



Monitoring Drought Tolerance Mechanisms of Sorghum and Maize Under Unevenly Distributed Precipitation

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Abstract

The study examines the morpho-physiological differences in leaf characteristics between two species of C4 plants: sorghum and maize. The research was conducted in field conditions where plants were rainfed. Both species different varieties were cultivated at two distinct sites that exhibited variations in soil texture and drought stress incidence according to the vegetation condition index (VCI). Samples were collected during various growth stages to analyze the relative water content (RWC), proline levels, and stomatal density. Sorghum plants displayed higher RWC, proline levels, and stomatal density than maize plants. In sorghum, the biochemical traits, such as the proline content, may play a more critical role in withstanding water-limited conditions than in maize in our experiment. Under the same water restriction period, sorghum showed higher RWC levels. Sorghum plants reduced stomatal density under more water-limited conditions, which proves its plasticity. Additionally, early maturation played a crucial role in both species. The early sorghum variety KWS Kallisto and maize variety Walterinio KWS had a more stable yield at both sites. Nevertheless, the highest yields were found in the later varieties, KWS Hannibal and KWS Inteligens. The higher proline levels and the relative water content are drought-tolerant mechanisms and may be used to indicate drought intensity in field conditions. Our findings spotlight the influence of genetic diversity and genotype-environment interactions in determining crop responses to drought stress, providing valuable information for future breeding programs to enhance drought tolerance in crops.

Keywords Water limitation · VCI · Rain distribution · Maize · Sorghum · RWC · Proline · Stomatal density

Introduction

Drought, abiotic stress, holds significant importance due to its adverse impact on plant physiology, biochemical processes (Per et al., 2017), and substantial anatomical alterations. Additionally, drought influences growth (Daryanto, et al., 2016), enzyme functionality, stomatal morphology (He et al., 2020), stomatal closure (Marček et al., 2019), photosynthesis, carbon fixation (Liu et al., 2015), and

relative water content (RWC) (Marček et al., 2019). As a result, drought poses a threat to agricultural production on a global scale, particularly in the face of current climate change conditions (Girvetz et al., 2009; Romm, 2015; Shrestha, et al., 2012; Xu, et al. 2016), which are projected to amplify the frequency and severity of drought occurrences (Středa et al., 2019).

In the Czech Republic, where the experiment was conducted (the South Moravian Region), soil moisture content during the growing season has been decreasing over the past six decades, and drought events have become significantly more frequent (Meitner, et al., 2023). More extended periods of drought alternate with one-off high rainfall totals. While it is impossible to precisely predict the timing of drought exposure for plants in field conditions, patterns in seasonal drought occurrence can be identified. Based on these patterns, farmers can make informed decisions regarding using early- or late-maturing varieties of their chosen crop species,

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taking advantage of drought escape. Therefore, to maintain the yield of maize and sorghum in this area, it is necessary to study drought tolerance under field conditions, and isolating the specific effects of various environmental factors and stresses on plant growth, yield quantity, and quality can be challenging. However, investigating drought tolerance adaptations in natural conditions can provide insights into natural selection processes and aid in identifying new stress tolerance mechanisms (Kooyers, 2015).

Understanding plant responses to drought involves a spectrum of morpho-physiological, biochemical, cellular, and molecular processes. These responses involve enhancements in root systems, leaf structures, osmotic adjustment, RWC, and stomatal regulation (Ilyas et al., 2021). Moreover, the differing reactions of distinct plant species to drought become apparent in their adaptive strategies. For instance, certain plant varieties employ drought escape as a mechanism to mitigate stress impact by completing their life cycles earlier, thereby avoiding the onset of drought (Fukai & Cooper, 1995; Taiz & Zeiger, 2010), which is particularly beneficial when late summer drought occurs (Bowers, 1995) in temperate climate zones.

Drought can lead to desiccation in susceptible cells, depleting compatible solutes like proline and sugars. The accumulation of proline, an osmolyte and amino acid, is one of the various metabolic responses of plants to salt and drought stress. Proline biosynthesis and subsequent signaling contribute to maintaining redox balance in both standard and stressful environments (Hayat et al., 2012; Per et al., 2017), and genes involved in proline metabolism are upregulated during water deficit (Badigannavar et al., 2018). The precise role of proline in stress tolerance remains a subject of debate. However, it is believed that, to stabilise subcellular structures, scavenge free radicals, buffering cellular redox potential, and initiate gene expression. Proline also exhibits cross-tolerance to different types of stress (Kaur & Asthir, 2015). Research indicates a correlation between heightened proline accumulation induced by stress conditions and intensified growth impediment, potentially serving as an indicative factor to eliminate the most vulnerable varieties (Arteaga et al., 2020; Fariaszewska et al., 2020).

Stomatal density may also play a crucial role in enhancing drought tolerance. Plants with fewer stomata tend to exhibit greater drought tolerance (Kusvuran et al., 2010) and are more efficient in water utilization (Caine et al., 2019). However, the relationship between stomatal density and drought response can vary among plant species. Contrary to certain findings, soybean plants have shown that higher stomatal density may enhance water supply by elevating stomatal conductance, particularly on the upper leaf surface, enhancing transpiration even during water stress (Buttery et al., 1993). Similarly, while wheat plants exhibit increased stomatal density under drought conditions, some

studies have reported contrasting observations (Öztürk & Korkut, 2018). Some investigations (Liao et al., 2005) have demonstrated that wheat plants with lower stomatal density showed improved water use efficiency, highlighting the complexity of the relationship. The density and size of stomata, along with guard cells, have been linked to water use efficiency in plants (Dunn et al., 2019; Xu & Zhou, 2008). Consequently, the response of stomatal density to drought stress is not solely determined by the plant species but is also influenced by the specific genetic makeup or genotype (Mansouri et al., 2016).

In this context, the hypothesis arises that plant species with varying origins and evolutionary adaptations might exhibit diverse responses to drought stress. Specifically, research indicates that maize, generally considered less drought-tolerant than sorghum (Hasan et al., 2017; Queiroz et al., 2019), could display dissimilar reactions under water stress compared to sorghum due to their inherent differences in drought tolerance mechanisms. Previous studies have highlighted variations in growth inhibition, gas exchange, and water use efficiency between maize and sorghum under drought conditions (Hasan et al., 2017). Sorghum, originating from Africa and adept at surviving dry seasons and high temperatures (Beyene et al., 2015), often demonstrates diverse responses and sensitivity levels among its genotypes (Abraha et al., 2015). These distinctions in maize and sorghum responses to water stress underscore the need for a comparative analysis of their reactions under drought conditions.

The study aims to fill the crucial gaps in understanding of how maize and sorghum respond differentially to agricultural drought, and evaluate the suitability and effectiveness of their cultivation by analyzing morpho-physiological traits such as proline levels, RWC, and stomatal density. The study is concurrently assessing the aboveground biomass yield of selected varieties across various sites differing primarily in soil texture and moisture content. The presented article describes the experimental plant material and the methods used, evaluates the course of the weather at the experimental locations, and presents the results of individual morpho-physiological properties and yield of maize and sorghum varieties. Such an investigation furthers our understanding of plant stress responses and holds potential implications for agricultural practices and breeding programs targeting drought resilience.

Materials and Methods

Plant Material

For this experiment, ten temperate sorghum varieties and ten temperate maize varieties were chosen, as outlined in

Table 1. The selection of varieties for both species was primarily based on their earliness, which served as the main criterion in the decision-making process. The categorization of earliness was determined based on information provided by the seed supplier. Among the sorghum varieties, the majority consisted of *Sorghum bicolor* (L. Moench) and *Sorghum × drummondii* (Nees ex. Steud.) hybrids, many of which are commonly cultivated in temperate climates for silage production due to their higher aboveground biomass. In the case of maize, earliness was characterized by the FAO number, which corresponds to the moisture content of the grain. The early maize varieties were identified by having the lowest FAO number.

Field Conditions

The plants were grown at four sites (1, 2, 3a and 3b), but each species was grown at only two different sites. These sites are located close to the South Moravian Region, Czech Republic (49.023N, 16.618E), and are classified as climate class Dfb. The average temperature for this location in which the sites are located is 10.3 °C with a precipitation sum of 491.1 mm per year (Orság et al., 2022). At the beginning of the experiment, sites 3a and 3b were considered more drought-stressed due to higher sand particle content (for more information, see **Supplementary Information**). A meteorological station located close to sites 1 and 2 recorded precipitation with a Met One 370 rain gauge (Met One Instruments, USA) and

temperature at the height of 2 m above ground with a Vaisala HMP155A Merici sensor (Vaisala, Finland).

Because the month's total precipitation creates a worse depiction of rainfall distribution, the conventional graphical representation of the quantity of monthly precipitation distribution (Fig. 1) leads to a poorer interpretation. As a result, we have developed a straightforward measure noted as the Rain Distribution Scale (RDS). The scale is based on monthly rainfall measurements expressed in millimetres, which are then converted into dimensionless values (Table 2). For instance, 1.2 mm of rain in one day is worth 2 on the scale.

This scale is based on the precipitation pattern in the given climatic conditions. The scale values for each day are then entered into the radar chart. The numbers on the outer line represent the day number. An average monthly scale value can be calculated from the sum of the monthly values of the scale, which also characterizes the given month.

The Vegetation Condition Index (VCI), derived from remote-sensing data (Jiao et al., 2016), effectively differentiates varying levels of water availability across diverse sites. Vegetation indices, such as the Normalized Difference Vegetation Index (NDVI), are extensively employed in agriculture due to their capacity to monitor changes in crop biomass and health (Becker-Reshef, et al., 2010; Dorigo, et al., 2007). These indices also serve in studying the impacts of drought (Liang et al., 2017; Liu & Kogan, 2007; Sholihah et al., 2016; Zambrano et al., 2016), exhibiting strong

Table 1 Basic characteristics of the chosen sorghum and maize varieties

Sorghum varieties	Earliness	<i>S. bicolor</i> x <i>Sorghum × drummondii</i> hybrid	EU country of admission	Maize varieties	FAO number (silage)	Earliness	EU country of admission
KWS Sole	Very Early	Yes	Portugal	Alombo	240	Early	Italy
Ruzrok	Early	No	Czech Republic	Celong	250	Early	Slovakia
Nutri Honey	Early	Yes	Spain	KWS Kidemos	270	Early	Czech Republic, France, Poland
KWS Kallisto	Early	Yes	Portugal	Walterinio KWS	280	Semi-early	Czech Republic, Germany
Latte	Early/Semi-early	Yes	Italy	KWS Koletis	290	Semi-early	Czech Republic, France
KWS Tarzan	Semi-early	No	Czech Republic, Germany, Hungary, Portugal	SY Ignis	320	Intermediate	Italy, Hungary
KWS Titus	Intermediate	Unknown*	Portugal	SY Solandri	360	Intermediate	Italy
KWS Hannibal	Intermediate	Unknown*	Italy, Portugal	SY Orpheus	370	Intermediate	France, Italy, Hungary, Slovakia
Big Kahuna	Late	Yes	France, Italy	SY Infinite	380	Intermediate	France, Italy
KWS Bulldozer	Late	Yes	Portugal	KWS Inteligens	390	Intermediate	Czech Republic, France, Italy, Slovakia

*More information at the seed provider

Fig. 1 Precipitation and air temperature at the studied sites during the growing season in 2022

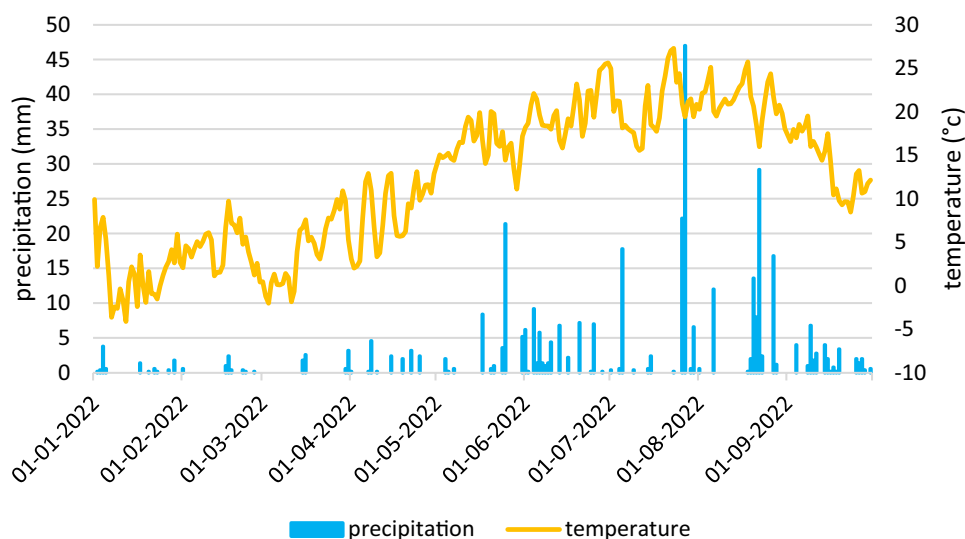


Table 2 Rain distribution scale to help interpret the monthly rainfall values

Recorded precipitation (mm)	Rain distribution scale
0–0.9	1
1–5	2
5.1–5.9	2.5
6–10	3
10.1–10.9	3.5
11 <	4

correlations with other drought indices such as the Palmer Drought Severity Index. This correlation underscores the NDVI's utility in effectively monitoring drought conditions (Jiao et al., 2016).

The vegetation condition index (VCI) was calculated using the geographic information system software ArcGIS (Esri, USA) to determine the per cent drought area during the chosen dates at the sites in 2022. Images recorded by a drone during the growing season of 2022 were used to calculate the VCI. First, the normalized difference vegetation index (NDVI) was calculated, and the VCI was then calculated as the per cent area of drought occurrence, as follows (Faridatul & Ahmed, 2020):

$$VCI = 100 * (NDVI_i - NDVI_{min}) / (NDVI_{max} - NDVI_{min})$$

$NDVI_i$ is the NDVI value for a specific pixel in the month, and $NDVI_{max}$ and $NDVI_{min}$ are the same pixel's maximal and minimal NDVI values for similar periods.

Sites 3a and 3b are at the exact location but consist of two fields with different crop rotations. The dimensions of the plots were 14.0 × 2.7 m for sorghum and 7.0 × 3.0 m for maize. The plants of each variety were grown in randomised

plots in two repetitions. The sowing depth was 0.08 m for maize with a row spacing of 0.75 m, and for sorghum, the sowing depth was 0.03 m with a row spacing of 0.45 m. The maize plants were fertilized with urea at a rate of 140 kg nitrogen/hectare at both sites in spring, two weeks before the sowing date; sorghum was not fertilized in the given field conditions, as it is not a practice and can grow well without fertilizers. All plants were solely rainfed. The preceding crop species were grain maize at sites 1 and 2, winter barley at site 3a, and spring poppy at site 3b. For details about soil chemical composition, see **Supplementary Information**.

Uneven Rain Distribution and The Vegetation Condition Index

The following charts (Fig. 2) graphically represent rain distribution in the growing season of 2022. According to the sum of precipitation for the given month, July seems to be the month with enough precipitation; however, the sum number of precipitation is affected by the storms and a few heavy showers of rain, and between these extremes of heavy rains, there were other extremes such as no rain and high temperatures. Therefore, the created scale (Rain Distribution Scale) gives us a sum of scale values of 1.4, which is lower than in June, in which the sum of month precipitation is lower, but the distribution is higher than in July.

The spring months, April and May, were the most drought-stressed. Meanwhile, the summer months of June, July and August had higher rain precipitation and scale values. However, it is mainly due to summer storms and heavy rains accompanying these storms. The spring months' precipitation mainly affects vegetative growth; meanwhile, the summer and autumn months affect reproductive stages such as apex development, flower and seed set, and the quality of seeds (maturation).

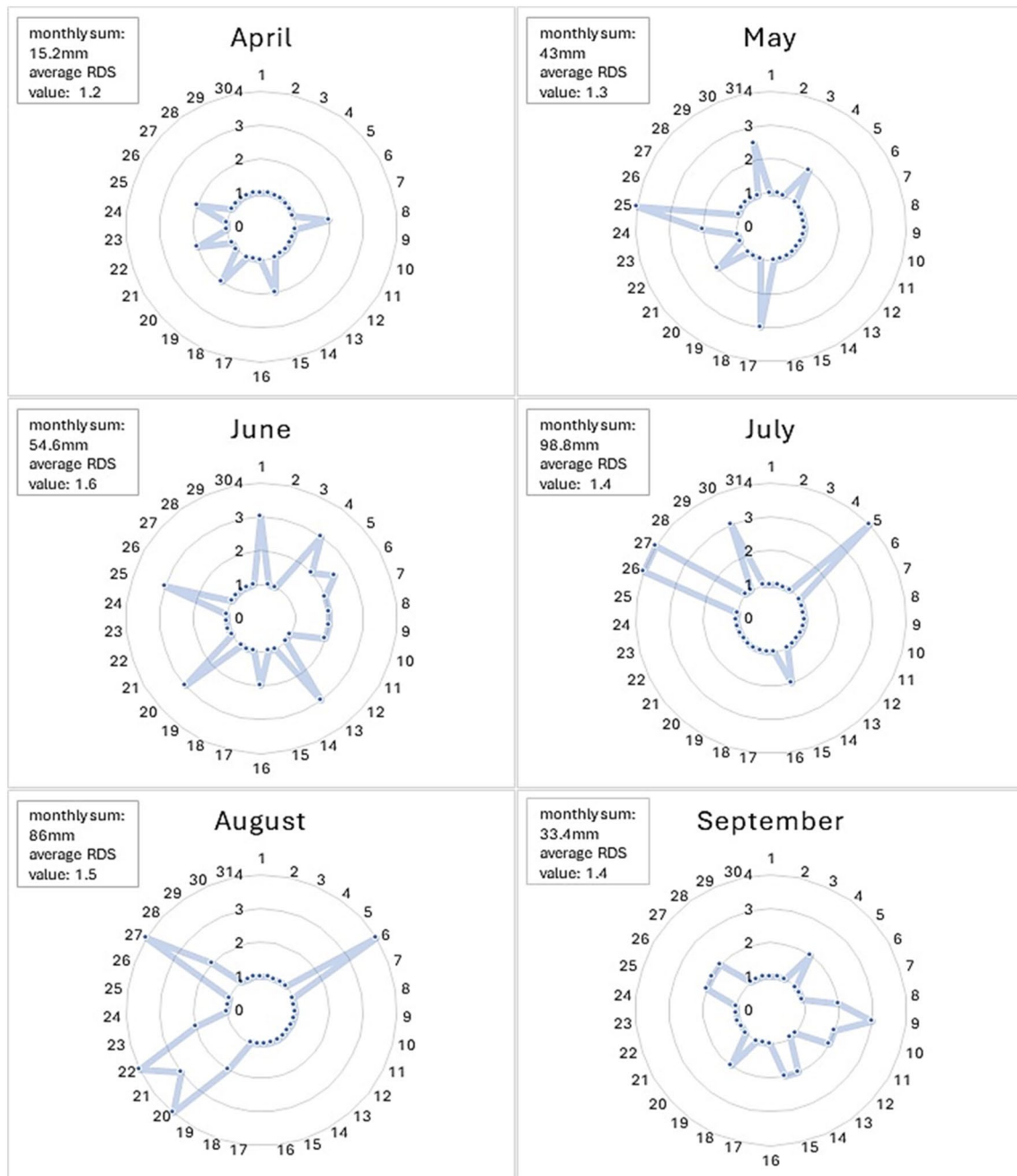


Fig. 2 Graphical representation of monthly rainfall distribution

Even though the selected sites were located relatively close to each other, the different soil structures and the different vegetation periods of the two investigated species affected the incidence of drought at these sites, which was calculated from the NDVI (Table 3). The NDVI results were graphically visualized (as shown in Fig. 3) and used to calculate the VCI (Table 4). The highest drought percentage incidence was recorded in May when the extreme values were recorded at site 1 (98.6% of extreme drought).

Aboveground Biomass Harvest and Other Observed Traits

The plants of sorghum and maize were harvested by hand in three replications at different times according to their dry matter content of aboveground biomass (in sorghum, about 28%; in maize, about 33%), which were monitored by continuous sampling. The plants were cut about 0.01 m above the ground. The size of the harvested area was for sorghum

Table 3 VCI values of the three study sites during the study period (numbers are rounded to two decimal places)

Date	Drought	Site 1	Site 2	Sites 3a and 3b
		Percentage		
03/05/2022	Extreme	98.56	87.92	94.68
	Severe	1.28	1.36	4.63
	Moderate	0.05	2.19	0.52
	No Drought	0.02	2.15	0.07
	Wet	0.08	6.38	0.09
15/06/2022	Extreme	63.54	13.57	42.28
	Severe	34.71	1.61	5.10
	Moderate	1.27	2.72	8.34
	No Drought	0.14	3.34	9.61
	Wet	0.31	78.76	34.70
30/08/2022	Extreme	0.04	1.69	0.00
	Severe	0.00	0.35	0.00
	Moderate	0.00	0.78	0.00
	No Drought	0.00	1.56	0.03
	Wet	99.97	95.31	99.97

(5.0×0.45 m) and maize (5.0×0.75 m). The harvested biomass was weighed, subsequently crushed and homogenised. This material was dried in two stages in an oven (BMT Venticell ECO, Czech Republic). In the first stage, the drying period was 24 h under 65 °C; in the second stage, the period was 4 h under 105 °C. Finally, the harvest of dry above-ground biomass was calculated for tonnes per hectare.

The sorghum height was also measured as the differences between the sorghum varieties were significantly greater than in maize. The height was measured before the harvest in three replications.

Proline Content Determination

The samples used for proline determination were collected at the times indicated in Table 4. In all cases, two replicates of approximately 1×5 cm leaf parts were cut from the middle part of the second oldest (second-to-bottom leaf) and second youngest (second-to-top leaf) leaves of two plants. The samples were collected in the field and transported to the laboratory in a portable cool

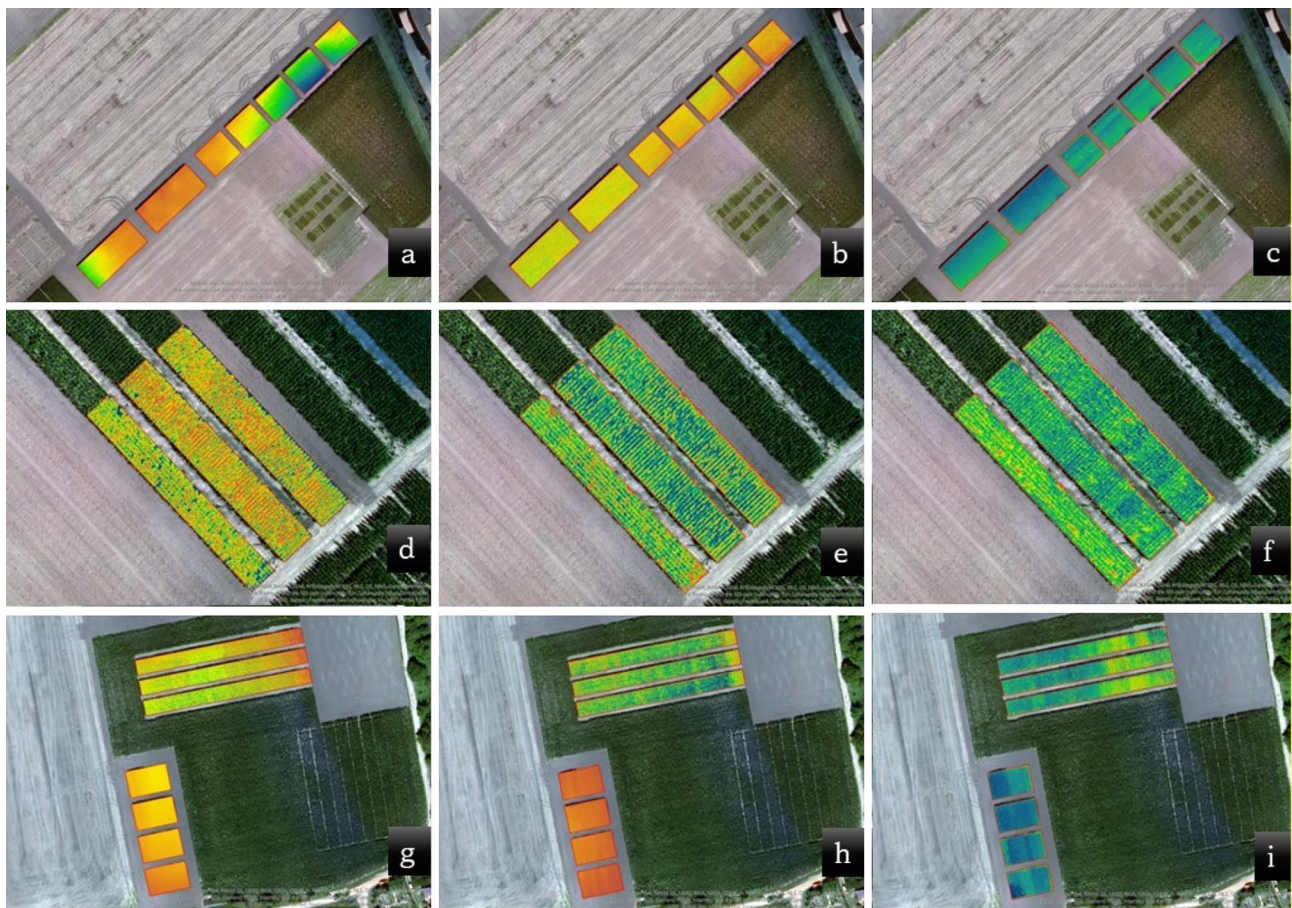
**Fig. 3** NDVI visualized at each site (a–c, site 1; d–f, site 2; and g–i, sites 3a (in the left corner) and 3b (at the top))

Table 4 Sampling dates and growth stages of sorghum and maize plants

Date	Days of vegetation	Site	Species	Growth stage (BBCH)	Analysis
May 31	36	2	Maize	16	Proline Determination RWC* Stomatal density
June 16	56	3b	Maize	32	Proline Determination RWC*
June 30	66	2	Maize	40	Stomatal density
	32	1, 3a	Sorghum	18	Proline Determination RWC* Stomatal density
July 18	84, 88	2, 3b	Maize	70	Proline Determination RWC*
	50	1, 3a	Sorghum	39	Proline Determination RWC*
August 10	107, 111	2, 3b	Maize	85	RWC* Stomatal density
	73	1, 3a	Sorghum	50 for later varieties 65 for KWS Sole, Nutri Honey, and Ruzrok, respectively	RWC* Stomatal density

RWC relative water content

box where the temperature was below 10° C. They were then stored at -80 °C in the laboratory until analysis.

With slight modifications, the samples were prepared for spectrophotometric determination (Magné & Larher, 1992). First, the extraction medium was prepared with 1% ninhydrin solution (prepared from ≥ 99% pure solid ninhydrin, Roth, Germany) dissolved in a 60:40 (v/v) glacial acid: water solution (99% pure glacial acid was diluted to 60%, Roth, Germany; and deionized water was used for the solution). Standards for proline spectrophotometric determination were prepared to obtain a gradient from 0.04 mM to 1 mM proline (L-proline, 98,5% pure, Roth, Germany; was dissolved in the extraction medium); the calibration set underwent the same procedure as did the samples from the field (excluding the steps of freezing and weighing). The field samples were taken from the freezer, weighed (average weight 100 mg), and ground in liquid nitrogen using a mortar and pestle. After the nitrogen had evaporated, the samples were transferred with a spatula to individual tubes, to which 1.5 ml of ninhydrin solution was added. The tubes were shaken by hand for 10 s and placed into a boiling water bath for 1 h. Then, the tubes were cooled to room temperature by placing them on ice packs; after cooling, 1.5 ml of toluene (99%; Lach-Ner, Czech Republic) was added for chromophore extraction. The tubes were vigorously shaken by hand for 15 s, then the phases were separated, and the absorbance

of the stable upper phase was measured at 520 nm after 24 h; toluene served as a blank sample.

Leaf RWC Determination

Leaf RWC is the percentage water content at the sampling time relative to the water content at full turgor (Alam et al., 2020). One prerequisite before each sampling time was the absence of rain for at least 3–4 days. As mentioned in Proline Content Determination, the samples of the same size were again collected at times indicated in Table 4 and placed into plastic tubes sealed with lids. The field samples were transported in the cool box to the field station as soon as possible, where all the samples were individually weighed (fresh weight). The leaves were subsequently cut into approximately 1 × 1 cm pieces and returned to the tubes, to which water was added until all parts of each sample were fully submerged. After 3 h, the water was poured from the tubes, and the samples were dried using filter paper, weighed (saturated weight), and then dried in an oven at 103 °C for 2 h (the drying time and temperatures were modified). After that, the samples were weighed again (dry weight), and the RWC was determined (González & González-Vilar, 2001).

$$RWC = \frac{\text{fresh weight} - \text{dry weight}}{\text{saturated weight} - \text{dry weight}} \times 100$$

Stomatal Density Determination

The samples for stomatal density determination were taken, as seen in Table 4. In the field, transparent nail polish was applied to the third youngest leaf, in the middle of the leaf on both the upper and the lower sides. After approximately 1 min, transparent adhesive tape was pressed onto the dry polish so that the polish adhered well to the tape. Then, the tape with the polish imprint on the leaf surface was peeled off and adhered to a glass slide. The stomatal density was then determined by light

microscopy. The number of stomata was counted within an area of 1 × 1 mm at 200 × magnification. Two replicates for each sample were included.

Statistical Analyses

Statistical analyses were performed in XLSTAT (Lumivero, USA), an MS Excel add-on software program. Differences between the results obtained for the two species, the different varieties and the different sites were analysed with the nonparametric Kruskal–Wallis test,

Fig. 4 Graphical representation of average relative water content (RWC) and proline content in sorghum under field conditions (100 mg of fresh leaf weight, for better clarity in the graph, the names of varieties have been shortened)

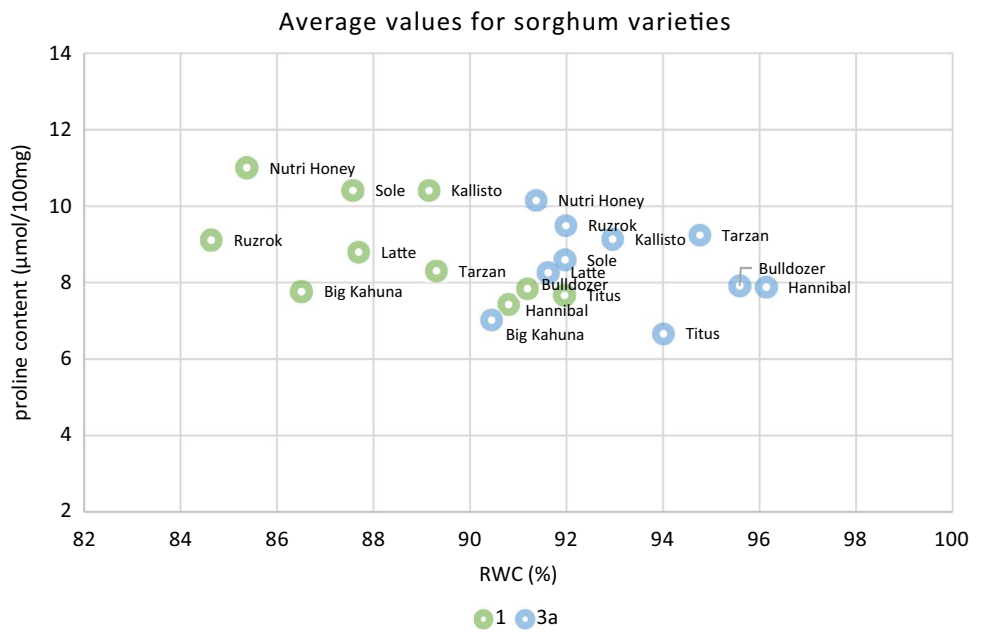


Fig. 5 Graphical representation of average relative water content (RWC) and proline content in maize under field conditions (for better clarity in the graph, the names of varieties have been shortened)

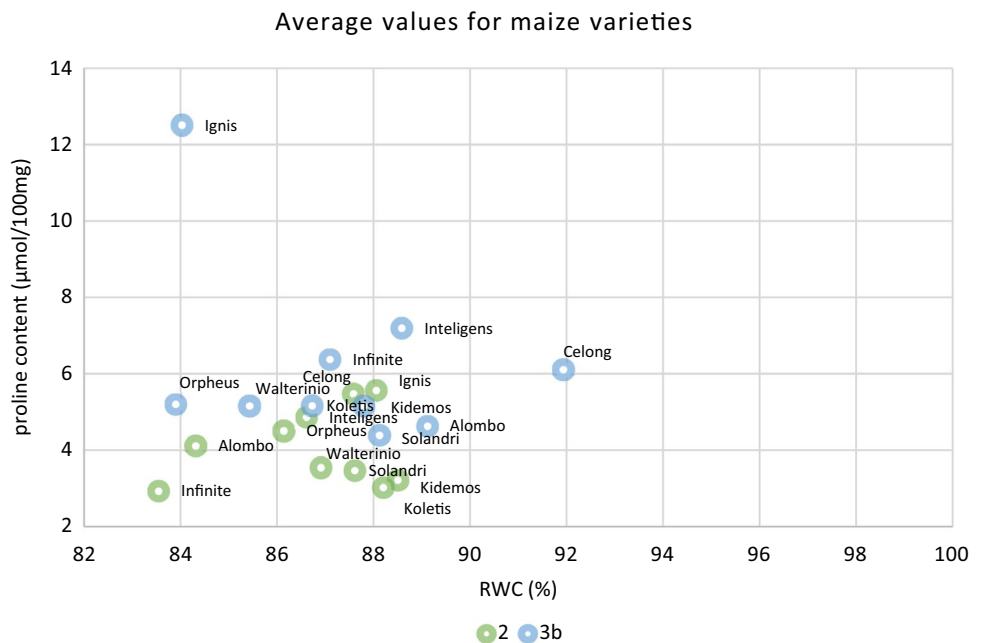


Fig. 6 Average values of RWC for each variety and site (error bars represent standard deviation values)

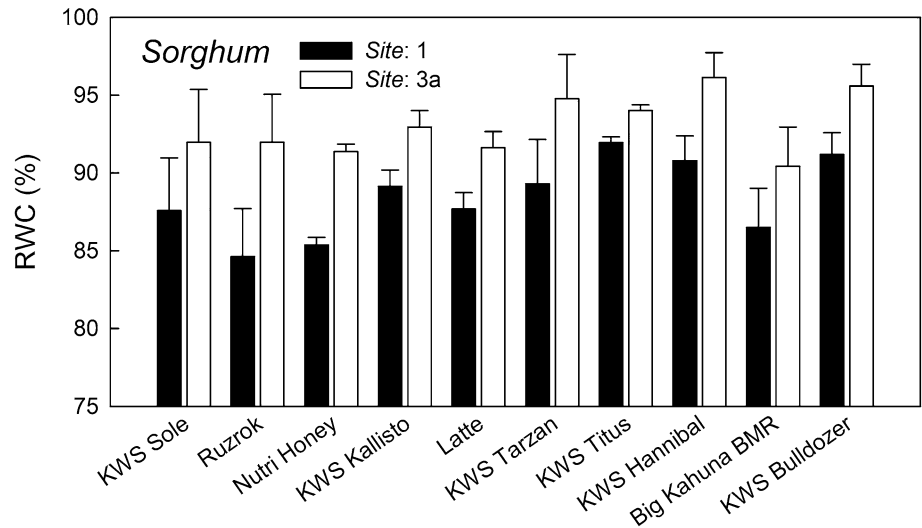


Fig. 7 Average values of RWC for each variety and site (error bars represent standard deviation values)

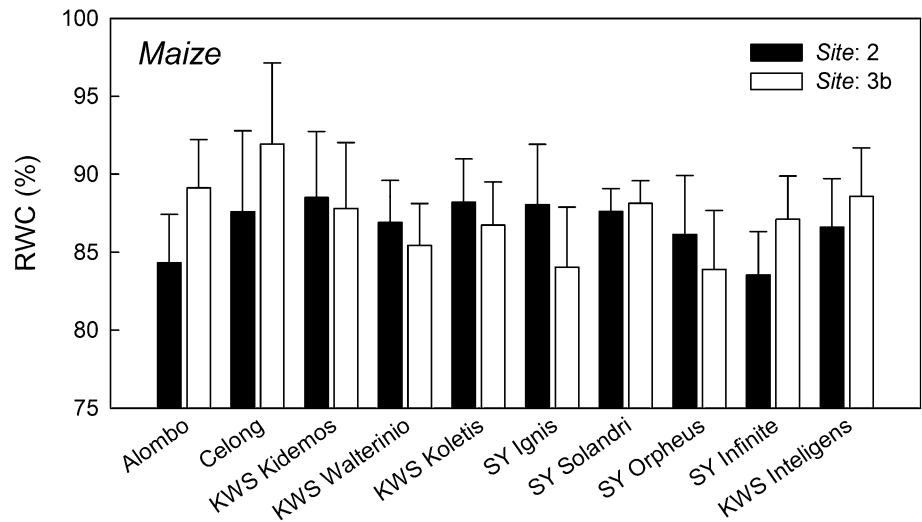


Fig. 8 Average values of proline content for each variety and site (error bars represent standard deviation values)

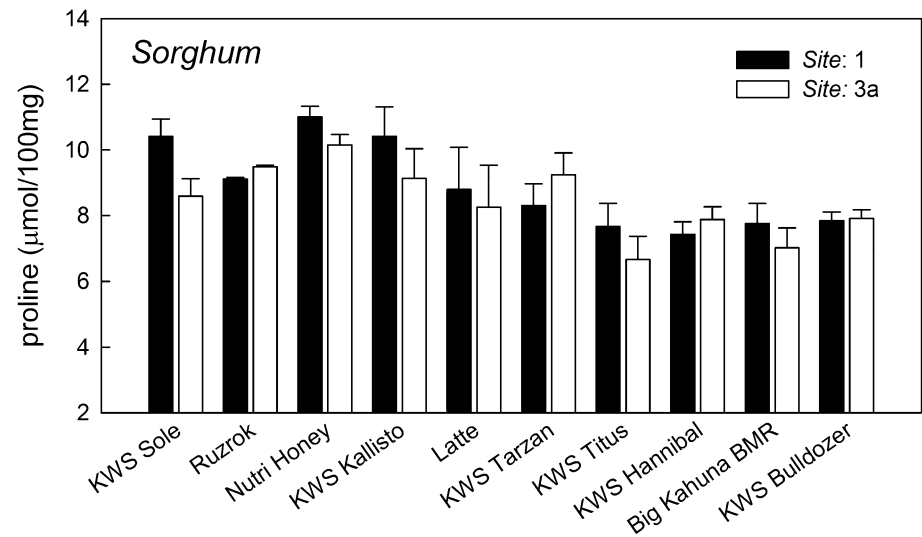


Fig. 9 Average values of proline content for each variety and site (error bars represent standard deviation values)

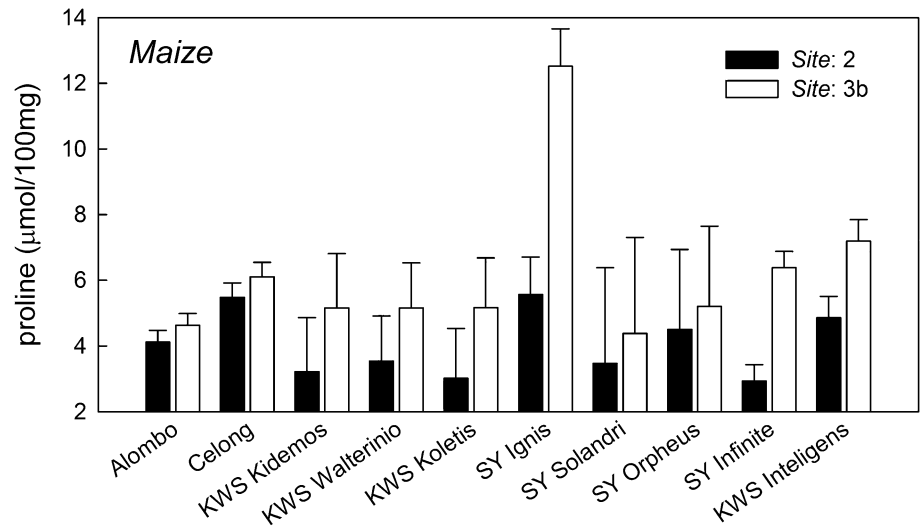


Fig. 10 Average values of stomatal density for each variety and site (error bars represent standard deviation values)

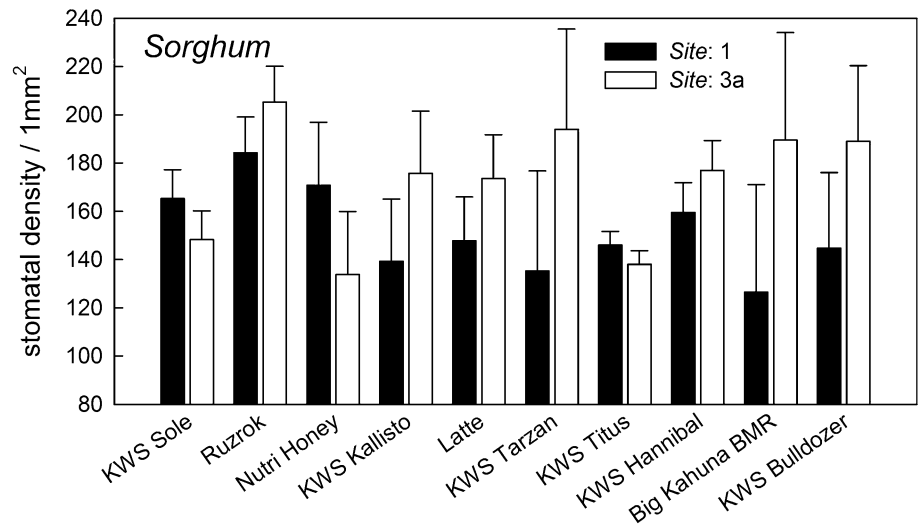
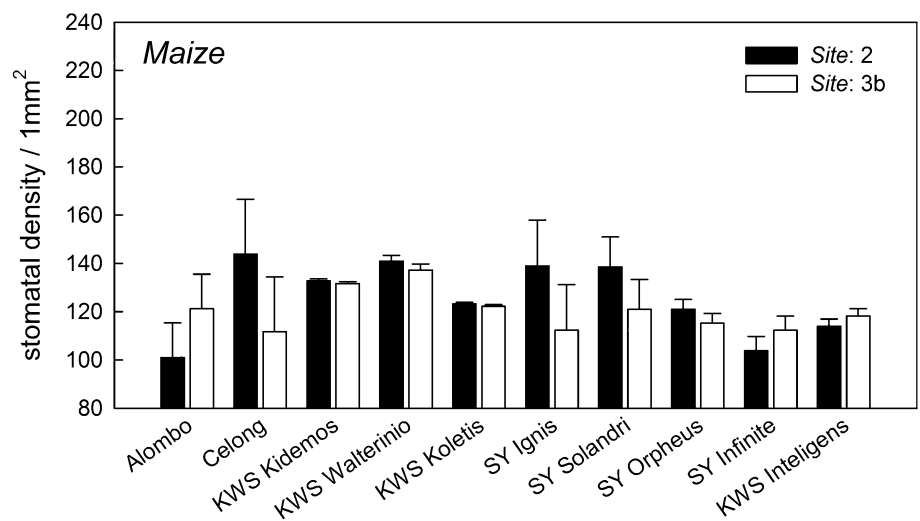


Fig. 11 Average values of stomatal density for each variety and site (error bars represent standard deviation values)



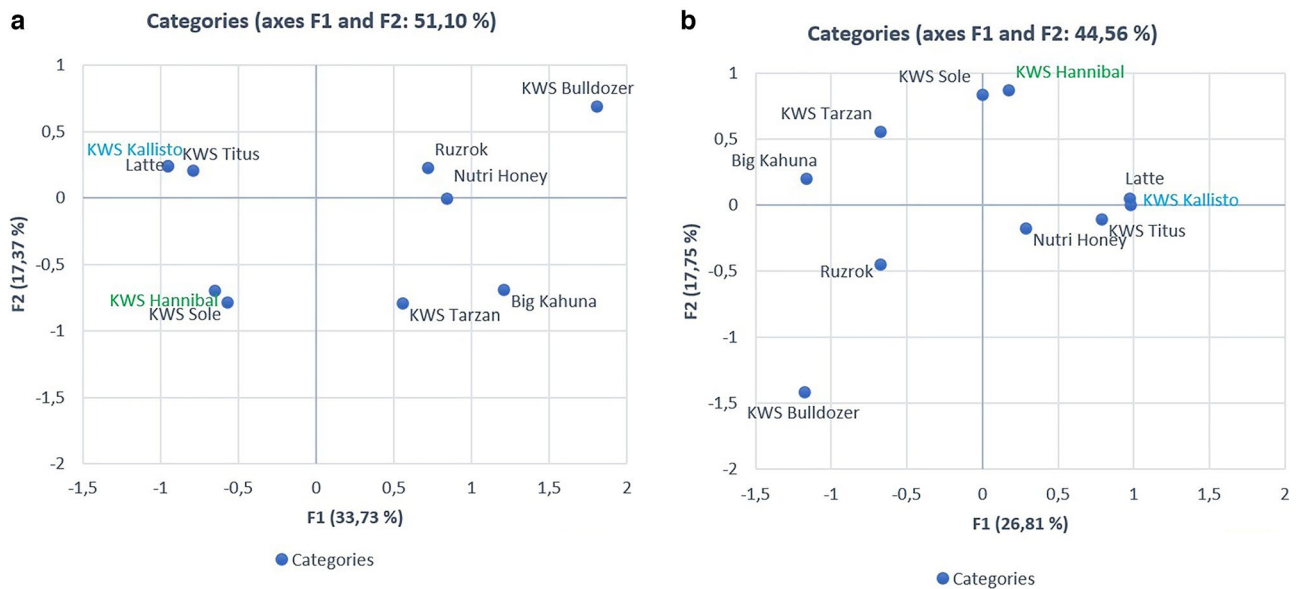


Fig. 12 Graphical representation of PCA analysis for sorghum at two sites (site 1 as a, site 3a as b): the variety with stable yield is marked lightly blue, the variety with highest yield is marked as turquoise green

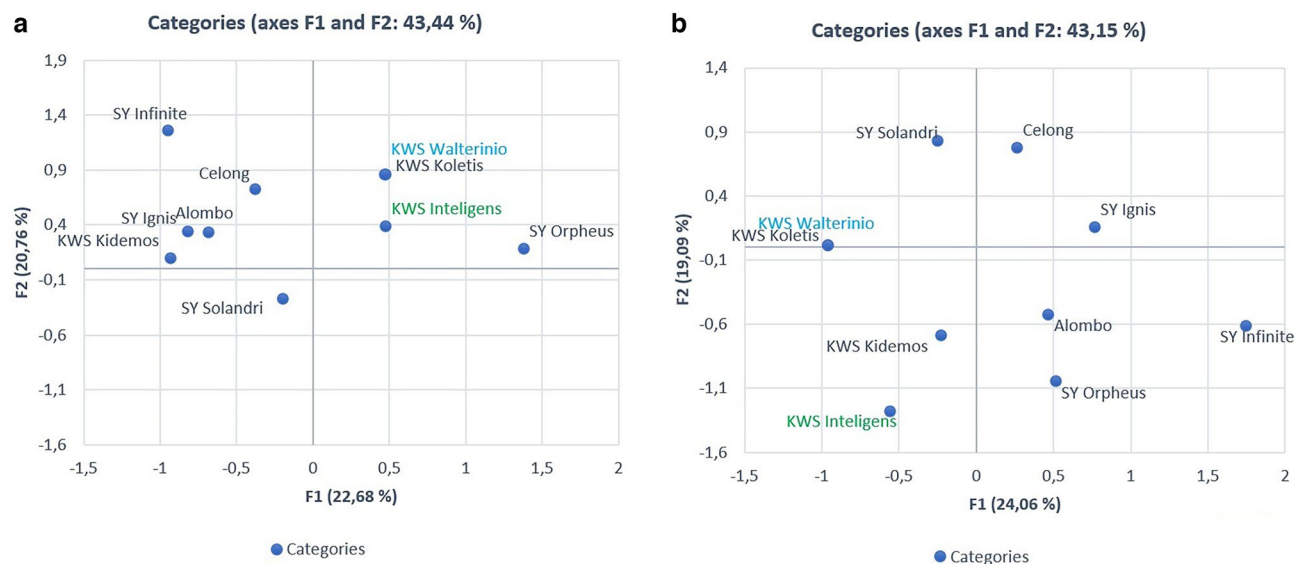


Fig. 13 Graphical representation of PCA analysis for maize at two sites (site 2 as a, site 3b as b): the variety with stable yield is marked lightly blue, and the variety with the highest yield is marked as turquoise green

and the Steel–Dwass–Critchlow–Fligner test was used for post hoc testing for multiple pairwise comparisons. Spearman correlation (r_s) was used to analyse the relationships among the observed traits, including the number of stomata, proline contents, RWC and aboveground biomass yield. The PCA (Principal Component Analysis) graphically represented the Spearman correlation values.

The graphical representations (Figs. 2, 4, 5) were made in Excel (version 2306, Microsoft, USA), and Figs. 6, 7, 8, 9, 10, 11, 14, 15 were made in SigmaPlot (Systat Software Inc., USA). Figures 12, 13 were made by XLSTAT.

Results and Discussion

The results obtained from the field experiment investigating the responses of maize and sorghum to agricultural drought exhibit several key insights that support the initial hypothesis regarding their differential reactions to drought stress.

Proline and RWC Relationship in Maize and Sorghum Under Field Conditions

Under the analysed field conditions, a negative correlation between the RWCs and the proline content of the leaves was found. Although the correlation between RWC and proline content in sorghum species was not statistically significant at site 3a ($r_s = -0.212$; $p = 0.560$), it was at site 1 ($r_s = -0.648$; $p = 0.049$). The association was extremely weak in maize (site 2 $r_s = -0.018$; site 3b $r_s = -0.200$), and the link was not shown to be statistically significant. In contrast to maize, the values of RWC and proline content of sorghum values are relatively widely dispersed in the graphical depiction of the data in Figs. 4, 5. One explanation for this is the diverse genetic background of the selected sorghum cultivars, some of which are hybrids of *Sorghum bicolor* and *Sorghum arundinaceum* (also known as *Sorghum × drummondii*, Sudan grass).

Sorghum bicolor has a greater drought tolerance than the Sorghum-Sudan grass hybrid, according to an experiment comparing the drought tolerance of maize, sorghum, and sorghum-sudan grass species (Schittenhelm & Schroetter, 2014). From the values of RWC and proline content, it can be inferred that proline content in sorghum has a more significant impact on water deficit than maize. However, it is essential to acknowledge that plants produce a variety of other chemicals, such as fructans (Benkeblia, 2022; Hendry, 1993; Vijn & Smeekens, 1999) and the phytohormone abscisic acid (Waterland et al., 2010; Sah, et al., 2016), which also

play a significant role in the response and survival of plants under water shortage. Determining their levels in field conditions may provide an interesting perspective on the mechanisms of drought tolerance of crops under field conditions.

The Selected Morpho-Physiological Parameters Differences in Both Species.

To investigate traits' statistical differences, we performed the Kruskal–Wallis test for each species and factor at each site, as plants under sub-optimal conditions at different sites may react differently to maintain their yield parameters (Table 5).

Relative Water Content (RWC) Analysis

The average values for each variety within the species are represented in Fig. 6 and Fig. 7. A statistically significant difference was found between the values of RWC in sorghum and maize (Table 5). The higher RWC values were found for sorghum, which showed an average value of 90.7% for the entire observed period. Meanwhile, the average value in maize was 87.0% under the studied field conditions.

RWCs of 100 to 90% are related to stomatal closure, cell expansion, and growth reduction as cells reach the maximal water content. At RWCs of 90 to 80%, changes in the tissue composition and alterations in photosynthesis and respiration rates occur (González & González-Vilar, 2001); hence, we assumed that some changes in photosynthesis may have occurred in maize plants, as the average value of RWC fell under 90%. The RWC below 80% is associated with a water potential of -1.5 MPa or less, which leads to metabolic changes, decreased photosynthesis, increases in respiration and proline content and accumulation of ABA (González & González-Vilar, 2001). The higher RWC observed in sorghum, a more drought-tolerant species than maize (Schittenhelm & Schroetter, 2014; van Oosterom et al., 2021), is intriguing. This finding suggests that sorghum potentially sustains higher water levels in its leaves over an extended

Table 5 *P*-values of the Kruskal–Wallis test for the selected parameters

	RWC		Proline contents		Stomatal density	
	Maize	Sorghum	Maize	Sorghum	Maize	Sorghum
Species	0.000466***		<0.0001***		<0.0001***	
Variety	0.703	0.001***	0.840	0.009**	0.319	0.851
Site	0.762949	<0.0001***	<0.0001***	0.281	0.537	0.013*
Date	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***
Leaf	<0.0001***	0.006**	0.833	<0.0001***	<0.0001***	0.001***
Leaves with higher values	Top	Top	Bottom	Bottom	Bottom part	Bottom part

The numbers represent *P*-values; the *symbol represents the statistical significance of the *P*-value. Each column represents a Kruskal–Wallis test (nonparametric ANOVA) for each factor, such as variety, site, date and leaf. The testing of statistical differences between two species is represented only by one column for both species in the third row

period, potentially due to improved water use efficiency (Danalatos, et al., 2009; Hasan et al., 2017) and enhanced water absorption through its more robust root system compared to maize (Schittenhelm & Schroetter, 2014).

Differences of statistical significance in RWC among varieties were found only in sorghum (Table 5). The highest average RWC values were found in varieties like Hannibal (93.4%), KWS Bulldozer (93.4%), and KWS Tarzan (93.1%). The observed varietal differences in RWC under drought conditions align with prior reports in various species such as maize, wheat, potato, soybean, and mungbean. (Hossain, et al., 2015; Nazran et al., 2019; Pokharel & Pandrey, 2012; Soltys-Kalina et al., 2016). The highest RWC values of the sorghum varieties were not affected by the time to maturity, as three different varieties presented the highest values and had different earliness. Nevertheless, the timing of drought in the early stage of development plays a vital role in this outcome, as the spring months of April and May seemed less precipitation-rich. Therefore, genotype and the interaction between genotype and environmental/field conditions played more prominent and essential roles in determining RWC than just the earliness of the sorghum varieties.

Environmental influence on RWC was evident between the sampled sites and different dates. Site-specific differences in sorghum RWC were profound, with site 3a recording the highest average values (92.9%) compared to site 1 (88.4%). These variations could be attributed to soil characteristics favouring plant growth at site 1 due to higher clay content, while the more favourable water regime at site 3a lessened drought severity. It is possible that during the drought period, the roots may have been coarser in clay loam, but in soils with a higher content of sand particles, fine roots may have formed easily, and it may have helped plants absorb water from the soil (Garg et al., 2020).

Temporal variations in RWC were observed for both sorghum and maize. The highest RWC values for both species were recorded in August (sorghum, 92.4%; maize, 93.2%), presumably influenced by abundant rainfall early that month. The lowest RWC values were recorded in June for sorghum (88.7%) and in July for maize (82.3%). Additionally, variations in growth stages between the two species, with maize sown earlier than sorghum in the given climate, contributed to differences in RWC levels across sampling months.

Proline Content

The data revealed significant differences in proline content between sorghum and maize (Table 5). Sorghum exhibited higher proline levels, averaging 8.7 micromoles/100 mg of fresh leaf weight, compared to maize, which had an average of 5.3 micromoles/100 mg of fresh leaf weight. These findings align with the observed higher Relative Water

Content (RWC) in sorghum under the specific field conditions (Figs. 8 and 9).

The variability in proline accumulation capacity among species has been found in previous studies (Yamada et al., 2005). Sorghum varieties exhibited distinct maximal proline accumulation abilities (Blum & Ebercon, 1976), potentially contributing to their ability to maintain higher RWC levels. Studies involving exogenous proline applications in plants, such as cauliflower, have shown enhanced RWC due to increased internal proline content (EL-Bauome et al., 2022). This exogenous proline application also increases chlorophyll content and crop yield through the increased activities of crucial enzymes like superoxide dismutase, peroxidase, and catalase (Hanif et al., 2021). Further evidence supports the link between proline accumulation and drought tolerance, with more drought-tolerant varieties showing higher proline levels (Quilambo, 2004). The opposite reaction was found in drought-resistant rice varieties that exhibited lower proline contents (Dien, et al., 2019). However, barley plants have also demonstrated proline accumulation under stressful conditions (Frimpong et al., 2021). From the results of our experiment, the plants of maize and sorghum can also achieve higher proline levels under drought conditions in the field. Nevertheless, sorghum plants exhibited higher levels of proline than maize, which may be one of many drought tolerance mechanisms of sorghum.

Significant variations in proline content were observed exclusively among sorghum varieties. Early sorghum varieties, specifically Nutri Honey (10.6 micromoles/100 mg of fresh leaf weight) and KWS Kallisto (9.8 micromoles/100 mg of fresh leaf weight), displayed the highest average proline content. This trend is characterized by relatively high proline levels in more drought-tolerant varieties, as found in other crops like wheat and sunflower (Ghosh et al., 2022; Keyvan, 2010; Manivannan et al., 2007). Compared to other sorghum varieties, KWS Kallisto stands out as potentially more drought-tolerant. In contrast, maize variety SY Ignis exhibited proline levels akin to certain sorghum varieties, yet it demonstrated a significant difference in aboveground dry biomass between the two sites.

Statistically significant differences in proline content between sites were found only for maize. Plants from site 2 displayed an average proline content of 4.1 micromoles/100 mg of fresh leaf weight, contrasting with the higher 7.9 micromoles/100 mg of fresh leaf weight in plants from site 3b. For sorghum, although average proline content differed slightly between sites as 8.9 micromoles/100 mg of fresh leaf weight in the samples from site 1 and 8.4 micromoles/100 mg of fresh leaf weight in those from site 3a, these variations were not statistically significant. The differences in maize proline content between sites suggest differential drought stress levels, particularly in June when maize at site 3b experienced higher stress than at site 2. The

consistently higher proline content in sorghum, regardless of site differences, points out a continuous strategy of proline production, possibly as a "prepare to drought strategy".

Regarding sampling dates, statistically significant differences in proline content were evident for both sorghum and maize. In sorghum, peak proline content coincided with the lowest RWC in June, correlating with the sowing of sorghum varieties, indicating higher stress during this initial growth stage. Conversely, maize exhibited its highest proline content and lowest RWC in July, a month marked by prolonged drought.

Stomatal Density

Differences in stomatal density between sorghum and maize, both belonging to the C4 species, were evident in this study. Sorghum exhibited a higher stomatal density, averaging 152.4 stomata/1 mm² leaf tissue, whereas maize showed a lower density with an average of 118.2/1 mm² (Figs. 10 and 11). This distinction in stomatal density could be attributed to an evolutionary trait, potentially representing an anatomical adaptation in response to differing levels of drought tolerance between these species.

The stomatal density in sorghum varied significantly across different sites. Site 3a displayed the highest stomatal density (161.2 stomata/1 mm²), followed by site 1 (143.6 stomata/1 mm²). Site 1 was identified as experiencing higher drought stress based on the Vegetation Condition Index (VCI). Our observations suggest a potential adaptive response in sorghum, indicating a possible decrease in stomatal density under more arid conditions. Analogous findings in other studies have identified a lower stomatal density in more drought-tolerant plant species such as barley, wheat, rice, and tomato (Caine et al., 2019; Hughes et al., 2017; Kulkarni & Deshpande, 2006; Lestari, 2016; Li et al., 2017). This aligns with our findings, suggesting that a reduction in stomatal density may be a mechanism sorghum employs to adapt to more severe drought stress.

Additionally, our study revealed fluctuations in stomatal density across different sampling dates, with the highest density recorded in both sorghum and maize in August, the final month of sampling. There was a consistent increase in stomatal density with plant growth. Future research could explore the growth stages at which stomatal density stabilizes in sorghum and maize by extending the sampling period beyond August, especially since the harvest of aboveground biomass continued until October. This prolonged study could provide valuable insights into the cessation of stomatal density increase during the growth cycle of these plant species.

Influence of Environmental Factors on Inter-parameter Relationships and Species Responses

A detailed correlation matrix can be seen in **Supplementary Information**. The results of PCA analysis can be seen in Figs. 12 and 13. The varieties and parameters located in the opposite quadrants of the chart reacted differently in our study. The investigated parameters were fresh aboveground biomass yield, aboveground biomass yield at 28% in sorghum and 33% in maize, height (sorghum), RWC, proline content and stomatal density. The varieties closest to each other in the graph reacted similarly in the given parameters.

Under conditions of reduced water availability, both sorghum and maize plants exhibited decreased leaf RWC, accompanied by elevated proline content, indicating heightened water deficit stress. This elevation in proline content revealed a significant negative correlation with yield, a correlation specifically observed in sorghum ($r_s = -0.806$ at site 1; $r_s = -0.733$ at site 3a).

In sorghum, increased plant height correlated positively with RWC at both sites ($r_s = 0.830$ and $r_s = 0.721$), suggesting a possible relationship between plant stature and root system development, thereby influencing water absorption. It has been shown in maize that varieties with the leafy trait, also characterized by taller plants, have longer roots and a greater root system surface area (Costa et al., 2002), but this theory needs to be tested in sorghum.

At site 1, more drought-affected according to the Vegetation Condition Index (VCI), sorghum plants exhibited reduced stomatal density, although the variety's response strongly impacted yield. A robust positive correlation emerged between earliness and yield, indicating that later maturing sorghum varieties demonstrated higher yields. In contrast, at site 3a, the plants' reaction to parameters differed, displaying lower proline content but higher RWC values. This suggests the existence of lower water deficit stress (as indicated by VCI); nevertheless, RWC exhibited an increase alongside plant height.

Exploring the response of sorghum varieties to different sites (Fig. 12), KWS Kallisto, exhibiting early maturity, demonstrated consistent and stable yields similar to Latte at site 1, displaying similar RWC values, dry mass yield, and dry matter content. The similarity in maturity rate and height between KWS Kallisto and Latte may elucidate their proximity in the graph. KWS Hannibal, a high-yielding variety, differed substantially in its reaction to the sites. It exhibited lower proline content, elevated stomatal density, and increased dry matter and fresh biomass. Therefore, KWS Hannibal has the genetic potential to yield the highest from the selected sorghum varieties; however, its yield and yield

characteristics are unstable and unpredictable in drought-stressed conditions.

Maize plants, likewise, showed diverse behaviours across the two sites. Increased proline content at site 2 correlated positively with the dry matter content of maize plants ($r_s = 0.794$), indicating a response to water-limited conditions. Enhanced stomatal density correlated with increased leaf dry matter content at site 2 ($r_s = 0.685$), likely resulting from expanded leaf dimensions. Conversely, at site 3b, maize plants exhibited increased stomatal density when proline content was decreased ($r_s = -0.676$), suggesting a reduction of stomatal density during increased proline levels, which can be a potential mechanism for drought tolerance in maize.

Analyzing the reaction of maize varieties to different sites (Fig. 13), at site 2, Walterinio KWS shared a quadrant with KWS Koletis and KWS Inteligens, where SY Orpheus

also resided. KWS Inteligens displayed similar proline and stomatal density content to Walterinio KWS, with comparable dry matter content observed in KWS Inteligens and SY Orpheus. However, at site 3b, Walterinio KWS diverged from KWS Inteligens by higher RWC values and lower proline content. These variations in response could suggest diverse drought response mechanisms among these varieties, including biochemical processes apart from proline content influencing RWC values.

The Yield Performance

The fresh aboveground biomass yield is a crucial trait for agronomists cultivating crops for fodder or silage purposes. Typically, sorghum plants exhibit superior yield under intensified drought-stress conditions compared to maize. While the quality of sorghum silage closely resembles that

Fig. 14 Average aboveground dry biomass yield in sorghum (error bars represent standard deviation values)

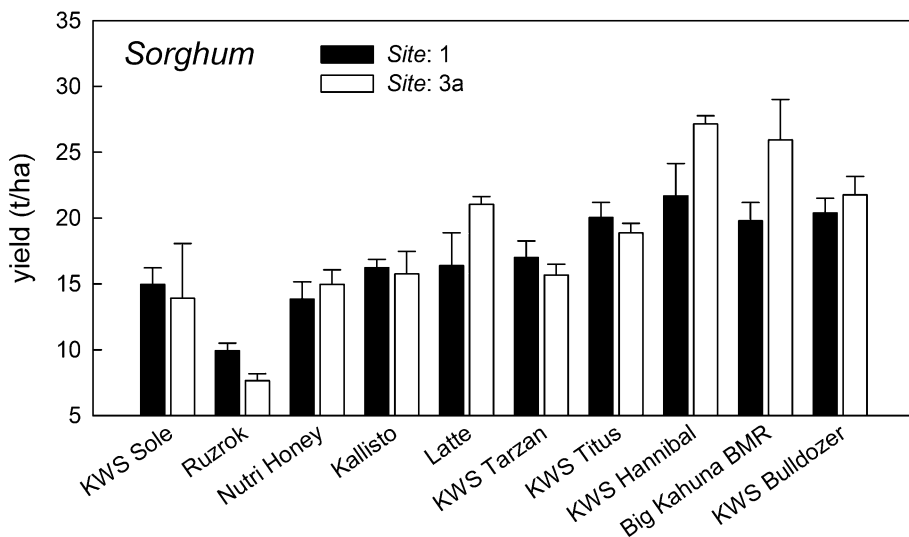
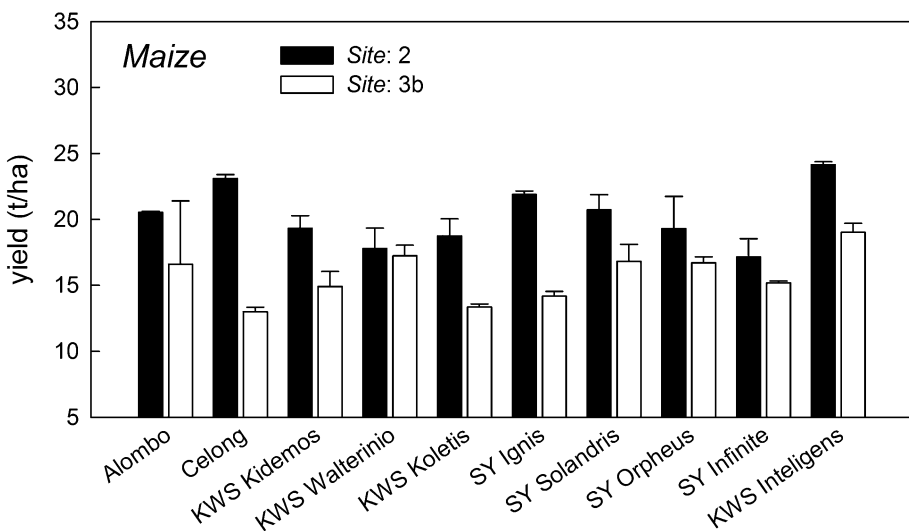


Fig. 15 Average aboveground dry biomass yield in maize (error bars represent standard deviation values)



of maize silage (Fazaeli et al., 2006; Nusrathali et al., 2021; Podkówka & Podkówka, 2011), sorghum's aboveground biomass comprises a higher lignin content relative to maize (Wahyuni et al., 2019; Zardin et al., 2017), potentially leading to decreased digestibility. Nevertheless, including the brown midrib (BMR) trait in sorghum varieties reduces lignin synthesis, enhancing digestibility. Consequently, a combination of sorghum and maize silage presents a promising alternative, especially when maize struggles to produce sufficient fresh biomass to feed animals (Cattani et al., 2017).

Therefore, it is desired that crop plants yield the highest yield possible. From this view (Figs. 14 and 15), the highest yield in sorghum was found in KWS Hannibal (intermediate; 21.7 t/ha at site 1 and 27.1 t/ha at site 3a) at both sites, followed by KWS Bulldozer (late; 20.4 t/ha) at site 1, and Big Kahuna (late; 25.9 t/ha) at site 3a. With the variety, Big Kahuna was a technical problem because its late earliness made it impossible to harvest the aboveground biomass at close to 28% in the given weather conditions in 2022. So, the plants were harvested at the lower dry matter before the plants could develop low-temperature damage in October. KWS Bulldozer, KWS Hannibal and Big Kahuna are quite tall plants, with KWS Hannibal being the tallest of the selected varieties (3.8 m at site 1; 3.1 m at site 3a) compared to the shortest Ruzrok plants (2.3 m at site 1; 2.0 m at site 3a). The height and good tillering ability were caused by genetic predisposition, which contributed to the highest yields in intermediate and late sorghum varieties. The highest yield in maize was found in KWS Inteligens (intermediate; 24.1 t/ha) at site 1 and (19.0 t/ha) at site 3b. In this case, the later varieties performed better than the earlier ones, suggesting that rate maturity also plays a significant role in maize.

These results support our hypothesis, because it implies that plant with varying origins are prone to exhibit diverse responses to drought stress. Despite the first view, when scientists look for a more drought-tolerant crop, the most stable yield is sought above the highest yield. For plant breeders, the combination of stable and high yield is the most required trait. Nevertheless, the complexity of this trait, with the high number of genes involved, makes it difficult to achieve by classical plant breeding methods. The most stable yield was found in the sorghum variety KWS Kallisto (early; 16.2 t/ha at site 1 and 15.7 t/ha at 3a) and the maize variety Walterinio KWS (semi-early; 17.8 t/ha at sites 2 and 17.2 at site 3b). The early varieties probably used a drought avoidance strategy (Shavrukov et al., 2017). The maturity rate affects the yield. The early varieties usually yield less (Degener & Kappas, 2015) due to the reduced time for yielding components. For more information on average values for each parameter and variety, see **Supplementary Information**.

Conclusion

The study revealed species-specific responses to drought stress in maize and sorghum, underlining sorghum's superior drought tolerance compared to maize under the studied field conditions. The sorghum plants presented higher RWCs, proline contents and stomatal density numbers than the maize plants. Moreover, there were differences in proline contents among the sorghum varieties, and some of the varieties yielded highly, perhaps because they modulated their RWCs and proline contents as drought tolerance mechanisms to maintain their yields. However, in maize, these parameters did not significantly affect yield. The sorghum variety KWS Hannibal (intermediate) yielded the highest yield. The most significant yield difference between the two sites (6.2 t/ha) was found in Big Kahuna (late). The maturity rate played a technical problem under the given weather conditions for the silage harvest of Big Kahuna. Therefore, the sorghum variety KWS Hannibal seems like a good candidate for our weather/climatic conditions, as its yield was among the highest, even with the second greatest difference in yields (5.5 t/ha), as the agronomists are interested more in the highest yield than the stable yield which was found in variety KWS Kallisto (early). In maize, the suitable variety was KWS Inteligens (intermediate), as the differences between yields were in the middle (5.1 t/ha) from the selected varieties. Nevertheless, the most adaptable variety seemed to be Walterinio KWS (semi-early). It may indicate that earliness still plays a decisive role in drought escape strategy in spring-sown crops grown in temperate climates. The findings also highlight the significance of genetic diversity and genotype-environment interactions in determining the morpho-physiological responses and yield performances of these crop species under agricultural drought, providing valuable insights for future breeding programs and strategies to enhance drought tolerance in crops.

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Data Availability All relevant data are in the paper and the Supplementary Information file, found at <https://doi.org/10.5281/zenodo.8206566>.

Declarations

Conflicts of Interest The authors declare that no competing interest exists.

Ethics Approval Not applicable.

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