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The effect of water deficit on leaf stomatal conductance, water relations, chlorophyll fluorescence and growth of rootstock-scion combinations of cacao

Ramón E. Jaimez [a,*], Geover Peña [b], Luigy Barragán [c], Eduardo Chica [d], Francisco Arteaga [a], George Cedeño [a]

[a] Universidad Técnica de Manabí, Facultad de Ingeniería Agronómica, Grupo de Investigación en Manejo, Nutrición y Ecofisiología de cultivos, Manabí, Ecuador
[b] Instituto Nacional de Investigaciones Agropecuarias. Estación Experimental Tropical Portoviejo, Manabí, Ecuador
[c] Mars La Chola MLCH C.L, Cerecita, Guayas, Ecuador
[d] Facultad de Ciencias Agropecuarias, Universidad de Cuenca, Cuenca, Ecuador

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ABSTRACT

Adverse effects caused by drought on cacao seedlings can produce significant yield losses and require a better understanding of plant-tolerance mechanisms in order to overcome them. The selection of drought-tolerant rootstocks is a key strategy to achieve greater resilience in water-limited environments. This study aimed to assess leaf ecophysiological responses and growth of four rootstocks and 24 rookstock-scion combinations to water deficit. Leaf stomatal conductance (g_s), leaf water potential (Ψ_{leaf}) and relative quantum yield of PSII (Φ_{PSII}) were measured in irrigated and non-irrigated seedlings. Data were recorded 14 and 20 days after irrigation was suspended in the non-irrigated seedlings. Biomass of leaf, stem and root as well root volume were determined 24 days after irrigation was withheld. The EET-400 rootstock showed significantly higher values of Ψ_{leaf} at both 14 and 20 days under water deficit. This rootstock and IMC-67 showed the highest g_s after 14 days without irrigation The relationship between Ψ_{leaf} and g_s showed that the combinations of rootstock EET-400 with scions EET-575, EET-576 and EETP-800 exhibited the highest values of g_s and Ψ_{leaf}. The lower root volumes found under the water deficit condition in EET-400 and IMC-67 demonstrate that tolerance to water deficit by cacao rootstocks is not influenced by a greater contact surface of the root with the soil.

1. Introduction

Water deficit is the one of main abiotic factors limiting plant growth and yield (Parkash and Singh, 2020). Drought affects the hydric state of plants, and the severity of its effects depends on air temperature and the evaporative demand of the atmosphere (Zandalinas et al., 2018). When exposed to water deficit (WD), plants respond by partially closing their stomata, which reduces water losses to the atmosphere, but leads to a decrease in diffusion of CO_2 to carboxylation sites, which reduces photosynthesis. Leaf stomatal controls vary between species and are associated with root distribution and hydraulic conductance (Martínez-Vilalta and Garcia-Forner, 2017). However, the response mechanisms of different crop species to drought have yet to be fully elucidated (Fahad et al., 2017). Therefore, the search for tolerance to WD through breeding programs is a priority (Nuccio et al., 2018), in order to mitigate the effects of future climate change scenarios that warn of increases in temperature and dry periods in various regions. Recent reviews have documented the different responses of plants in relation to their tolerance to WD, and physiological indices have been proposed for evaluating tolerance to WD (Sun et al., 2020).

Grafting is a technique whereby the functional union of two plants is achieved and a soil-root-stem communication established, which has been used to modify plant growth, confer resistance to pathogens and improve tolerance to abiotic factors (Martínez-Ballesta et al., 2010; Warschefsky et al., 2016). Under dry conditions, stomatal conductance (g_s) is regulated both by chemical signals (abscisic acid, ABA) derived from the root and by a hydraulic conductivity signal generated at the soil-root interface. Both mechanisms are considered to work together in stomatal control and are determined by the architecture of the root system, especially by the root length area (the ratio between root length

* Corresponding author.
E-mail address: ramon.jaimez@utm.edu.ec (R.E. Jaimez).

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and root surface area; Peccoux et al., 2018) and osmotic adjustment in the root (Miranda et al., 2020). The combination of traits of both the scion and the rootstock can influence the tolerance of the combination to WD; therefore, it is important to evaluate rootstock-scion combinations better adapted to local environmental conditions (Caruso et al., 2022; Opazo et al., 2020; Patil et al., 2019).

Although grafting is a widely used propagation method in cacao, knowledge about the physiological mechanisms involved in tolerance to WD in rootstock-scion interactions is limited (Medina and Laliberte, 2017) and it becomes currently more relevant in the selection of combinations with greater adaptation to WD (Osorio-Zambrano et al., 2021). Cacao is a tree species native to the Amazon region. Due to its importance in the production of chocolate and other products, it is cultivated mainly in most of the countries of the tropical strip of the world. It is estimated that it is cultivated by about 5 million growers and the cacao chain provides work for around 50 million people, mainly in developing countries, Tridge (2021).

Under conditions of WD, cacao seedlings have shown reductions in g_s, hence causing decreases in transpiration and CO_2 assimilation rates (Joly and Hahn, 1989). Reductions in leaf area, number of leaves, and dry mass also have been reported in response to WD, whereas ABA biosynthesis increased (Santos et al., 2014). However, progenies with tolerance to water deficit have been identified, whose dry biomass is not significantly reduced under conditions of water deficit (Santos et al., 2016). This has led to the fact that several growth parameters have also been proposed for the selection of genotypes tolerant to water deficit (Santos et al., 2016).Cacao clones have also been selected for drought tolerance based on their degree of osmotic adjustment (Almeida et al., 2002; Araque et al., 2012). The ability to maintain higher values of Ψ_{leaf} and intrinsic water use efficiency have also been suggested as variables for the selection of tolerance to WD (Osorio-Zambrano et al., 2021). Niether et al. (2020) reported that ABA and proline concentrations increased 4.5- and 1.5-fold, respectively, in cultivars grafted on the selected rootstoocks (e.g. IMC67) during short-term (21 days) WD. During longer WD (89 days), the concentration of both ABA and proline decreased, but it could not be determined whether these differences were related to the type of rootstock used. Although no differences were found in terms of biochemical responses, there were differences in growth, which suggests tolerance to WD in those cultivars with greater height. Relative quantum yield of PSII (Φ_{PSII}), which is directly related to the rate at which CO_2 is assimilated by the leaf, has been proposed as a rapid measurement parameter to evaluate tolerance to WD (Baker and Rosenqvist, 2004). The Φ_{PSII} has been used to evaluate the effects of the WD in cacao seedlings under greenhouse conditions, producing similar values in WD compared to irrigated seedling (De Almeida et al., 2016). However the latter authors found in field conditions values of Φ_{PSII} that were significantly lower in the dry compared to wet season in the 7-year-s-old hybrids evaluated. Similar results were obtained by Tezara et al. (2020). On the other hand, depending on the severity and duration of the WD, cacao seedlings can ultimately die (Niether et al., 2020).

This work has the purpose of testing the hypothesis that differences in tolerance to water deficit can be found in rootstocks commonly used in several Latin American countries. In addition to evaluating if the differences in tolerance between rootstocks are manifested in seedlings of rootstock-scion combinations, This work aimed to evaluate water relations, leaf stomatal conductance, chlorophyll fluorescence, growth and root volume in rootstock seedlings and rootstock-scion combinations of 'Nacional' cacao types under WD.

2. Materials and methods

2.1. Experimental conditions and plant material: rootstocks and scions

The trial was conducted in an area considered as tropical dry forest with annual rainfall averages of 700 mm. Experiments were done in a greenhouse at the Portoviejo research station of the Instituto Nacional de Investigaciones Agropecuarias (INIAP) (01°07′ S, 80° 24′ W, 54 m.a.s.l.) in Manabí province, Ecuador. The experiment was carried out between February and September 2019. The rootstocks evaluated were EET-399, EET- 400, IMC-67 and Pound-12. These rootstocks were selected because they have exhibited resistance to *Ceratocystis* wilt (Suárez, 1993). In addition to drought tolerance, the selection of rootstocks in cacao must be accompanied by resistance to *Ceratocystis cacaofunesta*, a pathogen causing *Ceratocystis* wilt, a disease initially reported in Ecuador that has already spread and caused considerable losses in Brazil (Ferreira et al., 2013). *Ceratocystis* wilt is considered among the eight most important diseases in cacao (Ploetz, 2007, 2016) and resistance to it is routinely included in rootstock breeding and selection programs.

Clones EET-400 and IMC-67 have been used as rootstock in several countries in Latin America, and EET-399 and Pound-12 are used in Ecuador. Seeds from pods of rootstocks produced on open pollinated flowers were sown in 16-kg bags filled with black soil (Andisol), a loamy type with 32, 54 and 14% of sand, silt and clay, respectively. Clones EETP-800, EETP-801, EET-575, EET-576, CCN-51 and INIAP-484 were selected as scions. With the exception of clone CCN-51, clones belong to the 'Nacional' type. The first two scions were recently released by INIAP (Loor et al., 2019), whereas EET-575 and EET-576, considered as local scions, have been recommended by INIAP for cultivation in Manabí province since 2009 (Amores et al., 2009). Clone INIAP-484 is currently under evaluation (Sotomayor et al., 2017). Clone CCN-51 was selected because of its generalized use in Ecuador and other countries (Boza et al., 2014; Jaimez et al., 2022). At 126 days after sowing the rootstock buds from the aforementioned scions were grafted onto the rootstocks seedlings using the bud grafting method. (4 rootstocks × 6 scions = 24 rootstock-scion combinations). At this time, the rootstocks' seedlings had 6 to 8 leaves. In addition to the 24 rootstock-scion combinations, un-grafted seedlings of the four rootstocks were included in the experiment. A completely randomized block design with three blocks was used. Each block had 6 seedling per treatment. The treatments were combinations of either roostock-scion and water status or ungrafted rootostock and water status. The seedlings were grown in a greenhouse and watered every two or three days (0.6 L per seedling) and fertilized with 15 g of 12:15:12 NPK + micronutrients fertilizer every 15 days until the start the experiment. During the period of the experiment in the greenhouse mean maximum and minimum temperatures were 31.0 and 22.3 °C, respectively. The average maximum and minimum relative humidity were 93 and 60%. A 25% shade cloth was placed inside the greenhouse above the seedlings providing a photosynthetic photon flux density (PPFD) of 780 μmol m^{-2} s^{-1} at noon. At noon, the maximum vapor pressure deficit (VPD) reached values of 1.9 kPa, a value that has been reported as not significantly influencing stomatal closure (Baligar et al., 2008). At 90 days after the grafting, irrigation was stopped in half of the seedlings of each combination, whereas the other half continued to receive irrigation as indicated before (80% of total available water in the soil). The WD treatment reduced the total available water in the soil from 80 to 40% by the 20th day without irrigation.

2.2. Leaf water potential, stomatal conductance, parameters of chlorophyll a fluorescence and root volume

On the 14th and 20th days of the experiment, leaf water potential at early morning Ψ_{em} (07:00–08:15 am) and midday Ψ_{md} (13:00–14:00) was measured using a Scholander pressure chamber (Model 615, PMS Instrument Company, Albany, USA). Three seedlings of each combination were measured. Simultaneously g_s and chlorophyll a fluorescence were measured on the same seedlings. The g_s was measured on fully expanded leaves using a portable leaf porometer (SC1, Decagon Devices, Pullman, Washington). On the 20th days Quantum efficiency of Photosystem II (Φ_{PSII}) were measured using a portable chlorophyll fluorometer (OS5p+, Opti-Sciences, Hudson, New Hampshire, USA) as $\Phi_{PSII} = (F'_m - Fs) / F'_m$, where F_s and F'_m are maximum fluorescence and

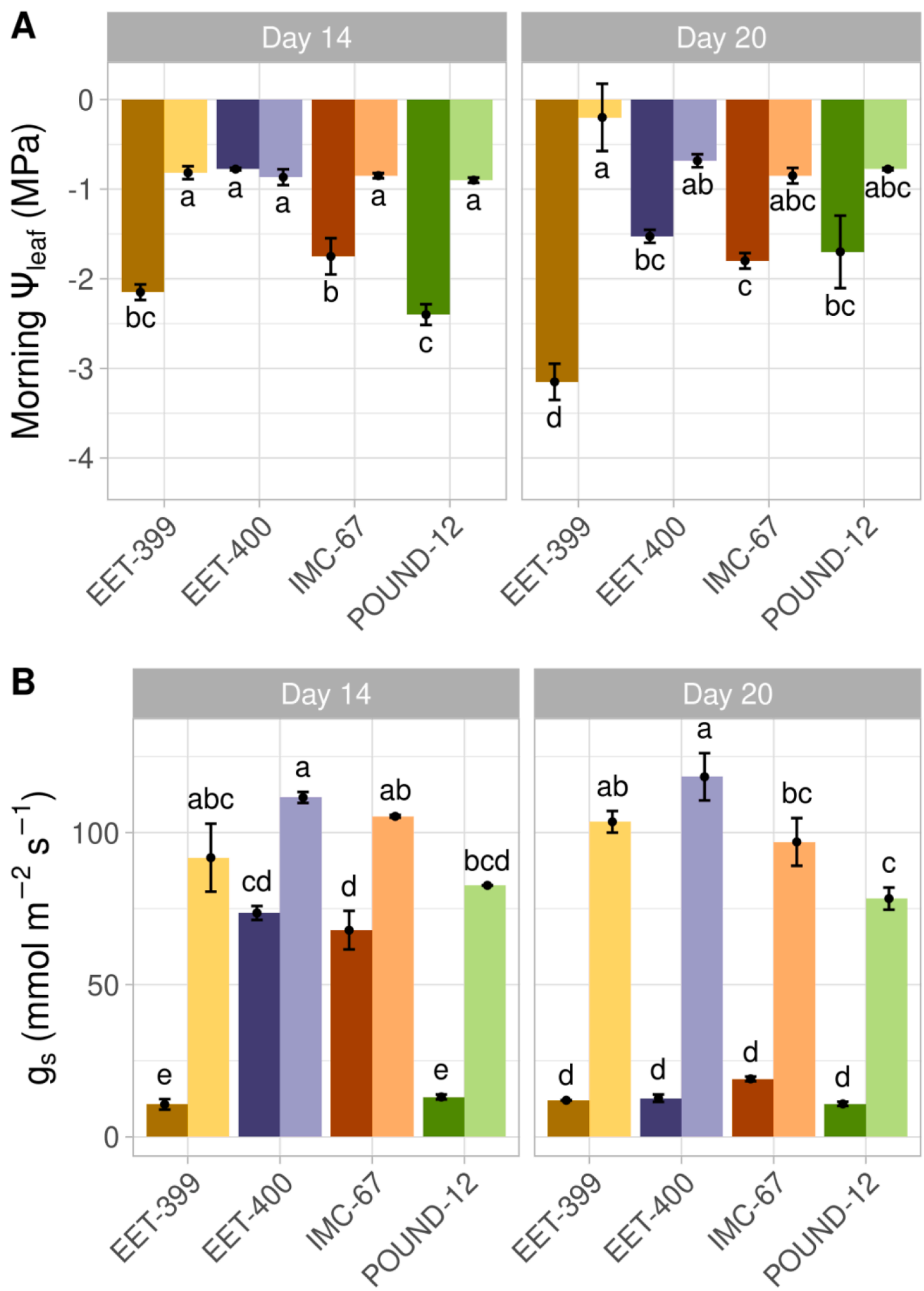

Fig. 1. Changes after 14 and 20 days of water deficit in: A, midday leaf water potential (Ψ_{leaf}), and B, stomatal conductance (g_s,) of cacao rootstocks. For each clone, the bar on the left corresponds to seedlings under WD and the bar on the right to watered seedlings. Different letters indicate statistically significant differences (Tukey's HSD) among rootstocks (both well irrigated and under water deficit) each day.

fluorescence at steady state in the light. Seedlings were not dark adapted and measurements were carried out between 12:00 h and 13:00 h. Four measurements were carried out by combinations and rootstock. Ambient sunlight was between 800–850 μmol $m^{-2}s^{-1}$.

At 20 days three seedlings per combination were harvested and separated into root, leaves and stem. Root volume was measured by removing the seedlings from the soil and washing the roots with water. The volume of the taproot and lateral roots was calculated by immersing the roots in a cylinder with a known volume of water and measuring the increase in volume once the sample was introduced (Santos et al., 2014). The leaves, stems and roots were oven dried at 65 °C for 48 h to a constant weight. Dry mass of leaves, stems, taproot and lateral root were determined for each seedling

2.3. Statistical analysis

The rootstock experiment was conducted using a completely randomized block design. In this experiment, each sampling date was analyzed separately. Differences among rootstocks x water status combinations were tested by ANOVA. When statistical differences were detected, differences among means were assigned using Tukey's test ($p < 0.05$). The rootstock-scion combination experiment was conducted also using a completely randomized block design with each sampling date and scion analyzed separately. Differences among rootstocks-scion x water status combinations were tested by ANOVA and Tukey's test as indicated before. Data are presented as means and standard errors. For all statistical analysis and graphs R software ver. 4.0.3 was used (R Core Team, 2020).

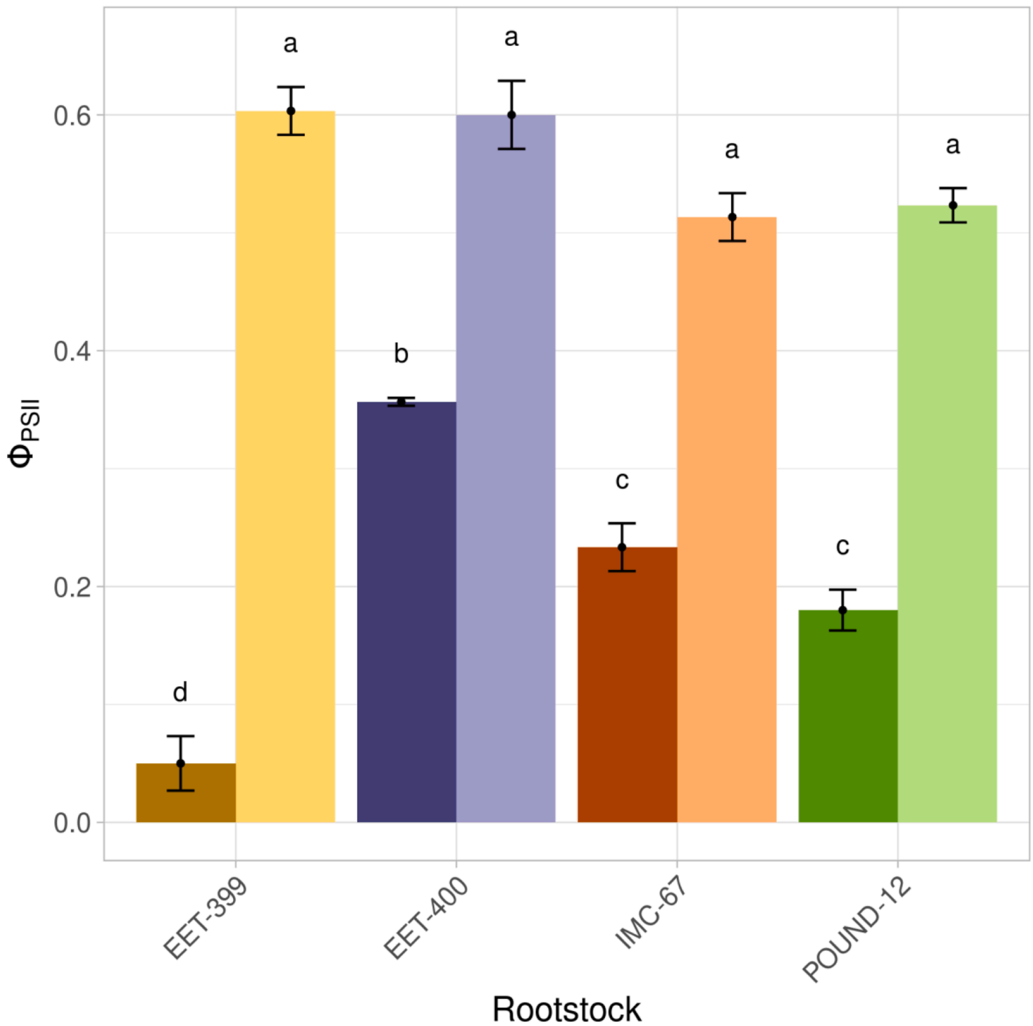

Fig. 2. Midday relative PSII quantum efficiency (Φ_{PSII}) of cacao rootstocks after 20 days of water deficit. For each clone, the bar on the left corresponds to seedlings under WD and the bar on the right to watered seedlings. Different letters indicate statistically significant differences (Tukey's HSD $P<0.05$) among rootstocks (both well irrigated and under water deficit) each day.

3. Results

3.1. Physiological responses of un-grafted rootstock seedlings

Water potentials in the eraly morning showed similar trends to those at noon (Append 1). After 14 days Ψ_{em} decreased to values between −0.9 and −2.4 MPa. After 20 days without irrigation Ψ_{em} decreased to values between −1.9 and −3.1 MPa. Under WD, all rootstocks showed more negative Ψ_{md} compared with irrigated seedlings. After 14 days, Ψ_{md} decreased to values between −2.1 and −3.1 MPa, while after 20 days Ψ_{leaf} declined to values between −2.3 and −3.6 MPa. Ψ_{md} in EET-400 and IMC-67 under WD was less negative than in the other rootstocks at 14 days, while after 20 days clone EET-400 had significantly less negative Ψ_{md} (Fig. 1A). Clones EET-400 and IMC-67 showed the highest g_s (between 67 and 74 mmol m^{-2} s^{-1}) after 14 days under WD but at 20 days all rootstocks under WD showed g_s below 20 mmol m^{-2} s^{-1} (Fig. 1B). Irrigated seedling showed g_s above 80 mmol m^{-2} s^{-1}. Clone EET-400 showed the higher g_s at 14 and 20 days.

The Φ_{PSII} of the rootstocks also decreased during the WD treatment compared to the well-watered controls (Fig. 2). After 20 days EET-400 had the highest values of Φ_{PSII}.

3.2. Physiological responses of rootstock-scion combinations to WD

All variables showed significant differences ($p < 0.05$) among the different combinations at both 14 and 20 days of WD at midday (Figs. 3-4). In most combinations, values of Ψ_{em} and Ψ_{leaf} at 14 days of WD were significantly less negative than at 20 days of WD (append 2 and Fig. 3). Similarly, g_s was significantly lower at 20 compared to 14 days of WD (Fig. 4). The Φ_{PSII} at 20 days of WD was significant lower than irrigated seedling in most of combinations (Fig. 5).

Rootstocks x scions interactions were detected in the response of Ψ_{em} Ψ_{md}, g_s and Φ_{PSII} in grafted seedlings. In the case of Ψ_{em} the mean value at 14 days and 20 days of WD were −1.0 MPa and −1.7 MPa, respectively while $\Psi_{md,}$ the mean value at 14 days of WD was −1.5 MPa, and at 20 days it decreased to −2.5 MPa (Fig. 3). At 14 days, neither CCN-51 nor EETP-800 showed differences among well irrigated and seedling under WD, whereas EET-575 grafted on EET-400 rootstock under WD had values of Ψ_{md} similar to those of well irrigated seedlings on the same rootstock. This was also found in EET-484 with Pound-12 and EET-801 grafted with EET-399, EET-400 and Pound-12.. At 20 days, mean Ψ_{leaf} was −2.5 MPa, while clones EET-575, EET 576 and EETP-800 showed less negative Ψ_{leaf} regardless of the rootstock they were grafted on. Less negative values of Ψ_{md} under WD were dependent on the specific rootstock x scion combination with no clear-cut differences among rootstocks.

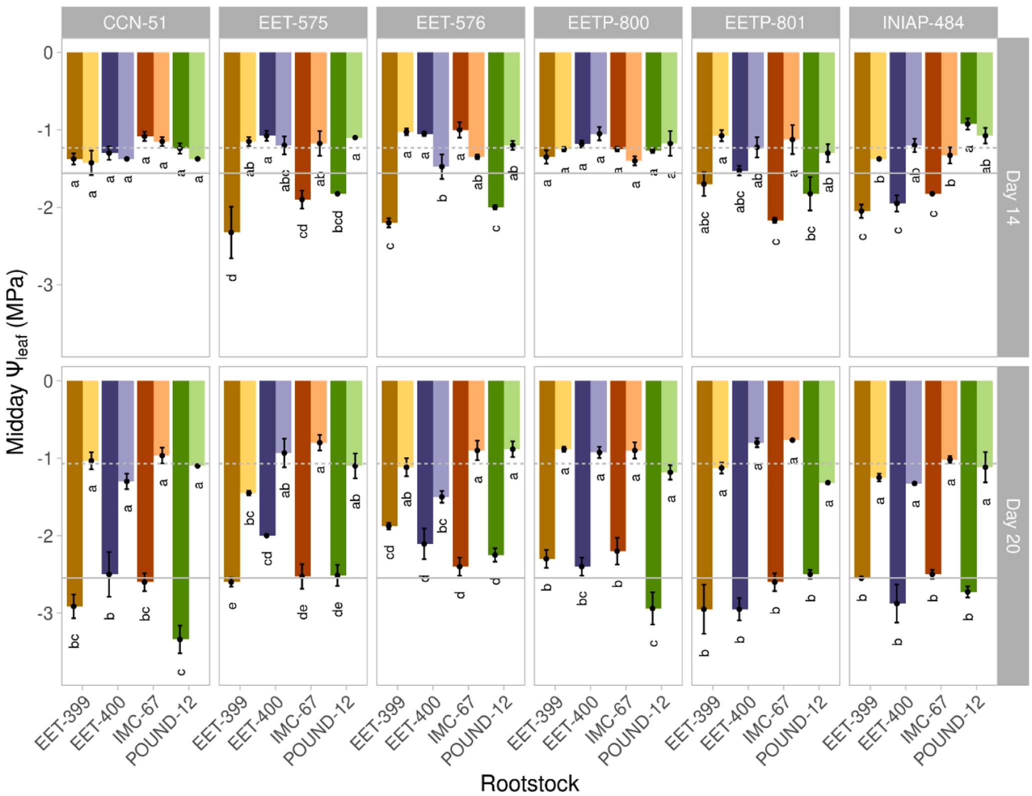

Fig. 3. Midday leaf water potential (ψ_{leaf}) of cacao rootstocks-scion combinations after 14 and 20 days of water deficit. For each clone, the bar on the left corresponds to seedlings under WD and the bar on the right, to watered seedlings. Different letters indicate statistically significant differences (Tukey HSDP<0.05) among all rootstocks within each scion (both well irrigated and under WD) each day. Dashed and solid horizontal lines represent the grand mean of well-irrigated and water-stressed seedlings, respectively, on each day.

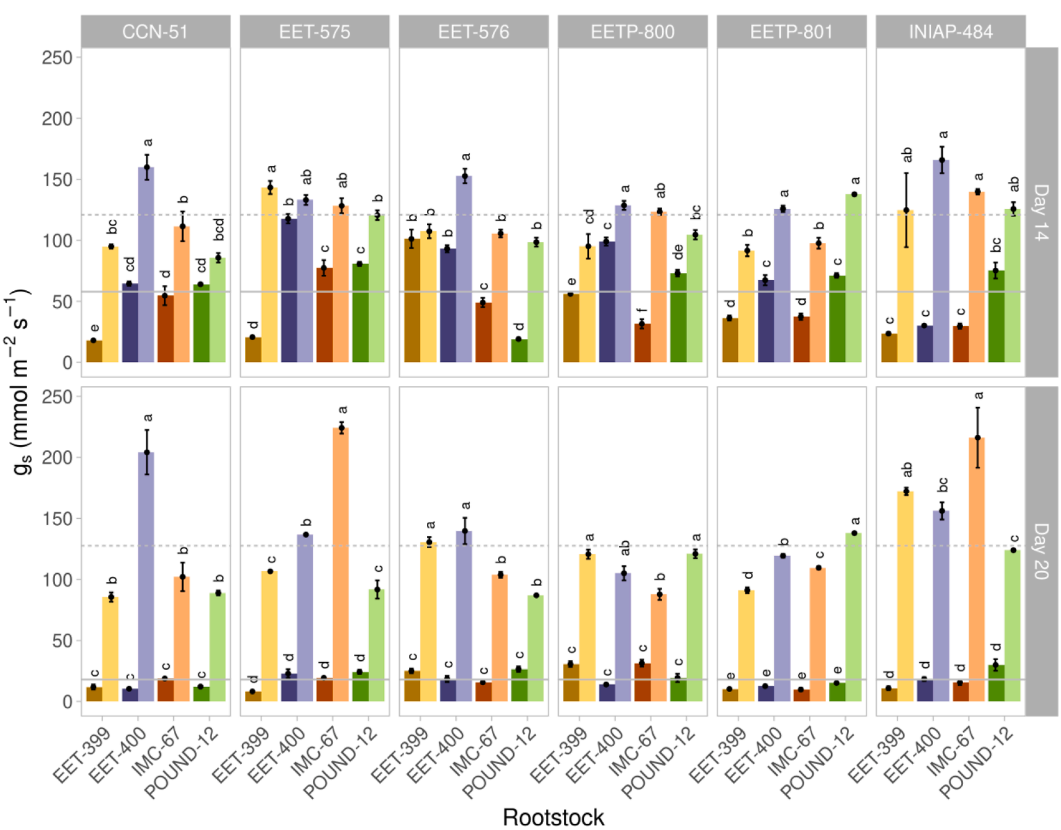

Fig. 4. Stomatal conductance (g_s) of cacao rootstocks-scion combinations after 14 and 20 days of water deficit. For each clone, the bar on the left corresponds to seedlings under WD and the bar on the right, to watered seedlings. Different letters indicate statistically significant differences (Tukey's HSDP<0.05) among all rootstocks within each scion (both well irrigated and under WD) each day. Dashed and solid horizontal lines represent the grand mean of well-irrigated and water-stressed seedlings, respectively, on each day.

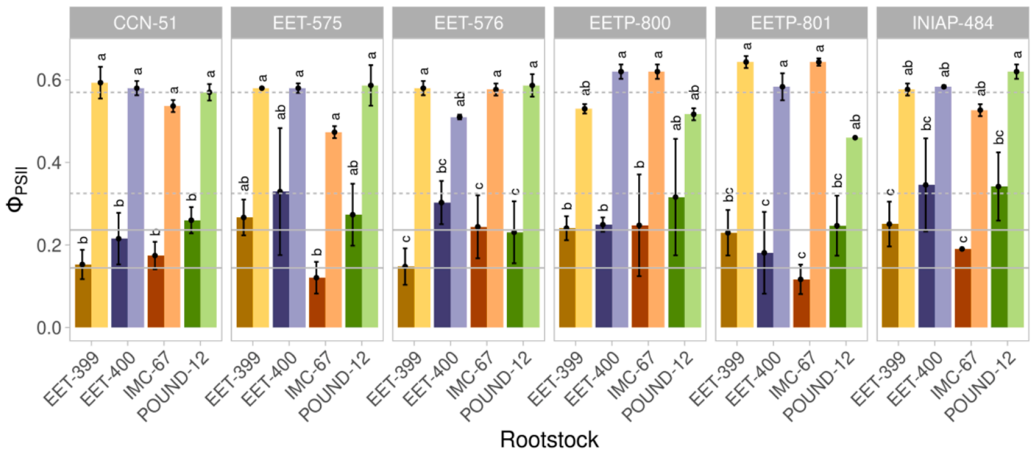

Fig. 5. Relative PSII quantum efficiency (Φ_{PSII}) of cacao rootstocks/scion combinations after 20 days of water deficit. For each clone, the bar on the left corresponds to seedlings under WD and the bar on the right, to watered seedlings. Different letters indicate statistically significant differences (Tukey HSD, $P<0.05$) among all rootstock with each scion (both well irrigated and under WD). Dashed and solid horizontal lines represent the grand mean of well-irrigated and water-stressed seedlings, respectively.

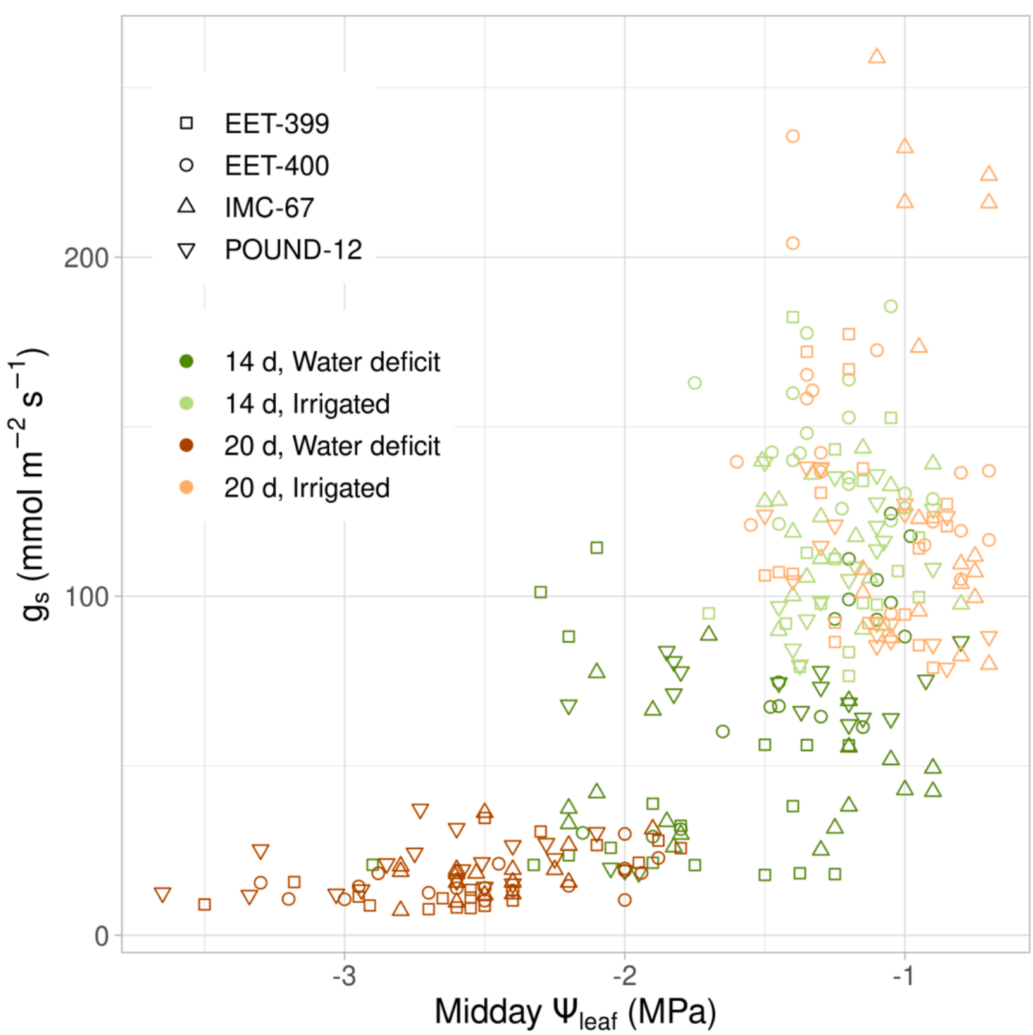

Fig. 6. Relationship between stomatal conductance (g_s) and midday leaf water potential (Ψ_{md}) of cacao rootstocks-scion combinations after 14 and 20 days of water deficit. Lighter shades of each color represent well irrigated controls of each rootstock/scion combination. Different symbols represent different rootstocks.

At 14 and 20 days irrigated seedlings had mean values of g_s of 128 mmol m^{-2} s^{-1}, while at 14 days under WD mean g_s was 60 mmol m^{-2} s^{-1} and at 20 days means dropped to 20 mmol m^{-2} s^{-1}. At 14 days of WD, the combinations of rootstock EET-400 with scions EET-575, EET-576 and EETP-800 and-EET 576 with the rootstock EET-399 had the highest values of g_s, which were around 110 mmol m^{-2} s^{-1}. At 20 days all combinations showed a marked stomatal closure, with values below 20 mmol m^{-2} s^{-1} (Fig. 4).

At 20 days the comparison between seedlings irrigated and WD revealed significant lower Φ_{PSII} in at least 17 combinations under WD (Fig. 5). No relationships were found between Ψ_{md} and g_s in irrigated seedling combinations, while under WD g_s decreased with Ψ_{md}(Fig. 6).

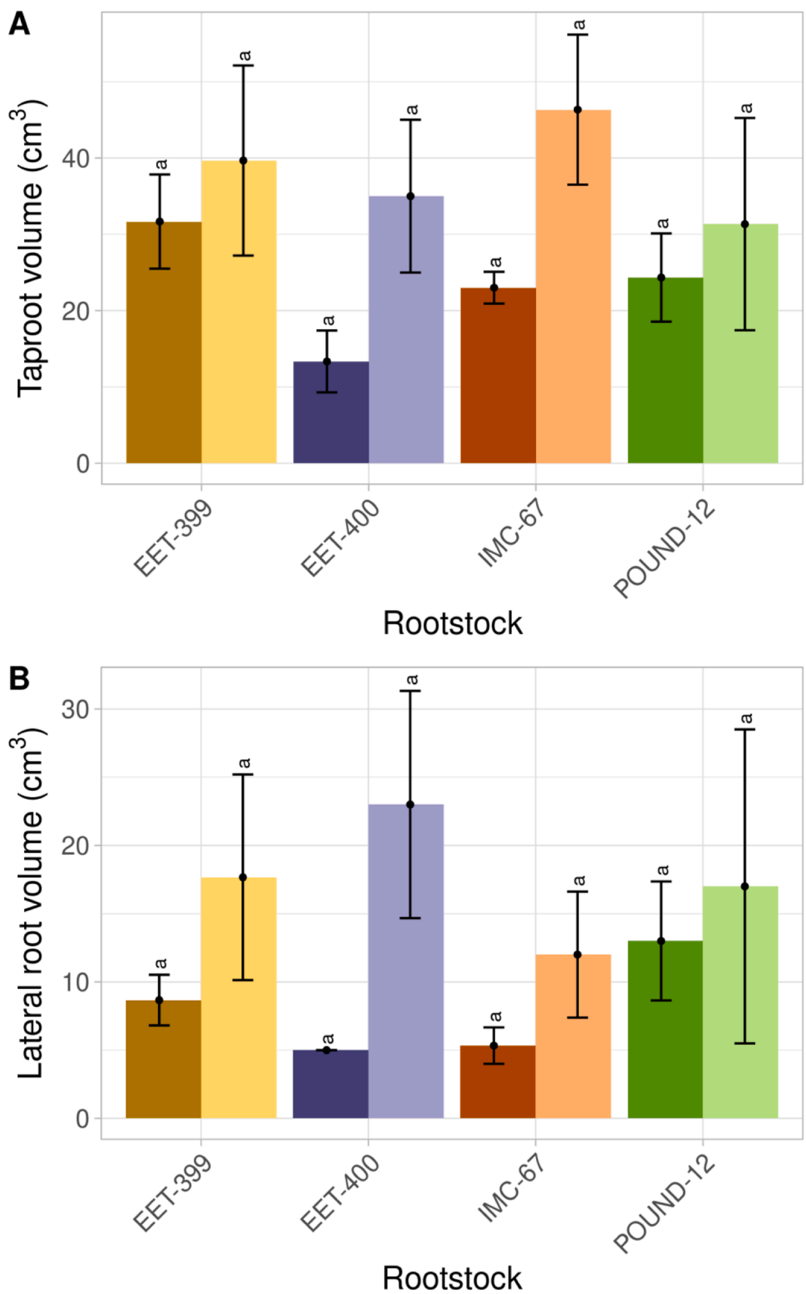

Fig. 7. Taproot and lateral root volume (A and B, respectively) of cacao rootstocks 20 days of water deficit. For each clone, the bar on the left corresponds to seedlings under WD and the bar on the right to watered seedlings. Different letters indicate statistically significant differences (Tukey's HSD, $p < 0.05$) among rootstocks (both well irrigated and under WD).

At 20 days values of g_s were very low, showing little change between the combinations despite Ψ_{lmd} decreasing to $-1.9 - -3.3$ MPa. At 14 days of WD, the combinations of rootstock EET-400 with scions EET-575, EET-576 and EETP-800 exhibited the highest values of Ψ_{md} and g_s. The more negative Ψ_{md} was -2.3 MPa with the combination EET-399xEET-575. At 20 days of WD, Ψ_{md} ranged from -1.9 to -3.3 MPa. ($p \leq 0.05$).

3.3. Root volume of taproot and lateral roots

Differences in either taproot or lateral root volumes among rootstocks were not statistically significant. (Figs. 7A and B). Lateral root volume of un-grafted rootstock seedlings under WD was at least 50% that of well irrigated seedlings, except for Pound-12, in which the reduction was only about 25% (Fig. 7B). No differences were detected in the taproot volume under WD among rootstocks in each scion. Only for scions EET-575 and EET-576 significant differences were found for some combinations between irrigated and WD seedlings. Other scions did not show differences among combinations (Fig. 8A). The lateral root volume was lower under WD in most combinations. Scion EETP-801 showed no differences in any of the rootstocks, whether irrigated or not (Fig. 8A). In only seven rootstock-scion combinations the reductions due to WD of lateral root volume were significant compared to the irrigated seedlings (Fig. 8B).

The WD caused a decrease in the dry weight of the leaf, stem, taproot and lateral roots in most of the rootstocks (Fig. 9) and combinations (Fig. 10). However, there were no significant differences between irrigated seedlings and those subjected to 20 days of WD (Fig. 9).

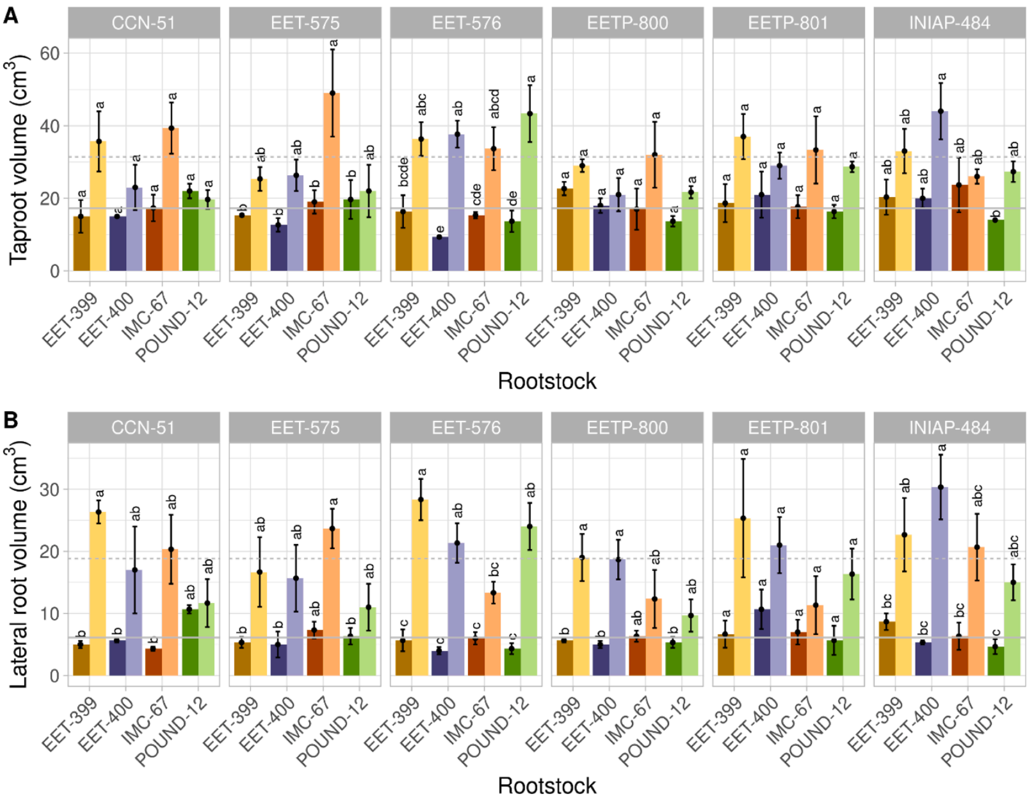

Fig. 8. Changes after 20 days of water deficit in: A. taproot volume, and B, lateral root volume of cacao rootstocks-scion combinations. For each clone, the bar on the left corresponds to seedlings under WD and the bar on the right to watered seedlings. Different letters indicate statistically significant differences (Tukey's HSD, $P<0.05$) among all rootstock/scion combinations (both well irrigated and under WD). Dashed and solid horizontal lines represent the grand mean of well-irrigated and water-stressed seedlings, respectively.

4. Discussion

In this study, four *Ceratocystis* wilt resistant rootstocks were evaluated for their tolerance to WD, revealing rootstock x scion interactions to have desirable physiological traits for cultivation under water limited conditions.

4.1. Roostocks

Among the rootstocks evaluated, EET-400 consistently maintained values of Ψ_{leaf} more similar to the well irrigated controls than the other rootstocks; however, after 20 days of WD, this rootstock was on a par with IMC-67. The highest Ψ_{md} measured in these rootstocks suggests a higher capacity for water intake by their root system than other rootstocks, which permits the maintenance of adequate values of g_s under WD. Nonetheless, g_s was higher under WD in these two rootstocks after 14 days, but no differences among rootstocks were detected after 20 days. These differences could be related to the ability of the roots of these rootstocks to continue absorbing water through osmotic adjustment, as reported for rootstocks in fruit crops (Dichio et al., 2006; Miranda et al., 2020). The low values of g_s found in our study at 20 of WD are close to the ones reported for cacao seedlings (Joly and Hahn, 1989; De Almeida et al., 2016; Lahive et al., 2018). This shows that at the seedling stage cocoa genotypes rapidly close their stomata as a mechanism to conserve water.

The lower root volumes found under the WD condition in EET-400 and IMC-67, agrees with results reported by Barrios-Masias et al. (2015) in grapevine scions and cacao (Jaimez et al., 2021a). Apparently a greater capacity to move water by having fewer but more conducive roots seems a better strategy than producing more roots. which may be the result of incomplete formation of apoplastic barriers. However, higher g_s and transpirations rate have been associated with a greater total root length area (ratio between root length and root surface area) in grapevine (Peccoux et al., 2018).

High yields have been obtained with different clones grafted on EET-400 (Goenaga et al., 2015) and its resistance to *C. cacaofunesta* allows suggesting this rootstock is suitable for growth in areas where water availability is limited. For example, according to our results, EET-400 and IMC-67 combined with productive scion should be evaluated under field conditions with longer dry periods (5 or 6 months) that occur on the Ecuadorian coast. Other variables such as osmotic adjustment, rate of CO_2 assimilation, transpiration and growth should be incorporated. Probably during the first year after transplant in conditions of high evaporative demand, EET-400 and IMC-67 rootstocks could confer enhanced tolerance to scions and would avoid the rapid wilting that was observed in the combinations with the other rootstocks and consequent seedling deaths.

4.2. Rootsotck-scion combinations

In general reductions in g_s as a mechanism to avoid high transpiration rates and maintain Ψ_{leaf} was the general response under WD, although low g_s may have led to a decrease in photosynthetic rate at the seedling stage, as suggested by results obtained by De Almeida et al. (2016). A decrease in Φ_{PSII} under WD is a consequence of the decreasing CO_2 concentration in the intercellular spaces producing a decrease in the consumption rate of ATP and NADPH, which would produce decreases in the electron transport rate and consequently lower Φ_{PSII} (Baker and Rosenqvist, 2004). In the case of cacao seedlings, Lahive et al. (2018) found decreases in Φ_{PSII} under WD and, despite increases in atmospheric CO_2 concentration under WD conditions, seedling exhibited lower g_s and ΦPSII compared to irrigated plants.

In our study, the combinations with higher Ψ_{leaf} also tended to show higher g_s, which can be associated to a higher hydraulic conductance and water uptake by the roots. The greater tolerance to WD of EET-400 and IMC-67 is also manifested in the combinations of this rootstock with several scions. The combinations of EET-400 with EET-576, EET-575 and EETP-800 scions show the best hydric states in terms of Ψ_{leaf} maintaining higher g_s, at 14 days of WD, which could lead to higher photosynthetic rates. In these combinations a greater water uptake

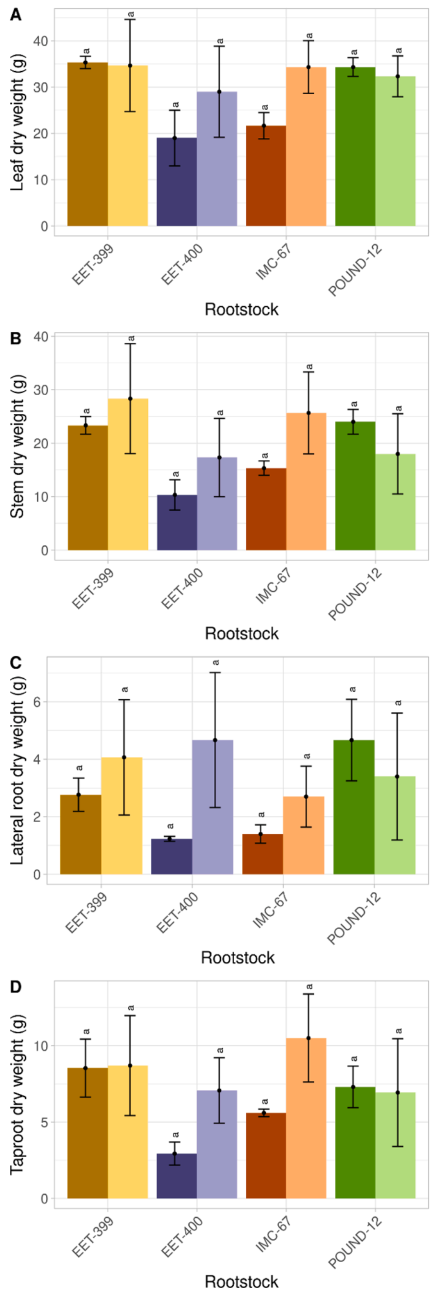

Fig. 9. Dry weight of leaf, stem, taproot and lateral root of cacao rootstocks after 20 days of water deficit. For each clone, the bar on the left corresponds to, seedlings under WD and the bar on the right to watered seedlings. For each variable different letters indicate significant differences (Tukey's HSD, $p < 0.05$) among all rootstocks (both well irrigated and under WD).

capacity by the roots apparently allows the maintenance of a greater stomatal opening.

Along with its lowest g_s, scion INIAP-484 also presented the lowest values of Ψ_{md} at 14 and 20 days of WD, which shows its sensitivity to WD. The other scions differed depending on the intensity of the WD; for example, CCN-51 in combination with the four rootstocks at 14 days of WD had a significant stomatal closure, leading to higher Ψ_{md} and sustained high Φ_{PSII}. Clones EET-575 and EET-576 showed higher Φ_{PSII}. These clones were selected for regions with mean annual rainfall below 1,100 mm and they have demonstrated their capacity to tolerate WD. Nevertheless, they have been less productive than EETP-800 and CCN-51. It would therefore be appropriate to evaluate their resistance to *C. cacaofunesta* and, in the case of showing resistance, they could be tested as rootstocks.

The selection of rootstock-scions combinations with greater tolerance to the WD is an important strategy that confers a greater possibility of avoiding greater losses of plants associated with lower water availability during longer periods without rain during two years after transplant (Araque et al., 2012). The use of rootstocks in cacao as a strategy to increase tolerance to WD has been little studied (Medina and Laliverte, 2017). Although there are studies carried out in greenhouse conditions (Joly and Hahn, 1989; De Almeida et al., 2016) and in the field (Araque et al., 2012; Ávila-Lovera et al., 2016) that show water deficit tolerance in some cultivars, it has not been demonstrated whether they could be used as rootstocks, due to their possible sensitivity to fungal diseases.

In the combinations evaluated, stomatal regulation is apparently associated with Ψ_{md} up to values between −1.3 to −1.7 MPa, which can vary among genotypes, as a mechanism to avoid cavitation. At the seedling stage, g_s is regulated in part by Ψ_{leaf}, and this regulation is established until Ψ_{md} reaching values of −1.7 MPa. Below this value, g_s is practically negligible, preventing loss of water through transpiration, and most probably leading to low or zero photosynthetic rate, as reported in other cacao cultivars in the seedling stage (Joly and Hahn, 1989; De Almeida et al., 2016). The reduction of photosynthetic rate, g_s, and Φ_{PSII} under WD in cacao seedlings has been also reported by Bae et al. (2008). However, under field conditions Araque et al. (2012) found similar values of Φ_{PSII} during the dry and the wet season for 15-months-old criollo cacao types. Almeida et al. (2002), found in 5-months-old seedlings that at $\Psi_{leaf} = -1$ MPa leaf relative water content was initially 90% and decreased to 55% when Ψ_{leaf} decreased to −3.5 MPa.

The g_s is also regulated by VPD. For example, Baligar et al. (2008) found in laboratory conditions a trend of decreasing g_s with increases in VPD but it was not significant and Acheampong et al. (2013) found in nursery house conditions stomatal regulation by high VPD under water deficit. In the case of the scions evaluated in this study, some differences in g_s shown by the scions may be due to responses to changes in VPD. In Ecuador, new plantations are being established with little or no shade and probably higher VPD in the dry period could induce greater stomatal closure (Jaimez et al., 2021b).

Although at 20 days in seedlings under WD the gradual decrease led to a lower accumulation of dry mass of leaves and stems, this was not significant in most of the combinations. Even though the differences in responses between combinations are appreciable, probably those combinations with the smallest decreases in dry mass subjected to WD, e.g., the combinations EET-400 / CCN-51, EET-399 / EET-575 and Pound-12 / EETP-801, could be selected. Santos et al. (2014) found differences in the decrease in dry mass of roots and leaves between 6-months-old non-grafted cultivars subjected to water deficit when values of Ψ_{leaf} reached −2.0 – −2.5 MPa. In some but not all cultivars the decreases were significant. However, our results show that a decrease in leaf mass under WD conditions can also imply a smaller transpiration area in some combinations that can consequently prevent lower Ψ_{leaf}, which does not occur in irrigated seedlings. Other studies have demonstrated that under WD the growth of cacao seedlings was significantly reduced when the soil moisture fell to between 15 and 20% in soils where field capacity is 40% (Lahive et al., 2018); or when soil moisture was 50% of field capacity (Hebbar et al., 2020).

Taproot and lateral root volume of rootstock-scion combinations were both reduced under conditions of WD. Under WD, significant relationships between g_s and volume of lateral roots did not occur.. Nevertheless, the high volume variation in the lateral roots of the irrigated seedling combinations suggests intraspecific variations of each combination.

Rootstock-scion combinations must necessarily be evaluated under field conditions to select those with the best performance. Under field conditions, evaluations of physiological traits, growth rate, yield and precocity of cultivars should be carried out under different climatic conditions, especially in regions with total rainfall less than 1,200 mm

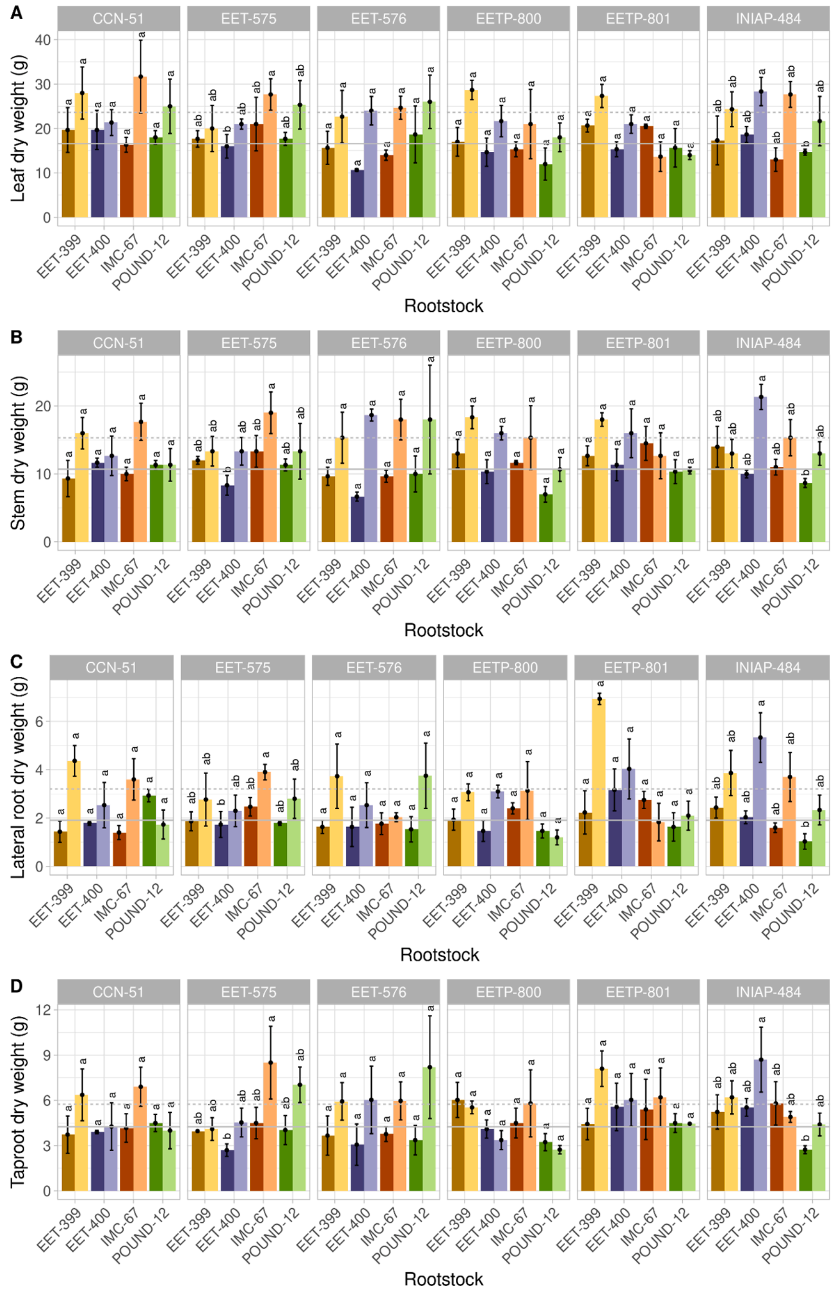

Fig. 10. Dry weight of leaf, stem, taproot and lateral roots of cacao rootstocks-scion combinations after 20 days of water deficit. For each clone, the bar on the left corresponds to, seedlings under WD and the bar on the right to watered seedlings. For each variable different letters indicate significant differences (Tukey's HSD, $p < 0.05$) among all rootstocks (both well irrigated and under WD).

yr^{-1}. Several years of information are required to record the variability of changes in yield of different rootstock-scion combinations and thus enable selecting the best rootstock-scion combinations.

5. Conclusions

This study shows that cultivars used as rootstocks in cacao exhibit different levels of water deficit tolerance, thus highlighting the convenience of continuing to evaluate the tolerance to WD of other rootstocks. The EET-400 and IMC-67 rootstocks exhibited a higher tolerance to WD (higher Ψ_{leaf} and Φ_{PSII}). These rootstocks have a greater capacity to move water by having fewer but more conducive roots The higher tolerance to WD presented by the rootstock EET-400 without grafting is also shown in scions EET-575, EET-576 and EETP-800 grafted onto this rootstock. These combinations exhibited the highest relationships between g_s and Ψ_{leaf} under WD, suggesting they should be evaluated in areas prone to develop water limited conditions. New research aimed at selecting cocoa genotypes with increased tolerance to WD should evaluate under field conditions rootstock-scion interactions rather than just the scion.

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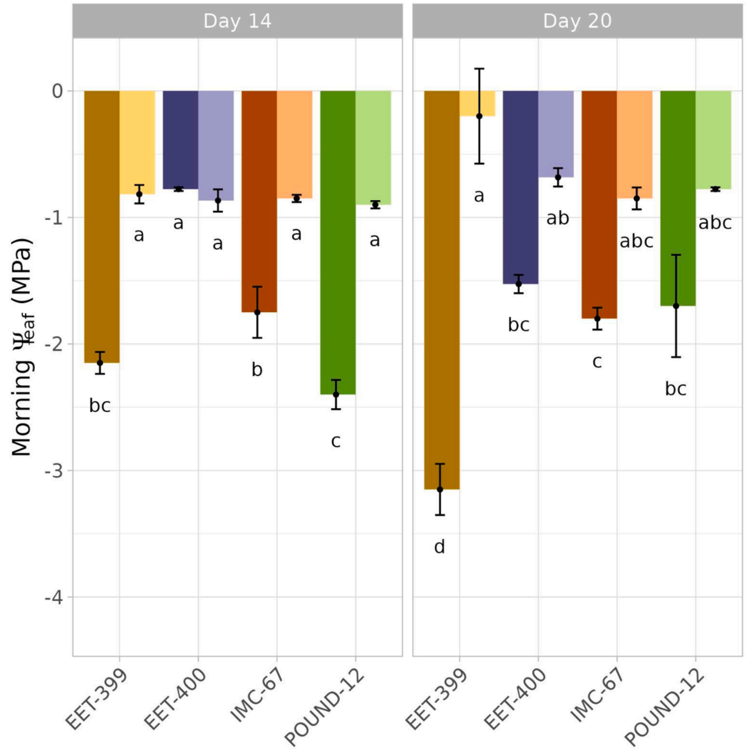
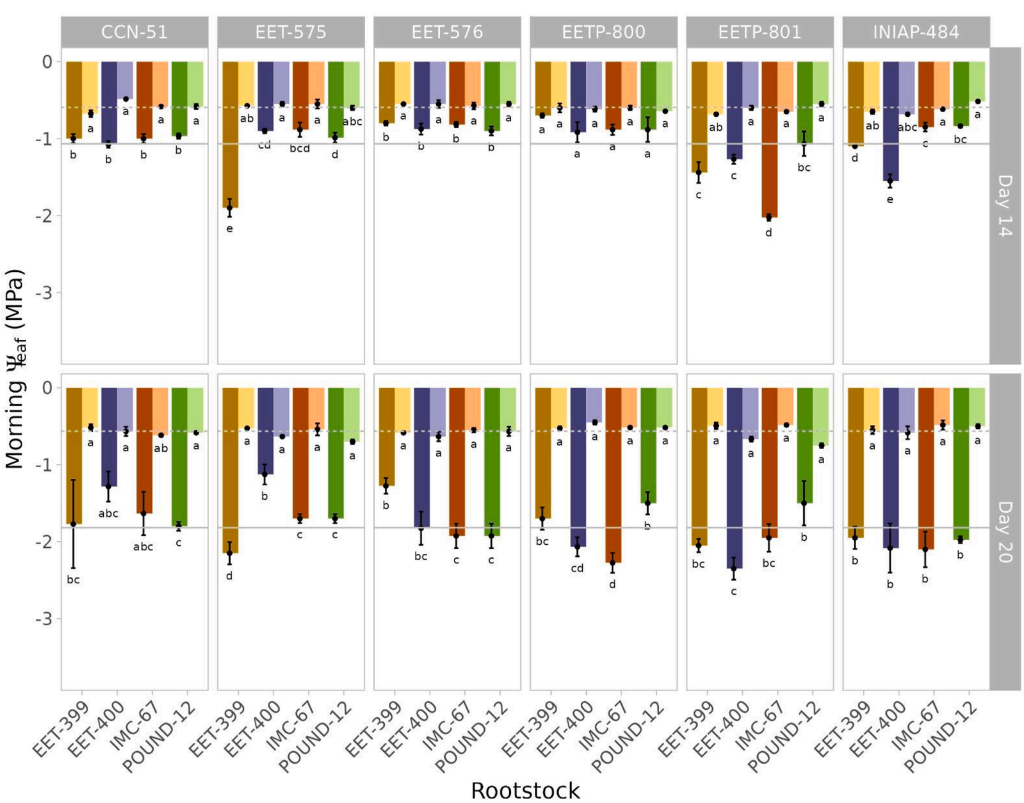


Append 1. Changes after 14 and 20 days of water deficit in: early morning leaf water potential (Ψ_{em}). For each clone, the bar on the left corresponds to seedlings under WD and the bar on the right to watered seedlings. Different letters indicate statistically significant differences (Tukey's HSD) among rootstocks (both well irrigated and under water deficit) each day.



Append 2. Fig. 3Early morning leaf water potential of cacao rootstocks-scion combinations after 14 and 20 days of water deficit. For each clone, the bar on the left corresponds to seedlings under WD and the bar on the right, to watered seedlings. Different letters indicate statistically significant differences (Tukey HSD$P<0.05$) among all rootstocks within each scion (both well irrigated and under WD) each day. Dashed and solid horizontal lines represent the grand mean of well-irrigated and water-stressed seedlings, respectively, on each day.

CRediT authorship contribution statement

Ramón E. Jaimez: Conceptualization, Funding acquisition, Methodology, Formal analysis, Writing – original draft. **Geover Peña:** Conceptualization, Funding acquisition, Methodology, Writing – original draft. **Luigy Barragán:** Formal analysis, Writing – original draft.

Eduardo Chica: Formal analysis, Writing – original draft. **Francisco Arteaga:** Methodology, Writing – original draft. **George Cedeño:** Methodology, Formal analysis.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ramon Jaimez reports financial support was provided by German Society for International Cooperation (GIZ) GmbH. Ramon Jaimez reports a relationship with Technical University of Manabi that includes: funding grants. There are no additional relationships to declare

Data availability

Data will be made available on request.

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Appends

Append 1, Append 2

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