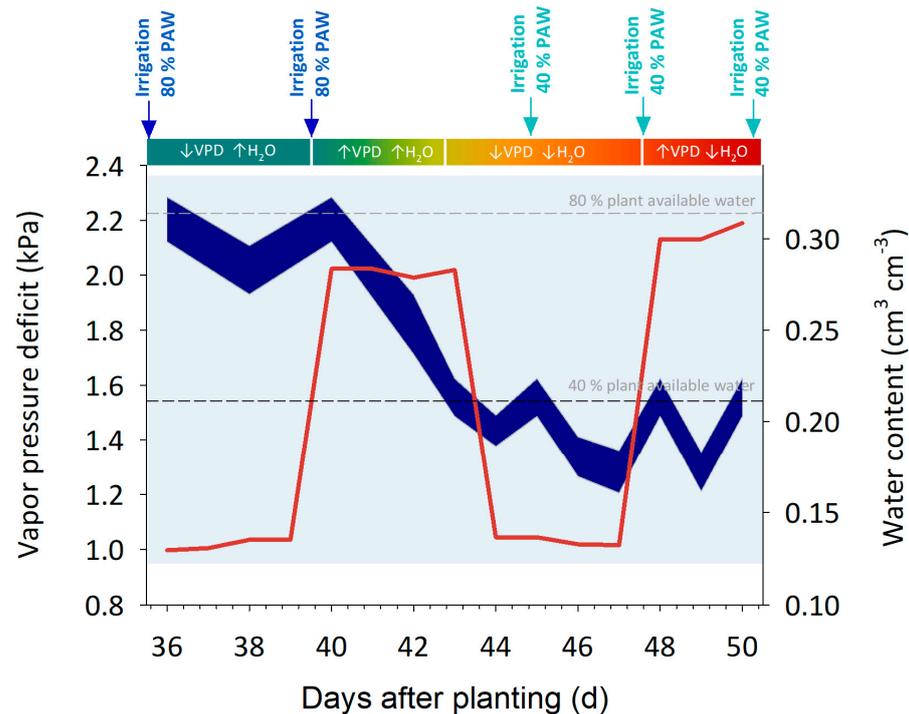
Seeds were pregerminated in Petri dishes in the dark at 20 °C until the emergence of the radicle, and one healthy seed of each cultivar was planted in each box at a 2 cm depth. Rhizoboxes were placed in a completely randomized design (CRD, and changing the position of the boxes after each weighing/irrigation; n = 5) in a climate room under a light intensity of 450  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ , a relative humidity (RH) of 70%, a day/night length of 14/10 h, and a 20 °C/15 °C temperature. Plant emergence was observed 14 ± 2 days after planting. Plants were fertilized when leaves started to expand, with water irrigation once per week with a commercial liquid fertilizer (SUBSTRAL® Pflanzen Nahrung, Mainz, Germany) at a concentration of 7 mL/liter to ensure the absence of nutrient stress. On average, rhizoboxes were weighed every 3 ± 1 days and irrigated to a water content corresponding to 80% plant available water (PAW;  $\theta = 0.32 \text{ cm}^3 \text{ cm}^{-3}$ ).

Plants were established under optimum water conditions (80% PAW) until their root systems were fully developed and arrived at the bottom (100 cm) of the rhizoboxes (BBCH 17, 36 days after planting (DAP); Figure 1).



**Figure 1.** Example of sugar beet root system extension (variety Ferrara) during the establishment phase of the plants. At 36 days after planting (DAP), when the first roots arrived at the bottom of rhizoboxes, (arrow at the bottom of the image at 36 DAP) the experimental phase was initiated to study the potential effects of distinct rooting patterns upon the transition from well-watered to successively intensifying drought stress conditions. Scale bars at the top indicate 2 cm.

At 36 DAP, the experimental phase started. Initially, the root systems of all plants were imaged, and the first measurement of stomatal conductance was recorded (cf. Section 2.3.) to characterize the non-stressed stage with plants under sufficient soil water supply (80% PAW) and moderate atmospheric demand (vapor pressure deficit (VPD) = 1 kPa). At 40 DAP, the plant water demand was increased to VPD = 2.0 kPa by raising the temperature to 27 °C and reducing relative humidity to 40% while keeping water supply to the soil at 80% PAW. This condition simulated a situation at the onset of a drought period, with the rising water uptake successively depleting soil water resources. At 44 DAP, a stage of supply-driven stress with limited soil water availability was established, with water content at 40% PAW, while again lowering the plant demand by reducing the VPD to the initial setting of 1 kPa. This condition was maintained until 47 DAP. Thereafter, the supply-related stress condition (40% PAW) was combined with high plant demand via a VPD of 2 kPa that was maintained until a final measurement at 50 DAP (Figure 2).



**Figure 2.** Ambient conditions (red line: vapor pressure deficit controlling plant water demand; blue area: soil water content controlling plant water supply) during the transition of rhizobox-grown sugar beet varieties from well-watered to successively intensifying stress conditions. Phase 1: well-watered; Phase 2: high atmospheric demand with sufficient soil supply; Phase 3: limited soil supply with moderate atmospheric demand; and Phase 4: limited soil supply with high atmospheric demand. The water supply settings (80% and 40% PAW) are indicated.

### 2.3. Measurement Methods

Roots were imaged following the method described by Bodner et al. (2017) [29]. In brief, rhizoboxes were mounted onto a custom-made imaging box to shield them from ambient light and to fix the camera for the acquisition of RGB images with a Canon EOS 6D digital camera (Canon Inc., Tokyo, Japan). RGB images from the top and bottom of the rhizobox were captured, merged into a single image ( $3648 \times 10,744$  pixels) using Corel Draw Version X7 (Corel Corporation, Ottawa, ON, Canada), and saved as TIFF files. Images were analyzed with Winrhizo Pro V.2013 (Regent, Québec, QC, Canada). Roots were separated from the background (root segmentation) based on the color thresholds between roots vs. soil (background). The visible root length was measured on the segmented binary image files in 10 cm slices, resulting in 10 length measurements characterizing the depth distribution of the roots.

The transpiration rate (TR;  $\text{mL d}^{-1}$ ) was obtained by weighing the rhizoboxes and dividing the respective difference between the final and initial mass ( $\Delta m$  in grams) by the respective time interval ( $\Delta t$  in days). The loss of mass between two time points thereby represents the water loss via plant transpiration, with rhizobox openings covered with aluminum foil to prevent soil evaporation and drainage losses from the bottom of the boxes being zero at the given water contents that were lower than field capacity. The TR was obtained for each of the four stages analyzed, i.e., non-stressed ( $\Delta m_{\text{DAP40-DAP36}}$ ), high demand–high supply ( $\Delta m_{\text{DAP44-DAP40}}$ ), low demand–low supply ( $\Delta m_{\text{DAP46-DAP44}}$  and  $\Delta m_{\text{DAP48-DAP46}}$ ), and low supply–high demand ( $\Delta m_{\text{DAP50-DAP48}}$ ). For Phase 3, two measurements of the transpiration rate were recorded to more accurately capture the transition from the 80% PAW during the previous stages to the 40% PAW in the subsequent stages.

Stomatal conductance (SC;  $\text{mmol m}^{-2} \text{s}^{-1}$ ) was measured using an AP4 V.4 leaf porometer. Three leaves per plant were measured, and the subsamples were averaged

for statistical analyses. Measurements were recorded eight times during the experimental phase, i.e., on DAP 36 (Phase 1), DAP 41 to 43 (Phase 2), DAP 44 and 45 (Phase 3), and DAP 48 and 50 (Phase 4).

At the end of the experiment, the plants were cut, and the leaves were scanned with a LICOR LI-3100C scanner to measure leaf area ( $\text{cm}^2$ ). Finally, the total aboveground biomass (g) was dried in an oven at  $105\text{ }^\circ\text{C}$  for 24 h to determine shoot dry matter.

#### 2.4. Statistical Evaluation

All statistical analyses were performed with SAS version 9.4 (SAS Institute, Cary, NC, USA). Treatment differences were analyzed with a mixed model analysis of variance (PROC MIXED). Within the model, repeated measures (i.e., time for transpiration rate and stomatal conductance, and depth for root length) were described using an unstructured model that resulted in the best overall fit based on the Akaike information criterion (AIC). Normal distribution was checked using the Kolmogorov–Smirnov test and homogeneity of variances using Levene’s test. Measurements not complying with the normality assumption were log-transformed. The mean comparison was performed with Tukey–Kramer HSD as a post hoc test.

Temporal relationships between the measurement series of stomatal conductance and transpiration rate were analyzed via cross-correlation using PROC TIMESERIES. The respective measurements were linearly interpolated to produce equally spaced data points.

The strength and significance of the relationships between the parameters were tested using Pearson correlation analysis (PROC CORR). Graphs were created using SigmaPlot version 14.5 (Systat Software Inc., Palo Alto, CA, USA).

### 3. Results

#### 3.1. Temporal Sequence of Sugar Beet Stress Response

The functional response of sugar beet after establishment for root and plant development during the 35 days until BBCH 17 (Figure 3a) was monitored for a two-week experimental phase using the transpiration rate and stomatal conductance (Figure 3b).

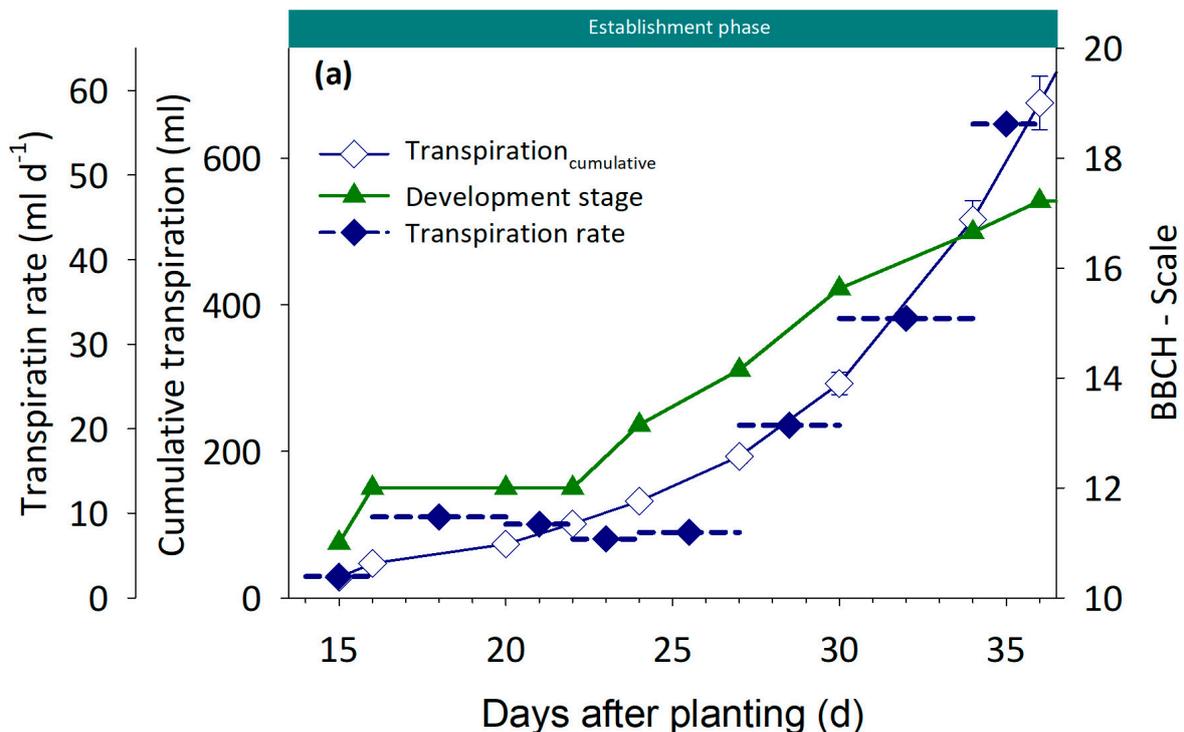
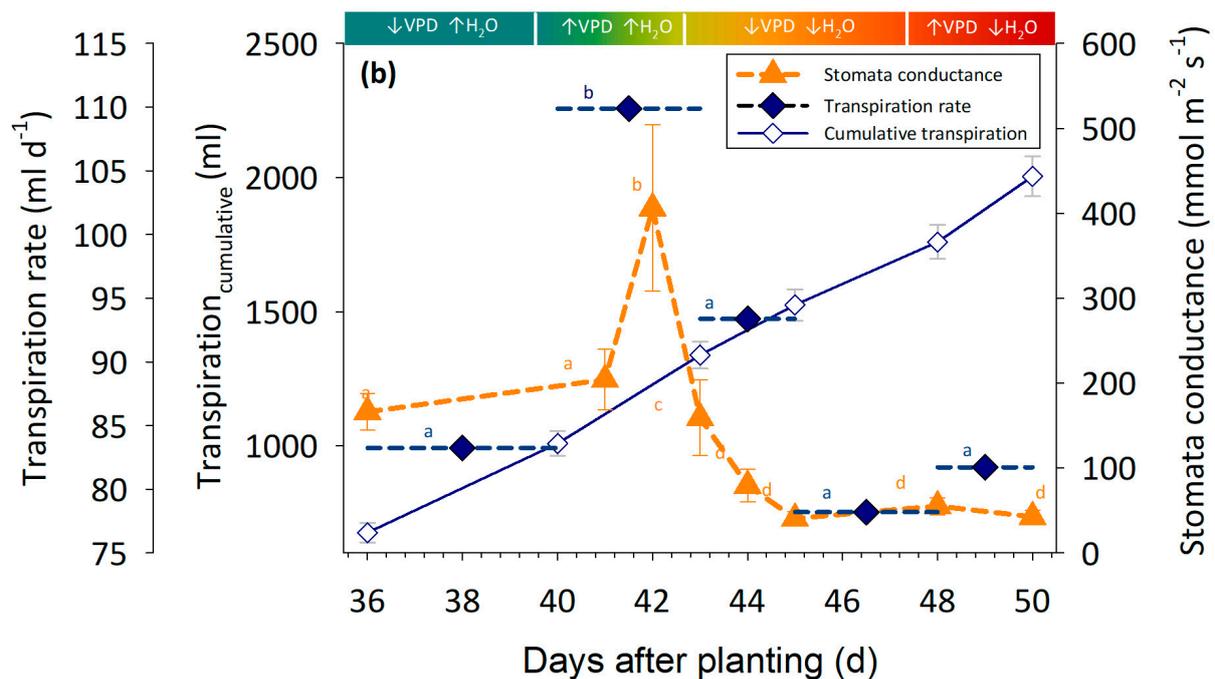


Figure 3. Cont.



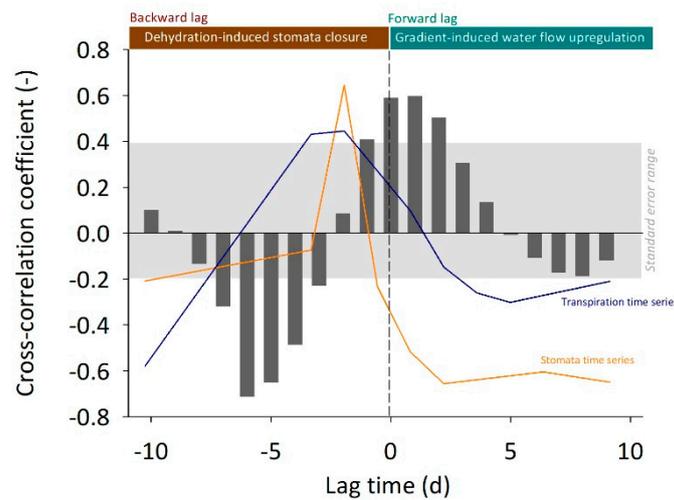
**Figure 3.** Plant development (BBCH and transpiration) during the establishment phase (a) and response (transpiration and stomatal conductance; means and standard deviation) during the experimental stages of the transition from an initial non-stressed condition (low VPD, high soil moisture) to a final drought stress stage with high plant demand (high VPD) and low soil supply (b). Lowercase letters indicate significant differences according to Tukey's HSD test at  $p < 0.05$  ( $n = 5$ ).

During the initial non-stressed stage with a water content at 80% PAW and an atmospheric demand driven by a VPD of 1 kPa, the average transpiration rate was  $83 \text{ mL d}^{-1}$  with stomatal conductance at  $166 \mu\text{mol m}^{-2} \text{ s}^{-1}$ .

Increasing the atmospheric water demand by increasing the VPD to 2 kPa while keeping the soil moisture availability at 80% PAW resulted in a significantly higher average transpiration rate (+32%). After its highest value, a significant decrease of 15% followed at the onset of water supply limitation. The decreasing trend continued (29% lower than peak value) during the stage at 40% PAW; finally (with higher VPD), it slightly increased again by 5% (ns) compared with that in the previous stage.

Stomatal conductance showed an initially slight increase of 22% (ns compared with the initial value) with rising VPD, to arrive at its highest value the day after (98%). Thereafter, it quickly decreased (60% lower than its peak value) during the transition to the stage with limited soil moisture supply (40% PAW), with no further significant changes occurring until the end of the experiment, leading to average values of  $54 \pm 18 \mu\text{mol m}^{-2} \text{ s}^{-1}$ .

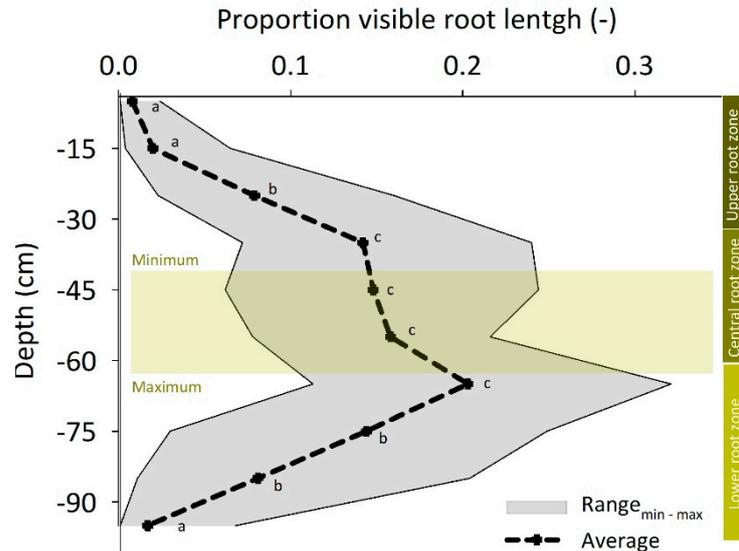
The results of the cross-correlation analysis of the temporal relationship between the transpiration rate and stomatal conductance are shown in Figure 4. Shifting the stomatal conductance in the forward direction produced a similar response pattern with the transpiration rate, with a positive correlation over a lag of two days ( $R = 0.50\text{--}0.60$ ). On the contrary, the backward lag showed a comparatively strong negative relationship that peaked at  $-6$  days ( $R = -0.71$ ), i.e., high previous transpiration rates result in lower stomatal conductance values (and vice versa).



**Figure 4.** Temporal relationship of sugar beet stress response variables revealed by the forward and backward shifting of a linearly interpolated time series of stomatal conductance and transpiration rate.

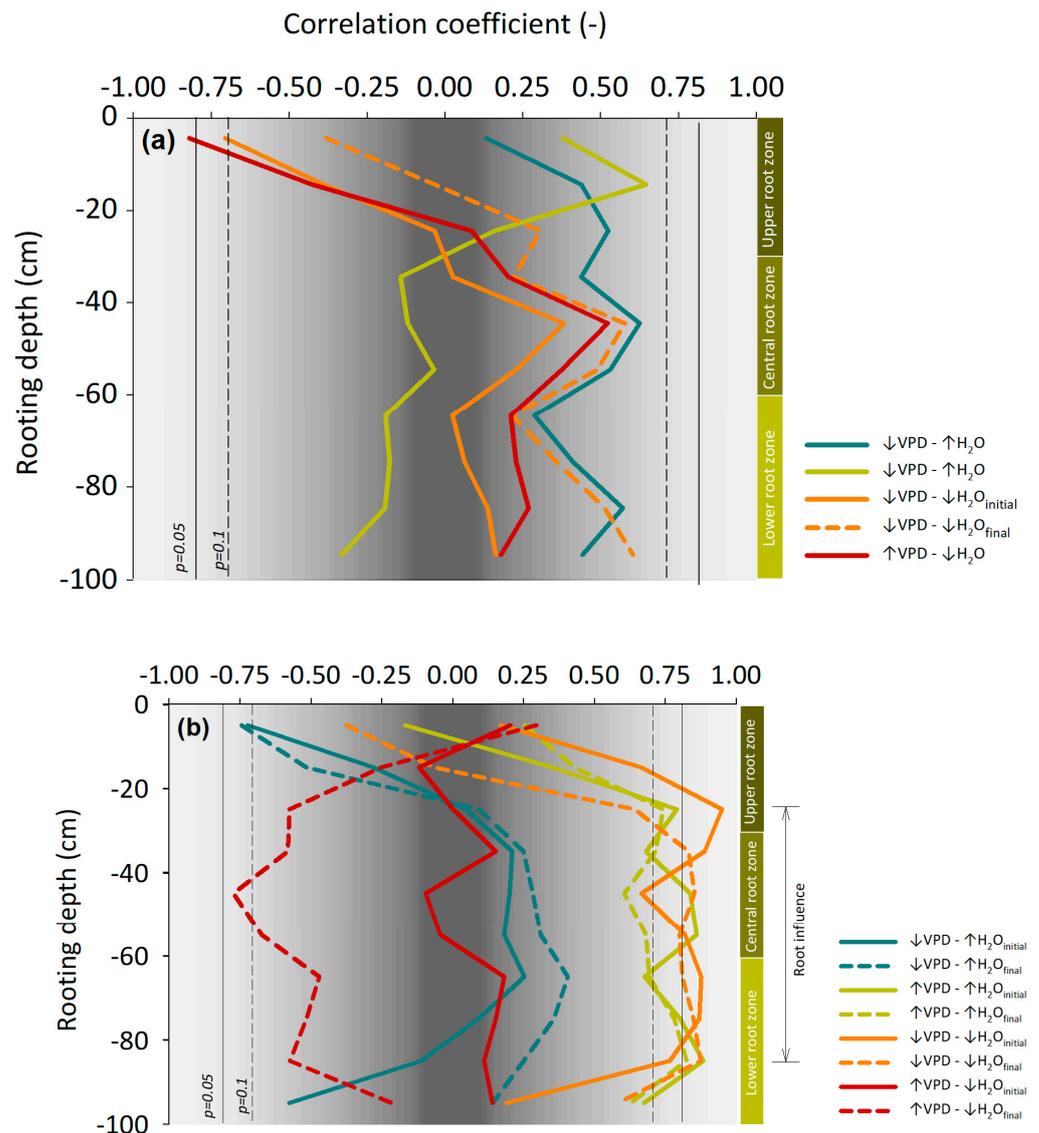
### 3.2. Root Influences on Transpiration and Stomatal Behavior

The average root depth distribution is shown in Figure 5. The allocation of visible root length over depth significantly increased up to the third layer (−30 to −40 cm); thereafter, it remained at similar (nonsignificantly different) values to −80 cm while decreasing to the bottom of the rhizoboxes (−100 cm). The depth of the 50% root length allocation (calculated according to Schenk and Jackson (2002) [31]) ranged from a minimum of 41 cm to a maximum of 63 cm.



**Figure 5.** Distributional pattern of root length over depth (minimum, maximum, and average). The yellow area shows the depth (minimum–maximum) where 50% of the root length was allocated. Lowercase letters show significant differences in root distribution between depth increments at  $p < 0.05$ .

Figure 6 reveals the influence of the rooting pattern on the transpiration rate (Figure 6a) and stomatal conductance (Figure 6b) at different time points during the dehydration experiment. For the transpiration rate only, the final measurement showed a significant influence of the upper root zone, with the negative relationship pointing to a topsoil depletion-related effect.

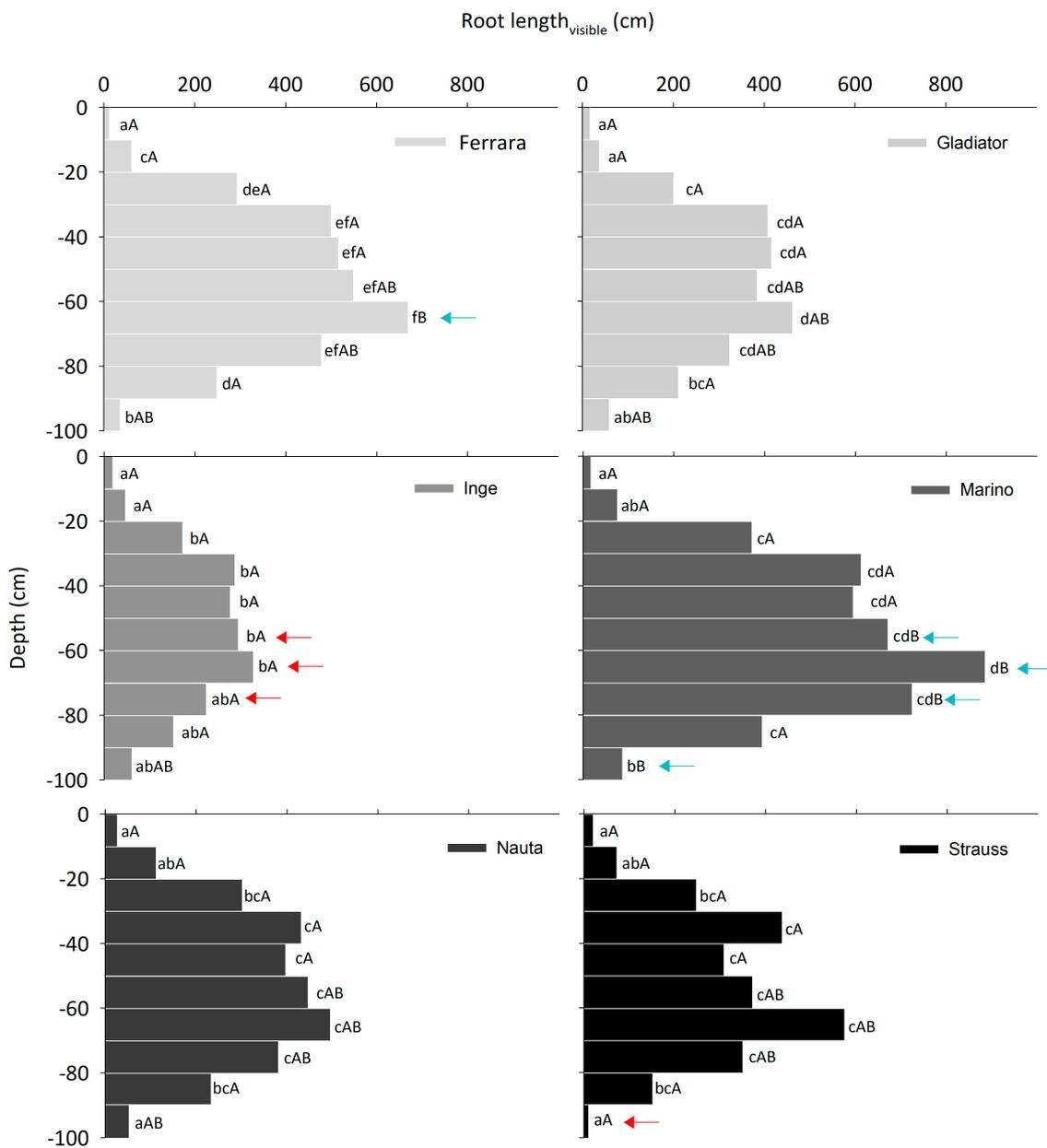


**Figure 6.** Relationship of rooting pattern with the temporal sequence of sugar beet stress response: (a) transpiration rate and (b) stomatal conductance (n = 6).

On the contrary, the influences of the roots on stomatal conductance extended over a wider root zone (−30 to −90 cm) and were expressed during the second (high VPD–high water content) and third (low VPD–low water content) stages of the dehydration process, whereas no significant correlations were found during the initial (no stress) and final (highest stress) phases.

### 3.3. Implications of Variety on Sugar Beet Dry-Down Response

The root system characteristics of the genotypes are shown in Figure 7. The results of the mixed-model ANOVA indicated a significant variety × depth effect ( $p = 0.011$ ). Generally, the differences in the visible root length of all genotypes were the highest between −60 and −70 cm. The central root zone between −40 and −80 cm significantly differed from the top-most (0 to −20 cm) and the bottom (−90 to −100 cm) zone for most varieties; except for Inge, which had the lowest average root length and nonsignificant differences between the central and bottom root zones. The layer with the highest root allocation (−60 to −70 cm) significantly differed in root length in the −20 to −30 cm as well as −80 to −90 cm layers for the varieties Ferrara, Gladiatore, and Marino. The other varieties showed less pronounced differences in the transition among the single layers.



**Figure 7.** Root depth distribution of sugar beet varieties. Lowercase letters show the differences between depth layers for each single variety, whereas uppercase letters indicate the variety differences at the same depth. Significant differences according to Tukey’s HSD test ( $p < 0.05$ ) are indicated by different letters. The colored arrows point to the depths where significant variety differences were found (turquoise arrows: highest values; red arrows: lowest values).

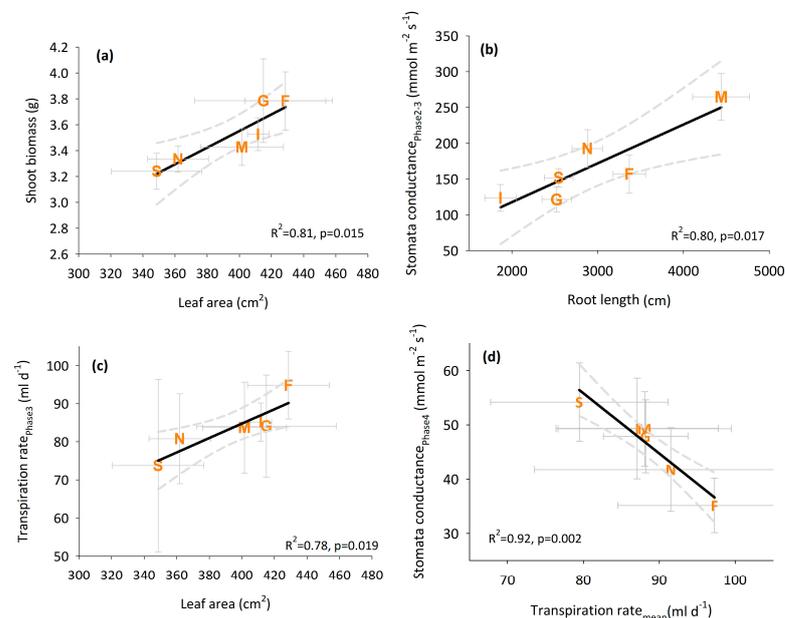
Variety differences in individual depth compartments were identified between Marino and Inge (−50 to −80 cm), Ferrara and Inge (−60 to −70 cm), and Marino and Strauss (−90 to −100 cm).

Table 2 shows the average values of the aboveground traits of the six sugar beet varieties analyzed in this study. We found no significant difference in the leaf area or shoot biomass. However, the varieties differed in their average stomatal conductance, with Marino having a significantly higher conductance (+56%) than most other varieties, except for Nauta, which had values in between Marino and the others.

**Table 2.** Aboveground traits of the investigated sugar beet varieties. Leaf area and shoot biomass represent values accumulated over the entire growth duration (50 days) and measured at the end of the experiment. Stomatal conductance represents the mean over the experimental phase (DAP 36—DAP 50). For traits with a significant variety effect, the lowercase letters indicate the differences among the single varieties according to Tukey’s HSD post hoc test ( $p < 0.05$ ).

Cultivar	Leaf Area (cm)	Shoot Biomass (g)	Stomatal Conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ )
Ferrara	428.7	3.75	138.0 a
Gladiator	415	3.79	113.3 a
Inge	411.6	3.53	120.6 a
Marino	401.6	3.43	197.7 b
Nauta	361.9	3.34	151.5 ab
Strauss	348.6	3.24	136.4 a
<i>p</i> -value	0.383	0.364	0.030

The relationships among the measured traits of the six varieties in responding to the applied dehydration sequence are shown in Figure 8. As expected, a close relationship was identified between the leaf area and shoot dry matter. The difference in the root system among the genotypes was a significant regulator of stomatal conductance, mostly during the stages of progressive drying (cf. Figure 6b). Genotypes with higher leaf area still maintained a high transpiration rate until stress Phase 3, with reduced soil water supply at a moderate VPD. The final stage, which had the most severe stress conditions due to the low water supply with a high plant demand, was mostly controlled by previous water uptake, i.e., varieties with an average high transpiration rate had the lowest stomatal conductance in the final experimental stage.



**Figure 8.** Predominant relationships (according to  $R^2$ ;  $n = 6$ ) between the traits of sugar beet varieties during different stages of stress incidence. (a) Average relationship of leaf area and shoot biomass; (b) influence of root length on stomatal conductance during the onset of stress with an initial high demand and progressive soil supply reduction; (c) leaf area relationship with transpiration rate at low soil water supply (40% PAW) with moderate atmospheric demand (VPD 1 kPa); and (d) average plant water uptake (transpiration rate) influencing stomatal conductance at the end of progressive water limitation. Orange letters are initials of the respective cultivars (F = Ferrara, G = Gladiator, I = Inge, M = Marino, N = Nauta, and S = Strauss).

#### 4. Discussion

The increase in the adaptation of crops to future climatic conditions is currently one of the key challenges in breeding. Understanding the physiological functioning of plants under variable environmental conditions can contribute to a more targeted trait selection and thereby support the breeding of climate-resilient crops [32]. In this study, six sugar beet cultivars were analyzed for their response to progressive drought stress from high atmospheric demand and/or limited soil supply, with a particular focus on root-induced differences in the dynamics of stomatal conductance and transpiration rate.

Generally, drought stress is the result of an imbalance between the evaporative demand of the atmosphere and the water supply from the soil to the plant root system [33]. The dehydration dynamics in this study followed a sequence of initially increased plant demand due to high VPD at sufficient soil supply, followed by supply limitation via reduced soil moisture availability, and finally a condition of severe imbalance between high plant demand and limited soil supply. Thereby, both conditions that can result in a demand–supply imbalance were simulated, as expected to occur in future crop production scenarios in central Europe, i.e., high temperatures and the, consequently, increased transpiration demand with more erratic rainfalls and, thus, limited supply [34]. Considering that, for sugar beet, relevant production areas in Europe are located in vulnerable regions, which are affected by heat as well as water stress, the sustainable raw material supply to the European sugar industry is at risk due to low and/or highly uncertain yields [12].

Overall, the physiological response of plants to water limitation is influenced by the specificity of the drought regime, i.e., intensity, duration, frequency, and timing [35]. In this context, a rhizobox experimental system with a constraint soil (rooting) volume ( $\approx 3000 \text{ cm}^3$ ) was used to simulate a rather extreme environmental situation: with a peak transpiration rate of  $\approx 100 \text{ mL d}^{-1}$  under high atmospheric demand, soil water availability dropped from sufficient (80% PAW;  $\theta = 0.31 \text{ cm}^3 \text{ cm}^{-3}$ ) to limited (40% PAW;  $\theta = 0.21 \text{ cm}^3 \text{ cm}^{-3}$ ) plant supply within three days. Assuming an average summer evapotranspiration rate of  $6 \text{ mm d}^{-1}$  for a central European Pannonian climate [36], the soil water depletion under field conditions over a similar drop in PAW would last between 10 and 14 days, assuming a rooting depth of 100 cm and depending on the specific soil–water retention characteristics.

The regulation of stomatal opening is the most important mechanism through which plants respond to a demand–supply imbalance to prevent metabolic damage [37]. However, the tightness of stomatal regulation to variable conditions along an isohydric–anisohydric continuum is plant-specific [38]. The decrease in sugar beet stomatal conductance measured in this experiment between the maximum values at high water supply and high atmospheric demand and the minimum value at the final stress phase was approximately one order of magnitude. The peak stomatal conductance (mean:  $406 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) was in a similar range to that reported by Ober et al. (2005) [25] for irrigated conditions ( $440 \text{ mmol m}^{-2} \text{ s}^{-1}$ ). The stressed values in our rhizobox setup, however, were substantially lower ( $-75\%$ ) than those in the drought field conditions reported in their study, while being similar to values obtained in a greenhouse drought stress experiment with sugar beet grown in columns ( $40 \text{ mmol m}^{-2} \text{ s}^{-1}$  [26]).

Stomata are the safety valve used by plants to prevent tissue dehydration and metabolic damage and, therefore, have a key function in drought adaptation. Notably, in an agricultural context, maximizing transpiration (and thus maintaining a high stomatal conductance) is the main focus for crop adaptation in water-limited environments due to the tight linkage of transpiration and dry-matter production [12]. The cumulative water uptake of sugar beet measured over the entire experimental duration was, on average, 2004 mL, ranging from 1853 mL (Inge) to 2175 mL (Nauta), with genotypic differences being nonsignificant over an experimental duration of 50 days, i.e., between  $32.4$  and  $42.6 \text{ mL d}^{-1}$ . For comparison, the daily values of sugar beet water consumption reported by Fitters et al. (2017) [39] for their single-plant column experiment in drought-stressed treatments, with an experimental duration of 94 days, were in a similar range ( $41.4 \text{ mL d}^{-1}$ ).

During the early stages of sugar beet development in this experiment (BBCH 17), leaf area was an important driver of both dry-matter accumulation (assimilating surface) as well as transpiration (transpiring surface; Figure 8a,c). However, stomatal and root characteristics were important mediators in this relationship (Figure 8b,d).

Analyzing the temporal interplay of stomatal conductance and transpiration rate can contribute to a better understanding of the water-spending vs. water-conserving dynamics in plant behavior during drought. The results of the cross-correlation analysis applied in this study for identifying these temporal linkages suggested two dynamics under the imposed stress conditions:

(1) Transpiration rates were maintained with a forward lag of a maximum of two days after the response (e.g., decrease or increase) of stomata to changing environmental conditions. The forward lag of the transpiration rate following the direction of stomatal conductance with some temporal delay is likely related to the effect of a concomitant change in the pressure gradient between the soil and leaves that drives the transpiration flux, together with conductance, e.g., a high stomatal conductance reduces leaf water potential and thereby induces a higher flux-driving gradient that, in the short term, compensates for the reduced conductance [33].

(2) Preceding water consumption inversely impacted subsequent stomatal behavior (i.e., higher depletion via transpiration lowering stomatal conductance and vice versa) with the time lag showing significant negative correlations between 3 and 7 days, pointing to the transpiration behavior during the previous phase(s) as a relevant driver of subsequent stomatal conductance. Withholding irrigation in pot experiments with a constrained rooting volume, as imposed in this experiment, leads to a comparatively quick depletion of soil moisture. Therefore, genotypes with more conservative use can save available plant water for subsequent stages, and the quickly depleting moisture results in a subsequently lower stomatal conductance to prevent dehydration damage [40].

The root system constitutes the key interface between the soil water supply and plant demand; therefore, the system is highly important for understanding crop stress dynamics. Dehydration avoidance, and, thus, sustained stomatal opening (screened, e.g., via leaf temperature or carbon isotope discrimination in field breeding trials), is strongly mediated via the effective root depletion of available water resources [41,42]. Sugar beet is considered a comparatively drought-tolerant species due to its ability to deeply root, thereby buffering severe drought conditions via the efficient exploitation of subsoil water [43]. Our results indicated that the observed stomatal conductance was significantly related to root characteristics. Specifically, the correlation pattern over soil depth suggested that the relevant root zone influencing stomatal behavior extended mostly between 30 and 90 cm in depth, with more intense rooting sustaining a higher stomatal conductance during the initial stages of drought. The active root zone, with relevance for sugar beet water uptake, is likely determined by two factors: root length allocation and soil water distribution over depth. Whereas some studies suggested the predominant water uptake of sugar beet from the top 30 cm of soil [19], other studies have indicated that under stress conditions and after younger deep-reaching roots have developed an active secondary xylem, subsoil resources essentially contribute to sugar beet water supply [39]. Additionally, our results point to the importance of root axes below 30 cm, extending toward deeper soil layers, to postpone stomatal closure under the impact of progressing water limitation.

From a breeding background, this linkage between root distribution and stomatal conductance qualifies as a drought-avoidance mechanism that could be targeted for increased sugar beet adaptation to drought environments. Importantly, our results demonstrated the substantial differences in the root characteristics among the investigated sugar beet genotypes (Figure 7). Overall, the average visible root length was  $2934 \pm 889$  cm (root length density:  $0.98 \pm 0.30$  cm cm<sup>-3</sup>), with the most intense rooting identified for Marino and the lowest values for Inge. The root length density of column-grown single sugar beet plants reported by Fitters et al. (2017) [39] was in a similar range ( $0.93$  cm cm<sup>-3</sup>). The depth

distribution revealed that most of the root length was allocated to the central root zone (40–80 cm depth), with less intense rooting in the topsoil and toward the bottom of the rhizoboxes. Similar root distribution patterns, with a peak length density in the central root zone, were described under field conditions [43]. In the region with the highest root allocation, the genotypic differences were also the most evident (statistically significant), mainly between Marino and Ferrara vs. Inge. Furthermore, Marino also significantly differed from Strauss in the lowest depth (90–100 cm).

More vigorous root systems were also found to contribute to a distinct resistance to beet cyst nematode (*Heterodera schachtii*). Although Inge (low resistance) vs. Marino (high resistance), which had the most distinctive differences in root length, also differ in their susceptibility to nematode root infestation, this rooting distinction (short vs. long roots), in line with the reported nematode resistance score, was not consistent with the other genotypes investigated here. Moreover, Wright et al. (2018) [27] did not find a clear relationship between root traits and nematode resistance. The authors, however, pointed to the actual nematode pressure as a relevant experimental background to clarify a potential root–nematode resistance relationship.

Concerning the response to successive drought stress, however, the rooting pattern constituted a key explanatory trait. Particularly, the delayed stomatal closure during the transition to soil water supply limitation (80% to 40% PAW) was sustained by more intense rooting (Figure 8b). Thus, the differences in root system characteristics were the essential morphological drivers of the observed stomatal behavior, with stomatal conductance being both a sensitive regulator of plant water status and a distinctive trait/function among the investigated genotypes. Other researchers have identified stomatal conductance [25] as well as carbon isotope discrimination (a time-integrated measure for stomatal opening [44]) as essential traits differing between sugar beet cultivars, thus qualifying for drought resistance selection. Other authors, however, using carbon isotope discrimination, only identified a general drought effect without any major distinction among genotypes [45,46].

Our study highlights that we must consider the differences in rooting patterns in order to explain differences in stomatal dynamics with relevance to stress resistance, such as the temporary buffering of the impact of water shortages expressed in sustained stomatal opening. Similar to other drought-resistance traits, desired root traits are also site-specific: the importance of deep rooting in sugar beet drought response that was found here is particularly relevant for the pedoclimatic conditions of deep soils with high water retention capacity, where available subsoil moisture strongly contributes to buffering intermittent summer drought periods [17].

Considering this environmental context and based on the identified statistically significant trait differences among the genotypes, our study suggests the root–stomata linkage as the most promising selection target. Other measured parameters, including aboveground dry matter, leaf area, and transpiration rate, did not show major variation in drought response. Generally, determining the statistical significance in single-plant experiments is challenging, although, in this study, similar-sized and vigorous seeds were selected to establish homogeneous plants, and might require a high number of replicates; however, this option was constrained by the number of available experimental units of the root phenotyping platform. Other authors did not find major genotypic differences in the physiological response patterns among sugar beet cultivars and their adaptation to water deficit [45,47]. This might point to an insufficient specific selection for drought-tolerance traits in sugar beet and, thus, comparatively similar physiological behavior within the current germplasm [26].

Our overall findings underline that distinctive rooting patterns qualify as essential drivers of a potential anisohydric behavior (the high fluctuation in leaf water potential with sustained stomatal opening in response to soil drying [48]) previously reported in sugar beet [23]. Therefore, enhancing the rooting vigor, either by breeding improvements and/or soil management measures, is essential (particularly for soils with a high water retention capacity such as chernozems) to foster the dehydration avoidance potential

of sugar beet and thereby better buffer the effects of intermittent drought periods that are expected to occur more frequently with climate change in major European sugar beet growing regions [49]. Future research should, therefore, focus on improving our current root phenotyping capabilities for the larger-scale screening of crop germplasm resources as well as integrating aboveground–belowground phenotyping platforms to better assess the linkages between rooting patterns and physiological responses, such as stomatal conductance, at the shoot level.

**Author Contributions:** Conceptualization, G.B.; formal analysis and data curation, M.A. and G.B.; writing—original draft preparation, G.B.; writing—review and editing, M.A. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was funded by the Federal Ministry of Agriculture, Forestry, Regions and Water Management (BML), grant number 101439.

**Data Availability Statement:** The data are available from the corresponding author on reasonable request.

**Acknowledgments:** We acknowledge the support of Herbert Eigner and Stefan Geyer from the AGRANA Research and Innovation Center (ARIC) for the donation of seeds and their valuable contributions in the contextualization of the results into the general situation of sugar beet production.

**Conflicts of Interest:** The authors declare no conflict of interest.

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