Physiological Responses of Contrasting Maize (*Zea mays* L.) Hybrids to Repeated Drought

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Abstract

Maize is the most important crop worldwide in terms of production and yield, but every year a considerable amount of yield is lost due to drought. The foreseen increase in the number of drought spells due to climate change raises the question whether the ability to recover quickly after a water pulse may be a relevant trait for overall drought resistance. We here address the following hypotheses: (i) different maize hybrids exhibit distinct physiological adaptive responses to drought stress and (ii) these responses affect the ability to recover from the stress. (iii) The relative biomass production of maize hybrids, which show severe drought symptoms but are able to recover quickly after a water pulse, is comparable to those hybrids, which invest more energy into tolerance mechanisms. The physiological responses of eight maize hybrids to repeated drought were elucidated employing physiological parameters such as electrolyte leakage, osmolality, relative water content, growth rate and gas-exchange measurements. Only one hybrid was able to maintain biomass production under drought conditions. Amongst the others, two hybrids with similar growth inhibition but contrasting physiological responses were identified by a PCA analysis. Both strategies, *i.e.* stabilization of leaf water content via resistance mechanisms *versus* high recovery potential were equally effective in maintaining aboveground biomass production in the scenario of a long drought intermitted by a water-pulse. However, each strategy might be advantageous under different drought stress scenarios. Overall, the recovery potential is underestimated in drought resistance under natural conditions, which includes periodic cycles of drought and rewatering, and should be considered in screening trials.

Abbreviations

A	Assimilation rate
EL	Electrolyte leakage
gs	Stomatal conductance
iWUE	Intrinsic water-use efficiency
PAR_{leaf}	Photosyntheticially active radiation on leaf surface
PC	Principal component
PCA	Principal component analysis
ROS	Reactive oxygen species
RWC	Relative water content
WHC	Water holding capacity of the pot

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Introduction

Drought stress is a major constraint in modern agriculture (IPCC, Olssen et al. 2019) and especially for C4 plants such as maize (*Zea maize* L.) with a high water demand and short growing season. With ongoing climate change, models predict increasing frequencies and severity of drought spells; therefore, a better understanding of the role of the recovery process in overall drought resistance is important (Lyon et al. 2016). Drought resistance is departed in drought avoidance and drought tolerance mechanisms (Levitt 1972). Drought avoidance strategies of plants are diverse and include (i)

developmental adaptations, for example an offset in the floral transition to avoid drought regimes at critical phases such as flowering (Shavrukov et al. 2017); (ii) morphological adaptations in order to enhance water uptake and to decrease transpirational water loss, for example by developing a deeper root system or an altered leaf shape or leaf anatomy (Price et al. 2002; Lipiec et al. 2013); and drought tolerance mechanisms comprise (iii) metabolic adaptations and enhanced antioxidant capacity for reactive oxygen species (ROS) scavenging (Farooq et al. 2009; Bhargava and Sawant 2013). Recently, rapid phenological drought recovery is increasingly in focus. Recovery-oriented plants are capable to restart growth quickly after the rewetting event, most likely due to hydraulic recovery, embolism repair (Martorell et al. 2014) and maintenance of photosynthetic capacity (Avramova et al. 2015) and higher chlorophyll content under stress (Chen et al. 2016). However, "phenological" recovery of growth is not always accompanied by a full return to the "pre-stress" metabolic or transcriptomic state (Wedeking et al. 2018; Schwalm et al. 2017). Some plants form a physiological, metabolomic, transcriptomic or epigenetic stress imprint or 'memory' during a priming event (Crisp et al. 2016; Wedeking et al. 2018), which may allow a more rapid response if the stress recurs. Whilst (i–iii) have been extensively investigated in drought stress research, the contribution of recovery to the overall drought resistance of a plant is still not well understood (Lyon et al. 2016). We here used a set of contrasting maize genotypes to elucidate this question. It is expected that the trait "recovery" may become increasingly important with the expected increased occurrence of repeated drought spells throughout the growing season.

The research aims of this study were (i) to identify contrasting physiological responses to repeated drought in a set of eight maize hybrids and to (ii) evaluate the influence of these responses on the recovery after rewatering. We hypothesize that hybrids, which show severe drought symptoms during a drought event, but are able to recover quickly, might by equally drought tolerant with respect to biomass production in a setting of recurrent drought events.

Materials and Methods

Plant Material and Cultivation

Z. mays seeds ($n=10$ replicates) from eight genetically diverse maize hybrids (see Table 1) were soaked overnight in 1 mM $CaSO_4$ on the 26th of June, 2017. On the following day, the seeds were sown in 7 L pots containing 4.5 kg of a 1:1 (v/v) mixture of sand and substrate (Greenworld Kultursubstrat Profi-Mix). The substrate composition was (v/v) 20% white peat 10–20, 20% white peat 20–40, 7% Perlite mixed with 3% clay and 50% sand, pH 5.8–6.2. The

Table 1 Abbreviation of hybrids

Abbreviation	Hybrid
AM	Amadeo
KW	KWS stabil
L2	LG30222
L5	LG30215
NT	Ammamonte
P1	P8589
TK	Tokala
TO	Torres

experiment was conducted in a greenhouse starting stress treatment at BBCH 16. Growth conditions are shown in Supplementary Fig. 2. After a total of 52 d, the plants were harvested at BBCH 51 on the 19th of August, 2017. Throughout the experiment, pots were randomly rearranged once a week in order to avoid random effects. The plants were fertilized on a weekly basis; the total amount was 1.25 g N_2, 0.5 g K, 1.23 g PO_4^-, 0.68 g Mg and 2.68 g SO_4^- (using NH_4NO_3, KH_2PO_4, $MgSO_4*7 H_2O$ as liquid solution). Fetrilon-combi micronutrient (AgNova Technologies Pty Ltd., Box Hill North, Australia) was given once as a liquid solution containing 0.8 mg Mg and 1.3 mg S, 1.5 mg B, 0.6 mg Cu, 4.0 mg Fe, 3.0 mg Mn, 0.05 mg Mo and 4.0 mg Zn.

Stress Treatment

After an initial cultivation for 33 d under control (well watered) conditions (55% of the water holding capacity of the pot (WHC)), the first stress period was induced at BBCH 16 in one half of the plants per hybrid ($n=5$) with a targeted soil moisture of 20% of the WHC (Fig. 1a) from d 33 (26th of July) to d 49. At this WHC, the plants still had water available but were at or below the permanent wilting point, based on the observation that the wilting phenotype of enrolled leaves was also present in the morning. To maintain the targeted water content in the pots, the weight of 10 control (well watered) and 10 drought-treated pots was determined two times per day, and the mean difference to the intended weight was then added to all pots. In addition, the electrical conductivity of the soil solution was measured daily for all pots to monitor the water content of the individual pots based on the correlation between electrical conductivity and pot water content (Supplementary Fig. 1). The electrical conductivity of potted soil was measured by a handheld conductivity meter device (Infield 7, München, Germany) equipped with a Theta Probe (ML2x, Cambridge, UK). On day 49, a singular water pulse was given by adjusting the water content to control conditions, followed by a second drought stress period at BBCH 41 from d 52–d 55 (Fig. 1a) lasting until harvest at BBCH 51. The second drought was slightly more severe

Fig. 1 a Experimental setup and timeline. The dark grey area represents the pot soil water content in drought-treated plants relative to control. **b** Scheme of harvested parts and corresponding measurements. **c** Photo of hybrids KW and L2 just before the second drought period compared with control
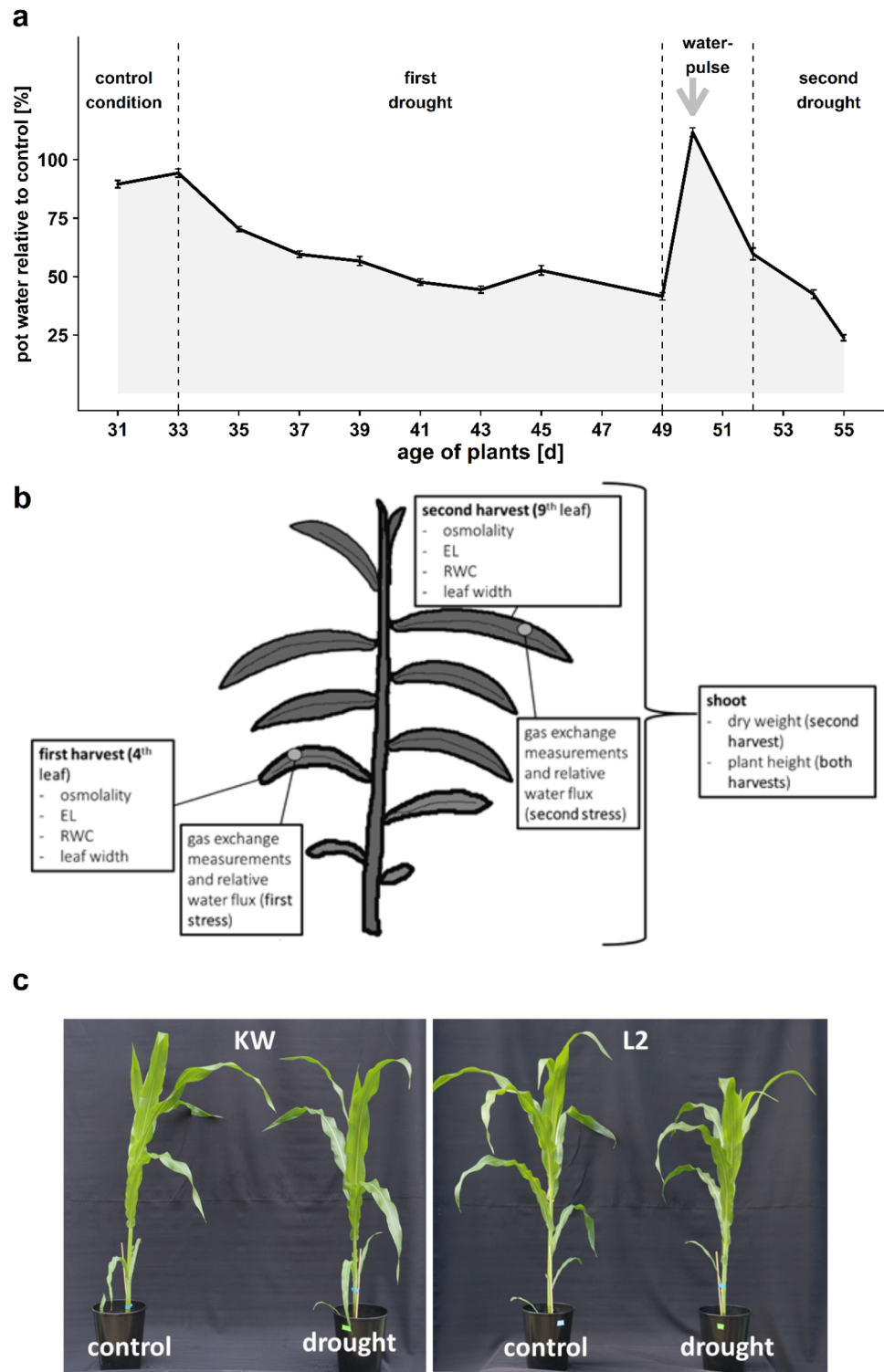
than the first drought because the water content of the soil was not maintained at a specific level, and due to the larger size of the plants and warmer temperatures, the soil dried out faster during the second drought event, reaching a final water content of 12% of the WHC.

Non-destructive Measurements

During the experiment, non-destructive measurements of leaf elongation rate (LER), leaf width, relative flux of leaf water content, gas exchange and photosynthetic rate were

conducted every two days, with a few exceptions (see time-scale, Fig. 1a), using leaf 4 (first stress) and leaf 9 (second stress) (Fig. 1b).

The LER was determined with a ruler by measuring the distance between the pot surface and the stretched tip of the respective leaf. The oldest leaf without collar was chosen for LER measurement (initially leaf 5). When this leaf was close to being fully expanded by visual estimation, the next youngest leaf was used. LER was expressed as leaf elongation in cm per h, calculated for every 2-day period. The maximum leaf width was determined with a ruler using leaf 4 and leaf 9, respectively.

For non-invasive measurements of relative fluxes of leaf water content, a novel water content sensor was employed (Zhang et al. 2019). The custom sensor is based on infrared light-emitting diodes and a photodiode connected to a custom device for digital recording. The principle is the simultaneous recording of transmission of two infrared wavelengths (1450 nm, 1050 nm) at an angle of 45 degrees to the leaf surface. These two wavelengths have different, contrasting absorption patterns of water (high absorption of water at 1450 nm, low absorption at 1050 nm), and the ratio of the transmission at these two wavelengths is linearly correlated with the leaf water content and is suitable for monitoring relative changes of water fluxes when calibrated against refraction patterns of the tissue observed (Zhang et al. 2019).

Photosynthetic rate (A) and stomatal conductance (gs) were determined by the use of a LCi Portable Photosynthesis System (ADC BioScientific Ltd., Hoddesdon, England). A broad chamber with a clamped leaf area of 625 mm^2 was used and the light was natural sunlight. The CO_2 flow into the leaf chamber was 400 ppm and H_2O flux was 0.17 µmol m^{-2} s^{-1}. A and gs were normalized by PAR$_{leaf}$ (Photosynthetically Active Radiation on the leaf surface) for principal component analysis (PCA). This normalization was done because of the high fluctuation of light conditions during the experiment (Supplementary Fig. 2). The intrinsic water-use efficiency (iWUE) was calculated as A/gs (Osmond et al. 1980).

Destructive Measurements

At the end of both stress periods, leaf 4 and leaf 9, respectively, were also used for destructive measurements of relative water content (RWC), electrolyte leakage (EL) and osmolality. The remaining plant shoot was used to determine fresh and dry weights as well as plant height.

For determination of the RWC, one leaf disc per plant was excised with a kork borer. Immediately after excision, the fresh weight (FW) of the leaf discs was determined, and the discs were floated on MilliQ water overnight at 4 °C for determination of turgid weight (TW). Leaf discs were then dried in an oven overnight at 70 °C and dry weight (DW) was measured. The RWC was calculated as the ratio $\frac{FW-DW}{TW-DW}$ in %.

Electrolyte leakage (EL) was measured by six leaf discs of 1 cm diameter each. The leaf discs were rinsed three times (4 s each) with MilliQ water and equilibrated with gentle shaking for 5 h in a 50 mL falcon tube containing 20 mL MilliQ water. The conductivity of the solution was determined using a conductometer (WTW LF90; WTW KLE1 cell, Weilheim, Germany). The leaf discs were then frozen overnight at −20 °C, thawed again and total conductivity was measured after equilibration to room temperature. EL was calculated as the ratio of the conductivity at 5 h and the total conductivity after thawing.

Osmotic potential was determined in leaf sap collected by squeezing the 4th or the 9th leaf blade, respectively, using a handheld press, and samples were stored at −20 °C until analysis. Osmolality of the samples was measured with a vapor-pressure osmometer (VAPRO® Vapor Pressure Osmometer, ELITechGroup, Paris, France). Each sample was measured in three technical replicates of the extracted leaf sap.

Dry weight (DW) of the shoot was measured after drying the plant material at 55 °C for 72 h in a ventilated oven until stability of weight. Plant height was measured as the distance between the pot surface and the youngest elongating leaf.

Statistical Analysis

All data are expressed as mean of $n = 4$ or 5 replicates ± standard error, if not specified otherwise. For analysis, the software R (Version 3.4.4) was used. To calculate significant differences between dry weights and evaluate whether there was an interaction between treatments and hybrids, a two-way ANOVA (results see Table S1) and subsequent post hoc Tukey HSD test were performed at the significance level of $p < 0.05$. For comparisons of timecourse measurements, a repeated measures ANOVA was performed and then t tests were performed between the treatments per time point and hybrid to analyse the time and treatment interactions at the significance level of $p < 0.05$. P-values were adjusted using "Benjamini Hochberg" correction for multiple testing. For PCA, absolute means were used, normalized and scaled with the R algorithm "prcomp()" and plotted using the package "ggbiplot."

Results

Growth and Biomass Accumulation

Whilst L5 accumulated significantly less DW, all other hybrids produced similar levels of aboveground biomass

under control conditions, in the order L5 < < L2, TK < KW, AM, NT, P1, TO (Fig. 2). Under drought, no significant differences in biomass were observed between all hybrids. Compared to controls, all hybrids except L5 showed a reduction in biomass production under drought, which was significant with the exception of TK. Relative to the control value, especially L5 had an almost negligible biomass reduction of 1.7%, followed by TK (22.3%), L2 (26.4%), KW (26.4%) and NT (27.7%), whilst the highest relative decrease was observed in AM (30.2%), P1 (31.1%) and TO (31.1%) (Table 2).

Electrolyte Leakage, Osmolality and Relative Water Content

The EL of leaves is shown in Fig. 3a. After the first stress period, only few hybrids showed a tendency towards increased EL, but none of these effects was statistically

significant (Fig. 3a). Differences were more pronounced after the second stress period, even though EL was significantly higher than in controls only for hybrids KW, AM, L5, TK and TO (Fig. 3a). Noteworthy, there was no tendency of increased EL after either stress period for hybrid L2, whilst KW had the largest increase relative to control at both time points with 99.9% in the first drought and 116.7% in the second drought, respectively (Table 2). Thus, L2 and KW are the most contrasting hybrids with regard to drought-induced membrane damage.

The relative water content of leaves (RWC) is shown in Fig. 3b. Whilst there were significant differences in RWC only in KW, AM and TO after the first stress period, all hybrids showed significant reductions in RWC after the second stress period (Fig. 3b). Relative to the control, this reduction was smallest in L2 (9%); intermediate for P1, AM, L5 (16.4–19.1%); and highest for KW, TO, NT and TK (21.6–26.2%).

Fig. 2 Dry biomass of hybrids at final harvest (d 55). The grey shaded area highlights the contrasting hybrids. Values represent means of $n = 5$ or 4 replicates and violin plots indicate the spread of values. Different letters indicate significant differences at the significance level of $p \leq 0.05$
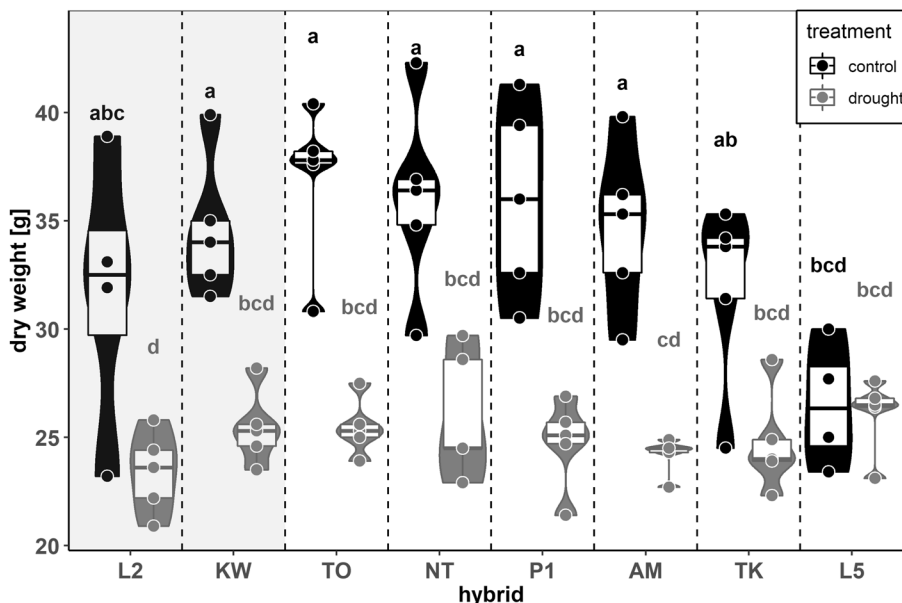
Table 2 Relative changes (compared to controls) of aboveground biomass, osmolality, RWC and EL in drought-treated hybrids at the end of both stress periods (d 447 and d 55, respectively)

| Hybrid | Relative change [% of control] (increase: positive value; decrease: negative value) of | | | | | | |
| | Biomass | Osmolality | | RWC | | EL | |
	Drought 2	Drought 1	Drought 2	Drought 1	Drought 2	Drought 1	Drought 2
L2	−26.4	16.4	19.4	−6.8	−9.0	8.9	16.2
KW	−26.4	12.4	22.8	−9.7	−22.7	99.9	116.7
TO	−31.1	5.4	34.5	−13.1	−23.1	63.1	89.6
NT	−27.7	15.7	69.7	−8.4	−21.6	31.0	98.2
P1	−31.1	18.3	45.0	0.45	−16.9	31.8	49.5
AM	−30.3	20	27.4	−10.1	−16.4	24.5	64.5
TK	−22.3	1.5	62.2	−3.4	−26.2	−3.8	107.0
L5	−1.7	3.1	55.1	−11.1	−19.1	34.0	102.4

Fig. 3 Electrolyte leakage (A), relative water content (B) and leaf sap osmolality (C) at the end of the first (left side) and the second (right side) stress period. For Hybrids' abbreviations, see abbreviations section. The grey shaded area highlights the contrasting hybrids. Values represent means of n = 5 or 4 replicates, asterisks indicate significant differences between treatments for individual hybrids at the significance level of adjusted $p \le 0.05$ (*), $p \le 0.01$ (**), $p \le 0.001$ (***)
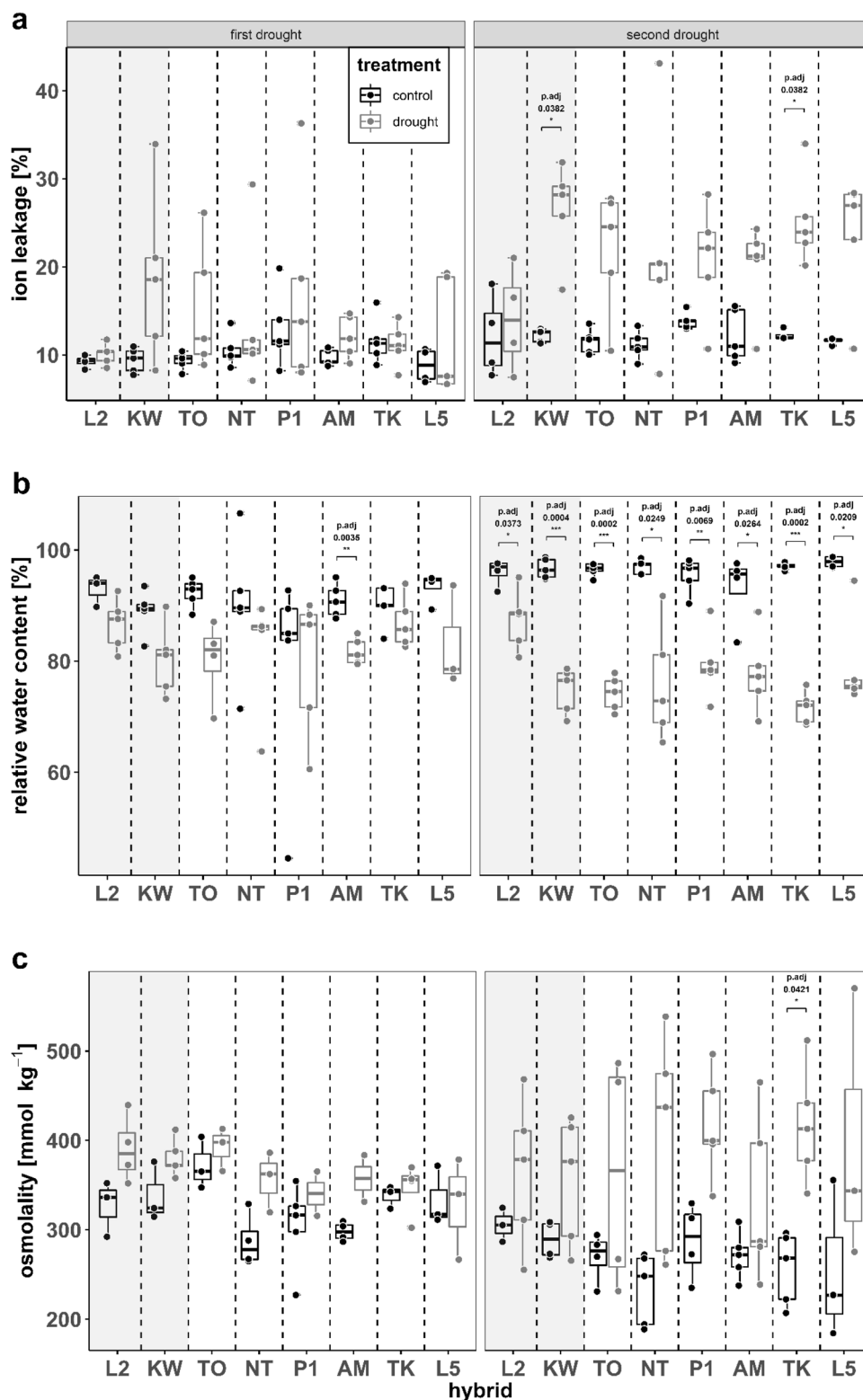
The effect of drought on osmolality was overall quite similar to that on electrolyte leakage, *i.e.* there were only very slight increases after the first drought stress and more pronounced increases (significant for NT, P1 and TK) after the second drought (Fig. 3c). Relative to controls, the osmolality in L2 and KW were increased to a similar extent in both drought periods (12.4 and 16.4 drought 1, 19.4% and 22.8% drought 2, respectively).

Principal Component Analysis

A principle component analysis (PCA, Fig. 4) was conducted using the factors osmolality, EL, RWC, gs/PAR$_{leaf}$, A/PAR$_{leaf}$, leaf elongation rate, plant height and leaf margin diameter, collected at both harvest dates. The first two principal components (PC) explained 50.6% (PC1) and 31.8% (PC2) of the total observed variance, respectively, together accounting for 82.4% of the total observed variability. PCA input data were clearly separated into four groups, which represent the two drought periods and the two different treatments (control, drought).

PC1 separated the control (positive side, Fig. 4) and drought-stressed plants (negative side, Fig. 4), indicating that PC1 is related to drought stress intensity. PC2 tended to separate the two drought periods (first period on the negative side, second period on the positive side), suggesting that PC2 is related to plant development. The loadings table showed that PC1 is mainly related to the physiological traits EL, osmolality, RWC, A/PAR$_{leaf}$, gs/PAR$_{leaf}$ and LER, whilst PC2 was mainly related to leaf width and plant height (Table S2).

Whilst all hybrids clustered together in both control treatments, the drought-treated hybrid L2 was separated from the other hybrids after both drought periods and ranged between control and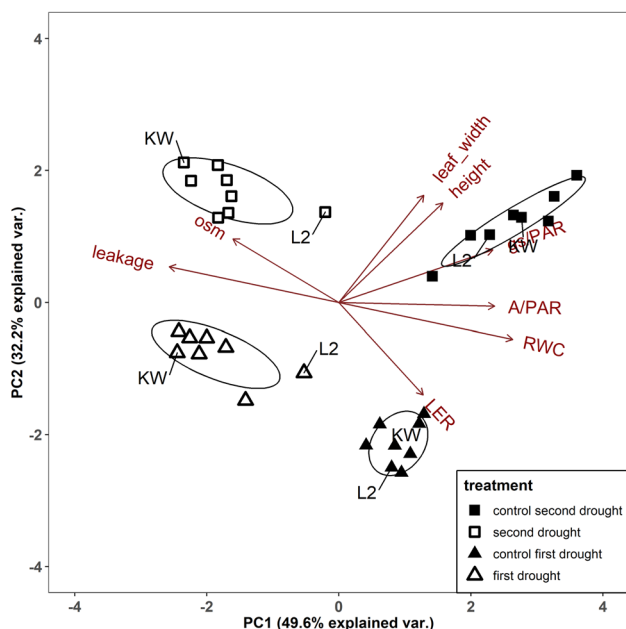 stress treatments. The hybrid KW represented the other extreme and ranged furthest away from controls after both drought periods (Fig. 4). This result was also obtained when the amount of input data for the PCA was reduced to electrolyte leakage, RWC and A/PAR$_{leaf}$ (Supplementary Fig. 3). We therefore identified hybrids "KW" and "L2" as contrasting genotypes for in-depth analysis.

Growth Rates, gs and A Dynamics and Water-Usage of Contrasting Hybrids

Figure 5a shows different patterns of LER amongst the two contrasting maize hybrids L2 and KW, which were selected based on the PCA (Fig. 4) and contrasting results for EL and RWC (Fig. 3). For hybrid KW, LER was significantly lower than controls during the first stress phase on d 39, d 41 and d 45. Following the water pulse (d 41), stressed KW plants rapidly resumed growth within 1 d, and the growth rate of stressed plants was initially even higher than that of controls (Fig. 5a). The growth rate of hybrid KW reached a maximum on d 52 (3 days after the water pulse), followed by a rapid decline during the second stress phase (significantly different from controls on d 54 and 55). L2 displayed a different behaviour, with less differences between controls and drought-stressed plants during the first stress period (differences not significant). However, after the water pulse on day 49, L2 resumed growth more slowly and reached control levels only 3 d after the water pulse. The subsequent rapid decline in LER was similar to hybrid KW and resulted in significantly lower values compared to controls on d 54 and d 55. There was also a Hybrid:time interaction on d 52 (Supplementary Fig. 4b, Table S1).

The very rapid growth response of KW after the water pulse cannot be explained by differential regulation of stomatal conductance, as the response of gs to the water pulse was similar in both hybrids (Fig. 5b, d 45–55). The decline of gs on d 45 and the increased growth rate in control plants of both hybrids on d 52 might be due to light and climate conditions (cloudy *vs.* sunny) during that time (Supplementary Fig. 2).

Kinetics of iWUE were also different between the two hybrids (Fig. 5c). Whilst iWUE was elevated in hybrid KW only during the first (until d 49), but not during the second drought (d 52–55), it was increased during both drought periods in hybrid L2 (d 39–41, d 52–55) (Fig. 5c). There was also a Hybrid:time interaction on d 50, but the effect was not significant with adjusted *p* (Supplementary Fig. 4a, Table S1).

Fig. 4 Results of the PCA analysis (hybrids KW and L2 are highlighted). Abbreviations: leaf_width: width of leaf; height: plant height; gs/PAR: PAR$_{leaf}$-normalized stomatal conductance; A/PAR: PAR$_{leaf}$-normalized assimilation rate; RWC: leaf relative water content; LER: leaf elongation rate; leakage: electrolyte leakage; osm: osmolality. Different symbols indicate the different treatment/harvest combinations

Discussion

Biomass Reduction Alone is Not Sufficient to Identify Drought-Tolerant Maize Hybrids

With drought being a severe adverse abiotic stressor, plants have evolved complex and diverse adaptation mechanisms
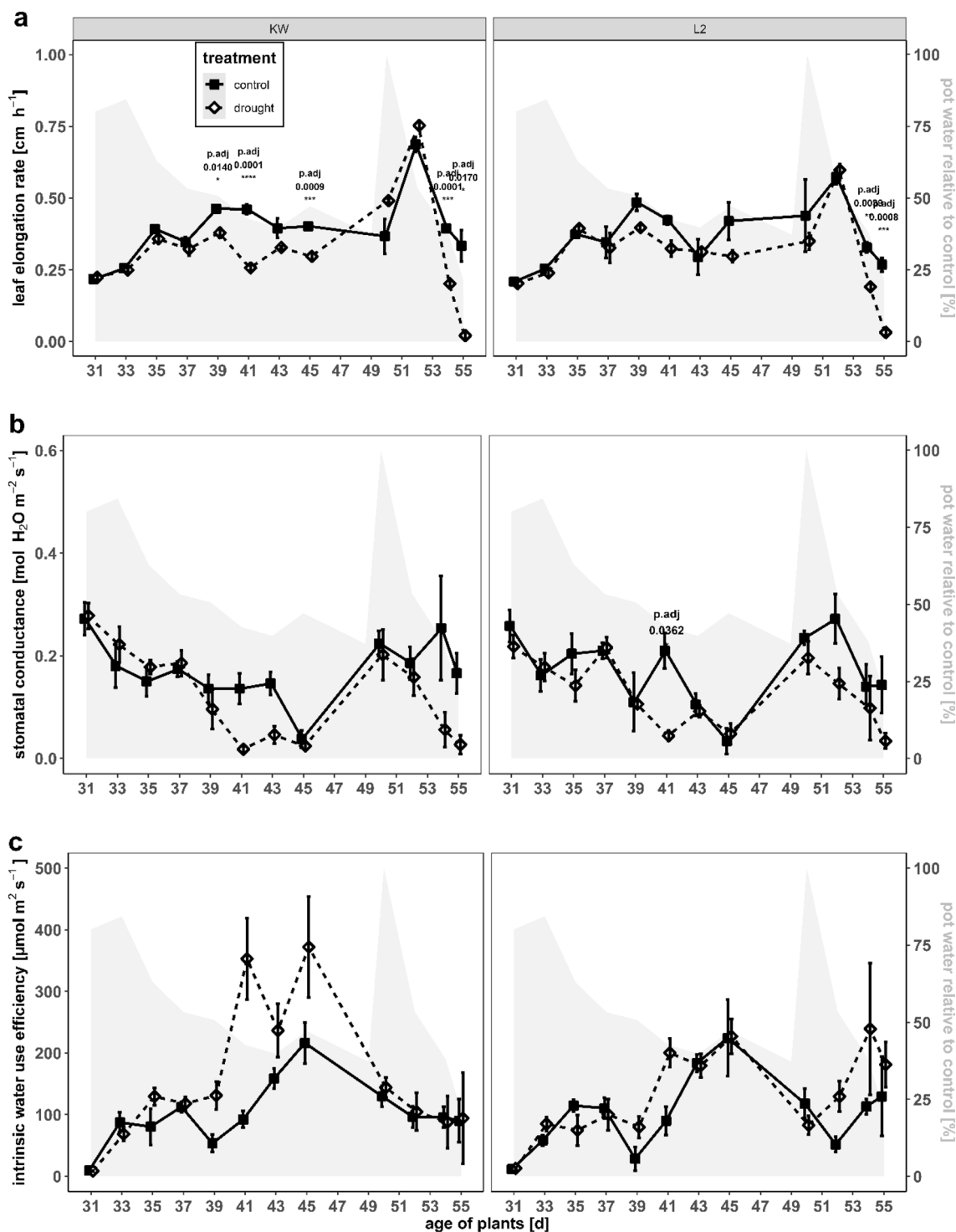
Fig. 5 Leaf elongation rate (**a**), stomatal conductance (**b**) and intrinsic water-use efficiency (**c**) during both stress phases in two contrasting hybrids KW and L2. The grey area indicates pot water content in drought-treated plants relative to controls. Asterisks indicate significant differences between treatments for individual days at the significance level of adjusted $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***)

in order to cope with the fluctuating environmental conditions (Levitt 1972). Consequently, different genotypes may explore these mechanisms to a different extent and drought resistance in crops is usually defined as the ability to

accumulate biomass under limited water supply compared to the well-watered control plants (Tardieu et al. 2018). Based on this, from the 8 maize hybrids used in the present study, only the hybrid L5 had a nonsignificant reduction in biomass

and would be considered drought resistant after two consecutive drought stress periods, whereas all other hybrids would be classified as more or less similar with respect to drought tolerance (Fig. 2). The fact that hybrid L5 was much smaller than the other hybrids under well-watered conditions, (Fig. 2) might support the observation that maize plants with higher yield under non-stress conditions are more sensitive towards stress (Su et al. 2019), but this is only partly true for the other hybrids studied in this experiment. However, analysing a set of physiological measurements, the drought-treated L2 was clearly separated in a PCA (Fig. 4) from the other hybrids and was located between stressed and control plants at both stress periods according to PC1, whilst the other hybrids clustered together into their respective treatment groups (Fig. 4). The PCA and the corresponding correlation matrix (Table S2) indicated that RWC, EL and A/PAR$_{leaf}$ were mainly responsible for the spacial separation of the control and drought treatments along PC1, suggesting that these physiological factors may represent the main difference between L2 and the other hybrids. This is also supported by the fact that the distinction of L2 from the other hybrids was also visible, when input data for the PCA were reduced to EL, RWC and A/PAR$_{leaf}$ (Supplementary Fig. 3), indicating that for rapid screenings, it might suffice to determine these factors in order to identify drought-resistant hybrids. The distinction of L2 was further supported by individual measurements (Fig. 3), which indicate that membrane damage and inhibition of photosynthesis were relatively less severe in this hybrid L2. Nevertheless, the biomass reduction of L2 was similar to most other hybrids, whereas hybrid L5 did not show any growth reduction, but was not specifically outstanding with respect to EL, RWC and osmolality. These results indicate that drought resistance is a multifaceted trait, which can be observed at many different levels in different maize genotypes. Moreover, within groups of hybrids with similar growth reduction under drought, few physiological factors can be used to identify candidates with a different (better) physiological adaptation to drought.

Two Hybrids with Similar Growth Reduction Under Drought Express Different Physiological Drought Stress Responses

In the present study, the most contrasting hybrids after both stress periods were L2 and KW (Fig. 4), even though both hybrids showed the same biomass reduction (Fig. 2, Table 2). This observation led us to suggest that KW and L2 might have different strategies for water management under drought, and/or different abilities to recover from an initial stress event. Both hybrids were nearly similar in their development (since the first drought stress treatment starting at BBCH 16) with only slight differences after two drought treatments; therefore, we think there is no remarkable

effect of different development on the physiology of both hybrids (see Fig. 1c development just before onset of second drought).

In the present study, L2 maintained a higher RWC than KW, especially after the second drought event (Fig. 3b, Table 2). Non-invasive measurements of relative fluxes of leaf water content during the first stress period (Supplementary Fig. 5) also confirm higher leaf water content for L2, possibly indicating a more water-saving strategy for this hybrid. According to their responses to declining soil water, plants can be categorized into isohydric or anisohydric behaviour. Whilst isohydric plants show a tighter stomatal limitation depended on fluctuations in soil water potential, leaf water potential and evaporative demand, anisohydric plants are characterized by greater fluctuations in leaf water potentials between the water treatments and lesser stomatal limitation (Tardieu and Simonneau 1998). In this regard, L2 seems to exhibit a rather isohydric and KW a more anisohydric behaviour. It is interesting that despite the observed differences in water content, no major (significant) differences were observed in stomatal conductance between the hybrids during the stress or after water supply, indicating that other mechanisms than closing of stomata must lead to the higher RWC in L2. One other option to maintain water relations might be the accumulation of osmotically active compounds, which would lower the osmotic potential of the cell sap and thus would lead to a higher water flux into cells. However, measurement of the cell sap osmolality did not indicate a major difference between both hybrids (Fig. 3c, Table 2). Indeed, compared to the other hybrids, L2 and KW maintained a low to intermediate level of osmotic potential especially after the second drought period (Fig. 3c, Table 2). It is possible that a better hydraulic conductivity through osmotic adjustment of the root–stem–leaf continuum (Westgate and Boyer 1985) might aid in maintenance of higher relative water content in the plant, but further measurements would be required to answer this question.

In addition, hybrid L2 maintained a low relative EL after both stress periods with only 9–16% increase compared to controls, whilst EL was elevated by 100–117% in hybrid KW (Fig. 3a, Table 2). Increased production of ROS under drought stress conditions can damage membranes and proteins leading to reduced photosynthetic rate and reduced growth and ultimately cell death (Carvalho 2008). It seems that L2 was able to protect its membranes much better from oxidative damage. Different tolerance mechanisms can contribute to such a protection, e.g. an overall better antioxidative capacity, but also a higher ability to redirect more resources to stress defence and repair mechanism (Crisp et al. 2016). The latter, however, comes at a cost because detoxification of ROS require large amounts of reductive power and energy (Pinheiro and Chaves 2011), and these resources are then not available for rapid regrowth after

cessation of the stress (Crisp et al. 2016). This was indeed observed in the present study, where the LER of hybrid KW was almost immediately resumed after a water pulse, whilst the LER of L2 recovered more slowly and reached control levels two days after KW (Fig. 5a).

Growth inhibition due to osmotic stress is one of the earliest stress-related symptoms of plants under drought (Chaves et al. 2003; Zörb et al. 2019) and can be witnessed even when wilting symptoms are not yet visible (Ribaut et al. 1997). The rate of growth resumption after rewatering reflects the capability of the plant to return to a phenological pre-stressed state and is relevant for recovery in situations where recurrent droughts might occur. With respect to this trait, hybrid KW can be considered to be well adapted to a short drought followed by rewatering (Xu and Zhou 2007; Avramova et al. 2015), and the fast recovery of the growth rate in KW after rewatering might indicate that this hybrid is likely resilient to xylem cavitation and embolism.

Overall, both hybrids had very different strategies to cope with drought stress, which might be beneficial in different environments and under different drought stress scenarios. Whilst L2 seems to be better adapted to resist a prolonged mild to intermediate drought stress event by investing into cell protection and maintenance of water relations, KW seems to be more prepared to recover rapidly from drought periods after rewatering by efficiently using small water pulses for growth. In the setting of the present experiment, both strategies were similarly successful with respect to biomass production after a series of two consecutive stress events. Even though this needs to be further tested in field studies, we conclude here that the investment of hybrid L2 into cell protection mechanisms might be beneficial during individual stress events, such as end of season drought, but rather ineffective in a setting of rapidly recurring drought events. Especially during early season growth, where drought periods are typically short but repeated, recovery-oriented genotypes such as hybrid KW might be able to produce more biomass during vegetative growth, which is relevant for later yield formation. A similar study conducted on maize seedlings of different inbred lines also suggests that the recovery trait is a key determinant of total drought adaptability (Chen et al. 2016). However, the question whether these observations hold true for subsequent drought-recovery events remains open.

In our study (Fig. 5b, c), the iWUE (defined as ratio between assimilation rate and stomatal conductance) was increased in both hybrids (but to a larger extent in KW) during the first drought stress, indicating that despite a reduction in stomatal conductivity, the assimilation rate was not or only slightly reduced. Such a modulation of the WUE to counteract inefficient CO_2 fixation under conditions of limited stomatal conductance is commonly observed under osmotic stress (Bramley et al. 2013) and can give rise to enhanced photosynthesis and even over-compensation of LER after rewatering (Xu et al. 2010). This effect was also observed in the present study for hybrid LW (Fig. 3a). However, during the second stress event, iWUE was increased only in hybrid L2, but not in KW. Since stomatal conductance decreased in both hybrids to a similar extent during the second stress, these results suggest that the assimilation rate was not maintained during the second drought period in hybrid KW. Given that KW resumed growth immediately upon rewatering, it seems possible that metabolic pools were rapidly exhausted upon rewatering, either to support growth or to maintain repair mechanisms needed to reinstall membrane integrity. It remains to be shown whether this effect is long-lasting and might negatively affect responses of this hybrid to subsequent drought events.

Conclusion

The data presented here suggest that different stress responses may be equally effective in providing resistance towards drought at least in a relatively short drought-rewatering cycle. With respect to relative aboveground biomass production, protection of membranes and stabilization of leaf water content might help to better endure an individual stress event, whilst a high recovery potential might be an advantage under recurring drought stresses.

Within a group of hybrids with similar growth reduction under drought, physiological factors such as EL, RWC and A/PAR$_{leaf}$ can thus be used to identify candidates with a different (better) physiological adaptation to drought.

Our results confirm the value of the recovery potential in overall drought resistance under natural conditions, which include periodic cycles of drought and rewatering. Nevertheless, a follow-up study is required to explore the possibility of metabolic exhaustion following the initial rapid re-growth after rewatering in the recovery-oriented strategy.

We suggest to consider local climatic conditions when screening for drought-resistant maize genotypes. In areas where short-term drought spells are predicted to increase during early season growth, repeated drought settings should also be included.

Author contributions CZ and CMG designed the project together with the help from MK. MK, XZ, BLF were making the experiment. MK, CMG, MW and CZ were doing the data analysis. MK, CZ, CMG, MW

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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