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Partitioning between primary and secondary metabolism of carbon allocated to roots in four maize genotypes under water deficit and its effects on productivity

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ABSTRACT

Plants may respond to drought by altering biomass allocation to shoots and roots or by changing the metabolic activities in these organs. To determine how drought changes the partitioning of carbon allocated to growth and secondary metabolism in maize roots and how it affects photosynthesis (A) and productivity in maize, we evaluated leaf gas exchange, yield componentes, root morphology, and primary and secondary metabolites including total soluble sugars (TSS), starch (S), phenolics (PHE), and lignin (LIG). Data were collected from pot-grown plants of four maize genotypes: BRS 1010 and 2B710 (sensitive genotypes) and DKB390 and BRS1055 (tolerant genotypes) under two soil water tensions: field capacity (FC, −18 kPa) and water deficit (WD, −138 kPa). WD was applied at the pre-flowering stage for 12 days and then the water supply was restored and maintained at optimum levels until the end of the cycle. For genotype BRS 1055 under FC, the greatest A did not result in greater grain biomass (DGB) because the accumulated photoassimilates had already filled the cells, and thus the excessive TSS synthesized in leaves was allocated to roots in large amounts. However, the sharp decrease in A caused by WD imposition in this genotype did not affect the influx pressure of leaf TSS, which was due largely to conversion of primary metabolites to PHE compounds to increase the length of fine roots. In leaves of DKB390 under WD, both S and TSS were reduced, whereas PHE were increased to prevent excessive water loss and xylem cavitation. Under WD, both BRS1010 and 2B710 genotypes displayed reduced allocation of biomass to shoots and roots and LIG content in leaves, as well as lower A and DGB values. In BRS1010 this response was coupled to S decrease in leaves and TSS increase in roots, whereas in 2B710 there was a concomitant S increase in roots.

1. Introduction

The threat of global climate change is causing concern in agriculture, given that climatic factors essential for crop development will be severely affected, reducing the production

and quality of food [1]. Given that most crops are produced in tropical regions characterized by low water availability and the occurrence of frequent and severe drought [2,3], research to identify the priority adaptive needs for investment in agriculture has become relevant to reduce the severity of the predicted impacts on production of crops [4] such as maize (*Zea mays* L.) in tropical regions.

Maize is an important cereal for human food and animal feed, and the severe WD experienced in Brazil has reduced the production and supply of this grain, affecting mainly small farmers and agro-industries in the northeast that use maize for animal feeding [5]. The main objectives of selection for increasing productivity in maize under drought include staygreen, anthesis–silking interval, and number of ears per plant, which are advantageous characteristics under WD. Thus, studies for phenotypic characterization and evaluation of the productivity of maize genotypes under WD are initial steps in elucidating mechanisms involved in the physiological differential behavior of some maize genotypes under this stress, and in providing indirect selection criteria for drought tolerance [6]. In this context, the search for new descriptors that reflect productivity, such as the characterization of roots is very important [7].

According to the theory of functional balance [8], plants increase the allocation of biomass to shoots if carbon gain is affected by limited resources aboveground, such as light and CO_2. Similarly, plants increase biomass allocation to roots in the presence of low levels of belowground resources, such as water and nutrients [9]. By altering their structure to increase the angle of root growth, plants using drought-avoidance strategies can exploit water in deeper layers of the soil, allowing marked improvement in grain yield [10]. Although the flexibility of biomass allocation to this organ of capture and storage of resources constitutes a key point in the determination of physiological changes in plants in environments with low soil water availability [11], little is known about this relationship [12]. The relationship between carbon allocation and water economy has been studied mainly in aboveground organs, with particular emphasis on leaves. Moreover, plants are likely to respond to drought not only by altering their allocation of biomass to shoots and roots, but also by changing the metabolic activities of these organs [13].

Primary metabolites in plants, such as sugars, starch, amino acids, and fatty acids, are synthesized mainly in the photosynthetic tissues of shoots, where the concentrations of these metabolites are higher than in roots [13]. Under drought conditions, however, the plasticity of the plants allows a shift to increased synthesis or allocation of several primary metabolites to roots and decreased allocation to shoots [13]. This shift may increase levels of plant secondary metabolites such as PHE and LIG, which are synthesized from primary metabolites and often referred to as compounds associated with stress response [13]. According to the hypothesis of compromise between growth and defense [14–16], the plant faces an energy competition between synthesizing defense compounds (which incur high metabolic costs for synthesis and storage) or investing their energy into other functions such as growth, maintenance and reproduction. In a pioneer study [16] with 41 tropical species, the energetic compromise between growth and defense was evident. Species with higher

growth rates showed leaves with lower concentrations of compounds associated with chemical defense, providing a greater amount of energy to growth. However, these species were more susceptible to defoliation by herbivores, suggesting that a negative relationship between growth rate and cost of synthesis holds.

The goal of this study was to determine how drought changes the partitioning of carbon allocated to growth and secondary metabolism in maize roots and how it affects A and productivity in four maize genotypes under WD.

2. Material and methods

2.1. Growth conditions, plant material, and experimental design

The experiment was conducted in a greenhouse at the National Maize and Sorghum Research Center (19°28′ S, 44°15′08″ W, 732 m.a.s.l.), and the plant material consisted of four maize genotypes with contrasting drought tolerance: two tolerant (DKB 390 and BRS1055) and two sensitive (BRS 1010 and 2B710). The choice of genotypes was based on results of previous field experiments performed by researchers from the breeding program of the National Maize and Sorghum Research Center, which, over the years, has accumulated experience in maize phenotyping for drought tolerance [17].

Plants were grown in plastic pots containing 20 kg of typical dystrophic Red Latosol soil. The water content in the soil was monitored daily between 9:00 a.m. and 3:00 p.m., with a moisture sensor (GB Reader N1535; (Measurement Engineering, Australia)) installed at the center of each pot with the aid of a screw auger at a depth of 20 cm. These sensors detect the water content in the soil based on electrical resistance and are coupled to digital meters. Water replacement by irrigation was based on the data obtained with the sensor and water was added to reach FC during the period preceding the imposition of the treatments. The water replacement calculations were performed with a spreadsheet and based on a soil water retention curve. In parallel, all necessary cultural and phytosanitary treatments were performed.

At the pre-flowering growth stage, half of each initial treatment was subjected to WD the other half continued to receive daily irrigation in order to maintain soil moisture close to FC, with a soil water tension of −18 kPa. WD was imposed by daily provision of 50% of the total available water until the soil water tension reached at least −138 kPa. After twelve days under this condition, the leaf water potential was determined with a Scholander-type pressure pump at midday (Ψ_{md}) [18]. Leaf gas exchange and chlorophyll *a* fluorescence were measured in leaves corresponding to the ear insertion with an infrared gas analyzer equipped with a fluorometer (LI-6400-40; LI-COR, USA) [18]. Samples of leaves and roots were collected and stored in liquid nitrogen for determination of the contents of TSS, S, PHE, and LIG. The water supply was then restored and maintained at optimum levels until the end of the cycle. At harvest, the agronomic parameters associated with productivity were analyzed according to the methodology detailed in the "Agronomic parameters" section. The
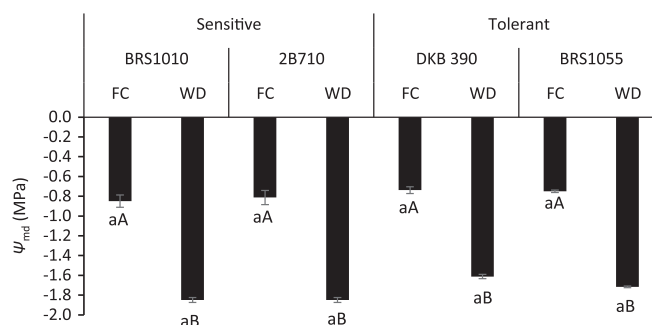
Fig. 1 – Leaf water potential at midday (Ψ_{md}) in four maize genotypes with contrasting characteristics for drought tolerance grown under different water levels in the soil. Drought-sensitive genotypes: BRS1010 and 2B710; drought-tolerant genotypes: DKB390 and BRS1055; FC: field capacity; WD: water deficit. Means followed by the same letter do not differ statistically. Lowercase letters denote comparisons between genotypes within the same level of water in the soil and uppercase letters indicate comparisons between the water levels in the soil within the same genotype. Scott–Knott test at 5% probability was applied.

experimental unit was the pot containing two plants, with six replications per treatment.

For the statistical analysis, the results were submitted to variance analysis and the means were compared by the Scott–Knott test at 5% probability.

2.2. Biochemical analysis

Tissues were macerated in 80% ethanol (v/v), incubated at 70 °C for 90 min, and subjected to two centrifugations (15,000 ×g, 10 min). In the insoluble fraction, S was analyzed enzymatically [19]. In the soluble fraction, TSS were determined [20]. The contents of total PHE was quantified by the Prussian Blue method [21], and LIG by the Klason method [22].

2.3. Agronomic parameters

Leaf number, ear number, leaf area, plant height, ear insertion height, and diameter and length of the ear were quantified [18], in six plants per treatment. Leaf area was measured with an area meter (LI-COR). The plants were then partitioned into roots, stems, leaves, tassel, ears (cob, straw, and grains), and dried in an oven with forced air circulation at 70 °C for 72 h. Based on the dry weights of the different parts, the harvest index, number of grains per ear, and weight of 100 grains were estimated [18].

A group of 50 kernels was subjected to morphometric characterization by measurement of the dimensions of the kernels (length, width and thickness) using a digital pachymeter, with three replications per treatment. These kernels were then soaked overnight in ethylenediamine (10%, w/v) and longitudinally cut with a knife to evaluate possible changes in embryo size, depending on the treatment. Photographs were obtained using a stereoscopic microscope and the ImageJ program was used to calculate the ratio between the areas of the endosperm and the embryo.

For the evaluation of the root system the computerized system WinRHIZO (WinRHIZO Pro, Regent Inc. Instr., Canada) was used to measure length, diameter, volume and surface area of roots by diameter class, as follows: very fine roots (Ø less than 0.5 mm), fine roots (>0.5 Ø <2.0 mm) and thick

roots (Ø > 2.0 mm) [7]. For this analysis, roots were collected from three replicates per treatment.

3. Results

All genotypes showed similar tolerance to leaf dehydration, with reduced leaf water potential at midday (Ψ_{md}) under WD compared to plants maintained in soil moisture at FC (Fig. 1).

Under FC, the A and stomatal conductance of water vapor (g_s) were different among genotypes, with higher values for BRS 1055, intermediate for 2B710 and DKB 390, and lower for BRS1010. Even under this condition, the transpiration rates (E) were higher for genotype 2B710, intermediate for BRS 1055, and smaller for BRS 1010 and DKB 390, and the internal concentration of carbon (C_i) unchanged (Table 1). In turn, irrespective of genotype, there was a significant reduction in A, g_s and maximum efficiency of photosystem II (F_v/F_m) in plants exposed to stress caused by WD compared to those for which the soil moisture was maintained close to FC, whereas C_i values increased (Table 1). Plants of the tolerant genotypes (DKB390 and BRS1055) showed average values of A and g_s 70.33% and 64.66% higher under WD, respectively, than the susceptible genotypes (2B710 and BRS1010) grown under the same condition. In addition, sensitive genotypes showed lower transpiration rates (E) compared to tolerant ones. Under WD, BRS 1055 showed larger values of F_v/F_m, than the other genotypes, and the BRS1010 genotype showed an A value 7.81 times lower than that of 2B710 but an E only 2.18 times lower, explaining the values of A/E for 2B710 similar to those for DKB 390 and BRS 1055.

The genotype BRS 1055 under FC showed an increase of 21.0% in A, with a 7.6% increase in total biomass (TDB) and 9.8% decrease in DGB compared to DKB 390 under the same condition (Table 2). Given that these two genotypes showed similar tolerance to WD, it was expected that higher A values would result in higher grain productivity. It is possible that in BRS 1055 under FC the largest A did not result in greater DGB because the cells that accumulate photoassimilates were already completely filled. In addition, the metabolic costs

Table 1 – Gas exchange parameters obtained in four maize genotypes with contrasting characteristics for drought tolerance grown under different water levels in the soil.

Parameter	Sensitive				Tolerant			
	BRS1010		2B710		DKB 390		BRS1055	
	FC	WD	FC	WD	FC	WD	FC	WD
A	23.20 cA	0.152 bB	28.06 bA	1.187 bB	27.72 bA	2.257 aB	33.62 aA	2.257 aB
g_s	0.102 cA	0.007 aB	0.145 bA	0.010 aB	0.138 bA	0.023 aB	0.189 aA	0.023 aB
E	2.107 cA	0.088 aB	3.886 aA	0.192 aB	2.347 cA	0.499 aB	2.979 bA	0.498 aB
C_i	75.27 aB	357.8 aA	31.64 aB	158.9 cA	43.38 aB	215.5 bA	67.94 aB	214.4 bA
F_v/F_m	0.803 aA	0.762 bB	0.790 aA	0.757 bB	0.800 aA	0.757 bB	0.801 aA	0.784 aA
A/E	11.99 aA	1.645 bB	7.315 bA	6.508 aA	11.81 aA	4.592 aB	11.28 aA	4.599 aB

Drought-sensitive genotypes: BRS1010 and 2B710; drought-tolerant genotypes: DKB390 and BRS1055; FC: field capacity; WD: water deficit; A: photosynthetic rate (μmol CO_2 m^{-2} s^{-1}); g_s: stomatal conductance (mol H_2O m^{-2} s^{-1}); E: transpiration rate (mol H_2O m^{-2} s^{-1}); C_i: internal CO_2 concentration (μmol CO_2 m^{-2} s^{-1}); F_v/F_m: maximum efficiency of photosystem II; A/E: efficiency in water use.
Means followed by the same letter do not differ statistically. Lowercase letters denote comparisons between genotypes within the same soil water level and uppercase letters indicate comparisons between soil water levels within the same genotype. The Scott–Knott test at 5% probability was applied.

associated with the highest root dry biomass (DRB) in BRS 1055 compared to the other genotypes offset the gains from A, thus reducing productivity.

Under WD, genotypes DKB 390 and BRS 1055 showed similar values of A and TDB, but the DGB was 28% higher in the DKB 390, resulting in a higher harvest index (HI) than that of BRS 1055 (Table 2). At first sight, the lower HI values in BRS 1055 might be interpreted as a low tolerance for WD. However, compared to the control (under FC) a decrease in HI of only 9.3% occurred for BRS 1055 under WD. The sensitive genotypes BRS 1010 and 2B710 under WD showed reductions of 22.0% and 24.0% in HI, respectively. In fact, plants from BRS 1010 and 2B710 showed reduced leaf area (LA), area of embryo relative to the endosperm (EM:E) and DGB under WD compared to FC, indicating the occurrence of a low photoassimilate flow to the grain in these two maize genotypes under WD, compared to genotypes DKB 390 and BRS 1055. Plants of BRS 1055 have a constitutively higher source capacity per se, associated with a root system specialized for water absorption. Thus large reductions in A had little effect on the availability of photoassimilates in the source under WD, and thus the flow of photoassimilates from source to sink organs (such as in grain filling) was similar to that in plants under FC.

Morphometric analysis of root images previously scanned with WinRHIZO showed no difference between genotypes under FC with respect to length, surface area, and volume in different diameter classes of (Table 3). However, under WD the BRS1055 genotype showed higher fine root length (FRL), surface area of fine roots (SAFR), total surface area of roots (TSAR), and volume of fine roots (VFR) than did the other genotypes (Table 3). In addition, plants of this genotype under WD showed greatly increased FRL, surface area of very fine roots (SAVFR), SAFR, TSAR, and FRV compared to those grown under FC, whereas in the genotypes BRS 1010 and DKB 390 these variables did not change. For the sensitive genotype 2B710 under WD, SAVFR, SAFR, and TSAR decreased in comparison with plants of the same genotype under FC (Table 3).

Plants of genotype 2B710 showed increased root S levels under WD compared to plants of the same genotype under FC, so that under WD this genotype showed significantly higher levels of S relative to the others under the same water condition (Fig. 2). Regardless of the water content in the soil, BRS 1055 had higher levels of TSS in roots than did the other genotypes (Fig. 2). Only plants of BRS 1010 showed increased TSS root levels under WD relative to plants under FC (Fig. 2). There was no significant variation in LIG levels in maize roots with the imposition of WD (Fig. 2). In turn, PHE levels in roots were greatly increased only in BRS1055 exposed to the stress caused by WD (Fig. 2). The concentration of PHE in BRS 1055 under WD was 2.57 times higher than that recorded in plants of this genotype under FC (Fig. 2). In leaves of DKB390 under WD, both S and TSS were reduced, whereas PHE was increased. Under WD, both BRS1010 and 2B710 genotypes showed reduced LIG content in leaves and S decreased in leaves of BRS1010.

4. Discussion

Higher maize yield is not always associated to higher values of A [23]. In the present study we found that BRS1055 genotype showed higher A values than DKB390, BRS1010, and 2B710 under FC, but did not use the additional photosynthate to increase DGB. It is possible that the largest A did not result in greater DGB because the cells that accumulate photoassimilates were already completely filled, and as a result the excess of TSS synthesized in leaves was allocated and used in large amounts in roots. Grain size and the potential to accumulate photoassimilates in maize are probably determined by the number and size of endosperm cells [24]. In addition, the metabolic costs associated with the highest DRB in BRS 1055 compared to the other genotypes may contribute to this response.

In maize, stomatal closure is one of the first events that occurs with declining leaf water status, and invariably coincides with reduced A and E [18]. The ameliorative effect

Table 2 – Agronomic production in four maize genotypes with contrasting characteristics for drought tolerance grown under different water levels in the soil.

| | Sensitive | | | | Tolerant | | | |
| | BRS1010 | | 2B710 | | DKB 390 | | BRS1055 | |
	FC	WD	FC	WD	FC	WD	FC	WD
PH	1.764 bA	1.712 cA	1.840 bA	1.728 cA	1.820 bA	1.883 bA	2.190 aA	2.157 aA
EIH	0.951 aA	0.913 bA	0.744 bA	0.732 cA	0.981 aA	1.111 aA	0.927 aA	0.915 bA
SLA	15.58 aA	15.77 aA	17.82 aA	15.48 aA	16.86 aA	16.79 aA	16.56 aA	15.90 aA
LA	0.529 bA	0.480 bB	0.567 bA	0.486 bB	0.559 bA	0.553 aA	0.630 aA	0.576 aA
ILA	0.043 bA	0.040 bA	0.047 bA	0.040 bA	0.046 bA	0.046 aA	0.052 aA	0.048 aA
LN	12.17 aA	11.92 aA	12.00 aA	11.92 aA	12.00 aA	12.00 aA	12.17 aA	11.92 aA
NGE	440.6 bA	291.1 cB	563.4 aA	387.9 bB	388.4 bB	456.3 aA	508.5 aA	426.2 aA
DRB	30.09 bA	24.96 bA	25.09 bA	19.90 bA	28.55 bA	21.04 bA	46.61 aA	43.39 aA
DGB	90.74 bA	53.83 cB	104.0 aA	58.33 cB	99.87 aA	98.92 aA	90.02 bA	71.63 bB
TDB	261.0 aA	197.1 bB	261.2 aA	192.1 bB	259.4 aA	243.9 aA	279.3 aA	244.9 aB
HI	0.349 bA	0.270 bB	0.401 aA	0.303 bB	0.384 aA	0.405 aA	0.324 bA	0.294 bA
P100G	20.66 bA	18.42 bA	18.49 bA	15.43 bA	26.06 aA	21.87 aA	17.75 bA	16.80 bA
ED	49.67 bA	45.26 bB	54.86 aA	48.83 aB	53.80 aA	49.51 aA	46.28 cA	43.31 bB
EL	17.66 aA	13.60 cB	18.03 aA	15.75 bB	15.38 bB	17.42 aA	18.11 aA	17.80 aA
NGR	34.00 aA	23.16 cB	35.00 aA	22.33 cB	31.17 aA	34.67 aA	35.17 aA	28.67 bB
EM:E	0.200 aA	0.156 aB	0.225 aA	0.165 aB	0.229 aA	0.228 aA	0.194 aA	0.187 aA

Drought-sensitive genotypes: BRS1010 and 2B710; drought-tolerant genotypes: DKB390 and BRS1055; FC: field capacity; WD: water deficit; PH: plant height (m); EIH: ear insertion height (m); SLA: specific leaf area ($m^2 kg^{-1}$); LA: leaf area (m^2); ILA: individual leaf area (m^2); LN: number of leaves (N°); NGE: number of grains per ear; DRB: dry roots biomass (g); DGB: dry grain biomass (g); TDB: total dry biomass (g); HI: harvest index ($g\ g^{-1}$); P100G: weight of 100 grains (g); ED: ear diameter (mm); EL: ear length (cm); NGR: number of grains per row (No.); "EM:E": embryo: endosperm relationship.

Means followed by the same letter do not differ statistically. Lowercase letters denote comparisons between genotypes within the same soil water level and uppercase letters indicate comparisons between soil water levels within the same genotype. The Scott–Knott test at 5% probability was applied.

of A on tolerant maize genotypes exposed to early-term drought is believed to occur largely via counteracting oxidative stress by modulating ABA-mediated antioxidant enzymes such as catalase (CAT) [25]. However, with long-term drought exposure an A decline becomes even more evident with failure of the ABA-mediated antioxidant defense system, mainly when other mechanisms for counteracting oxidative stress are lacking. In the present study, after long-term drought exposure all genotypes showed severely reduced A values. In fact, a four- to six-day episode reduces maize A to near zero and can reduce yield [26], as seen mainly in the sensitive genotype BRS1010.

In WD plants of BRS1055, the sharp decrease in A did not affect the influx pressure of leaf TSS through the plasmodesmata in the phloem into kernel cells; the values of DGB and EM:E remained unchanged compared to FC. Under WD, the S, PHE, and LIG values in leaves of this genotype did not change in comparison with FC, and the values of these compounds were lower in leaves than in roots. In plants of BRS1055 under WD only PHE in roots were increased in comparison with FC, suggesting that large TSS primary metabolites in the roots were transglycosylated and used in PHE compounds of secondary metabolism other than LIG, such as quinic, chlorogenic, cinnamic, ferulic, coumaric, caffeic, and salicylic acids and/or tocopherol (also associated with mechanisms of protection against damage caused by exposure to WD). In fact, WD plants of BRS1055 showed increases in FRL without change in DRB. The largest amount of water absorbed by the root system is due to fine and very fine roots [27], and only

plants with conservative characteristics in arid environments show improvement in water absorption efficiency for producing very fine roots [28].

Plants of the DKB390 genotype showed decreased A values under WD compared to FC conditions, and despite their lack of mechanisms for increasing the absorption of water by roots, their yield was maintained. Plants of DKB390 show a smaller distance between the vascular bundles in the leaf blade under WD than under FC and high phenylalanine ammonia lyase (PAL) activity [18]. In the present study, we also showed that leaves of DKB390 under WD showed increased PHE, whereas S and TSS decreased and LIG did not change. We observed neither oxidative damage nor CAT activity increase in WD plants of this genotype (data not shown), suggesting diversion of the H_2O_2 accumulated in the leaf to biosynthesis of PHE compounds other than LIG, to prevent excessive water loss and xylem cavitation [29]. It may be that PAL activity increase [18] is more highly expressed than LIG formation, suggesting formation of PHE compounds of secondary metabolism other than LIG. HI was also correlated with root features such as increases in the formation of aerenchyma [18]. In field studies with maize under WD conditions, genotypes with a higher proportion of aerenchyma in their roots presented better performance (higher root growth and higher biomass of the above-ground part) [30]. A higher proportion of aerenchyma can allow enhanced soil exploration and water intake, since these structures decrease the metabolic cost of growing roots, due to a decrease in the number of cells that are undergoing

Table 3 – Morphometric characterization of roots in four maize genotypes with contrasting characteristics for drought tolerance grown under different water levels in soil.

| | Sensitive | | | | Tolerant | | | |
| | BRS1010 | | 2B710 | | DKB 390 | | BRS1055 | |
	FC	WD	FC	WD	FC	WD	FC	WD
VFRL	356.4 aA	469.5 aA	417.0 aA	420.0 aA	404.2 aA	552.1 aA	469.1 aA	687.8 aA
FRL	415.9 aA	364.2 bA	379.2 aA	317.2 bA	386.7 aA	390.6 bA	382.5 aB	509.7 aA
TRL	84.40 aA	88.28 aA	82.49 aA	71.24 aA	90.88 aA	85.83 aA	67.62 aA	89.37 aA
TLR	856.7 aA	822.0 aA	878.7 aA	808.5 aA	881.8 aA	1029 aA	919.2 aA	1286 aA
SAVFR	33.89 aA	36.86 aA	48.52 aA	29.81 aB	36.95 aA	41.77 aA	33.17 aB	51.11 aA
SAFR	121.6 aA	106.0 bA	114.7 aA	90.14 bB	130.5 aA	112.8 bA	107.6 aB	149.4 aA
SATR	104.9 aA	108.2 aA	98.88 aA	92.38 aA	113.2 aA	115.2 aA	96.17 aA	112.7 aA
TSAR	260.4 aA	251.0 cA	262.1 aA	212.3 cB	280.6 aA	269.8 bA	236.9 aB	313.2 aA
VFRV	2.457 aA	2.346 aA	2.761 aA	1.861 aA	2.390 aA	2.151 aA	1.615 aA	2.471 aA
FRV	3.253 aA	2.905 bA	3.161 aA	2.563 bA	3.351 aA	3.049 bA	2.832 aB	4.114 aA
TRV	13.4 4 aA	14.51 aA	12.65 aA	16.18 aA	14.31 aA	17.24 aA	16.50 aA	16.92 aA
TVR	19.15 aA	19.77 aA	18.58 aA	20.60 aA	20.05 aA	22.44 aA	20.94 aA	23.50 aA

Drought-sensitive genotypes: BRS1010 and 2B710; drought-tolerant genotypes: DKB390 and BRS1055; FC: field capacity; WD: water deficit; VFRL: very fine roots length (cm); FRL: fine roots length (cm); TRL: thick roots length (cm); TLR: total length of roots (cm); SAVFR = surface area of very fine roots (cm^2); SAFR: surface area of fine roots (cm^2); SATR: surface area of thick roots (cm^2); TSAR: Total surface area of roots (cm^2); VFRV: very fine roots volume (cm^3); FRV: fine roots volume (cm^3); TRV: thick root volume (cm^3); TVR: total volume of roots (cm^3).
Means followed by the same letter do not differ statistically. Lowercase letters denote comparisons between genotypes within the same soil water level and uppercase letters indicate comparisons between soil water levels within the same genotype. The Scott–Knott test at 5% probability was applied.

cellular respiration. However, WD plants from DKB390 genotype showed no significant variation in growth, surface area and root volume and DRB, leading us to believe that the increase in the amount of aerenchyma reported in [18] was used only for the maintenance of the physiological processes in the root, which although beneficial, does not improve water capture in this genotype.

Drought reduced the amount of carbon allocation to shoots and roots, as well as DGB and LIG content in leaves in plants of BRS1010 and 2B710 in comparison with drought-tolerant lines. For example, roots of genotype 2B710 under WD showed substantial reductions in SAVFR, SAFR, and TSAR. Considering that the root cap acts in geotropism and protects the root meristem from damage by mucilage secretion at the edges of the cells [31], it is possible that under WD the area of these roots was reduced, leaving the meristem unprotected. As a result, there was a smaller replacement of the damaged cells of the root cap by the quiescent center, which led to a restriction in SAVFR, SAFR, and TSAR without affecting DRB.

LIG occupies the spaces in the cell wall between cellulose, hemicellulose, and pectin, especially in xylem tracheids and vessel elements, creating crosslinkages between polysaccharides that support the cell wall [32]. The mechanical strength of cell walls in leaves of these maize drought-sensitive lines was low, as evidenced by reduced LIG deposition. In fact, a previous study found that WD decreased the level of lignifying enzymes and consequently the LIG level in maize leaves [33]. We also observed an S decrease in leaves and a TSS increase in roots of BRS1010, whereas in 2B710 there was only a concomitant S increase in roots. In leaves, osmotically active compounds have been shown to originate mainly in S degradation, and the same mechanism was suggested to underlie the osmotic adjustment in roots of plants under WD

[34]. Our data indicate that drought increases the partitioning of recent assimilates to osmotic adjustment in roots and that S breakdown in leaves contributes to a drought-induced increase of root TSS in BRS1010. The increased S in 2B710 plants under WD relative to FC suggests that either drought was not causing carbon starvation, or if plants were starving, they would not use S to address any carbon imbalance.

Few studies have examined how root traits are associated with A and yield in plants grown under WD [10]. The main hindrance to these studies is the difficulty of root phenotyping in field-grown plants. Several methods have been developed to study root growth under both field and controlled environmental conditions. Under controlled environments, root scanning based on WinRHIZO is one of the most efficient methods, allowing image analysis and examination of root morphological traits [7,35]. A reasonable compromise to avoid the difficulty of studying roots in the field is also offered by growing plants in large pots with soil. Pot experiments allow precise measurement of the amount of water provided to each plant and estimation of the capacity of roots to penetrate a wax layer of high mechanical impedance mimicking a soil hardpan, often the main constraint to access of roots to soil moisture in deeper soil layers [36].

The results of the present study provide clear evidence that genotypes DKB 390 and BRS1055 are more tolerant to WD than BRS 1010 and 2B710. However, these two tolerant genotypes have different mechanisms for overcoming the stress generated by WD. DKB 390 uses mainly physiological mechanisms at the shoot level for the maintenance of productivity, by minimizing water loss and escaping xylem cavitation, a response that reduces dependence on metabolic adjustments in the root system to increase the absorption of water. BRS 1055 drought tolerance, in contrast, is associated with increase in water uptake or transport.
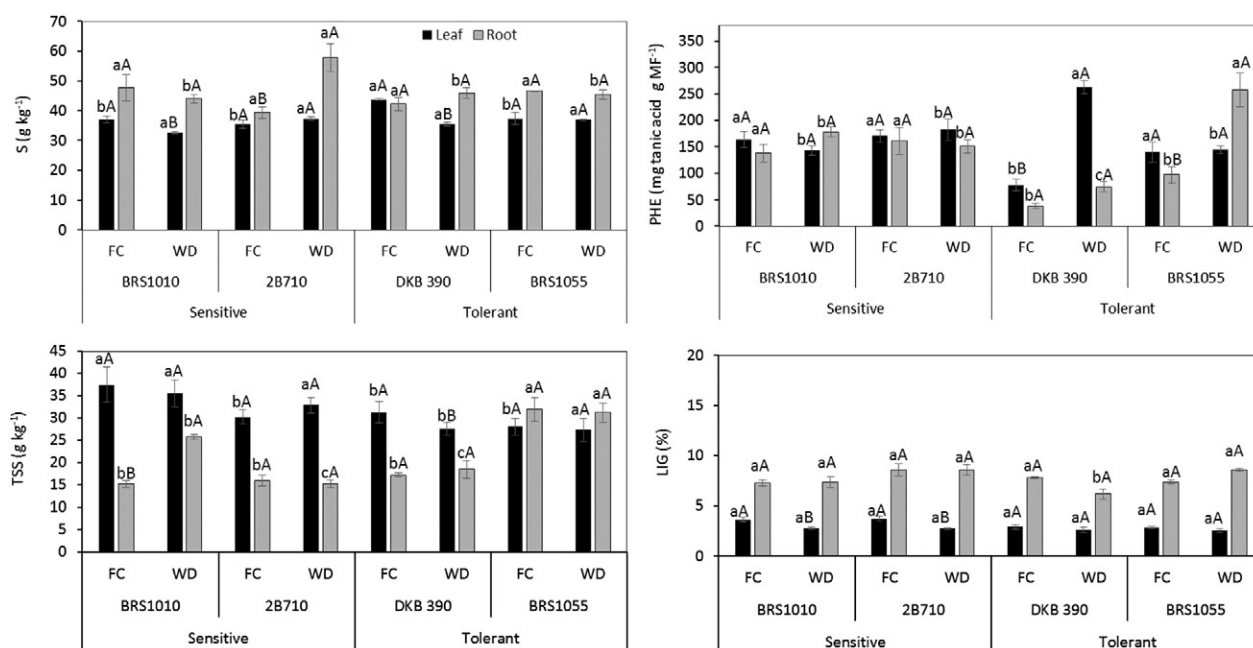
Fig. 2 – Starch content (S), total soluble sugars (TSS), lignin (LIG), and phenols (PHE) in roots and leaves of four maize genotypes with contrasting characteristics for drought tolerance grown under two water levels in the soil. Drought-sensitive genotypes: BRS1010 and 2B710; drought-tolerant genotypes: DKB390 and BRS1055; FC: field capacity; WD: water deficit. Means followed by the same letter do not differ statistically. Lowercase letters denote comparisons between genotypes within the same soil water level and uppercase letters indicate comparisons between soil water levels within the same genotype. The Scott–Knott test at 5% probability was applied.

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