



ELSEVIER

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Yield-related phenotypic traits of drought resistant maize genotypes

Mateus Vilela Pires^a, Evaristo Mauro de Castro^a, Bethânia Silva Morais de Freitas^a,
Jean Marcel Souza Lira^a, Paulo Cesar Magalhães^b, Marcio Paulo Pereira^{a,*}

^a Department of Biology, Structural Botany Sector, Federal University of Lavras, 37200-000 Lavras, Minas Gerais, Brazil

^b National Maize and Sorghum Research Center, EMBRAPA, 35701-970, Sete Lagoas, Minas Gerais, Brazil

ARTICLE INFO

Keywords:

Relative distance plasticity index
Rhizotrons
Leaf anatomy
Water deficit
Zea mays L.

ABSTRACT

The aim of this study was to evaluate two maize genotypes with contrasting drought-resistant phenotypic traits and to characterize how the plastic phenotypic traits contribute to yield potential under drought. The maize genotypes DKB 390 and BRS 1010 present contrasting yield traits in relation to drought and were treated with two water regimes: well-watered plant (WW, ≈ -2 kPa) and water deficit (WD, ≈ -100 kPa). In WD conditions, DKB 390 had a greater capacity to accumulate biomass in shoots than BRS 1010, indicating that shoot traits are important in increasing yield gain in the DKB 390 drought tolerant genotype and therefore, an important phenotypic trait for breeding for high yield in drought conditions. However, neither the total root dry mass nor its ratio to the shoot was related to increased yield. A large reduction in gas exchange and increased water use efficiency was observed in BRS 1010 compared to DKB 390. These traits are likely related to yield loss mainly due to decreased transpiration rate and stomatal conductance, which led to low photosynthetic rates and reduced growth. The root angle between genotypes was a constitutive trait and can be primarily responsible for the differences in yield. However, this trait in the DKB 390 genotype was affected by WD, as indicated by narrowing of the root angle. Our work describes the relationship between the narrow root growth angle in DKB 390 and its association with an increased capacity for water uptake in deeper regions, which may result in higher yield under drought. Regarding leaf anatomy we hypothesized that both osmotic adjustment and cell wall properties can influence anisotropic cell expansion under WD conditions and result in leaf tissue with higher yield potential in DKB 390. The high plasticity of vascular tissues and wide vessels could indicate a greater propensity for embolism and a slower response to WD, leading to yield loss in BRS 1010. The results of the phenotypic plasticity of maize genotypes show a negative relationship between high plasticity and yield potential and suggest that high plasticity can result in higher yield potential only when are present in phenotypic traits responsible for higher water uptake and drought tolerance.

1. Introduction

Soil resource acquisition is a primary limitation to crop production (Lynch, 2013) and drought stress is one of the main limitations of global agricultural production due to the complexity of water limitation and climate change. Plants have evolved a series of mechanisms at the morphological, physiological, biochemical, cellular, and molecular levels to overcome water deficit and drought stress conditions (Fang and Xiong, 2015).

Drought resistance in its physiological context is defined according to Levitt (1972) as being determined by 'dehydration avoidance' and/or 'dehydration tolerance'. Dehydration tolerance is the ability of a plant to function in a dehydrated state, whereas dehydration avoidance is defined as the capacity for plants to sustain a high water status or

cellular hydration under the effects of drought. Dehydration-avoidant phenotypes are rarely compatible with high yield because these strategies such as stomatal closure, reduced leaf area, and senescence of older leaves function to minimize water loss (Blum, 2005; Lopes et al., 2011). There is inherent conflict between biomass accumulation and stress avoidance because reduced transpiration rates affect photo-assimilate acquisition which is dependent on stomatal aperture and leaf area (Araus et al., 2008; Blum, 2011, 2009; Lopes et al., 2011). Another reason for a negative relationship between yield potential and drought resistance (mainly dehydration avoidance) is the fact that at least in cereals, high yield and a large sink constitute a load on the shoot in terms of its water status and turgor maintenance under drought stress (Blum, 2005). Therefore, while improved yield potential can translate into better performance under stress, it also places a greater demand on

* Corresponding author.

E-mail address: marciopaulo@hotmail.com (M.P. Pereira).

<https://doi.org/10.1016/j.envexpbot.2019.103962>

Received 2 September 2019; Received in revised form 2 December 2019; Accepted 5 December 2019

Available online 11 December 2019

0098-8472/ © 2019 Elsevier B.V. All rights reserved.

water and other resources. Thus, in conditions with limited soil water availability, a higher capacity for water uptake by the plant can sometimes result in an increased frequency of stress experience. Accordingly, a higher yield potential should be accompanied by enhanced stress tolerance (Lopes et al., 2011).

Currently targeted approaches to drought tolerance should consider root traits such as soil moisture capture for transpiration, root architecture, and improvement of effective use of water through increased stomatal transpiration rates or stay green (Hausmann et al., 2002; Blum, 2009; Lopes et al., 2011). Certain genetic or developmental constraints can be functionally overcome by changes in other, more plastic traits. Consequently, evolution of architectural features cannot be interpreted without a minimum understanding of the plasticity of involved phenotypic traits in response to the environment (Pugnaire and Valladares, 2007). In this context, studies that evaluate contrasting phenotypic traits to drought resistance may help identify plastic phenotypic traits that are responsible for yield gain in drought-tolerant maize genotypes and can improve breeding programs focused on increasing plant yield in drought conditions. It is of critical importance to evaluate these crop strategies under different droughts/management scenarios and include genotypes with well-understood and contrasting physiological and structural characteristics would assist this effort (Gleason et al., 2019). In addition, it is important to show the relationship between phenotypic plasticity and yield potential under drought to understand the role of trait plasticity in yield gain in different maize genotypes. Therefore, the aim of this study was to evaluate two maize genotypes with contrasting drought-resistant phenotypic traits and to characterize how the plastic phenotypic traits contribute to yield potential under drought.

2. Materials and methods

2.1. Plant material

The maize genotypes DKB 390 and BRS 1010 present contrasting yield traits in relation to water deficit (Souza et al., 2013a, 2013b; Lavinsky et al., 2015; Magalhães et al., 2015; Avila et al., 2016), including grain yield and yield gain (Table 1). The seeds for these maize genotypes were made available by the National Maize and Sorghum Research Center, EMBRAPA - Sete Lagoas MG, Brazil.

2.2. Experimental design

The experimental design was completely randomized in a 2×2 factorial scheme with eight replications and an experimental unit of one maize plant. The two maize genotypes were treated with two water regimes: well-watered plant (WW = 24% of moisture) and plant under

Table 1

Yield of maize genotypes with contrasting traits for grain yield under water deficit.

Grain yield traits	(Lavinsky et al., 2015)		(Avila et al., 2016)		Yield gain DKB 390 (%)
	DKB390	BRS1010	DKB390	BRS1010	
NGE	456.30 a	291.10 b	—	—	36.20
DGW (g)	98.92 a	53.83 b	111.78 a	57.99 b	46.92
ED (mm)	49.51 a	45.26 b	36.88 a	21.83 b	22.34
NGR	34.67 a	23.16 b	21.00 a	10.00 b	40.43
W100 (g)	21.87 a	18.42 b	—	—	15.77
EL (cm)	17.42 a	13.60 b	15.93 a	7.25 b	37.48
HI (g g^{-1})	0.40 a	0.27 b	0.51 a	0.32 b	35.51

Yield gain under water deficit in genotype DKB 390 was calculated in relation to BRS 1010 in the same water condition. NGE: number of grains per ear, DGW: dry grain weight, ED: ear diameter, NGR: number of grains per row, W100: weight of 100 grains, EL: ear length, HI: harvest index. Means with the same letters are not significantly different by the Scott-Knott test ($P \leq 0.05$).

water deficit (WD = 10% of moisture). The matric potential of the compost was ≈ -2 kPa for WW and ≈ -100 kPa for WD.

2.3. Plant growth conditions

Maize seedlings were germinated on filter paper until primary roots reached a length of ~ 1 cm (about 5 d at $22\text{--}25^\circ\text{C}$). Seedlings of both genotypes with approximately the same size were selected and transferred into rhizotrons (size: $42.0 \times 29.7 \times 3.0$ cm) containing 2.6 L compost (1:1 washed sand and commercial substrate). The rhizotrons were tilted 43° towards the horizontal plane with the transparent plate facing downward to direct root growth toward the transparent plate and improve root visualization.

The plants were grown in a greenhouse at $25 \pm 2^\circ\text{C}$ with a relative humidity of 50%, photosynthetically active radiation (PAR) of $\approx 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 12 h/12 h day/night light conditions.

On the first day of the experiment, both treatment groups were well watered with a nutrient solution (Hoagland and Arnon, 1950) at 40% ionic strength. At the end of the first week, the water regimes were applied at the V1 maize growth stage. During the following 23 d, the compost moisture was monitored using resistive moisture sensors with an LM393 voltage comparator module and Arduino Mega 2560 microcontroller that could automatically trigger the irrigation pumps as soon as the compost moisture reached a predetermined resistance value specific to each water regime. The moisture sensor probes were positioned and two dripping stakes at the bottom and top of the rhizotron, respectively. Therefore, the maize plants were maintained in the same conditions from sowing to harvesting for a total of 30 d.

2.4. Plant growth analysis

Plant growth assessment was performed 23 d after application of the water regimes. The plant height was measured using a tape measure from the compost surface to the highest point of the arch of the uppermost leaf whose tip was pointing down. The root system was washed in water to remove the compost before sampling. The leaves were scanned in an A3 Scanner (1200S, Mustek, China) so that the leaf area of all leaves was determined using image analysis with ImageJ software. Roots, stems and leaves were dried at 60°C until they reached a constant weight and then dry mass was determined with an analytical balance (AY220, Shimadzu, Sao Paulo, Brazil). The root/shoot ratio (g g^{-1}), leaf area ratio (LAR) ($\text{cm}^2 \text{g}^{-1}$), and leaf mass ratio (LMR) (g g^{-1}) were calculated.

2.5. Leaf water potential (Ψ_w)

Leaf water potential (LWP) was taken on the twenty-eighth day of the experimental period, which corresponded to 21 d of WD. A Scholander portable pressure chamber (model 1.000; PSM Instrument Company, Corvallis, Oregon, USA) was used to estimate LWP. The pressure chamber used N_2 gas to apply the required pressure. Evaluations were made using the fifth fully expanded leaf with apparent ligules. The equilibrium pressure required to bring water to the cut made in the midrib was recorded as leaf water potential.

2.6. Gas exchanges analysis

Gas exchange measurements were recorded using an infrared gas analyzer (IRGA) model LI-6400XT (Li-COR Biosciences, Lincoln, Nebraska, USA), a 6 cm^2 cuvette and a red/blue LED light source (LI-6400-02B, Li-Cor, Lincoln, Nebraska, USA). These evaluations were made on the fourth fully expanded leaves with apparent ligules. The measurements were made between 08:00 and 11:00, and the photosynthetic photon flux density (PPFD) was standardized at $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ in an equipment cuvette. The leaf vapor pressure deficit was 1.63 kPa, the pump flow was $500 \mu\text{mol s}^{-1}$ and the block

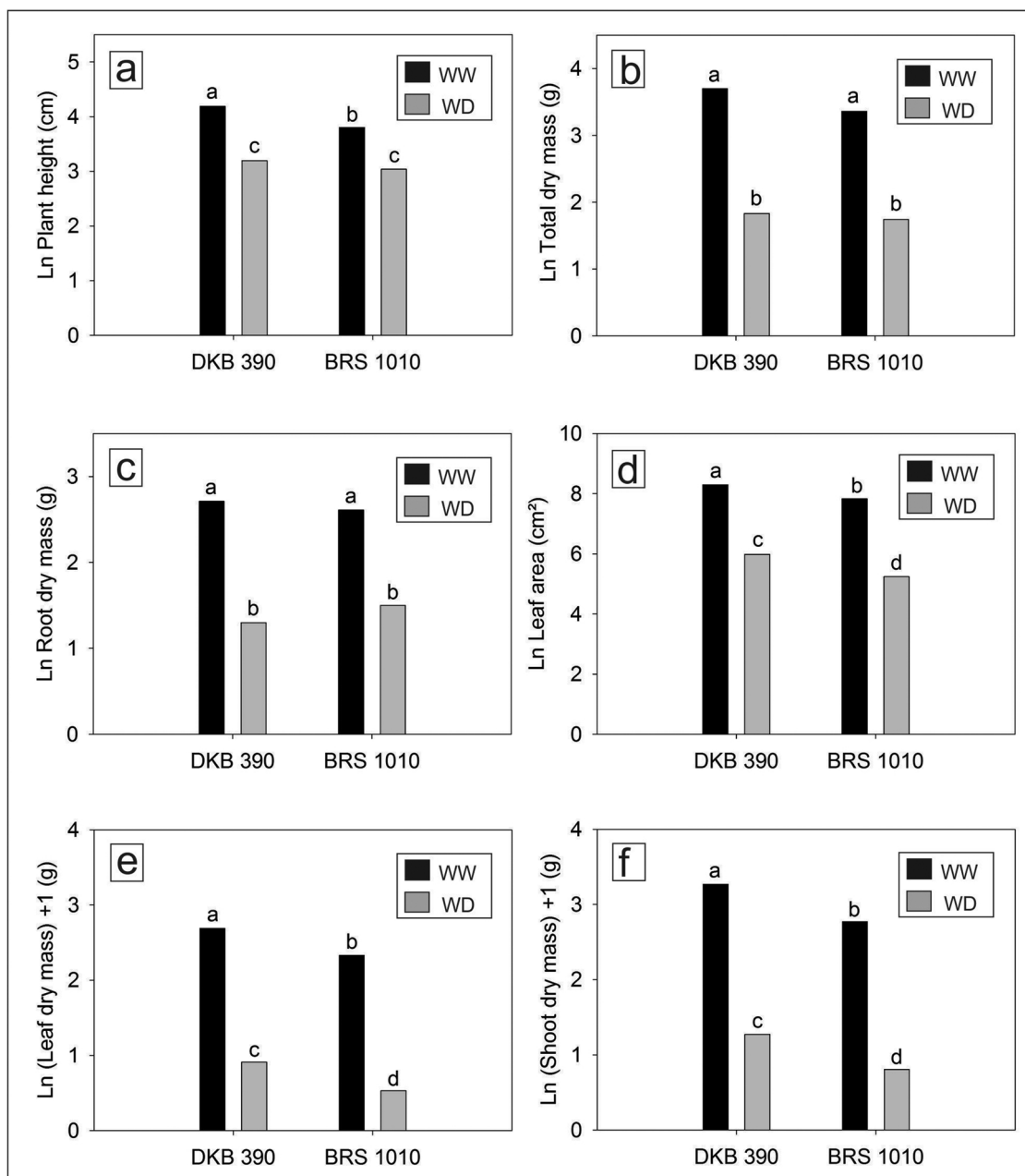


Fig. 1. Plant growth traits in DKB 390 and BRS 1010 maize genotypes in the well-watered (WW) and water deficit (WD) regimes. All data were normalized (Ln) and units refer to untransformed data. Means with the same letters are not significantly different from one another by the Tukey's test ($P \leq 0.05$).

temperature was 28.26 °C. In addition, the mixer function used carbon dioxide capsules to provide 380 ppm of CO₂ during the analyses.

The net photosynthesis rate (A), stomatal conductance to water vapor (g_{sw}) and transpiration rate (E) were evaluated, and water use efficiency (WUE) was calculated.

2.7. Moisture content and root system angle

Rhizotrons were imaged using an A3 Scanner (1200S, Mustek, China), and ImageJ and Corel Photo Paint X7 software were used for image processing. In ImageJ, the images were stacked and a Z-stack (median) was constructed to obtain mean gray-scale intensity values from 70 images per treatment. The moisture content in the rhizotrons was visualized by differences in gray-scale intensity values from the Z-stacks and improved visualization of moisture distribution using a 16 color look-up table (LUT) (Rellán-Álvarez et al., 2015).

The root system architecture was made with eight stacked images (frame), and a Time-Lapse Color Coder (LUT-Spectrum) was used to obtain images of the root system architecture. The root system angle was measured with ImageJ software.

2.8. Plant anatomy

Anatomical assessment was conducted on the fourth fully expanded leaves with visible ligules. Leaves were fixed at 70% FAA (Johansen, 1940) and dried with increasing ethanol concentrations (70, 80, 90, and 100%) at 2-h intervals and embedded in historesin according to the manufacturer's instructions (Leica Microsystems, Wetzlar, Germany). The histological cross sections (8- μ m thick) were made using a semi-automated rotary microtome Yidi YD-335 (Jinhua Yidi Medical Appliance Co., LTD, Zhejiang, China). The sections were stained with 1% toluidine blue (w/v) (Feder and O'Brien, 1968) and mounted on slides

with Entellan (Merck, Darmstadt, Germany).

Paradermal sections were made freehand with the help of a steel blade and then clarified with 50% sodium hypochlorite and washed in distilled water. The sections were stained with safranin and mounted on semi-permanent slides with 50% glycerol (Johansen, 1940).

The slides were imaged using a microscope with an attached image capture system (Eclipse E100-LED; Nikon, Tokyo, Japan), and quantitative anatomical analysis was performed in ImageJ.

The following anatomical traits were evaluated in the leaf cross section: xylem area, phloem area, vascular bundle area, metaxylem diameter and mesophyll thickness. In the paradermal sections: stomatal density and epidermal cells density were evaluated in both sides of the epidermis.

2.9. Relative distance plasticity index

The relative distance plasticity index (RDPI) was calculated as previously described (Valladares et al., 2006). We tested eight individuals of each maize genotypes ($j = 1, 2, 3, 4, 5, 6, 7$ and 8) in each water regime ($i = 1, 2$). The phenotypic plasticity for a given variable x can be related to the difference of x between two individuals (j and j') of the same genotype grown under different water regimes (i and i') (Marchiori et al., 2017). RDPI was calculated as $\Sigma(d_{ij} \rightarrow i'j') / (x_{ij'} + x_{ij}) / n$ where n is the number of distances and has been described previously (Valladares et al., 2006). The RDPI was calculated with the data for a given variable x obtained 30 d into the experimental period (from sowing to plant harvest).

2.10. Data analysis

Statistical analyses were performed using R software version 3.5.1 (R Core Team, 2018). The data were submitted to tests of normality (Shapiro-Wilk), a homoscedasticity test (Breusch-Pagan) and independence (Durbin-Watson). Non-normal data were transformed (log and box cox). For genotype comparisons, water regime levels, and interaction effects, a two-way ANOVA was used. The means were compared with the Tukey's test and significant difference values were reported when the F test was significant at $P \leq 0.05$.

3. Results

3.1. Plant growth

All plant growth traits were significantly different in the WD regime compared to WW conditions; however, the genotypes were not affected similarly by drought conditions. The plant height in the WD regime did not differ between genotypes, although DKB 390 was taller in WW conditions than BRS 1010 (Fig. 1a). The root dry mass and total dry mass was not significantly different between the genotypes in WD conditions (Fig. 1b and c); however, the BRS 1010 had a smaller leaf area, lower leaf dry mass and shoot dry mass than DKB 390 in WD (Fig. 1d, e and f).

The root/shoot ratio increased in the WD regime and BRS 1010 showed a significant increase compared to DKB 390 (Fig. 2a). The BRS 1010 genotype in the WD regime had the lowest LAR and LMR of the two genotypes (Fig. 2b and c).

3.2. Physiological traits

Leaf water potential decreased in the WD treatment; however, there was no significant difference between the maize genotypes in this regime (Fig. 3).

Leaf gas exchange was strongly influenced by WD. The BRS 1010 genotype in the WD treatment had the lowest net assimilation rate (A), transpiration (E) and stomatal conductance (g_{sw}), but higher E and g_{sw} under WW. However, the DKB 390 genotype in WD conditions did not

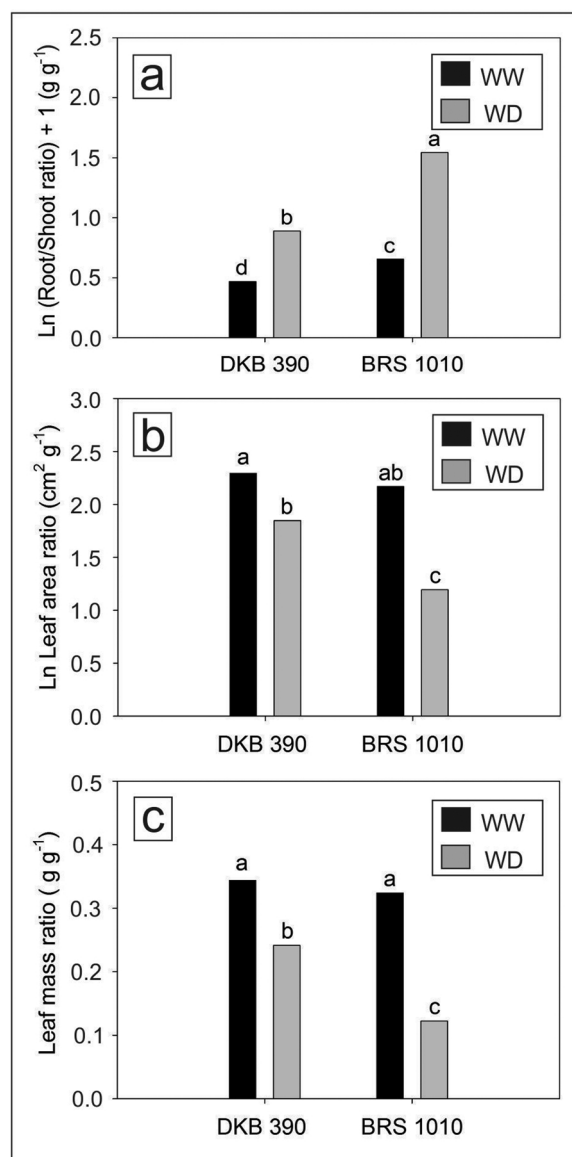


Fig. 2. Physiological growth indexes of DKB 390 and BRS 1010 maize genotypes in the well-watered (WW) and water deficit (WD) regimes. Root/shoot and leaf area ratios were normalized (Ln) and units refer to untransformed data. Means with the same letters are not significantly different from one another by the Tukey's test ($P \leq 0.05$).

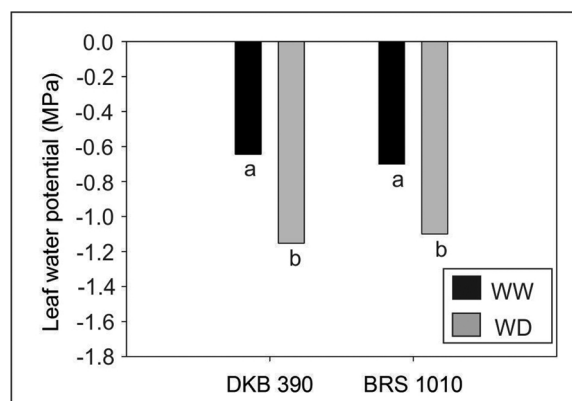


Fig. 3. Leaf water potential in DKB 390 and BRS 1010 maize genotypes in well-watered (WW) and water deficit (WD) regimes. Means with the same letters are not significantly different from one another by the Tukey's test ($P \leq 0.05$).

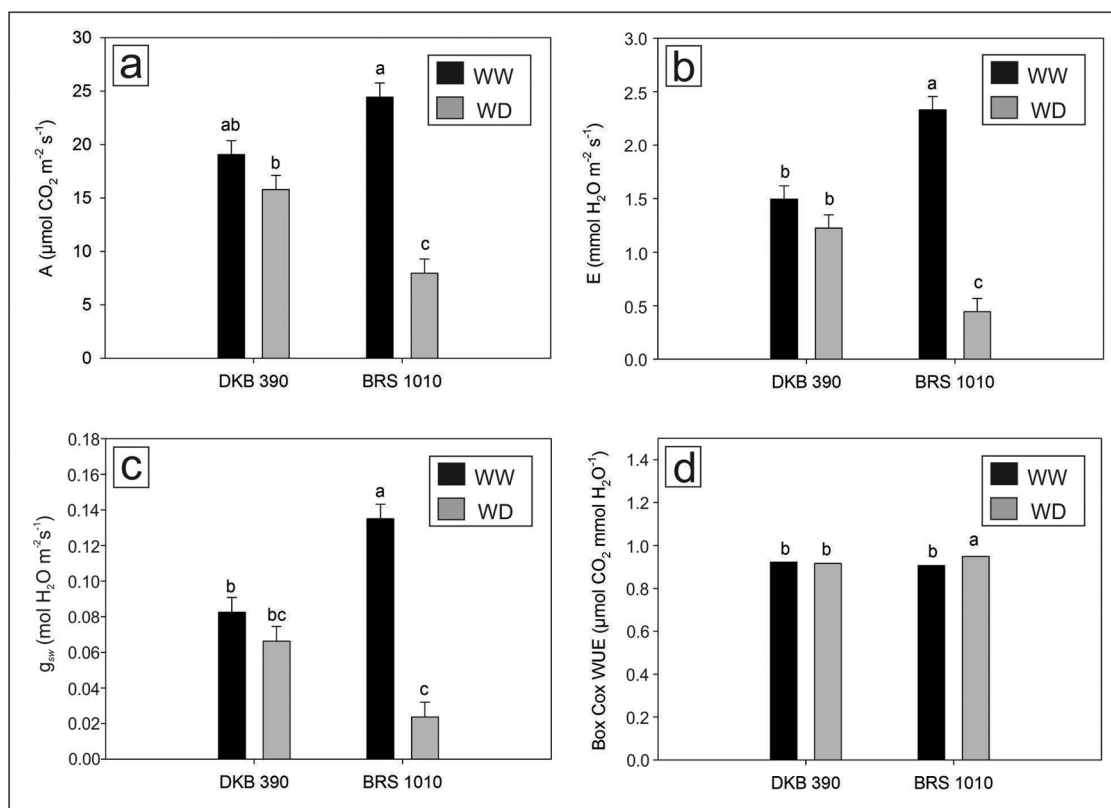


Fig. 4. Leaf gas exchange in DKB 390 and BRS 1010 maize genotypes in well-watered (WW) and water deficit (WD) regimes. Water use efficiency data was normalized (box cox) and units refer to untransformed data. A: net assimilation rate, E: transpiration, g_{sw} stomatal conductance to water vapor and WUE: water use efficiency. Means with the same letters are not significantly different from one another by the Tukey's test ($P \leq 0.05$).

show a significant decrease in A, E and g_{sw} compared to the WW regimen (Fig. 4a, b and c).

Water use efficiency (WUE) was higher in BRS 1010 in WD conditions compare to DKB 390, while WUE as a function of the WD regime did not change in the DKB 390 genotype relative from the WW condition (Fig. 4d).

3.3. Moisture mapping and root system architecture

Moisture mapping results of the water uptake pattern of genotypes indicated that BRS 1010 in both water regimes presented a greater capacity for water uptake from middle and superficial regions of the rhizotron compared to DKB 390 (Fig. 5b and d). However, DKB 390 genotype under WD presents greater water uptake in deep regions and maintained greater moisture in the middle and lateral regions (Fig. 5a and c).

The root angle of the DKB 390 genotype was characterized by a narrow root angle while BRS 1010 had a wide root angle (Fig. 5). The root angle is a constitutive trait between these genotypes and was only modified by WD in the DKB 390 genotype. (Figs. 5 and 6). Moreover, the WD regimen was able to further narrow the angle of the DKB 390 root system (Fig. 5 and 6).

3.4. Leaf anatomy traits

The WD regimen strongly influenced cell expansion and resulted in a reduction in mesophyll thickness in the BRS 1010 genotype (Fig. 7). However, mesophyll thickness in DKB 390 did not significantly change as a function of WD (Fig. 7)

Leaf epidermis cell traits increased in the BRS 1010 genotype under WD (Fig. 8). The stomatal density (SD) and epidermal cell density (ECD) on both leaf epidermal surfaces were higher in BRS 1010 (Fig. 8).

In contrast, water regime did not affect these epidermal cell traits (SD and ECD) in the DKB 390 genotype (Fig. 8).

All quantitative vascular tissue traits were reduced by the WD in both genotypes (Fig. 9). An important anatomical trait was observed in BRS 1010 grown in the WW condition; this genotype had a larger xylem area, vascular bundle area and metaxylem diameter compared to DKB 390 (Fig. 9). Similar to the effects of water treatment on root angle, in the WD regimen, the DKB 390 genotype has narrow xylem vessels while the BRS 1010 wide xylem vessels, indicating that xylem morphology is a constitutive trait difference between these genotypes that is affected by WD conditions (Fig. 9a, c and d).

3.5. Phenotypic plasticity

The BRS 1010 genotype presented higher RDPI for 79.1% of the rated phenotypic traits. However, DKB 390 showed higher RDPI only in 20.9% of the phenotypic traits. High RDPI was observed mainly in strictly drought-related phenotypic traits (e.g., leaf dry mass, shoot dry mass, leaf area, xylem area, and transpiration (Fig. 10). However, the BRS 1010 genotype showed higher RDPI for these traits. The DKB 390 presented higher RDPI for only a few phenotypic traits that may be related to increased water uptake and drought tolerance (e.g., root dry mass, plant height, root system angle and leaf water potential) (Fig. 10).

4. Discussion

The plant traits that provide drought resistance can be approached in two ways: (a) plant traits that improve crop yield during predictable seasonal soil moisture supply and (b) plant traits that contribute to plant survival in very limited soil water holding capacity (Blum, 2009). Therefore, we will discuss the phenotypic traits that may contribute to higher yield potential during water deficit (WD). We also define yield

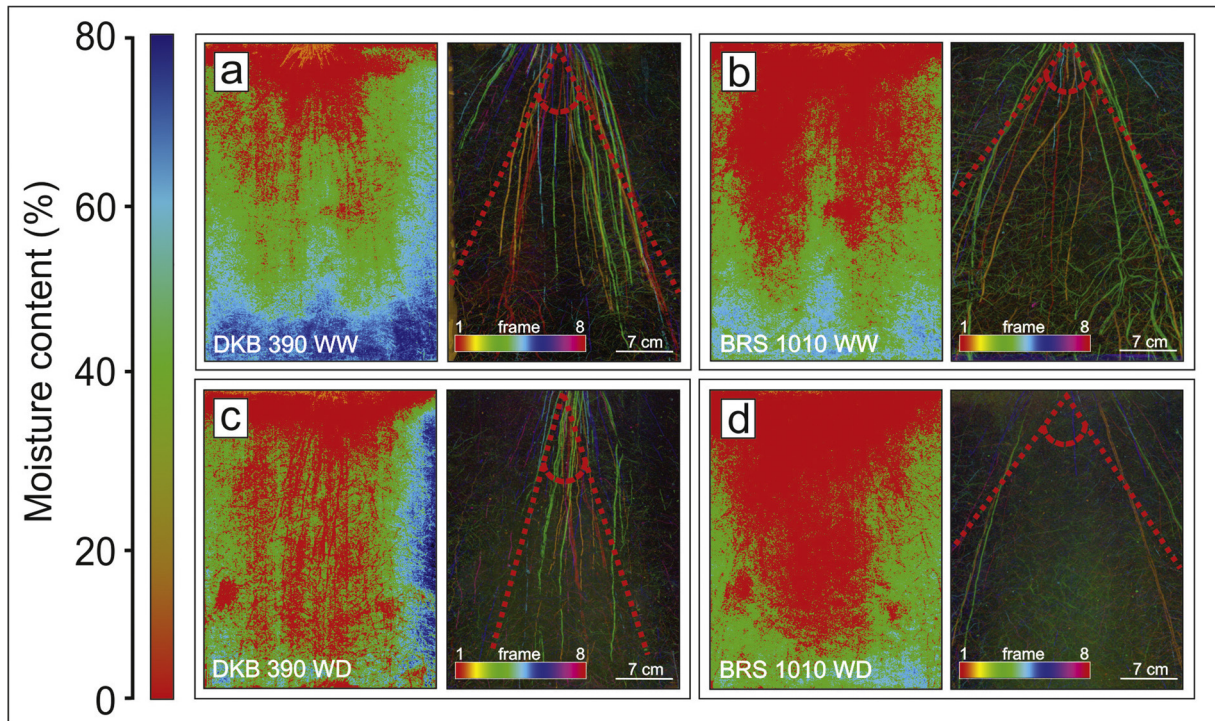


Fig. 5. Moisture content mapping and root angle architecture of DKB 390 and BRS 1010 maize genotypes in well-watered (WW) and water deficit (WD) regimes.

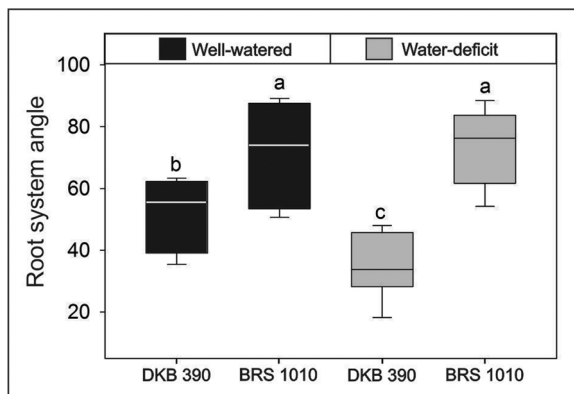


Fig. 6. Root system angle in DKB 390 and BRS 1010 maize genotypes in well-watered (WW) and water deficit (WD) regimes. Means with the same letters are not significantly different from one another by the Turkey's test ($p \leq 0.05$).

potential as the traits that can support yield gain when drought occurs in the maize reproductive stage (e.g., stem reserves for grain filling under drought stress; Blum, 1998).

There was no difference in the total yield between the genotypes under WD (Fig. 1a, b and c), however, there was a differential partitioning between root and shoot dry mass (Fig. 1d, e, f, and Fig. 2a). This differential mobilization of resources has important implications for crop production systems and selection for yield alone (as done historically) puts a selective pressure on morphology and the assimilated partitioning process, but not on basic plant production (e.g., biomass; Blum, 2011). The DKB 390 under WD had a greater capacity to accumulate biomass in the shoot than the BRS 1010 genotype as indicated by its larger leaf area and dry mass of leaves and shoots. In addition, LAR and LMR (Fig. 2b and c) indicated the efficiency with which the plants used their leaves to produce biomass (Allaby, 2006). The shoot traits act as a force that drives the water uptake (by E) through a plant and root system properties determine the plant access to water (Comas et al., 2013). Therefore, our study shows that shoot traits are important

for increasing yield gain in the DKB 390 drought tolerant genotype and are important phenotypic traits for breeding a high yield under drought. Opposing traits like those in the BRS 1010 genotype, illustrate a dehydration avoidance mechanism, where the shoot represents a large sink in terms of its water status and turgor maintenance under drought stress (Blum, 2005).

Root/shoot dry mass ratio increases under drought stress, not because of increased root mass but a relatively greater decrease in shoot mass (Figs. 2a and 1c and f; Blum, 2005). Root mass rarely increases under stress (Blum, 2005); however, root length and depth may increase in a drying soil even at a reduced total root mass (Figs. 5 and 6). Hence, total root dry mass or its ratio to shoot dry mass is not appropriate information for genotypes selection (Figs. 1c and 2a; Blum, 2005).

The genotypes presented different gas exchange patterns and in general, gas exchange in the DKB 390 genotype was not affected by the WD condition compared to the WW regime (Fig. 4). However, the opposite was observed in BRS 1010, which had a significant decrease in A, E and g_{sw} in WD compared to WW regimes (Fig. 4a, b and c). Because stomatal transpiration rate and g_{sw} is related to carbon uptake by stomatal opening and dehydration avoidance is defined as the capacity to sustain high plant water status, the decrease of E and g_{sw} in BRS 1010 may explain the reduction in A and yield loss in this genotype in response to drought conditions (Figs. 3 and 4a). Constitutive whole-plant traits play a major role in water use and plant dehydration avoidance under stress. These traits largely determine the negative relationship between yield potential and the ability to sustain yield under severe water shortage (Blum, 2005).

According to Blum (2005), effective use of water (EUW) and not water use efficiency (WUE) is the target of crop yield improvement under drought stress. Exploiting high instantaneous water-use efficiency (A/T) to breed for greater agronomic water-use efficiency is complicated for cereals due to the association between high A/T and slow crop growth rate (Condon et al., 2004). Improvements in leaf-level water-use efficiency may not always translate into higher crop water-use efficiency or yield. (Condon et al., 2004). Therefore, enhanced biomass production in the DKB 390 drought tolerant genotype (Figs. 1d,

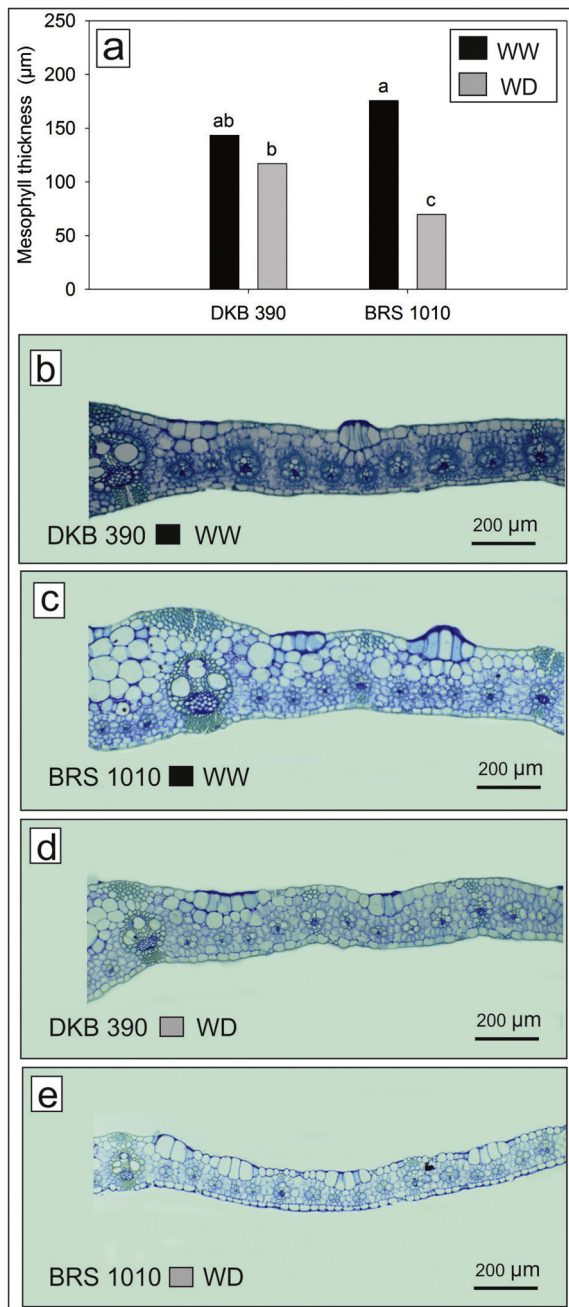


Fig. 7. Mesophyll thickness in DKB 390 and BRS 1010 maize genotypes in well-watered (WW) and water deficit (WD) regimes. Means with the same letters are not significantly different from one another by the Tukey's test ($P \leq 0.05$).

e, f, 2 b and c) can be achieved primarily by maximizing soil water capture while diverting the largest part of the available soil moisture towards stomatal transpiration (Figs. 4b, 5 c and 6 ; Blum, 2009). This action is the major engine for agronomic or genetic enhancement of crop production under a limited water regime (Blum, 2009).

The LWP was the same in both genotypes under WD (Fig. 3); however, the RDPI results showed that the DKB 390 genotype had higher phenotypic plasticity than BRS 1010 (Fig. 10), indicating that LWP is subject to greater changes between WW and WD regimes and this higher plasticity may be an important drought tolerance trait. Bolaños et al. (1993) reported the lack of direct and correlated changes in traits related to plant water status due to selection, which suggests a low heritability of such traits or a weak association with grain yield under severe water stress in the studied maize population. A

physiological reason for different susceptibility to drought between sensitive and resistant genotypes is the increased efficiency of tissue water status protection in resistant genotypes (Grzesiak et al., 2006).

The root angle between the tested genotypes was a constitutive trait significant for improved breeding. However, this trait in the DKB 390 genotype became narrower in the WD conditions compared to WW (Figs. 5 and 6). The optimal root system architecture (RSA) of a crop is context-dependent and critical for efficient resource capture in the soil (Alahmad et al., 2019). Narrow root growth angle promotes deeper root growth and is often associated with improved access to water and nutrients in deep soils during terminal drought (Alahmad et al., 2019). Therefore, our work in controlled conditions shows the relationship between DKB 390 narrow root growth angle and a greater capacity for moisture uptake in deeper soil regions (Figs. 5 and 6). Thus, the narrow angle root system is a useful phenotypic trait for the selection of drought tolerant genotypes and can prevent yield reduction during terminal drought.

The major plant adaptive response to drought at the cellular level, which has an effect on yield under drought stress, is osmotic adjustment (OA). In response to water stress, plants accumulate a variety of organic and inorganic substances such as sugars, polyols, amino acids, alkaloids, and inorganic ions, that get concentrated in the cytochylema, reduce the osmotic potential and improve cell water retention. (Blum, 2005; Fang and Xiong, 2015). As OA helps to maintain higher leaf relative water content (RWC) at low leaf water potential (LWP), OA contributes to sustained growth (i.e., cell expansion) by reducing its LWP so that the plant meets its transpiration demand (Blum, 2011). OA sustains turgor maintenance and hence, yield-forming processes during moderate and severe water stress (Blum, 2005; Ali and Talukder, 2008) and is a component of dehydration avoidance (Blum, 2011). However, in plant cell walls with low expansion resistance, OA does not function in dehydration avoidance and turgor maintenance, but promotes cell expansion in drought tolerant plants (Figs. 7 and 8). In our study, OA may have occurred in both genotypes, indicating that OA influences on cell expansion are dependent on the mechanical properties of the cell wall.

Drought restricts both cell division and expansion, and the molecular mechanisms involved in decreasing cell expansion under drought are an active area of research (Bhaskara et al., 2017; Farquharson, 2017). Factors that regulate cortical microtubule organization and stability likely affect plant growth in drought conditions. The EGR phosphatases and MASP1 protein regulate cortical microtubule stability during drought stress; EGR reduces and MASP1 promotes microtubule stability and anisotropic cell expansion (Bhaskara et al., 2017; Farquharson, 2017). Fine-tuning the activity of these two opposing sets of proteins may improve plant growth and yield during moderate drought (Bhaskara et al., 2017; Farquharson, 2017) and therefore, our results on the quantitative features of leaf anatomy (Figs. 7 and 8) that show that both OA and cell wall properties can influence anisotropic cell expansion under water deficit. Furthermore, our results also show that leaf tissues with high yield potential (DKB 390) or leaf tissues that require low water status (BRS 1010) can be strongly linked to gas exchange and yield results of maize genotypes.

Water transport occurs under high tension within xylem conduits. If this tension becomes too high, the adhesion and cohesion forces holding the water column together may break, forming a quickly expanding embolism and thus rendering the conduit useless for water transport (Gleason et al., 2019). Wide vessels tend to be longer than narrow vessels and more susceptible to cavitation (Comstock and Sperry, 2000). Because of the functional dependence of drought tolerance on xylem cavitation resistance (Lopez et al., 2005), the drought tolerant genotype DKB 390 even under WW has a constitutive narrow vessel, which confers greater hydraulic safety and lower anatomical plasticity compared to BRS 1010 (Figs. 9 and 10). We hypothesized that high plasticity in vascular tissues and wide vessels could indicate a greater propensity for embolism and a slower response to WD that

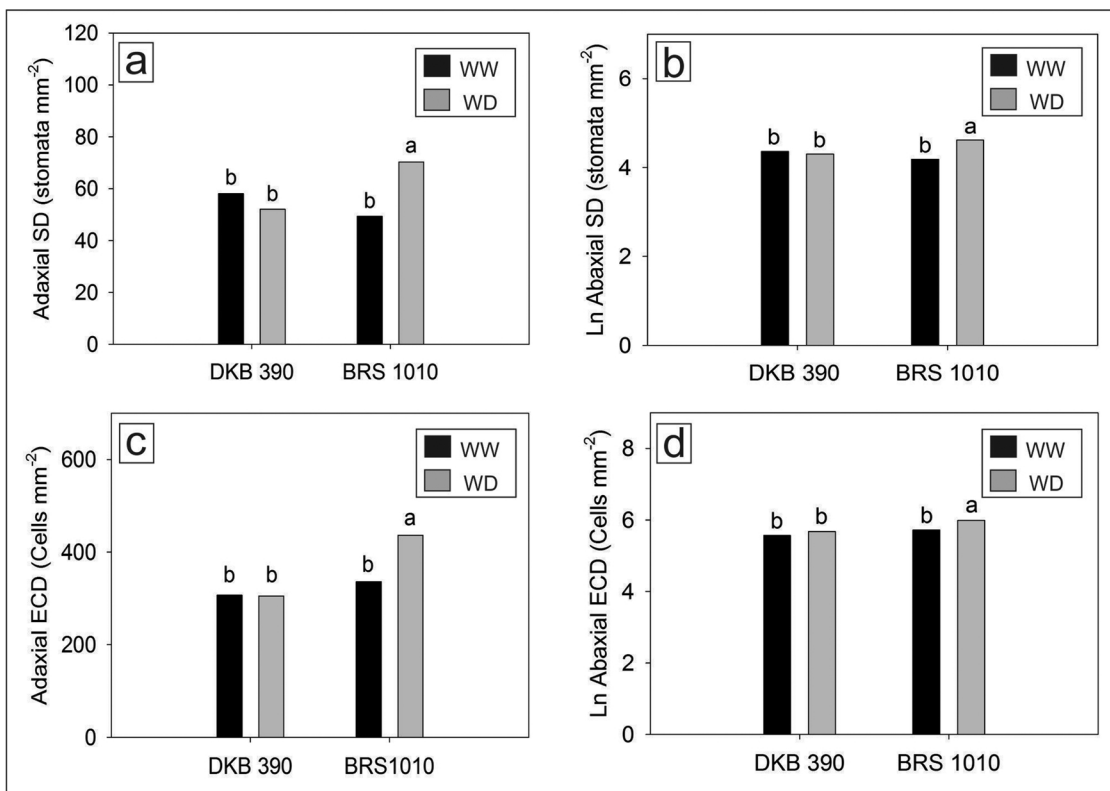


Fig. 8. Leaf epidermis cell traits of DKB 390 and BRS 1010 maize genotypes in well-watered (WW) and water deficit (WD) regimes. Abaxial data were normalized (Ln) and units refer to untransformed data. SD: stomatal density, ECD: epidermal cells density. Means with the same letters are not significantly different from one another by the Tukey's test ($P \leq 0.05$).

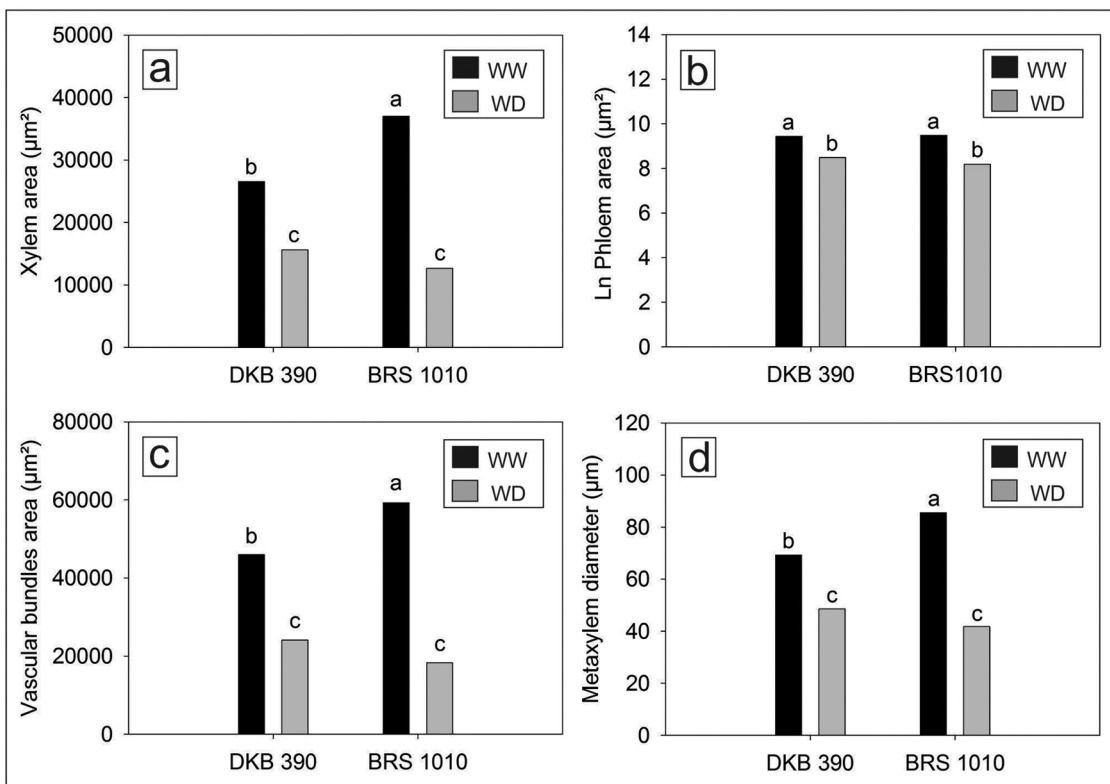


Fig. 9. Vascular tissue traits of DKB 390 and BRS 1010 maize genotypes in well-watered (WW) and water deficit (WD) regimes. Phloem area data were normalized (Ln) and units refer to untransformed data. Means with the same letters are not significantly different from one another by the Tukey's test ($P \leq 0.05$).

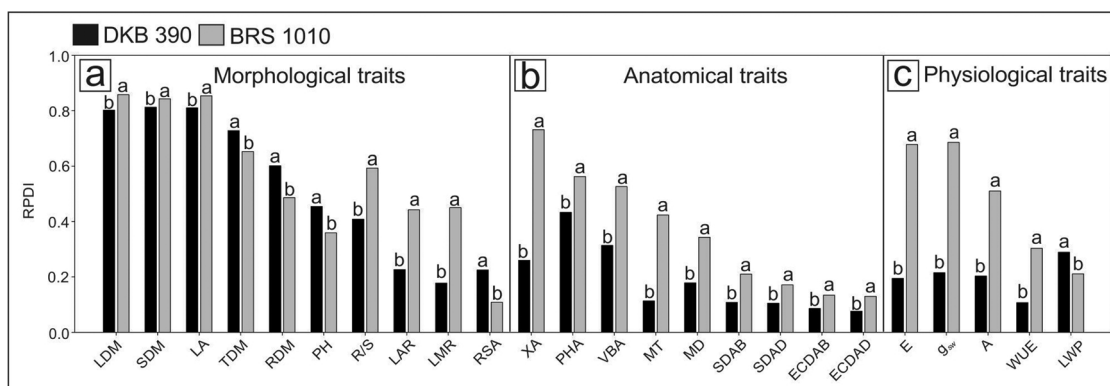


Fig. 10. Relative distance plasticity index (RDPI) in DKB 390 and BRS 1010 maize genotypes. (A) Morphological traits, (B) Anatomical traits, (C) Physiological traits. Means with the same letters between genotypes are not significantly different from one another by the Tukey's test ($P \leq 0.05$). LDM: leaf dry mass, SDM: shoot dry mass, LA: leaf area, TDM: total dry mass, RDM: root dry mass, PH: plant height, R/S: root/shoot ratio, LAR: leaf area ratio, LMR: leaf mass ratio, RSA: root system angle, XA: xylem area, PHA: phloem area, VBA: vascular bundles area, MT: mesophyll thickness, MD: metaxylem diameter, SDAB: abaxial stomatal density, SDAD: adaxial stomatal density, ECDAB: abaxial epidermal cells density, ECDAD: adaxial epidermal cells density, E: transpiration, g_{sw} : stomatal conductance, A: net assimilation rate, WUE: water use efficiency, LWP: leaf water potential.

would lead to yield loss in BRS 1010.

The current interest in plasticity results from a need to predict species responses to global climate change (Potvin and Tousignant, 1996; Rehfeldt et al., 2001) and the importance for understanding trait-mediated species interactions (Callaway et al., 2003; Valladares et al., 2006). Many studies have hypothesized that plasticity functions as a way for plants to adapt to variable environmental conditions. Therefore, plasticity allows a certain degree of adaptation without a need for genetic changes (Potvin and Tousignant, 1996; Valladares et al., 2006).

Wild species are exposed to natural selection for continued survival, while domesticated species are subjected to artificial selection that emphasizes optimal yield (Vilela and González-Paleo, 2015). The literature suggests that plasticity may have changed substantially during the process of domestication; however, most studies focus on yield plasticity, yield components and phenological development (Sadras et al., 2009; Peltonen-Sainio et al., 2011; Mohammadi, 2014; Vilela and González-Paleo, 2015). It is still unclear if selection has led to increased or decreased plasticity in traits indirectly associated with yield (Nicotra and Davidson, 2010). Additionally, a large body of controversial literature exists on the influence of the environment on plasticity (Alpert and Simms, 2002). The maize genotype BRS 1010 has higher plasticity for most phenotypic traits (Fig. 10) and this higher phenotypic plasticity is potentially related to water conservation and high water status maintenance. On the other hand, DKB 390 presented lower plasticity for most phenotypic traits (Fig. 10). However, this genotype has greater plasticity in a few essential phenotypic traits that maintain higher water uptake and drought tolerance to sustain higher yield potential in relation to BRS 1010. The results for phenotypic plasticity of maize genotypes indicated a negative relationship between high plasticity and yield potential and suggest that high plasticity can result in higher yield potential only in combination with phenotypic traits related to higher water uptake and drought tolerance (e.g., root dry mass, plant height, root system angle and leaf water potential).

In conclusion, under water deficit the traits related to high water uptake capacity and high shoot growth in the DKB 390 genotype is responsible for the yield gain of this genotype compared to BRS 1010. These phenotypic traits are important only in drought tolerant genotypes because the higher yield potential of DKB 390 may also be related to its ability to function in a dehydrated state. Dehydration avoidance is incompatible with high yield potential, indicated by the reduction in shoot growth and gas exchange in BRS 1010 genotype, which maintained high water status but is probably related to potential yield loss in drought conditions. Constitutive traits between genotypes that can be enhanced under WD (e.g., narrow root angle and narrow xylem vessels) are traits that may be strongly related to the yield gain in the DKB 390

compared to BRS 1010 genotype. Phenotypic plasticity in traits related to water uptake and drought tolerance is more important to maintain high yield potential than phenotypic plasticity in traits responsible for high water status.

Author contributions

Mateus Vilela Pires: experimental work, anatomical analysis and manuscript review. Evaristo Mauro de Castro: advised the experimental work, technical support, and manuscript review. Bethânia Silva Moraes de Freitas: experimental work, anatomical analysis and manuscript review. Jean Marcel Souza Lira: statistical analysis of data and manuscript review. Paulo Cesar Magalhães: provided the maize genotypes and manuscript review. Marcio Paulo Pereira: co-advisor, experimental work, anatomical, physiological, and phenotypic plasticity analysis and manuscript review.

Funding

This work was supported in part by Coordination for the Improvement of Higher Level Personnel, Financed Code 001 (Capes/PNPD) and the National Council of Technological and Scientific Development (CNPq/PQ and PDJ).

Declaration of Competing Interest

The authors declare no conflict of interest

References

- Alpert, P., Simms, E.L., 2002. The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? *Evol. Ecol.* 16, 285–297. <https://doi.org/10.1023/A:1019684612767>.
- Alahmad, S., El Hassouni, K., Bassi, F.M., Dinglasan, E., Youssef, C., Quarry, G., Aksoy, A., Mazzucotelli, E., Juhász, A., Able, J.A., Christopher, J., Voss-Fels, K.P., Hickey, L.T., 2019. A major root architecture QTL responding to water limitation in durum wheat. *Front. Plant Sci.* 10, 1–18. <https://doi.org/10.3389/fpls.2019.00436>.
- Ali, M.H., Talukder, M.S.U., 2008. Increasing water productivity in crop production-A synthesis. *Agric. Water Manag.* 95, 1201–1213. <https://doi.org/10.1016/j.agwat.2008.06.008>.
- Allaby, M., 2006. *A Dictionary of Plant Sciences*. Oxford University Press, Oxford. <https://doi.org/10.1093/acref/9780198608912.001.0001>.
- Araus, J.L., Slafer, G.A., Royo, C., Serret, M.D., 2008. Breeding for yield potential and stress adaptation in cereals. *Crit. Rev. Plant Sci.* 27, 377–412. <https://doi.org/10.1080/07352680802467736>.
- Avila, R.G., Magalhães, P.C., Alvarenga, A.Ade, Lavinsky, Ade O., Campos, C.N., Souza, T.Cde, Gomes Júnior, C.C., 2016. Drought-Tolerant Maize Genotypes Invest in Root System and Maintain High Harvest Index During Water Stress. *Rev. Bras. Milho e Sorgo* 15, 450–460. <https://doi.org/10.18512/rbms.v15i3.842>.

- Bhaskara, G.B., Wen, T.N., Nguyen, T.T., Verslues, P.E., 2017. Protein phosphatase 2Cs and microtubule-associated stress protein 1 control microtubule stability, plant growth, and drought response. *Plant Cell* 29, 169–191. <https://doi.org/10.1105/tpc.16.00847>.
- Blum, A., 2011. *Plant Breeding for Water-Limited Environments*. Springer-Verlag, New York.
- Blum, A., 2009. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Res.* 112, 119–123. <https://doi.org/10.1016/j.fcr.2009.03.009>.
- Blum, A., 2005. Drought resistance, water-use efficiency, and yield potential - are they compatible, dissonant, or mutually exclusive? *Aust. J. Agric. Res.* 56, 1159–1168. <https://doi.org/10.1071/AR05069>.
- Blum, A., 1998. Improving wheat grain filling under stress by stem reserve mobilisation. *Euphytica* 100, 77–83. <https://doi.org/10.1023/A:1018303922482>.
- Bolaños, J., Edmeades, G.O., Martinez, L., 1993. Eight cycles of selection for drought tolerance in lowland tropical maize. III. Responses in drought-adaptive physiological and morphological traits. *Field Crops Res.* 31, 269–286. [https://doi.org/10.1016/0378-4290\(93\)90066-V](https://doi.org/10.1016/0378-4290(93)90066-V).
- Callaway, R.M., Pennings, S.C., Richards, C.L., 2003. Phenotypic plasticity and interactions among plants. *Ecology* 84, 1115–1128. [https://doi.org/10.1890/0012-9658\(2003\)084\[1115:PPAIAP\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2003)084[1115:PPAIAP]2.0.CO;2).
- Comas, L.H., Becker, S.R., Cruz, V.M.V., Byrne, P.F., Dierig, D.A., 2013. Root traits contributing to plant productivity under drought. *Front. Plant Sci.* 4, 1–16. <https://doi.org/10.3389/fpls.2013.00442>.
- Comstock, J.P., Sperry, J.S., 2000. Theoretical considerations of optimal conduit length for water transport in vascular plants. *New Phytol.* 148, 195–218. <https://doi.org/10.1046/j.1469-8137.2000.00763.x>.
- Condon, A.G., Richards, R.A., Rebetzke, G.J., Farquhar, G.D., 2004. Breeding for high water-use efficiency. *J. Exp. Bot.* 55, 2447–2460. <https://doi.org/10.1093/jxb/erh277>.
- Fang, Y., Xiong, L., 2015. General mechanisms of drought response and their application in drought resistance improvement in plants. *Cell. Mol. Life Sci.* 72, 673–689. <https://doi.org/10.1007/s00018-014-1767-0>.
- Farquharson, K.L., 2017. Fine-tuning plant growth in the face of drought. *Plant Cell* 29, 4. <https://doi.org/10.1105/tpc.17.00038>.
- Feder, N., O'Brien, T.P., 1968. Plant microtechnique: some principles and new methods. *Am. J. Bot.* 55, 123–142. <https://doi.org/10.2307/2440500>.
- Gleason, S.M., Cooper, M., Wiggans, D.R., Bliss, C.A., Romay, M.C., Gore, M.A., Mickelbart, M.V., Topp, C.N., Zhang, H., DeJonge, K.C., Comas, L.H., 2019. Stomatal conductance, xylem water transport, and root traits underpin improved performance under drought and well-watered conditions across a diverse panel of maize inbred lines. *Field Crops Res.* 234, 119–128. <https://doi.org/10.1016/j.fcr.2019.02.001>.
- Grzesiak, M.T., Grzesiak, S., Skoczowski, A., 2006. Changes of leaf water potential and gas exchange during and after drought in triticale and maize genotypes differing in drought tolerance. *Photosynthetica* 44, 561–568. <https://doi.org/10.1007/s11099-006-0072-z>.
- Hausmann, B.I.G., Mahalakshmi, V., Reddy, B.V.S., Seetharama, N., Hash, C.T., Geiger, H.H., 2002. QTL mapping of stay-green in two sorghum recombinant inbred populations. *Theor. Appl. Genet.* 106, 133–142. <https://doi.org/10.1007/s00122-002-1012-3>.
- Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soil. *Calif. Agric. Exp. Stn.* 347, 1–34.
- Johansen, D.A., 1940. *Plant Microtechnique*. McGraw-Hill, New York.
- Lavinsky, A.O., Magalhães, P.C., Ávila, R.G., Diniz, M.M., de Souza, T.C., 2015. Partitioning between primary and secondary metabolism of carbon allocated to roots in four maize genotypes under water deficit and its effects on productivity. *Crop J.* 3, 379–386. <https://doi.org/10.1016/j.cj.2015.04.008>.
- Levitt, J., 1972. *Responses of Plants to Environmental Stresses*. Academic Press, New York.
- Lopes, M.S., Aarus, J.L., Van Heerden, P.D.R., Foyer, C.H., 2011. Enhancing drought tolerance in C 4 crops. *J. Exp. Bot.* 62, 3135–3153. <https://doi.org/10.1093/jxb/err105>.
- Lopez, O.R., Kursar, T.A., Cochard, H., Tyree, M.T., 2005. Interspecific variation in xylem vulnerability to cavitation among tropical tree and shrub species. *Tree Physiol.* 25, 1553–1562. <https://doi.org/10.1093/treephys/25.12.1553>.
- Lynch, J.P., 2013. Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. *Ann. Bot.* 112, 347–357. <https://doi.org/10.1093/aob/mcs293>.
- Magalhães, P.C., Lavinsky, A.O., Melo, H.F., Alves, J.C., Souza, T.C., Barbosa, A.P., Ribeiro, C., 2015. Relationship between nitric oxide, enzymatic antioxidant system and aba in maize under long-term drought. *Rev. Bras. Milho e Sorgo* 14, 155–166. <https://doi.org/10.18512/1980-6477/rbms.v14n2p155-166>.
- Marchiori, P.E.R., Machado, E.C., Sales, C.R.G., Espinoza-Núñez, E., Magalhães Filho, J.R., Souza, G.M., Pires, R.C.M., Ribeiro, R.V., 2017. Physiological plasticity is important for maintaining sugarcane growth under water deficit. *Front. Plant Sci.* 8, 1–12. <https://doi.org/10.3389/fpls.2017.02148>.
- Mohammadi, R., 2014. Phenotypic plasticity of yield and related traits in rainfed durum wheat. *J. Agric. Sci.* 152, 873–884. <https://doi.org/10.1017/S0021859613000580>.
- Nicotra, A.B., Davidson, A., 2010. Adaptive phenotypic plasticity and plant water use. *Funct. Plant Biol.* 37, 117–127. <https://doi.org/10.1071/FP09139>.
- Peltonen-Sainio, P., Jauhainen, L., Sadras, V.O., 2011. Phenotypic plasticity of yield and agronomic traits in cereals and rapeseed at high latitudes. *Field Crops Res.* 124, 261–269. <https://doi.org/10.1016/j.fcr.2011.06.016>.
- Potvin, C., Tousignant, D., 1996. Evolutionary consequences of simulated global change : genetic adaptation or adaptive phenotypic plasticity. *Oecologia* 108, 683–693. <https://doi.org/10.1007/BF00329043>.
- Pugnaire, F., Valladares, F., 2007. *Functional Plant Ecology*. CRC Press, Boca Raton.
- R Core Team, 2018. R software: Version 3.5.1. R Found. Stat. Comput. <https://doi.org/10.1007/978-3-540-74686-7>.
- Rehfeldt, G.E., Wykoff, W.R., Ying, C.C., 2001. Changing climate on *Pinus contorta*. *Clim. Change* 50, 355–376. <https://doi.org/10.1023/A:1010614216256>.
- Rellán-Álvarez, R., Lobet, G., Lindner, H., Pradier, P.L., Sebastian, J., Yee, M.C., Geng, Y., Trontin, C., Larue, T., Schrager-Lavelle, A., Haney, C.H., Nieu, R., Maloof, J., Vogel, J.P., Dinnyen, J.R., 2015. GLO-Roots: an imaging platform enabling multi-dimensional characterization of soil-grown root systems. *Elife* 4, 1–26. <https://doi.org/10.7554/eLife.07597>.
- Sadras, V.O., Reynolds, M.P., de la Vega, A.J., Petrie, P.R., Robinson, R., 2009. Phenotypic plasticity of yield and phenology in wheat, sunflower and grapevine. *Field Crops Res.* 110, 242–250. <https://doi.org/10.1016/j.fcr.2008.09.004>.
- Souza, T.Cde, Castro, E.Mde, Magalhães, P.C., Lino, L.de O., Alves, E.T., Albuquerque, P.E.Pde, 2013a. Morphophysiology, morphoanatomy, and grain yield under field conditions for two maize hybrids with contrasting response to drought stress. *Acta Physiol. Plant.* 35, 3201–3211. <https://doi.org/10.1007/s11738-013-1355-1>.
- Souza, T.Cde, Magalhães, P.C., Castro, E.Mde, Albuquerque, P.E.Pde, Marabesi, M.A., 2013b. The influence of ABA on water relation, photosynthesis parameters, and chlorophyll fluorescence under drought conditions in two maize hybrids with contrasting drought resistance. *Acta Physiol. Plant.* 35, 515–527. <https://doi.org/10.1007/s11738-012-1093-9>.
- Valladares, F., Sanchez-Gomez, D., Zavala, M.A., 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *J. Ecol.* 94, 1103–1116. <https://doi.org/10.1111/j.1365-2745.2006.01176.x>.
- Vilela, A.E., González-Paleo, L., 2015. Changes in resource-use strategy and phenotypic plasticity associated with selection for yield in wild species native to arid environments. *J. Arid Environ.* 113, 51–58. <https://doi.org/10.1016/j.jaridenv.2014.09.005>.