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Morphological, physiological, and biochemical responses of yerba mate (Ilex paraguariensis) genotypes to water deficit

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Abstract

Severe drought is expected to increase in frequency and intensity in South America, likely causing decreased plant productivity and altering species distributions. Yerba mate (*Ilex paraguariensis*) is a tree species native to South America and has an ecological, social, and economic importance in several countries. Natural forests and cultivated areas of yerba mate will be endangered by climate change because of the expected reduction in water availability. Here we determined how four clonal genotypes of yerba mate (BRS BLD Yari, BRS BLD Aupaba, BRS 408, and EC40) respond to water deficits by evaluating the plants' morphophysiological and biochemical characteristics when subjected to four levels of soil water holding capacity (WHC at 100%, 80%, 60%, or 40%) over 60 days. We observed a reduction in plant height and biomass accumulation related to the decrease in water availability; physiological and biochemical parameters indicated that water-deficit stress reduced the plants' C assimilation and increased their production of bioactive compounds. BRS BLD Yari and BRS BLD Aupaba had a higher tolerance to low water availability, whereas the EC40 and BRS 408 genotypes experienced a more pronounced reduction in growth and biomass accumulation. Understanding how different yerba mate genotypes respond to water deficit is essential for favoring species conservation and developing climate-adapted breeding programs.

Introduction

Climate change is altering forest ecosystems around the world (Seidl et al. 2017; Girona et al. 2023). Recent projections of climate change indicate that South America a greater variability in rainfall patterns and with longer and more intense periods of drought (Llopart et al. 2020; Gomes et al. 2022). These unpredictable drought cycles will have potentially devastating effects on forest and agricultural plantations, reducing production capacity, and causing considerable economic losses (Grüter et al. 2022; Jiménez et al. 2023). Production capacity will be reduced mainly because of water deficits that will affect cellular processes, leaf expansion, stomatal conductance, transpiration rates, foliar abscission, and photosynthetic activities (King and Purcell 2017; Pappas et al. 2022). How plants will respond to these changing conditions is a critical question, and understanding the survival and growth mechanisms of various groups of plants and genotypes will serve to guide future management strategies and breeding programs (Scolforo et al. 2017, 2019)

Yerba mate (*Ilex paraguariensis*) is a tree species native to the South American humid subtropical forest of Brazil, Paraguay, and Argentina. It is planted in monocultures, in agroforestry systems, or exploited in native forests (Carvalho 2003; Gottlieb et al. 2005). In 2021, annual yerba mate production in Brazil was 1,064,121 tons (worth approximately $300 million US). It is the most important forest product in southern Brazil (IBGE 2022). The leaves and young shoots of yerba mate are used in South America mainly to produce beverages such as chimarrão, tereré, and tea; it therefore has an important social and cultural aspect related to its consumption (Duarte et al. 2020; Tomasi et al. 2021). Recently, its potential has been explored for use in food, pharmaceutical, and chemical industries because of its bioactive compounds as well as its antioxidant and anti-inflammatory properties. Moreover, the consumption of

yerba mate has increased in nontraditional countries, including the United States, Germany, and Syria (Gullón et al. 2018; Vieira et al. 2021; Duarte et al. 2022; Gerber et al. 2023).

The distribution patterns of yerba mate are determined mainly by temperature and rainfall, and climate change will likely negatively affect the productivity of commercial plantations and alter its distribution by reducing its range to higher altitudes and more humid regions (Da Silva et al. 2018). The latest IPCC report indicates that southern South America will experience significant changes in the regional water regime, and extreme temperature and rainfall events will become more frequent (IPCC 2022).

Climate change affects the morphological, physiological, and anatomical characteristics of plants as well as plant biomass production (Madani et al. 2018; Girona et al. 2023). The magnitude of the water deficit determines the strategy adopted by plants to overcome this stress. Initially, when soil moisture reaches a value lower than the soil water holding capacity, plants maintain turgor to ensure growth continuity; however, after an extended period of water deprivation, photosynthesis decreases, and several mechanisms, e.g., the production of abscisic acid and stomatal closure, are activated to avoid water loss and ensure osmotic adjustment (Tardieu and Simonneau 1998; Brunner et al. 2015).

Recent studies have characterized the molecular mechanisms associated with the drought response in yerba mate, such as leaf osmotic adjustments and alterations of the plant's metabolic profile; the nature of these responses indicate that changes in water regimes may be the most significant factor influencing on yerba mate biomass production in the coming years (Acevedo et al. 2019). Drought resistance and recovery in yerba mate are regulated by gene expression; thus a marked genetic effect will determine how different genotypes respond to climate change (Acevedo et al. 2016, 2018). Therefore, it is important to identify how genotypes used in breeding programs respond to drought. Such genotype–climate influence studies have not been undertaken for the Brazilian breeding program of yerba mate.

Here we evaluate the response of four yerba mate clonal genotypes to water deficit, as extensive dry periods are projected in South America in the future. We hypothesize that water availability alters plant growth and physiology and that yerba mate genotypes differ in their respective responses to water deficit given that breeding programs select yerba mate genotypes for various non-climate-related purposes and from different regions.

Material and methods

Experimental design and treatments

The study was conducted in Colombo, Paraná, Brazil (25°19'15" S, 49°09'31" W; 934 m) in a plastic-sided greenhouse. The experiment was established as a completely randomized design, in a double factorial arrangement (four genotypes × four water availabilities); each treatment was distributed in four replicates comprising six plants for each treatment. Thus our study involved a total of 384 plants. We set our water availability treatments at 100%, 80%, 60%, and 40% water holding capacity of the soil. We used

four genotypes of yerba mate propagated via a mini-cutting technique in 110 cm^3 polypropylene tubes; plants were on average six months old and 15 ± 3 cm in height. We selected four genotypes from the yerba mate breeding program of the Brazilian Agricultural Research Corporation (Embrapa – Florestas). Three genotypes (BRS BLD Yari, BRS BLD Aupaba, and BRS 408) are used at a commercial scale and are already registered with the Brazilian Ministry of Agriculture, Livestock, and Food Supply (MAPA), and one (EC40) is still undergoing tests. The breeding program selects yerba mate genotypes on the basis of leaf biomass productivity and leaf taste (Sturion et al. 2017; Wendling et al. 2018).

Each seedling was transplanted into a 3.8 L pot filled with 3.4 L of a ferralsol, a soil commonly underlying yerba mate plantations (Table S1; IUSS Working Group WRB 2015). Five days post-transplanting, the plants were subjected to different water availabilities depending on the soil water holding capacity. For 60 days, between 8:00 and 9:00 AM, we measured the amount of water applied during each treatment by weighing the sample pots and plants and then subtracting the initial weights.

Morphological analysis

For each sample, we measured plant height, diameter, and dry biomass 60 days after the beginning of water availability treatments. For height, we measured plants from the base to the apex with a millimetric-scale ruler, and stem diameter was measured using a digital caliper. For dry biomass, we separated the aerial part and roots of three plants of each replicate. Both parts were packed in identified paper packages and dried in an oven with forced ventilation at 60°C until a constant weight was attained, generally after 72 h. Dry biomass was weighed using a precision balance (0.002 mg Accuris™ Precision Balance, EuroPlug). We used these data to calculate total dry biomass and the shoot:root (SDB:RDB) dry biomass ratio.

Physiological analysis

Gas exchange was evaluated using an infrared gas analyzer (IRGA, Model LI 6400, LI-COR®) at the end of the experiment. Readings were obtained between 9:00 and 11:00 AM on a sunny day. We used the environmental CO_2 concentration as a reference, which varied from 380 to 400 µmol CO_2 mol^{-1}. We also recorded carbon assimilation (A, µmol CO_2 $m^{-2}{\cdot}s^{-1}$), transpiration (E, mol H_2O $m^{-2}{\cdot}s^{-1}$), stomatal conductance (g_s, mol H_2O $m^{-2}{\cdot}s^{-1}$), and intracellular carbon concentration (C_i, µmol·CO_2 mol air^{-1}). From these data, we calculated water use efficiency (WUE, µmol CO_2 mol H_2O^{-1}), determined by the A/E ratio, and the carboxylation efficiency, determined by the A/C_i ratio.

Preparation of plant material and aqueous extracts

After 60 days of water availability treatments, we collected mature leaves—only leaves showing no apparent damage—for biochemical analysis and immediately dried the collected material in a microwave for five minutes. The samples were then ground and stored at − 4 ºC until analysis, following the methodology of Tomasi et al. (2021). We used 0.50 g ± 0.01 g of ground leaves and 50 mL of boiling water (98 ºC) to prepare an aqueous extract. The extract was vortexed for 30 s. The solutions were

subjected to ultrasound at 30 ºC for 30 min and then filtered through a qualitative filter paper, and the extract was made up to 100 mL by adding destilled water. Sample moisture was determined by weighing 0.50 g of material, drying it in a forced-air oven at 100 ºC for 24 h, and then reweighing the material. All laboratory analyses were performed in triplicate at the Non-Timber Forest Products Laboratory (LPPFNM) of Embrapa Florestas.

Total phenolic compounds

We quantified the total phenolic compounds using the Folin–Ciocalteau spectrophotometric method (Singleton and Rossi 1965). Briefly, using a 10 mL volumetric flask, we added 0.1 mL of aqueous extract, 6.0 mL of distilled water, and 0.5 mL of Folin–Ciocalteau reagent, followed by 1 min of vortex mixing. We then added 2 mL of 15% aqueous Na_2CO_3 solution, again vortex mixing for 30 s, and we adjusted the final volume to 100 mL by adding distilled water. After 2 h of reaction in a dark room at 23 ºC, sample absorbance was measured at 760 nm in a Shimadzu-1800 UV/VIS spectrophotometer (Shimadzu Corp., Japan). We used gallic acid within the range of 0.25–13 mg L^{-1} to produce a reference analytical curve. Our results were expressed in mg gallic acid equivalent per gram of sample (mg GAE g^{-1}) on a dry basis.

Antioxidant activity (ABTS and DPPH free radicals)

Antioxidant activity of the aqueous extracts was determined using the free radicals DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] (Brand-Williams et al., 1995; Re et al., 1999). DPPH was determined by adding 0.1 mL of sample to 3.9 mL of DPPH methanolic solution (0.06 mmol·L^{-1}). This reaction occurred in a dark environment for 30 min, and the absorbance was measured at 515 nm. Antioxidant activity of ABTS was determined by reacting 10 mL of ABTS (7 mmol·L^{-1}) with 176 µL of potassium persulfate (140 mmol·L^{-1}) in a dark room at 23 ºC for 16 h. An aliquot of 1 mL of ABTS solution was added to 100 mL of sodium acetate buffer (48.5 mmol·L^{-1}) pH 4.5, with the absorbance adjusted to 0.7 ± 0.05. Then, 3 mL of ABTS buffered solution was added to 30 µL of extract. After 2 h, the absorbance was measured at 734 nm. All antioxidant activities assays were performed in a spectrophotometer UV–VIS Shimadzu-1800 UV/VIS (Shimadzu Corp., Japan). Results were compared against a standard curve (Trolox 0–1000 µmol·L^{-1} for DPPH and 0–2500 µmol·L^{-1} for ABTS) and expressed in µmol Trolox equivalent per gram of sample (TEAC µmol·g^{-1}) on a dry basis.

Statistical analysis

To explain the variability in genotype response to water availability and differentiate the morphological, physiological, and biochemical responses of the genotypes, we applied a generalized linear model (GLM) having a Gaussian error distribution and an identity link function. We set water availability and genotypes as fixed effects. When we detected a significant effect, we used a Tukey test to differentiate between treatments. We ran all our analyses in R software version 4.2.2 (R Core Team 2020).

Results

Changes in the water holding capacity (WHC) produced significant differences in the morphological variables among the genotypes (Figs. 1 and 2). Height was the only variable with significant interaction between factors, and the highest mean values were observed for the Yari genotype (36.1 cm) for all water availability treatments, although its height was similar to that of Aupaba for the 60% WHC treatment. For shoot, root, and total dry biomass, we found no interaction between genotypes and WHC, and the highest dry biomass values were observed in the Aupaba and Yari plants (respectively 6.43 and 6.68 g for shoots, 4.11 and 4.34 g for roots, and 10.53 and 11.03 g for total dry biomass), whereas for WHC treatments, the highest biomass values were observed in plants subjected to 100% WHC (7.25 g, 4.50 g, and 11.75 g, for shoot, root, and total dry biomass, respectively). Stem diameter and shoot:root ratio were not influenced by the treatments.

Carbon assimilation (A) increased 28% as water availability rose from 40–100% for all clones without any interactions between genotypes and WHC (Fig. 3). Water use efficiency was highest in the lower water availability treatments, and the Yari genotype had the highest water use efficiency (8.58 µmol CO_2.mol H_2O^{-1}); Aupaba and EC40 did not differ significantly from each other. There was no difference in carboxylation efficiency for any of the factors. Aupaba,

BRS 408, and Yari presented similar transpiration responses for all WHC, with the highest values observed in plants subjected to 100% WHC (0.92, 0,79, and 0.88 mol $H_2O \cdot m^{-2} \cdot s^{-1}$, respectively) (Fig. 4). EC40 plants had the highest transpiration values for plants subjected to 60%, 80%, and 100% WHC (0.93, 0.92, and 1.12 mol $H_2O \cdot m^{-2} \cdot s^{-1}$, respectively), showing no significant difference from Yari plants at 80% WHC. We also observed that stomatal conductance (g_s) and intracellular carbon concentration (C_i) were reduced or did not differ with increased water availability.

Phenolic compound content and antioxidant activity decreased 25–33% for all clones as water availability increased from 40–100% (Fig. 5); however, the interactions between factors indicate that each genotype responded differently to WHC. For phenolic compounds, the EC40 seedlings presented the highest levels when subjected to 40% WHC (98.15 mg GAE$\cdot g^{-1}$), although this genotype did not differ from Yari seedlings for any of the WHC treatments. When the genotypes were subjected to 80% and 100% WHC, we observed no difference in total phenolic compound content among genotypes. EC40 seedlings had the highest mean antioxidant activity when evaluated for ABTS and DPPH. Antioxidant activity varied between 1741.49 and 2048.84 TEAC µmol$\cdot g^{-1}$ for ABTS and between 440.32 and 545.11 TEAC µmol$\cdot g^{-1}$ for DPPH.

Discussion

Drought resilience is an important property for cultivated plants, particularly in the context of expected future climate change. Studies of various plant species have elucidated the mechanisms related to drought resilience, and genotypes of the same species can differ in their strategies to cope with water deficits (Scolforo et al. 2019; Krzyżak et al. 2023; Toro et al. 2023; Perera-Castro et al. 2023). Using the

regulation of leaf water potential by stomata, plants can be classified as having either an isohydric or anisohydric behavior. Isohydric plants have a fast response to water deficits and close their stomata to avoid water loss. Anisohydric plants maintain water potential even under water deficit situations, being insensitive to decreases in soil water potential (Tardieu and Simonneau 1998; Pou et al. 2012; Martínez-Vilalta et al. 2014). Under minimal water deficit conditions, anisohydric plants may have an advantage in terms of biomass production by keeping their stomata open, although more severe and long-lasting water deficits will cause more damage to these plants than to isohydric species (Pou et al. 2012). The selection of genotypes that tend to have an isohydric behavior is therefore highly desirable in breeding programs for species that will likely encounter more severe drought situations in the field.

Yerba mate is a typical isohydric plant, which explains the growth stagnation observed in our study; these plants close their stomata at a relatively high plant water status to avoid dehydration by limiting water loss (Tardieu and Simonneau 1998; Acevedo et al. 2019). This behavior is important for the commercial plantations of yerba mate that use the plant's leaves as the main product, considering that this drought resilience strategy avoids leaf abscission. We observed reduced stomatal conductance, especially for Yari plants, although all genotypes used in our study experienced reduced photosynthesis and/or transpiration in the low water availability treatments, indicating stomatal closure (Chen et al. 2020). The maximal isohydric behavior of Yari plants among the studied genotypes may be an important characteristic of this genotype for its use in areas subjected to frequent droughts, given that this genotype also presented higher biomass accumulation. Lower stomatal conductance and photosynthesis rates have also been observed as part of the physiologic and metabolic changes triggered by drought in other yerba mate genotypes (Acevedo et al. 2019). The Yari and EC40 plants showed a higher water use efficiency when subjected to reduced water availability, indicating a high tolerance of this pair of genotypes to water deficits. Water use efficiency is defined as the quantity of carbon assimilated per unit of water used by the plant; a higher water use efficiency is an advantage to plants subjected to drought (Toro et al. 2023). Hakamada et al. (2020) observed significant differences in the water use efficiency among *Eucalyptus* clones subjected to various planting density and drought treatments, indicating that this physiological characteristic is primarily inherited. The BRS 408 plants presented lower growth rates even for seedlings at 100% water availability; these results are supported by the observed physiological variables that indicate a lower C assimilation and water use efficiency.

Our results demonstrate that water availability in the substrate significantly affects the morphological, physiological, and biochemical characteristics of yerba mate genotypes. Moreover, we observed that genotypes respond differently to water availability; the Yari genotype, for instance, showed a higher resilience to water deficits. It should be noted that the seedlings were not subjected to a constant water level. Water availability in the soil was adjusted once a day, and water loss due to evapotranspiration was not controlled. In general, Yari and Aupaba seedlings had a higher tolerance to low water availability, indicated by the higher biomass accumulation in the 40% and 60% WHC treatments relative to the other genotypes and by their increased water use efficiency in the lower water availability treatments. Although we observed a reduced growth and symptoms of water stress in seedlings subjected to a lower water availability, the treatments used in our study and the duration for which seedlings were subjected to this

water availability (60 days) were insufficient to cause plant death. Decreased growth in plants subjected to water deficit has been widely demonstrated for arboreal species and has also been observed in other yerba mate genotypes (Gortari et al. 2020). In the latter study, water deficits reduced plant height, and both seedlings and mini-cuttings responded similarly to water deficit after 50 days.

Biomass accumulation is often the main morphological characteristic affected by water deficit (Amaral et al. 2023; Leite et al. 2023). We observed that all yerba mate genotypes at a higher water availability tended to increase their biomass accumulation. Griebeler et al. (2021) obtained similar responses after subjecting *Cedrella fissilis* and *Eucalyptus saligna* to various irrigation regimes (continuous irrigation and daily irrigation at different intervals). The authors observed increased biomass in both species when the plants had a higher water availability. Griebeler et al. (2021) also noted that the root/shoot ratio differed between species; *E. saligna* experienced an increased ratio when subjected to water restrictions, whereas the ratio for *C. fissilis* did not differ between treatments. Increased root biomass and reduced shoot biomass (higher root:shoot ratio) are expected when plants are subjected to a water deficit as a strategy to reduce water loss and increase water absorption capacity. In our study, however, we observed that instead of increased root growth, yerba mate demonstrated a stagnated growth in both its shoots and roots when subjected to a water deficit. Similar results were obtained by Gortari et al. (2020) after subjecting yerba mate seedlings and mini-cuttings to simulated drought periods.

Yerba mate is known for its high content of phenolic compounds and its antioxidant activity (Vieira et al. 2021; Duarte et al. 2022). Environmental factors such as temperature, water and nutrient availability, and solar radiation, as well as the plant development stage can influence total phenolic compound levels and antioxidant activity in yerba mate (Croge et al. 2021). We observed that yerba mate subjected to a lower water availability had a higher phenolic compound content and increased antioxidant activity. EC40 plants presented the highest levels of antioxidant activity and total phenolic compounds, especially for the lower water availability treatment—an increase of 27% for the ABTS radical and 32% for the DPPH radical in the treatment with 405 WHC—indicating a higher stress response of these plants to reduced water availability. EC40 plants submitted t 40% WHC showed the highest increase in phenolic compound content (32%) relative to plants submitted to 100% WHC, followed by Yari (20%), Aupaba (19%), and BRS 408 (18%) in the same treatments. Phenolic compounds are the largest group of secondary metabolites in plants and have several physiological and metabolic functions. The accumulation of phenolic compounds in plants subjected to abiotic stresses is a known adaptative response to unfavorable conditions (Sharma et al. 2019). The action of phenolic compounds in plants under drought stress is related mainly to inhibiting the production of reactive oxygen species and their accumulation, a phenomenon observed in several plant species (Nina et al. 2023; Shao et al. 2023; Shohani et al. 2023).

The expected increase in the frequency, duration, and severity of drought events requires selecting water deficit–resistant genotypes of yerba mate for both production plantations and genetic conservation purposes (Acevedo et al. 2016, 2019). In Brazil, the yerba mate breeding program focuses primarily on leaf productivity, the main product obtained from yerba mate plantations (Wendling et al. 2016; Sturion et al. 2017); however, as we found, these genotypes do not respond similarly to water deficit. In general, the

Yari and Aupaba genotypes presented the best biomass accumulation among the different water availabilities, indicating that these genotypes have an effective response to water deficit. New studies and breeding programs must focus on selecting and developing water deficit–resistant genotypes and genotypes adapted to other consequences of climate change. Subjecting different yerba mate genotypes to a controlled water restriction helps predict a genotype's behavior to water deficits in the field and guides future decisions in yerba mate breeding programs.

Conclusion

Decreased water availability to yerba mate plants reduces plant growth and biomass accumulation, alters physiological activities, and enhances the accumulation of phenolic compounds and antioxidant activity. Among the evaluated genotypes, BRS BLD Yari and BRS BLD Aupaba showed a higher tolerance to low water availability, whereas EC40 and BRS 408 experienced more severe negative effects in regard to their morphological and physiological parameters. We recommend the use of drought-resistant genotypes in yerba mate plantations for maintaining commercial production, conserving the species, and adapting to climate change. New research is necessary to detail the mechanisms used by yerba mate for favoring drought resistance and to understand how breeding programs can select drought-resistant genotypes.

Declarations

Competing interests: 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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Figures

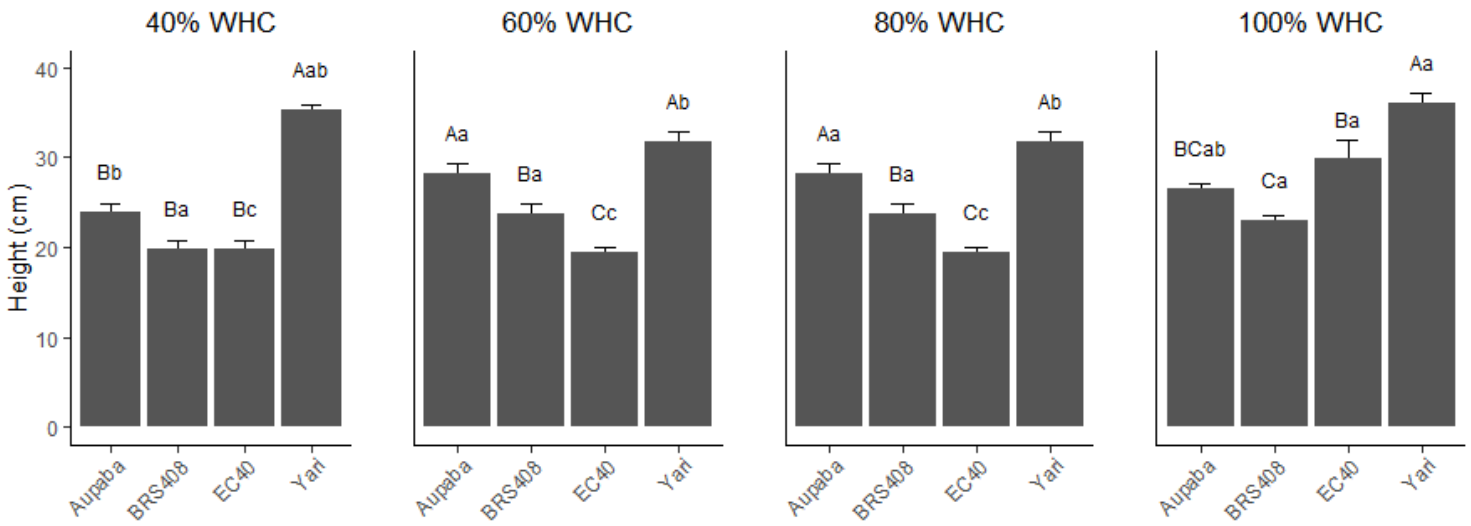


Figure 1

Height (cm) of *Ilex paraguariensis* genotypes subjected to different water availabilities (WHC).

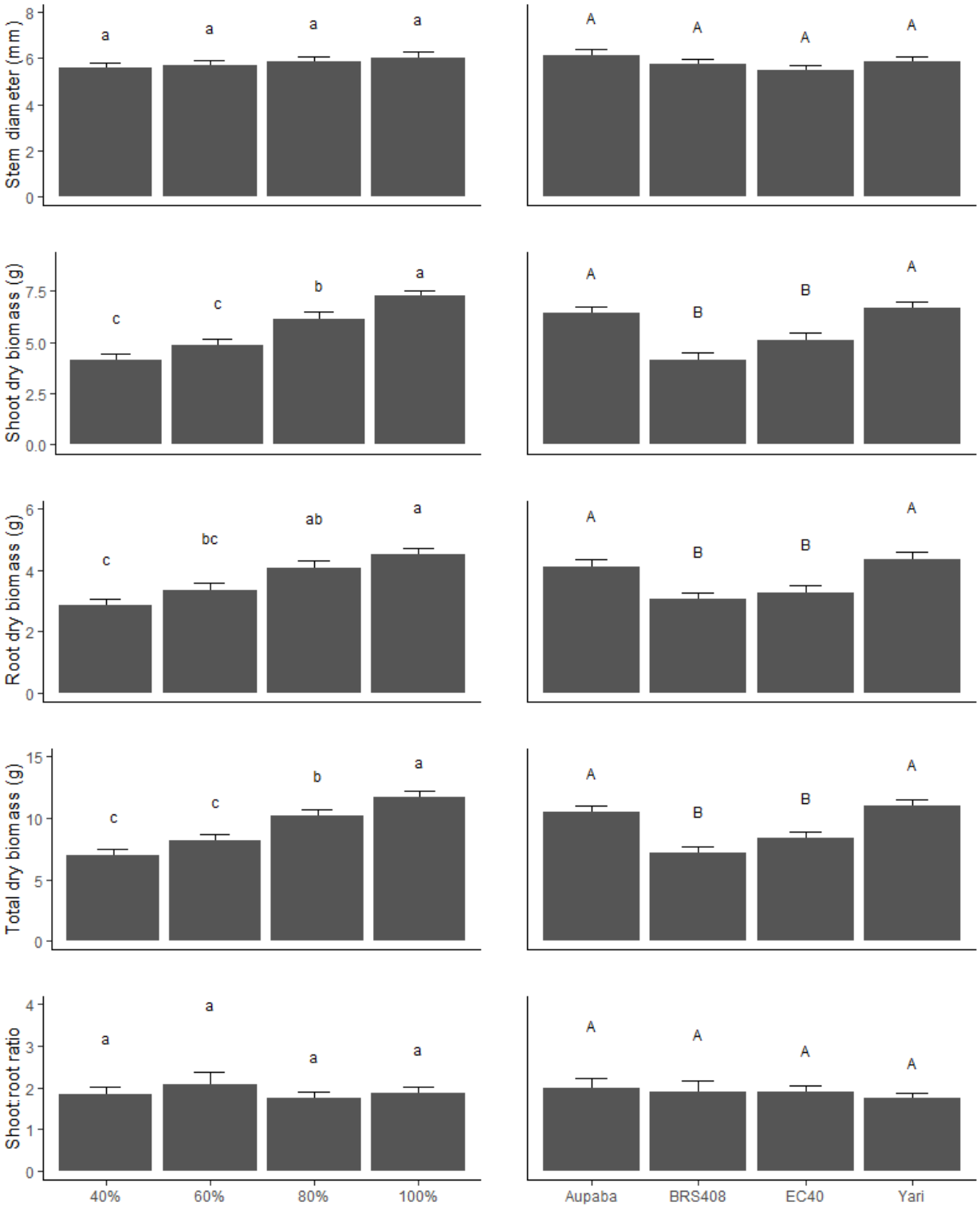


Figure 2

Stem diameter (mm), shoot dry biomass (g), root dry biomass (g), total dry biomass (g), and shoot:root ratio (SDB:RDB) of *Ilex paraguariensis* genotypes subjected to different water availabilities (WHC).

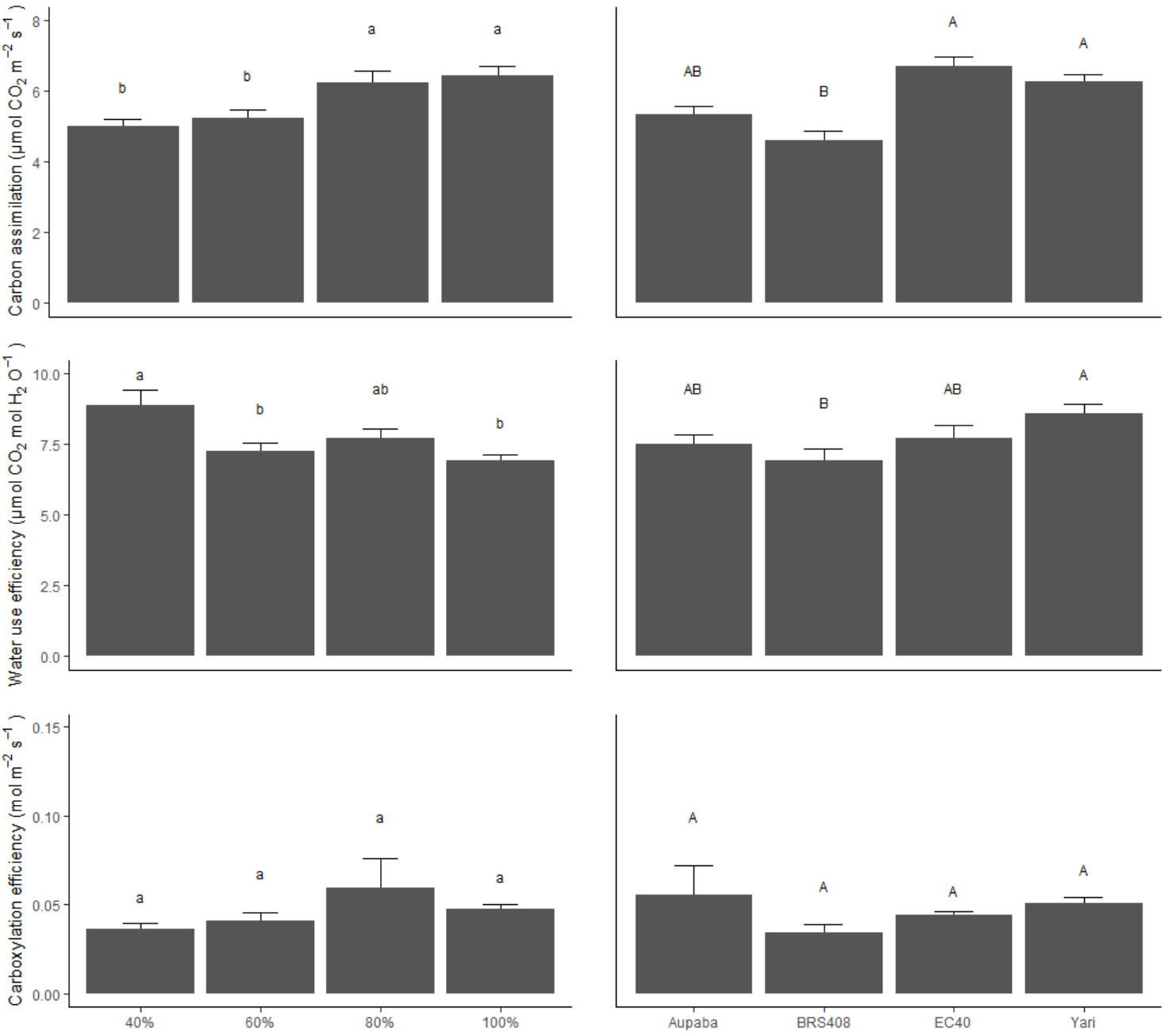


Figure 3

Carbon assimilation (A, μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), water use efficiency (WUE, μmol $CO_2 \cdot mol\ H_2O^{-1}$), and carboxylation efficiency (A/C_i, mol $m^{-2} \cdot s^{-1}$) of *Ilex paraguariensis* genotypes subjected to different water availabilities (WHC).

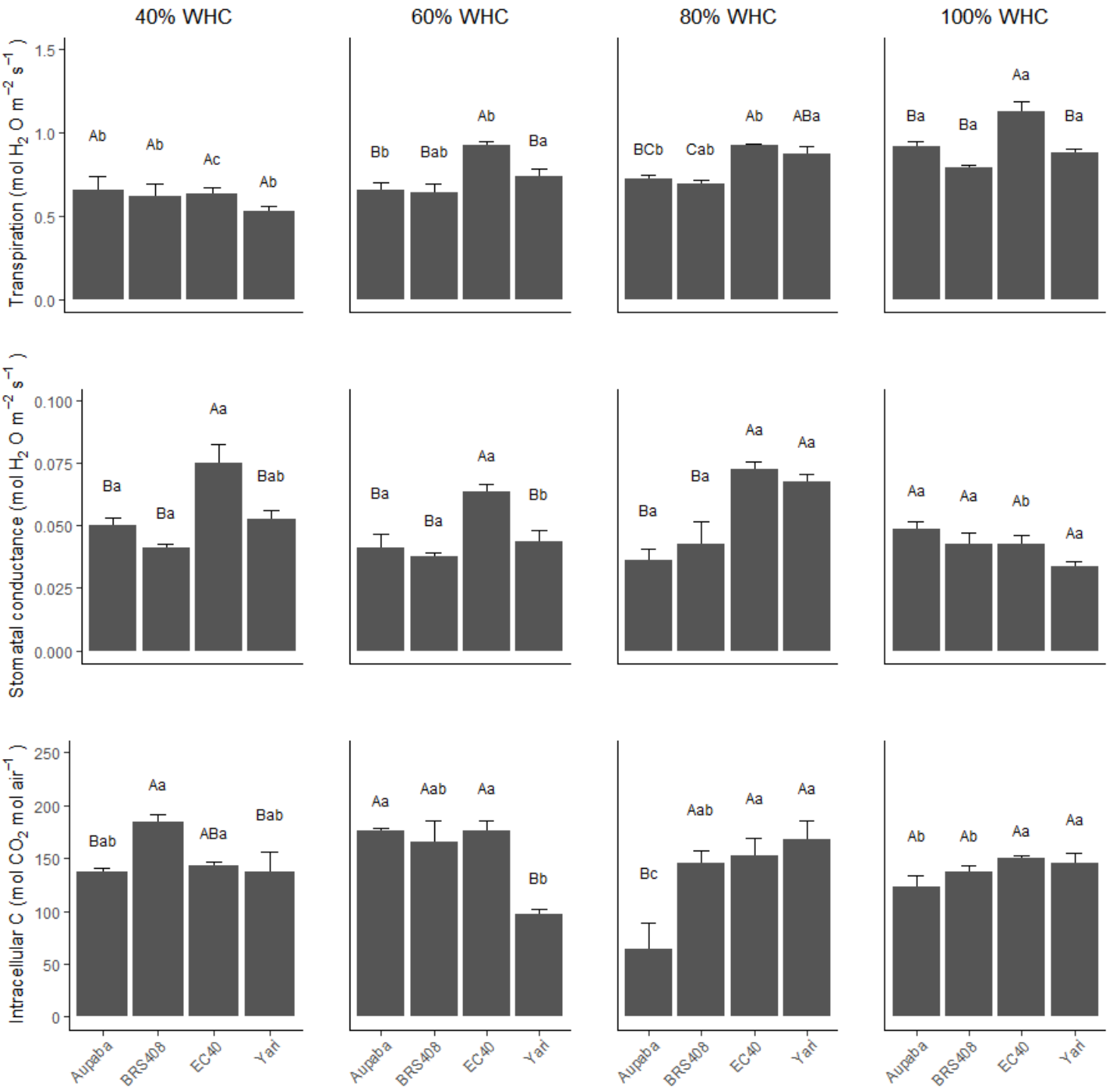


Figure 4

Transpiration (E, mol $H_2O \cdot m^{-2} \cdot s^{-1}$), stomatal conductance (g_s, mol $\cdot H_2O \cdot m^{-2} \cdot s^{-1}$), and intracellular carbon concentration (C_i, µmol $CO_2 \cdot$mol air^{-1}) of *Ilex paraguariensis* genotypes subjected to different water availabilities (WHC).

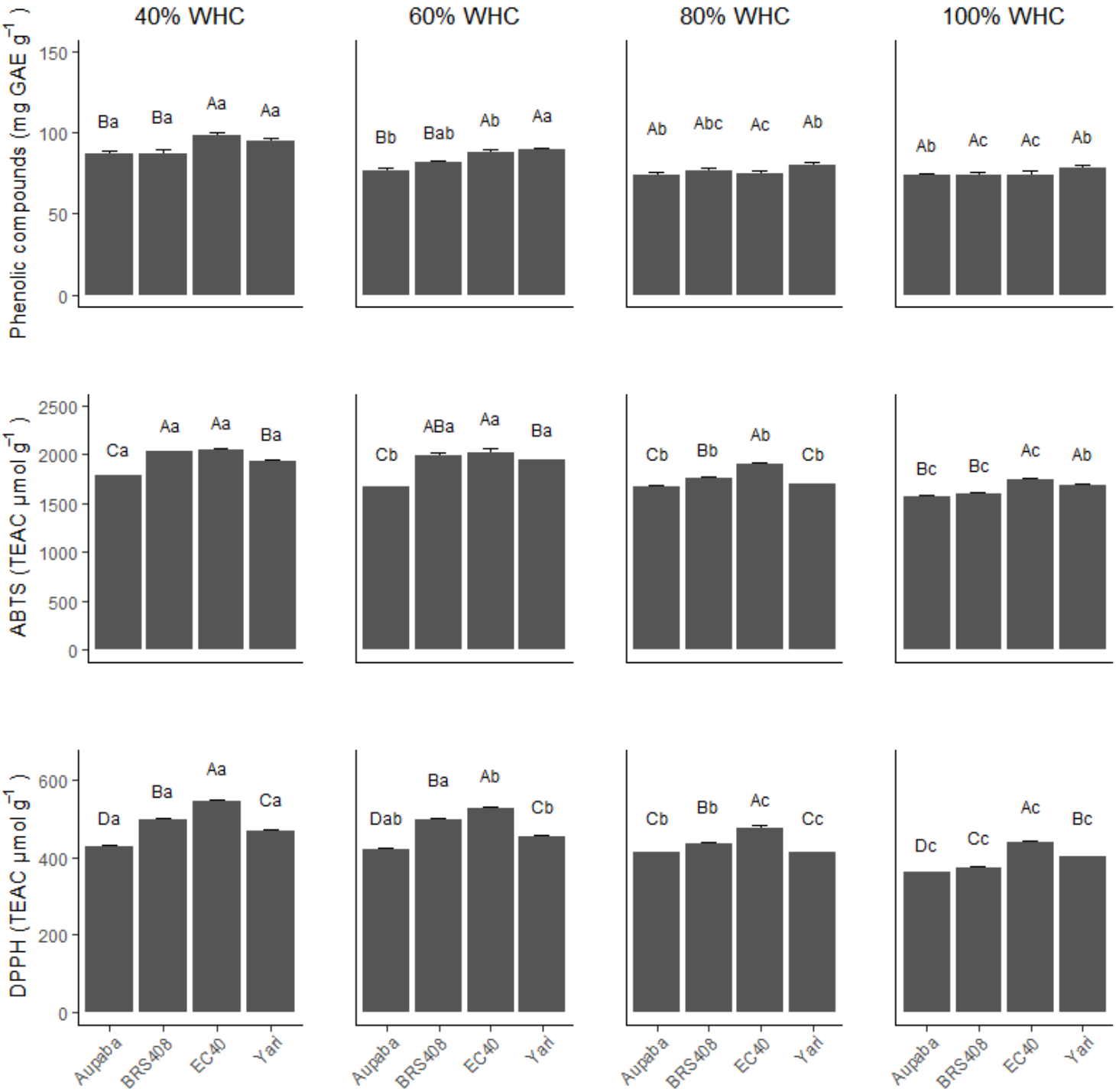


Figure 5

Content of total phenolic compounds (mg GAE g^{-1}) and antioxidant capacity of the free radicals DPPH and ABTS (TEAC µmol g^{-1}) of *Ilex paraguariensis* genotypes subjected to different water availabilities (WHC).

Supplementary Files

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- SMTableS1.docx