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Genotypic differences in water deficit effects on leaf and crown traits in mature field-grown cocoa

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ABSTRACT

Cocoa trees are highly sensitive to water stress but these negative effects may differ genetically and may be mitigated by potassium (K) application. We studied these effects on six-year-old adult trees, five genotypes produced by somatic-embryogenesis, and one by cross pollination (Hybrid), grown under sub-optimal field conditions in central Côte d'Ivoire. Trees were subjected to two soil water treatments (with or without dry season irrigation) and two K fertilization levels (with and without K). We assessed interactive effects of treatments on several leaf and crown traits: sap flux density (SF), leaf water potential (LWP), stomatal conductance (Gs), leaf size (LS), specific leaf area (SLA), leaf water content (LWC), leaf area index (LAI), light interception, litterfall and a visual index of whole-plant water stress (WSI). Mixed-effects model results revealed that withholding irrigation negatively affected SF, LWP, Gs, LS, SLA, LAI and light capture and increased WSI scores. Potassium application did not significantly mitigate the negative effects of withholding irrigation. A significant effect of genotype was observed for most of the monitored leaf physiological, morphological and crown traits. Furthermore, we found significant, positive interactions between genotype and irrigation for SF, LWP, leaf area, LS, SLA, LWC, LAI and intercepted light, suggesting that the differences in observed responses to drought conditions are genotype-specific. These results provide insights into the acclimation strategies of cocoa and genetic variation therein, and can be used to select drought-tolerant genotypes.

1. Introduction

Through increased temperature and shifts in rainfall patterns, climate change is projected to result in increased incidence and severity of extreme climate events such as drought and high temperatures (IPCC, 2022). Many parts of the tropics are hence projected to experience increases in the frequency and the extent of water scarcity events. These changes will likely affect agricultural production in West Africa (Sultan and Gaetani, 2016), including production of important commodity crops, such as cocoa (Läderach et al., 2013). In West Africa, where 70 % of global cocoa is produced, water availability is the main limiting factor for cocoa growth and yield (Anim-Kwapong and Frimpong, 2005; Läderach et al., 2013), resulting in a significant, projected reduction of the area suitable for cocoa production by 2050 (Schroth et al., 2016). Therefore, there is a need to develop climate-smart agricultural adaptation strategies for cocoa production (Lipper et al., 2014; Vaast et al., 2016; Nasser et al., 2020).

Cocoa evolved in habitats (Amazon rainforest) that are not typically water limited, probably explaining its drought sensitivity (Wood and Lass, 1987). Cocoa does not tolerate long periods of drought stress

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Abbreviations: AIC, Akaike's Information Criterion; CEC, Cation exchange capacity; DAP, Di-Ammonium Phosphate; Gen, Genotype; Gs, Stomatal conductance; Irrig, Irrigation; LAI, Leaf area index; LMM, Linear mixed-effect models; LS, Leaf size; LWC, Leaf water content; K, Potassium; KCl, Chloride of potassium; LWP, leaf water potential; MLWP, midday leaf water potential; N, Nitrogen; P, Phosphorus; PLWP, Predawn leaf water potential; PtT, Potassium application; SLA, Specific leaf area; SF, Sap flux density; TDR, Time Domain Reflectometer; WSI, Water stress index.

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(Abdulai et al., 2018a; Bae et al., 2008; Raja and Hardwick, 1988) and possesses large leaves, shallow rooting systems and wide xylem vessels, that allow optimal growth under moist conditions (Antwi, 1994; Meinzer et al., 1992). In cocoa, brief episodes of water shortage can decrease stomatal opening, photosynthesis, and transpiration and may thus reduce yields (Carr and Lockwood, 2011; Gattward et al., 2012; Wessel, 1971). Studies on sap flow density for cocoa showed transpiration was reduced during dry spells (Della Sala et al., 2021; Moser et al., 2010; Abdulai et al., 2018b). Nonetheless, little is known about cocoa transpiration responses to limiting water availability.

The availability of potassium (K) is believed to play an important role in plant responses to drought stress. It regulates cell water potential and affects plant stomatal opening (Jordan et al., 2008; Peiter, 2011), which in turn plays a key role in the way plants deal with water limitation. K application can help to mitigate the negative effect of water deficit on cocoa seedling growth (De Almeida and Valle, 2007; Djan et al., 2017). Hence, K nutrition may also mitigate this effect in mature cocoa, particularly because cocoa has a high demand for K for pod production (Medina and Laliberte, 2017; van Vliet and Giller, 2017). Yet, the interactive effects of water availability and potassium application are poorly understood, and have remained untested in cocoa under field conditions.

Experiments on cocoa drought effects revealed strong genotypic variation to drought tolerance (Daymond and Hadley, 2011), suggesting scope for more drought tolerant cocoa types. However, currently it is not fully assessed how these mechanisms differ across genotypes under field conditions in cocoa plantations and whether some of these genotypes are better adapted to drought stress than others. Therefore, the genetic variation in responses to drought, K and their interaction needs to be further explored, especially in adult trees under field conditions, to support breeding efforts for drought tolerant varieties (Lahive et al., 2018). In this study, we evaluate the influence of water deficit, K application and their interaction on leaf physiology of mature field-grown trees of six cocoa genotypes. We address the following research questions:

(i) How do irrigation and K application and their interaction alter cocoa leaf physiology and morphology?
(ii) How do irrigation and K application and their interaction impact crown-level traits of cocoa?
(iii) Do the effects of irrigation and K application differ among cocoa genotypes?

To this end, we conduct a field experiment in which we test responses to water deficit and K application for adult field-grown trees of six cocoa genotypes: five clones and one hybrid. We hypothesize that: (1) withholding irrigation in the dry season will reduce sap flux density, leaf water potential, stomatal conductance and leaf morphological traits (leaf size, SLA, LWC, leaf area index, intercepted light), and that K application will mitigate these effects for non-irrigated trees; (2) withholding irrigation will result in greater leaf fall and a reduction in the percentage of intercepted light and leaf area index over the different seasons over the year; and (3) more drought tolerant genotypes will exhibit smaller reductions in sap flux density, water potential and stomatal conductance as well as smaller changes in leaf morphology and smaller increases in litter production when irrigation is withheld.

2. Material and methods

2.1. Study site and experimental design

The experiment was conducted at the Nestlé Research station in Zambakro, in the Central-Eastern part of Côte d'Ivoire (6°49′13.98″N, 5°16′36.26″W) from 2020 to 2021. The mean annual temperature at the station was 26.3 °C and mean annual precipitation was 1120 mm, which is considered as very dry cocoa producing area (Ehounou et al., 2019). Typically, there are six dry months (precipitation < 100 mm) distributed over a long (November to February) and a short (July to August) dry season. Soil analysis of the experimental site (February 2020 and 2022) showed that the upper soil layer (0 – 20 cm) was acidic, and of sandy loam texture. Soil cation exchange capacity (CEC) ranged between 1 and 5 (meq 100 g^{-1}) typical for relatively sandy soils. Soil organic carbon tended to be less than 1 mg g^{-1} in the topsoil with 0.83 mg g^{-1} in 2020 and 0.42 mg g^{-1} in 2022.

Planting material was produced through somatic embryogenesis which offers morpho-agronomic heterogeneity in the fields. This method gives significant technological advantages to obtain a large quantity of disease-free planting material with good agronomic characteristics and genetic stability (Tapi et al., 2020; Garcia et al., 2018). For each genotype, five subplots of eight trees per row were established in 2015, randomly placed within the overall stand, which contained 240 trees planted at a distance of 2.5 × 3 m (Fig S 4). Around the stand, one row of border trees was included.

2.2. Experimental treatments

The cocoa stand was divided into two blocks, one irrigated and one not irrigated, and each block was divided into two sub-plots, with one fertilized with potassium and one not. The irrigation and potassium treatments were initiated in January 2020 when the trees were six years old. Water was supplied through drip irrigation. Each tree was located between two emitters (flowrate 1 L h^{-1}) placed on a single drip line. Emitters were located 80 cm away from the trunk and at 80 cm from each other. Irrigation was applied four times per week during dry periods only (Dec-Mar = major dry period and Aug-Sept = minor dry period). It consisted of 9.3 mm day^{-1}, thus a total of 893 mm water supplied per year.

N and P fertilizer was applied either with or without K fertilizer, to increase the probability of K being the main limiting major nutrient. All the genotypes received the same total amount of N and P in the form of Nitrabor (167 kg ha^{-1}) and of Di-Ammonium Phosphate (DAP, 54 kg ha^{-1}). For the potassium treatment, the genotypes received the same amount of K in the form of KCl (71 kg ha^{-1}). Three doses of Nitrabor, DAP and KCl were applied in March, July and September in 2020 and 2021. Each tree received an amount of 0.125 kg N, 0.04 kg P and 0.053 kg K per fertilizer application.

Every month, soil moisture content was recorded from 0 - 20 cm depth at 40 cm from each tree with a time-domain reflectometer (TDR350, FIELDSCOUT Spectrum Technology, Inc.). Daily weather conditions, i.e. rainfall, relative humidity, solar radiation, and air temperature, were automatically recorded by the weather station located near the experimental plot during the experiment from January 2020 to March 2022. For both years, leaf trait and gas exchange measurements (Gs) were done in the middle of both the major and minor dry and wet seasons (December-March, April-July, August-September, October-November) for each genotype over four consecutive days.

2.3. Data collection

2.3.1. Leaf water potential

Leaf water potential (LWP) at predawn and at midday were measured using a Scholander-type pressure chamber. This was done for four central trees per subplot, per treatment, for two genotypes only, the hybrid Mercedes and the clone CI03 from somatic embryogenesis. Trees were relatively homogeneous, but the two genotypes differed in the way they react phenologically to drought conditions based on field observations. M maintains a green crown, whereas CI03 undergoes an intense crown defoliation. Per tree, six fully developed mature leaves from sun-exposed branches were measured. For measuring midday leaf water potential (MLWP), leaves were excised between 13.00 and 14.00 h, and placed into plastic bags in a cooler, until measurements were done within 10minutes. For measuring pre-dawn leaf water potential (PLWP), ten sun-exposed mature leaves per species were pre-bagged at sunset the day prior to measurement, with both plastic sheet and aluminum foil bags to deflect solar radiation (Choné et al., 2000) and at dawn of the next day measurement was done. Before measurements, the petiole was cut and the bag was closed after removing the air.

2.3.2. *Sap flux measurements*

Thermal dissipation probes (Granier, 1987) were applied to continuously measure sap flux density in cocoa trees every week over four dry months, from February to May 2022. Sap flow was monitored on six cocoa trees in two adjacent genotypes: three central clonal trees (CI03) and three central hybrid trees (M) in each treatment. Each tree was equipped with a pair of needle sensors in holes of 2 mm diameter and about 23 mm depth, below the average jorquette height (~1.3 m) before the first branches. The upper probe of the sensors was heated with a constant power of 12 V, and was placed at a vertical distance of 5 cm from the lower, unheated probe. Probes were diagonally installed. Probes were first coated with heat-conducting silicon paste and placed into aluminum tubes pre-inserted in the sapwood. Probes were sealed with reflective bubble wrap and plastic bags to protect the sensors from environmental influences, such as rain and direct solar radiation. Differential voltages of the sensors were measured every 60 s and averaged every 30 min, using an AM16/32 multiplexer and CR1000X data logger (Campbell, Scientific Instruments, Logan, UT). Because of limited capacity of the data logger, measurements could not be done for all trees at the same time. The data logger was transferred from irrigated to non-irrigated plots and vice versa weekly. Sap flow density was calculated following Granier (1987):

$$U = 0.714 \times K \wedge 1.231 \tag{1}$$

where U is sap flux density (ml cm^{-2} min^{-1}), and K was determined as:

$$K = (\Delta T_M - \Delta T)/\Delta T \tag{2}$$

where ΔT is the temperature difference between two needles (mV) and ΔT_M is the maximum value of nighttime ΔT (mV) when there is no sap flow (zero set value).

2.3.3. *Stomatal conductance*

Stomatal conductance (Gs) was measured between the veins of the abaxial surface of three sun-exposed, green, healthy, mature leaves per tree for the four central trees per subplot for the M and CI03 genotypes. For both years, Gs was measured in the middle of both major and minor dry and wet seasons (December-March, April-July, August-September, October-November) for each genotype over four consecutive days. Stomatal conductance was measured with a portable leaf porometer (SC-1 Steady State Leaf Porometer; Decagon Devices, Pullman, WA, United States), which measures the amount of water transpired from the leaves through the stomata. Leaves were allowed to equilibrate, and a reading was recorded after approximately 30–120 s. Measurements on leaves that took longer than 3 min to equilibrate were discarded. Measurements were performed between 7:00 am and 9:30 am at dew point, before the increase in air temperature.

2.3.4. *Other leaf traits*

Additional leaf traits were measured for each of the six trees for all six genotypes per treatment. Leaf trait measurements were done for the two dry and two wet seasons, in the middle of the season. Four leaves were randomly sampled in four directions within the crown of each tree. Leaf fresh weight and leaf thickness were determined. Leaf area was measured by scanning the leaves with a flatbed scanner, and by analyzing the images in Image J software (Perez-Harguindeguy et al., 2016). Leaf dry weight was determined after oven-drying the leaves at 65 °C to constant mass. Leaf water content was calculated, which is the amount of water per unit leaf dry mass (LWC; in %), and specific leaf area, the amount of leaf area per unit leaf dry mass (SLA; in cm^2 g^{-1}).

2.3.5. *Light interception*

Light interception was measured monthly using a HOBO light sensor (HOBO, USA) on sunny days from 10:30 am to 14:30 pm. Firstly, the incident light intensity (I) was measured below the crown (I_{below}) at two positions within each subplot with the instrument (mEssfix 6 m, Switzerland) surface horizontal upward, facing the sky. Secondly, incident light intensity (I) was measured above the crown (I_{above}) at the same location. Percent light interception was calculated as follows: % Interception = [100 - (I_{below} x 100/I_{above})] Eq. (3), where I_{below} = global incident radiation below the crown, and I_{above} = global incident radiation above the crown. Measuring light availability simultaneously above and below the crown allowed the leaf area index to be calculated by inverting the Beer-Lambert radiation extinction law (Monsi and Saeki, 1953) as follows: $LAI = -1/k \ln (I_{below}/ I_{above})$ Eq. (4). The light extinction coefficient (k) was taken as 0.6 (Zuidema et al., 2005).

2.3.6. *Litterfall sampling*

Litterfall was estimated by randomly placing two 1 m × 1 m litter traps with a 2-mm nylon mesh in each subplot. Litter traps were placed 1 m above the ground. Litter was collected from the 60 traps at monthly intervals, but at weekly intervals in periods of heavy rainfall and/or wind. The collected litter samples were separated into leaf and non-leaf components, and oven-dried at 65 °C for two days to constant mass, and weighed. The number of leaves were counted in each sample.

2.3.7. *Visual water stress index*

During dry periods, cocoa leaves typically turned yellow, dried out and were dropped, to reduce water loss. We used a visual index of water stress based on the degree and severity of leaf discoloration and crown defoliation. A score of 0 – 5 was assigned to indicate water stress levels (0 not stressed and 5 most stressed): 5 indicated fully defoliated trees, 4 indicated 80–100 % of the leaves dried, 3 indicated 75–80 % of the leaves dried, 2 indicated 50–75 % of the leaves dried, 1 indicated 25–50 % of the leaves dried, and 0 indicated <25 % of the leaves dried. In addition, we monitored crown phenology over the months. For each tree, the presence or absence of each of the following stages was recorded in the first week of each month: leaf flushing, mature, dark green leaves and senesced leaves (yellow or turning yellow), flowers, and fruits.

2.4. *Statistical analysis*

In the analysis, the dry period was defined as the period in which irrigation was turned on, and the wet period was the period in which irrigation was turned off. We used linear mixed-effects models (LMMs) in order to assess the effect of irrigation, K application and their interaction on SF, Gs, PLWP, MLWP, the water stress index, litterfall rates and leaf and crown traits, and how effects differed between genotypes. In a first step, we tested whether there was an effect of the period (dry vs. wet), irrigation and their interaction, based on a mixed-effects model comparison. We included the period (dry/wet), irrigation and the two-way interaction between period and irrigation as fixed effects, with tree, and litter trap in the case of litterfall, as random effect. We compared models with all possible combinations of the fixed effects using maximum likelihood estimation, and selected the best model based on Akaike's Information Criterion (AIC_c), adjusted for small sample sizes. The model with the lowest AIC_c value was selected. Model assumptions of the LMMs were checked by inspecting residual plots for homogeneity and quantile-quantile plots for normality. Marginal and conditional R squared values were calculated for the best model (Nakagawa and Schielzeth, 2013), where the marginal R squared indicates variation explained by the fixed effects only, and the conditional R squared indicates variation explained by both the fixed and the random effects.

In a second step, we included the dry period only to evaluate the effects of irrigation, potassium application and genotype, using linear mixed-effects models. Genotype, potassium application, irrigation treatment and the interactions between genotype and potassium, genotype and irrigation, irrigation and potassium, and genotype and irrigation and potassium were included as fixed effects. Tree, and litter trap in the case of litterfall, were included as random effects. Similar to the first analysis, a model comparison was conducted, and we selected the best model based on AIC$_c$. Effects were considered significant, if bootstrapped 95 % confidence intervals of the model coefficients did not overlap with zero. PostHoc tests were only performed in case of a significant interaction. All statistical analyses were performed using R Statistical Software 3.5.1 (R Core Team, 2018). LMMs were performed using the “glmmTMB” package (Brooks et al., 2017).

3. Results

3.1. Microclimate variation

Rainfall generally showed a bimodal pattern typical for this region, with high values during March - June and August – September, and low values during November - February (Fig. 1A). During January 2021, however, exceptionally high rainfall was reported (496 mm). The mean relative humidity was lowest in the dry periods in 2019 (on average 81.7 ± 4.5 %), in 2020 (on average 81.3 ± 5.5 %), and in 2021 (on average 76 ± 3.8 %) and highest in wet periods (Fig. 1A). The monthly relative amount of time under sunny conditions ranged from 20 to 30 % to 85 % and tended to be higher in the period between February and August than in the other months (Fig. 1B). The average diurnal air temperature pattern over the course of the day followed that of the solar radiation and ranged between monthly minimum temperature 26.5 ± 1 °C (mean ± SE) to maximum 30 ± 2 °C (Fig. 1B). Soil moisture content (VWC) was highest in March and November 2020, and in April and December 2021 VWC ~35 % (Fig. 1C). VWC was higher in wet periods and in irrigated plots and lower in the non-irrigated plots (Fig. 1C).

3.2. Effects of irrigation in the dry and the wet period

We first tested whether there was an effect of the period (dry vs. wet), irrigation and their interaction on different traits combining the data for different genotypes and potassium treatments. Leaf water potential values were significantly lower (more negative) in the dry than in the

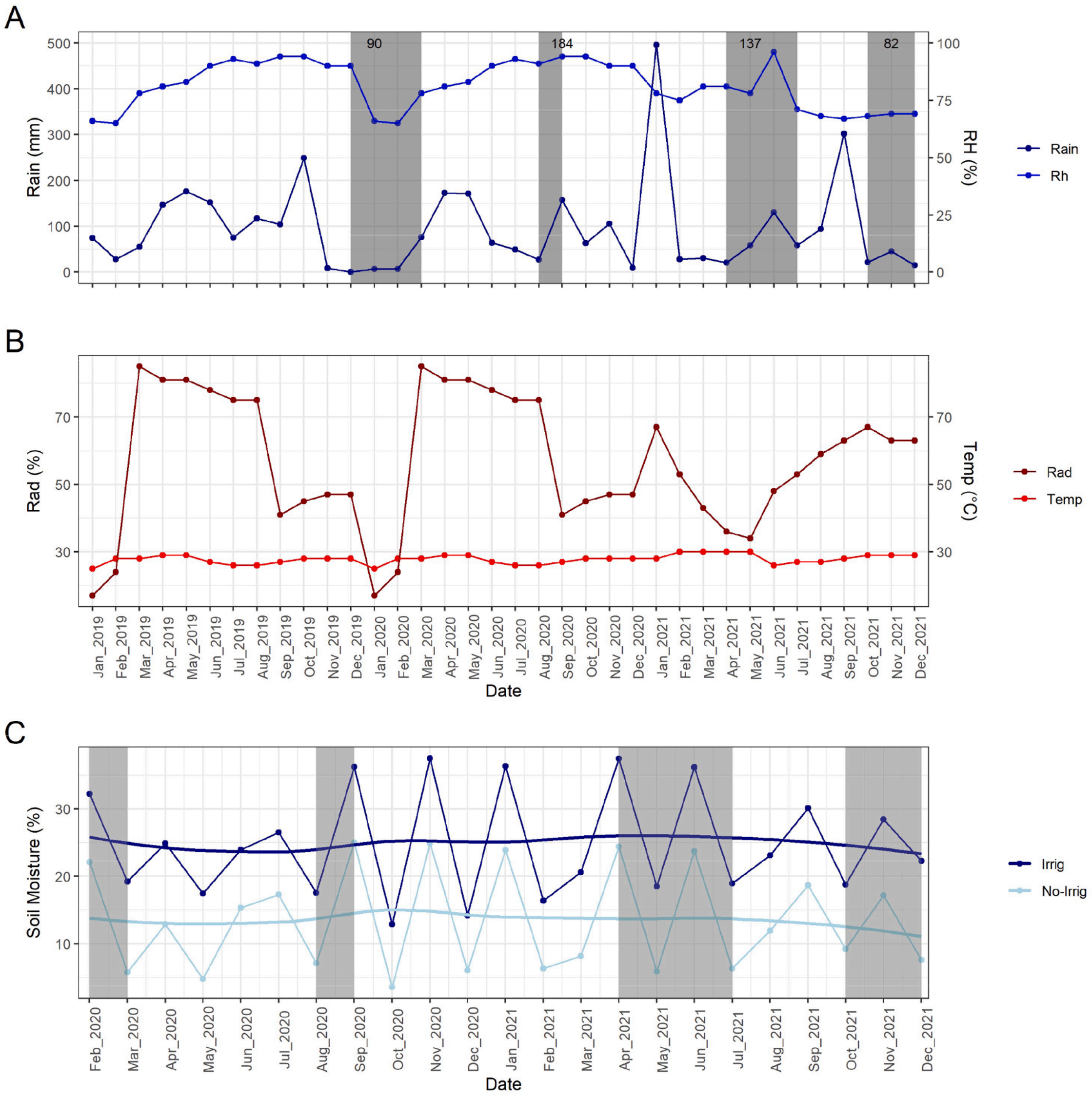

Fig. 1. Monthly averages of microclimate variables throughout the experiment. (A) Rainfall (Rain; mm) on the left y-axis and relative humidity (RH; %) on the right, (B) Solar radiation (the percentage of time with sunshine Rad; %) on the left y axis and air temperature (Temp; °C) on the right axis, (C) Soil volumetric water content (%) for irrigated (Irrig) and non-irrigated (No-Irrig) plots. The gray areas indicate the dry months in which the irrigation treatment was applied; the numbers in the gray area indicate the total rainfall received in that period.

wet period, and irrigation significantly increased leaf water potential values in the dry season. This effect extended into the wet period even though there was no irrigation supply in the wet season (Fig. A1). Stomatal conductance was significantly lower in the dry than in the wet period, and higher under irrigation than without. The negative effect of dry periods on Gs was mitigated by irrigation (as indicated by the significant period by irrigation interaction) (Fig. A1). Leaf area was significantly higher in the wet than in the dry period and higher in irrigated than non-irrigated trees (Fig. A2). Notably, there was a significant interaction between irrigation and period, with a slightly larger irrigation effect in the wet than in the dry period. Specific leaf area (SLA) and leaf water content (LWC) were both significantly influenced by the effect of irrigation and the effect of period separately, while leaf thickness was only influenced by period, being larger in the wet than in the dry period (Table A1). Irrigation had a positive effect on SLA and LWC. During a wet period, both SLA and LWC increased due to the availability of water, while during a dry period, they decreased as a response to water stress. Most of the crown traits responded to irrigation with irrigated plants having higher LAI, light interception and lower WSI (less stressed) than non-irrigated ones. LAI, light interception and WSI were lower in the dry than in the wet period (Table A1). Litterfall was larger in the dry period than in the wet period for non-irrigated trees, as indicated by the significant interaction between period and irrigation (Fig. A3). Hereafter, we include analyses for the dry period only.

3.3. *Effects of irrigation, potassium application and genotypes on leaf physiological traits*

We assessed how leaf water potential and stomatal conductance in cocoa trees responded to the irrigation and potassium treatments during the dry period for genotypes M and CI03 only. Results revealed a significant effect of genotype, irrigation as well as a significant interaction between genotype and irrigation on leaf water potential at predawn (pLWP) and midday (mLWP). The irrigation treatment made the leaf water potential significantly less negative. However, no significant effect of potassium and no interaction between the potassium and irrigation treatments was found (Table 1, Fig. 2A, B). The water potentials of the two genotypes responded differently to irrigation. With mean values of −15.11 to −16.8 Bar, the genotype M had higher (less negative) average leaf water potential (pLWP and mLWP) values compared to CI03 (−16.3 to −18.06 Bar), and this difference was larger in the irrigated than in the non-irrigated trees (Fig. 2A, B, Table A1). This indicates that genotype M exhibited both overall less negative LWPs, and a greater change in LWP in response to variation in water supply, with probably a faster recovery from water stress than CI03.

As expected, irrigation significantly increased stomatal conductance (Gs), and it did so in both genotypes (Fig. 2C). Potassium application did not affect stomatal conductance, and we did not find any significant interaction between potassium and irrigation. Stomatal conductance significantly differed among the two genotypes, being smaller for CI03 (114.18 mmol m^{-2} s^{-1}) than for M (146.15 mmol m^{-2} s^{-1}). Responses to irrigation were similar for both genotypes, as the interaction between genotype and irrigation was not significant.

3.4. *Sap flux density*

We assessed whether sap flux density in cocoa trees responded to the potassium and irrigation treatments during the dry period in the genotypes M and CI03, as sap flux measurements were done in the dry period only. We found a significant effect of genotype, irrigation, and potassium application on the mean water flux density. The irrigation treatment significantly increased mean water flux density (Table 1, Fig. 3), but there was no significant interaction between irrigation and potassium. The mean water sap flux density was significantly reduced from 0.450 to 0.416 ml cm^{-2} min^{-1} under non-irrigated conditions. Genotype M had higher average flux density values than CI03 (Fig. 3). There was no significant genotype by irrigation interaction. Potassium increased mean water flux density by about 11 %, in M and 22.22 % in CI03, and this response did not differ significantly between genotypes.

3.5. *Leaf morphological traits*

We assessed the effect of irrigation and potassium application on leaf morphological traits for all six cocoa genotypes during dry periods. Generally, leaf area (the mean area of a leaf) significantly increased in response to irrigation, but the response differed among genotypes (Fig. 4A), as there was a significant interaction between genotype and the irrigation treatment. There was a significant effect of genotype on specific leaf area (SLA) with a significant interaction between genotype and potassium application, and between genotype and irrigation (Table 1). Overall, there was no clear response to irrigation and potassium application in SLA values (Fig. 4B), as responses strongly differed among genotypes. Irrigation and potassium application did not have a significant effect on leaf thickness (Table 1, Fig. 4C), and there was no difference between genotypes. We did find a significant effect of genotype, irrigation, and potassium application on leaf water content (LWC), as well as interactions between genotype and potassium, between genotype and irrigation, and between potassium and irrigation (Table 1).

Table 1

Linear mixed-effects model results testing the effects of genotype, irrigation, potassium application and their interactions on cocoa tree physiology. Included variables were predawn leaf water potential (pLWP), midday leaf water potential (mLWP), stomatal conductance (Gs), sap flux density (SF), leaf area, specific leaf area (SLA), leaf thickness, leaf water content (LWC), leaf area index (LAI), intercepted light percentage, water stress index (WSI), and litterfall. Fixed effects that were tested in the model comparison are indicated: genotype (Gen), potassium treatment (PtT), irrigation treatment (Irrig), and the interaction between predictors (:). The marginal (m) and conditional (c) R squared values are indicated. + indicates that the predictor was included in the best model. ^{Sig} indicates that the effect of the predictor was significant.

Variables		Unit	Gen	PtT	Irrig	Gen: PtT	Gen: Irrig	PtT: Irrig	Gen: PtT: Irrig	R^2m	R^2c
Leaf physiological traits	pLWP	Bar	+ ^{Sig}	+	+ ^{Sig}		+ ^{Sig}	+		0.37	0.37
	mLWP	Bar	+ ^{Sig}	+	+ ^{Sig}		+ ^{Sig}	+		0.57	0.60
	Gs	mmol s^{-1}	+ ^{Sig}	+	+ ^{Sig}		+			0.02	0.02
Sap flux density	SF	ml cm^{-2} min^{-1}	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}	+	+			0.21	0.09
Leaf morphological traits	Leaf area	cm^2	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}		+ ^{Sig}	+		0.13	0.21
	SLA	cm^2 g^{-1}	+ ^{Sig}		+	+ ^{Sig}	+ ^{Sig}	+		0.04	0.15
	Thickness	mm	+	+	+	+	+			0.11	0.17
	LWC	%	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}		0.04	0.15
Crown traits	LAI	_	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}			0.08	0.32
	Intercepted light	%	+ ^{Sig}	+	+ ^{Sig}	+ ^{Sig}	+ ^{Sig}			0.23	0.23
	WSI	_	+ ^{Sig}		+ ^{Sig}					0.11	0.11
	Litterfall	kg ha^{-1}	+ ^{Sig}		+					0.11	0.54

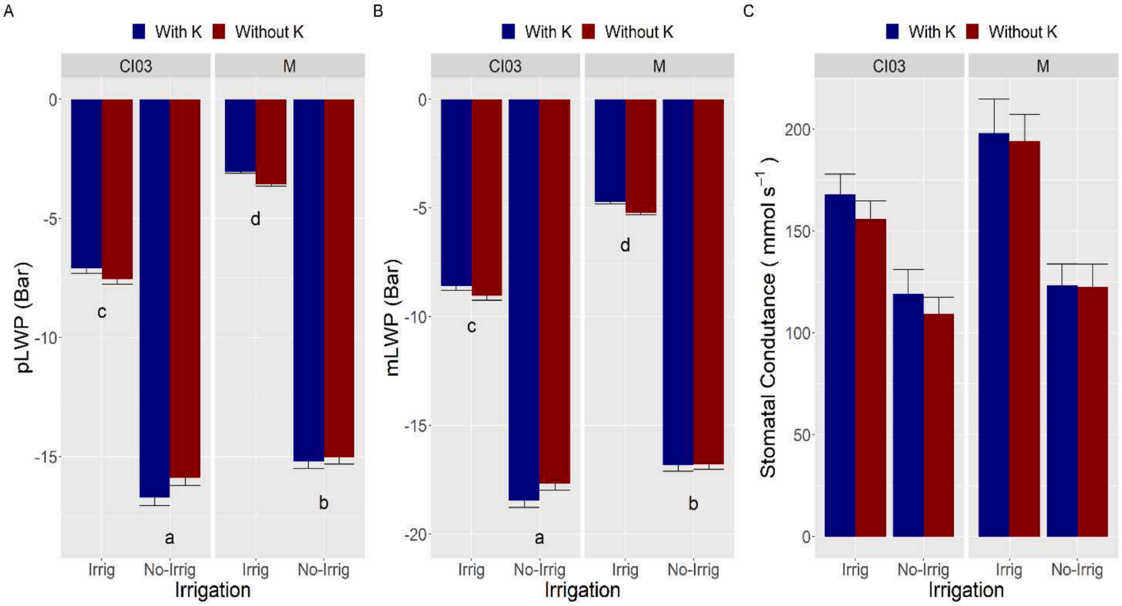

Fig. 2. Effects of irrigation, potassium application, and genotype on leaf water potential and stomatal conductance for mature trees of two cocoa genotypes. Genotypes refer to one clonal variety CI03 and one hybrid variety M. (A) Predawn leaf water potential (pLWP, Bar), (B) Midday leaf water potential (mLWP, Bar), (C) Stomatal conductance (mmol m^{-2} s^{-1}). “Without K” indicates without potassium application, “with K” indicates with potassium application, “Irrig” indicates the irrigated treatment and “No-Irrig” indicates the non-irrigated treatment. Values represent means ± standard error. Different letters below the bars indicate a significant difference except in panel C.

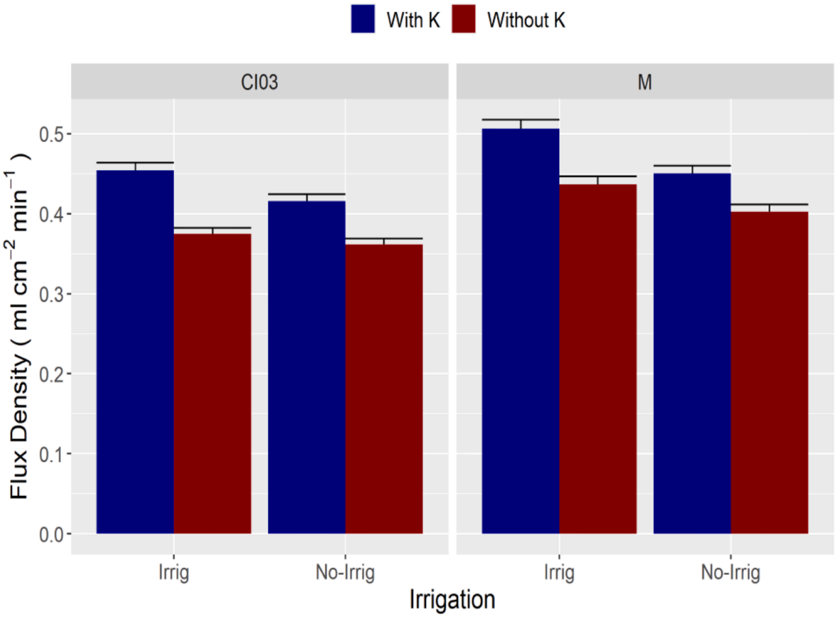

Fig. 3. Effects of irrigation, potassium application, and genotype on sap flux density (SF) for two cocoa genotypes. Genotypes refer to one clonal variety CI03 and one hybrid variety M. Without K indicates without potassium application, with K indicates with potassium application, Irrig indicates with irrigation and No-Irrig indicates that no irrigation was applied. Values represent means ± S.E.

Generally, responses were rather weak and differed across genotypes (Fig. 4A, Fig. 4D).

3.6. Crown dynamics

We evaluated performance of cocoa genotypes in response to irrigation and potassium application at the crown level. Leaf area index (LAI) was significantly influenced by genotype, potassium and irrigation, with significant two-way interactions between genotype and potassium application, and between genotype and irrigation (Table 1). Generally, LAI declined significantly when irrigation was withheld as shown by a significant main effect (Table 1). But when testing per genotype, the irrigation effect was mostly not significant (Fig. 5A). The percentage of intercepted light was significantly affected by genotype, irrigation, the interactions between genotype and potassium application, and between genotype and irrigation. As with LAI, the non-irrigated trees had lower light interception values than the irrigated trees, but this effect was weak, and was not present for most of the genotypes (Fig. 5B). The effects of potassium on LAI and light interception were fairly inconsistent being positive in some but negative in other

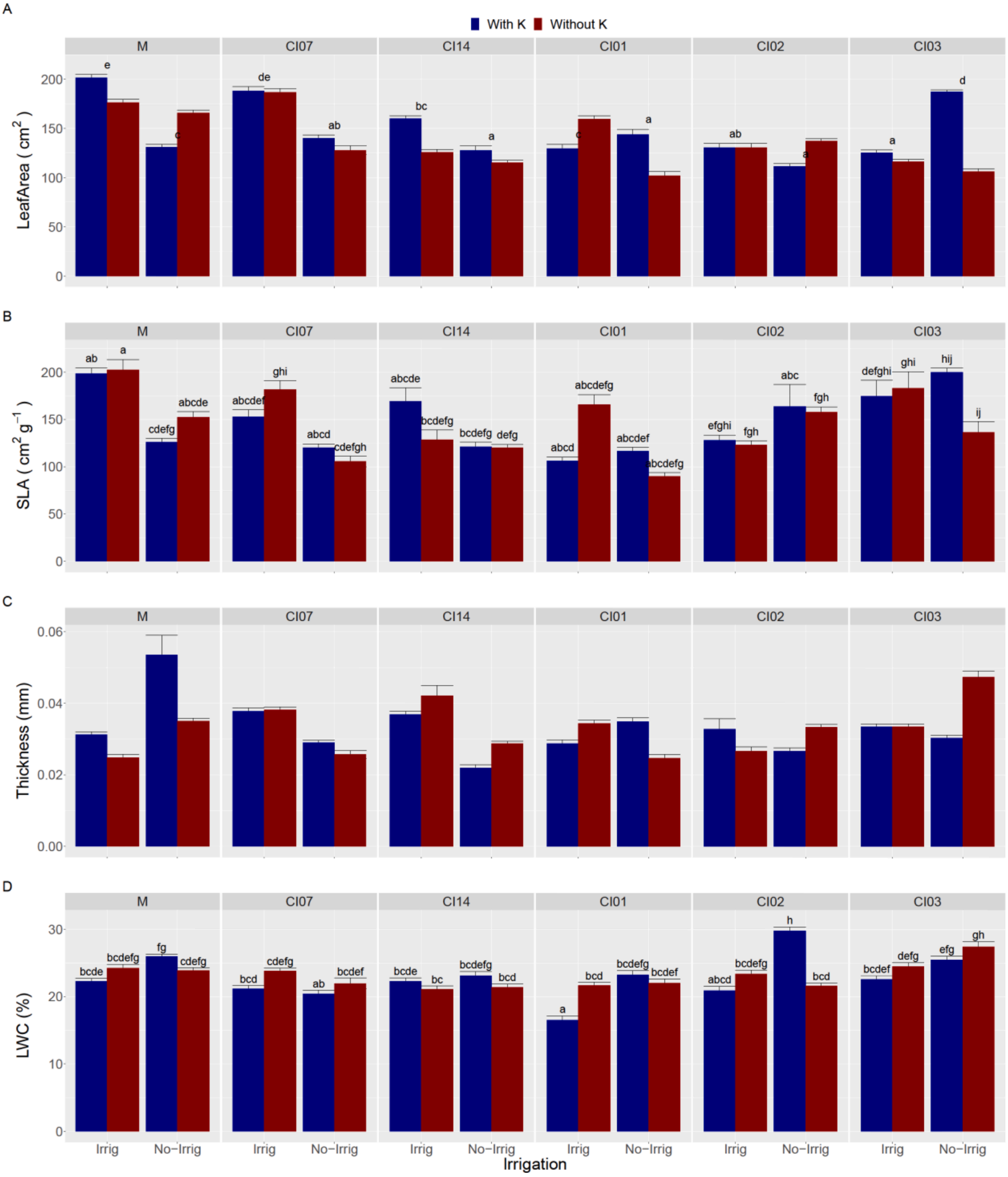

Fig. 4. Effects of irrigation, potassium application and genotype on leaf morphological traits: (A) Leaf area (cm^2), (B) Specific leaf area (SLA, cm^{-2} g^{-1}), (C) Leaf thickness (mm), (D) and leaf water content (LWC, %) for six genotypes. Genotypes refer to five clonal varieties CI07, CI14, CI01, CI02, CI03 and one hybrid variety M. Treatment abbreviations as in Fig. 2. Different letters above the bars indicate a significant difference.

genotypes (Fig. 5A, B).

WSI was significantly increased by withholding irrigation, indicating more drought stressed plants, and differed among genotypes (Table 1). This increase in WSI tended to be larger in the CI02 and CI03 clones than in the others, but the genotype by irrigation interaction was not significant (Fig. 5C). There was neither a significant effect of potassium nor a significant interaction between irrigation and potassium application on WSI (Table 1).

Defoliation occurred during dry periods, and lasted between 4 and 11 weeks (depending on the year) in all genotypes, leading to increased litterfall. Subsequently, re-growth of leaves occurred immediately at the onset of wet periods (data not shown). Litterfall significantly differed among genotypes, but we did not find significant effects of irrigation, potassium application, or their interaction (Table 1). M and CI01 produced significantly less litterfall than CI07, CI03, CI14 and CI02 (Fig. 5D).

4. Discussion

Physiological responses of cocoa genotypes to variation in irrigation

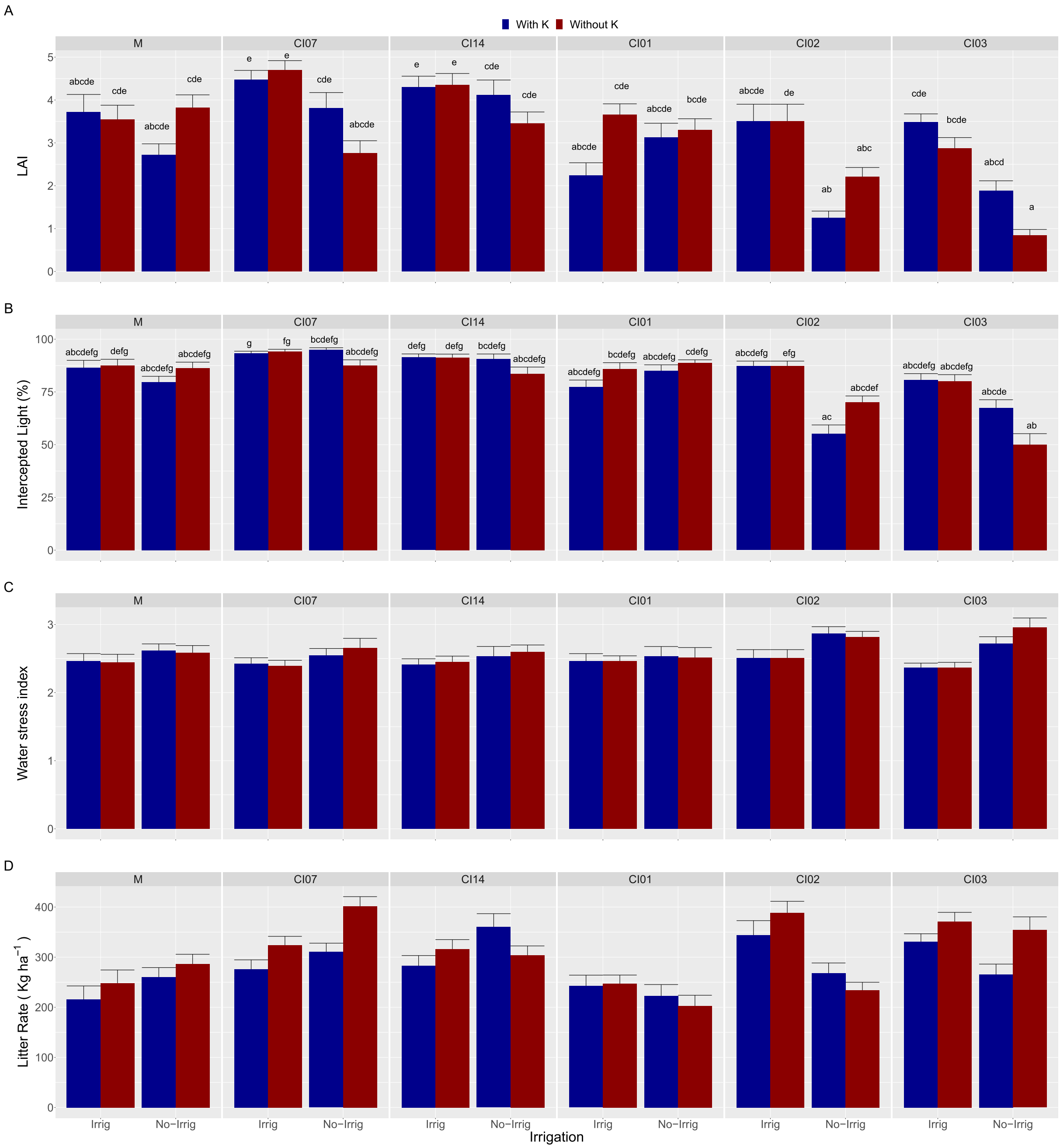

Fig. 5. Effects of irrigation and potassium application on the performance of cocoa genotypes on (A) leaf area index (LAI), (B) Percentage of light intercepted (%) by the crown, (C) water stress index (WSI) and (D) monthly litterfall. Treatment abbreviations as in Fig. 2.

and potassium application were evaluated under field conditions to test how leaf and crown characteristics of mature field-grown trees respond to water deficit, and whether these responses were modified by potassium application. We also explored whether these responses differed across genotypes.

Overall, we found that withholding irrigation had clear negative effects on cocoa sap flow density (SF), several leaf physiological water-related traits (pLWP, mLWP, Gs), on leaf area, as well as on crown size (LAI). Furthermore, withholding irrigation increased the overall tree water stress index (WSI). The strongest LAI reductions in the non-irrigated treatment were observed for genotypes CI02 and CI03, suggesting these to be less drought tolerant than the other genotypes. We also found that the hybrid M was able to maintain higher levels of LWP both under irrigated and non-irrigated conditions than the apparently less drought tolerant CI03 clone. This was not the case for sap flux and Gs, which did not show interactions between genotype and irrigation.

Together these results indicate a clear genetic variation in drought tolerance that is reflected at both leaf and crown levels. However, contrary to our expectations, there were no consistent interactive effects between irrigation and potassium application for any of the included traits at the leaf, crown and whole-tree level. We did find that potassium application increased sap flux density, which may indicate a role for potassium in relieving drought stress.

4.1. How cocoa trees deal with water deficit at leaf level

As hypothesized, withholding irrigation had negative effects on leaf physiological and leaf morphological traits. Our findings showed a significant reduction of withholding irrigation on daily sap flux density (SF) and on predawn and midday leaf water potential (Table 1). Similar findings were obtained for clonal cocoa seedlings (Santos et al., 2018; Osorio-Zambrano et al., 2021) at a soil volumetric water content (VWC) below 6 %. In another study, Tezara et al. (2020) noted that drought caused a 40 % decrease in LWP of cocoa seedlings. Because LWP predominantly reflects the ability of plants to acquire water, lower values are an indication of drought stress (Lambers et al., 1998) and inhibit leaf physiological functioning. To put our results in context, the LWP values under non-irrigated conditions in the dry season were about −16 bar, which for cocoa is considered the threshold for cessation of leaf photosynthesis (De Almeida and Valle, 2007). In addition to indicating drought stress, the reduction in LWP may also (but to a much lesser extent) be due to accumulation in leaf cells of osmolytes resulting in a more negative osmotic potential. As water moves into the plant along a pressure gradient, this osmotic response facilitates water uptake under drought conditions. However, we did not measure leaf osmotic potential and hence cannot assess the role that variation herein might have played.

Results show that Gs was significantly reduced under withholding irrigation in both genotypes M and CI03 (Fig. 2C), which was also found in studies on other cocoa genotypes (Acheampong et al., 2013; Araque et al., 2012; Baligar et al., 2008; Daymond et al., 2011; De Almeida et al., 2016; Tezara et al., 2016). Stomatal closure helps reducing water loss and preventing leaf dehydration. In addition, as transpiration (E) declines more steeply with a reduction in Gs than photosynthesis (A), it may increase photosynthetic water-use efficiency (A/E) (Chaves et al., 2002; Lahive et al., 2019; Tezara et al., 2020). On the other hand, it also entails a reduction in photosynthesis and transpiration, possibly resulting in an increase of leaf temperature.

LWC was only slightly lower in the dry than in the wet periods (Fig. A) and, surprisingly, there was no consistent effect of irrigation (Fig. 3D). LWC reflects the balance between water availability (indicated by LWP), water transport to the leaves (indicated by SF) and loss through transpiration (indicated by Gs and SF). Inconsistent effects of withholding irrigation on LWC likely indicated that water loss prevention through stomatal closure matched the reduction in water availability such that leaves were not significantly dehydrated (DaMatta, 2004).

Our results did show reductions in SLA and leaf size in dry compared to wet periods (Fig. A). Furthermore, responses in these traits to irrigation were inconsistent among genotypes, perhaps as a result of the way we conducted our experiment. We only withheld irrigation during the dry season when trees do not produce many leaves unless soil water content increases (Miyaji et al., 1997a, 1997b). As leaf size and SLA are largely determined at leaf initiation and formation, our trait values measured in the dry season may reflect the conditions in the wet season when the leaves were formed. In this respect, our experimental set-up differed from studies which did find a negative effect on these traits, as those studies applied constant drought and leaf formation was thus certainly under drier conditions than in their wet treatments (Ayegboyin and Akinrinde, 2016; Baligar et al., 2017).

4.2. *Crown-level responses to drought*

We expected that drought would result in greater litterfall, WSI, and lower LAI and hence light capture in cocoa trees. Our results confirm this hypothesis for WSI and LAI, but not for light capture and litterfall, which did not clearly differ between irrigation treatments. The latter could be associated with the fact that litter production is a function of both the fraction of leaves that are dropped and the size of the crown (i.e. LAI). Drought resulted in a considerably (~40 %) lower LAI, which may result in lower transpiration and maintenance respiration (Gupta et al., 2020; King, 1990; Santos et al., 2014), and increased the water stress index. Results revealed that the reduction in LAI together with the reduction in Gs was largely responsible for the lower SF values observed in non-irrigated plants, which was also found in other studies (Dos Santos et al., 2014; Villalobos et al., 2000). Lower LAI and light capture while preventing dehydration during drought events may limit the ability of plants to quickly recover once drought conditions end.

4.3. *Potassium fertilizer did not mitigate water stress*

Contrary to our expectations, application of potassium fertilizer did not generally mitigate the negative effects of withholding irrigation on most of the leaf and crown traits or on the overall stress index. This also contrasts with other studies where a drought mitigation effect of potassium fertilizer application was observed in cocoa, albeit in seedlings (Anokye et al., 2021; De Almeida and Valle, 2007; Djan et al., 2017; Kaba et al., 2022). The only significant potassium effect we found was on daily sap flux density (SF), which increased with potassium application. In a study on *Eucalyptis grandis* trees, sap flow was positively related to whole-plant transpiration and hence to LAI and Gs (Asensio et al., 2020). In our study, LAI did increase with potassium application in the clone CI03, but not in the hybrid M, while Gs was not affected by potassium in either genotype. Regarding the latter it is worth noting that we only measured Gs in the morning while SF reflects daily transpiration, it could thus be that potassium application may have reduced midday stomatal closure (Oddo et al., 2020).

As noted by Lahive et al. (2019), in cocoa the interactive responses to soil water and potassium availability could be genotype-specific, i.e., the effect being more apparent in some genotypes than in others. It could thus be that the presently selected genotypes happened to be ones that do not show this response very strongly. Another explanation for our findings could be a reduction in root hydraulic conductance that may have reduced potassium ion transport from the soil into the plant (Qi et al., 2019; Steudle, 2000). Finally, and possibly more importantly, our experiment was initiated five months after cessation of fertilizer application to all plots in our study site. It is possible that trees may still have had stored potassium reserves and that use of these reserves masked any potassium fertilizer effect in our study. Yet, this does not explain the higher sap flux rates in the potassium treatment. Unfortunately, we were not able to obtain a sufficient amount of samples to test for effects of the treatment on potassium concentrations in soil and leaves. In line with Lahive et al. (2019), we stress that more and longer-term research is needed to explore the potential of potassium application as a drought stress mitigating strategy in adult field-grown cocoa.

4.4. *Genotypic differences in responses to drought*

We observed clear genotypic differences in the effects of withholding irrigation on LAI, namely that the increment in these values was significantly lower in the genotypes CI02 and CI03 than in the other genotypes. The whole-plant stress indicator WSI tended also to be significantly larger for these two genotypes. This suggests that CI02 and CI03 were less drought tolerant than the other four genotypes. This result is consistent with that of other studies documenting cocoa genetic variation in drought tolerance (Araque et al., 2012; Ávila-Lovera et al., 2016; Daymond et al., 2011). Genotype by irrigation interactive effects were also observed on leaf physiological and morphological traits. The apparently more drought tolerant hybrid M maintained less negative LWP values under non-irrigated conditions and exhibited a larger LWP difference between irrigated and non-irrigated conditions than the apparently less drought tolerant CI03, with similar trends being observed for Gs. A relatively high (less negative) midday LWP during drought has been proposed as a proxy for drought tolerance in cocoa (Araque et al., 2012). The ability to maintain less negative LWP values under drought conditions can be associated with the presence of deep, dense root systems; and/or resistance to losses in stem water conductance (De Almeida and Valle, 2007; Dos Santos et al., 2016; Dos Santos et al., 2014).

Together, the results for SF, LWP and Gs indicate that the genotype M tends to maintain better access to water when irrigation is withheld and shows a stronger positive response in these traits when irrigated. The latter suggests a faster recovery and hence greater resilience under drought stress of the hybrid M than clonal variety CI03. Generally, SF was lower in CI03 than in M (Fig. 4.). This lower SF under water limitation aligns well with a previous study (Abdulai et al., 2018b) that demonstrates a reduction in daily maximum cocoa sap flux density in full sun conditions at the end of the dry period. The studies by Dierick et al. (2010) and Dierick and Hölscher (2009) demonstrated that daily cocoa sap flux density declined in response to high levels of vapor pressure deficit (VPD), decrease soil water content and strongly differ among species monitored (Köhler et al., 2009). Differences among genotypes could be driven by differences in stem xylem vessel features such as vessel area, density, and diameter that could be smaller in CI03 than in M. In apple, for example, lower SF was associated with smaller xylem vessel features (Bhusal et al., 2019). Overall, our results suggest that cocoa genotypic differences in drought tolerance reflect plasticity differences in traits determining drought responses.

5. Conclusions

Physiological responses to water deficit and potassium application were evaluated in field conditions for six cocoa genotypes derived from somatic embryogenesis. Withholding irrigation significantly affected cocoa sap flux density, leaf physiology, leaf morphology and crown traits, while potassium application did not mitigate the negative effects of water deficit. Therefore, the putative, added benefit of potassium application to mitigating drought stress needs to be further investigated, in long-term fertilizer trials. Cocoa responses to irrigation were strongly genotype-specific, Mercedes (M) followed by the clone CI 07 seems to have greater adaptive ability to water deficit than the other genotypes. Results imply that the observed genotypic variation in responses to irrigation provides useful input for developing cocoa varieties specifically suitable for restricted (limited to the dry period) irrigation practices. Together these results contribute to the body of knowledge on how strategic combinations of dry-period irrigation and use of drought tolerant genotypes could help in making cocoa production more drought resilient.

CRediT authorship contribution statement

Lucette Adet: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. **Danaë M.A. Rozendaal:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Arthur Tapi:** Resources, Writing – review & editing. **Pieter A. Zuidema:** Methodology, Investigation, Writing – review & editing. **Philippe Vaast:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Niels P.R. Anten:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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Appendices

Table A1 and Figs. A1-A4.

Table A1

Linear mixed-effects models (lowest AICc) testing the effects of seasons, withholding irrigation, and their interactions on mature cocoa trees physiological responses, the marginal (m) and conditional (c) R squared values. + indicates that the predictor was included in the best model. ^{Sig} indicates that the effect of the predictor was significant.

Variables		Unit	Period	Irr	Period: Irr	R^2m	R^2c
Leaf physiology traits	pLWP	Bar	$+^{Sig}$	$+^{Sig}$	$+^{Sig}$	0.53	0.57
	mLWP	Bar	$+^{Sig}$	$+^{Sig}$	$+^{Sig}$	0.56	0.59
	Gs	mmol s^{-1}	$+^{Sig}$		$+^{Sig}$	0.06	0.06
Leaf morphology traits	Leaf Area	cm^2	$+^{Sig}$	$+^{Sig}$	$+^{Sig}$	0.21	0.44
	SLA	cm^2g^{-1}	$+^{Sig}$	$+^{Sig}$		0.02	0.17
	Thickness	mm	$+^{Sig}$			0.04	0.33
	LWC	%	$+^{Sig}$	$+^{Sig}$		0.03	0.43
Sap flux density	SF	ml cm^{-2} min^{-1}	$+^{Sig}$	$+^{Sig}$	$+^{Sig}$	0.003	0.008
Crown traits	LAI	_		$+^{Sig}$		0.24	0.32
	Intercepted Light	%	+	$+^{Sig}$		0.06	0.22
	WSI	_	+	$+^{Sig}$		0.11	0.11
	Litterfall	kg ha^{-1}	$+^{Sig}$	+	$+^{Sig}$	0.11	0.54

$*^{Sig}$ means significant effects of the predictors associated with each modeling inclusion.

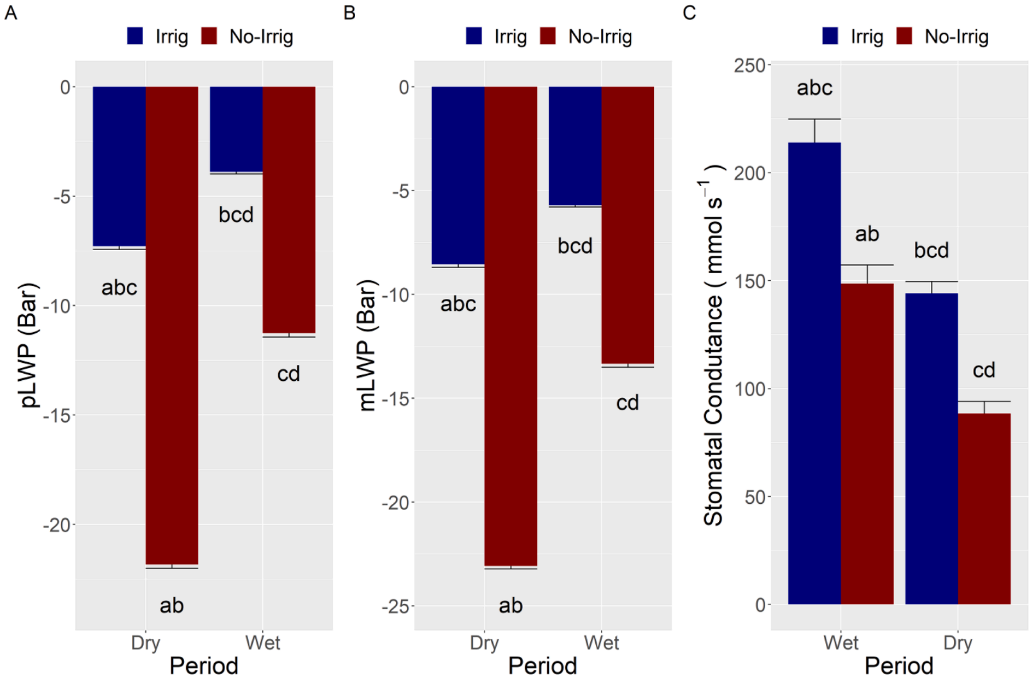


Fig. A1. The effect of irrigation, period (dry vs. wet seasons) and their interaction on leaf physiological traits (A) Predawn leaf water potential (pLWP, Bar), (B) Midday leaf water potential (mLWP, Bar), (C) Stomatal conductance (Gs, mmol s^{-1}). in the irrigated treatment (Irrig) and the non-irrigation treatment (No-Irrig). Different letters above and below the bars indicate a significant difference.

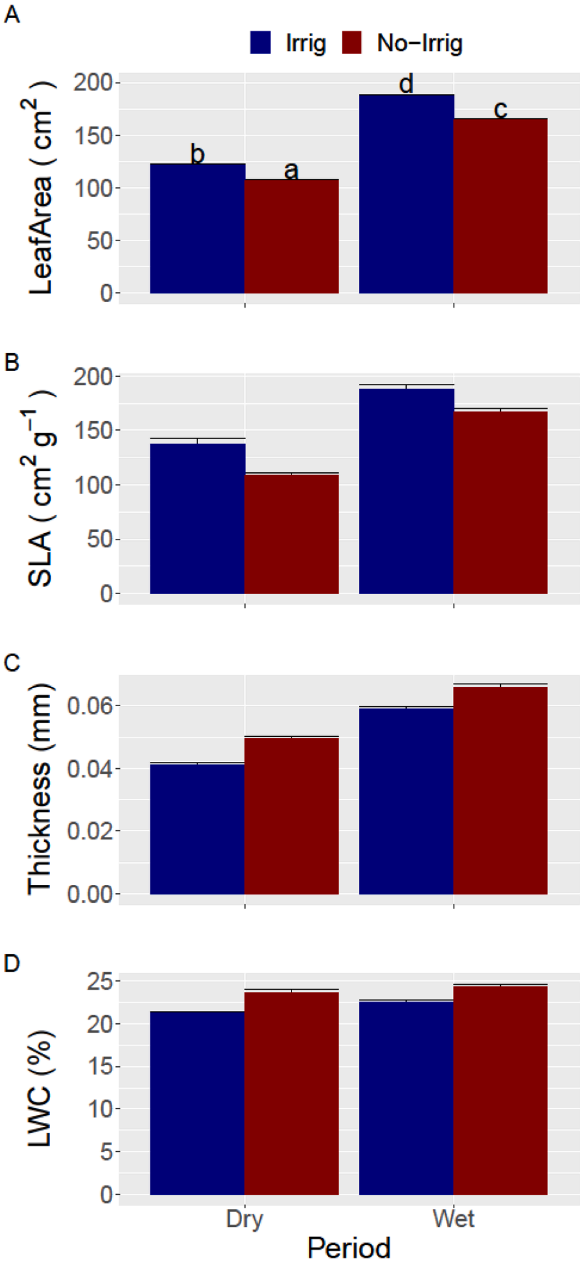


Fig. A2. The effect of irrigation, measurement period (dry vs. wet seasons) and their interaction on cocoa leaf morphological traits: (A) Leaf area (cm^2), (B) Specific leaf area (SLA, cm^2 g^{-1}), (C) Leaf thickness (mm), (D) leaf water content (LWC, %) for each of the six genotypes in the irrigated treatment (Irrig) and the no-irrigation treatment (No-Irrig). Errors bars represent standard errors. Tukey PostHoc letters represent significant interaction effect between irrigation, period (Table A). Different letters above the bars indicate a significant difference.

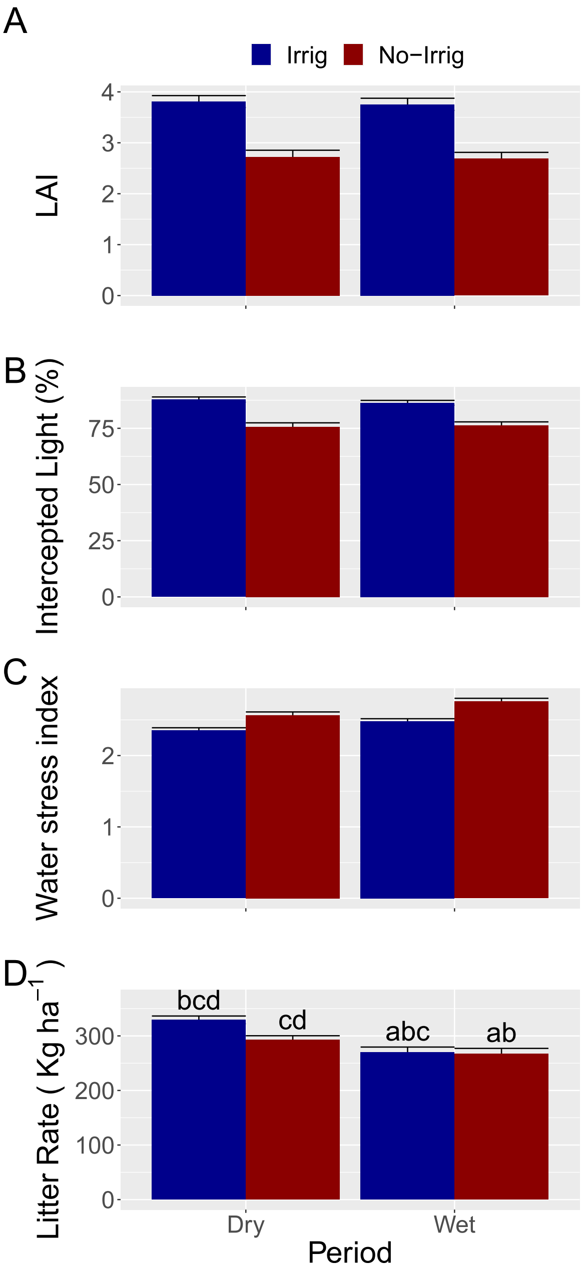
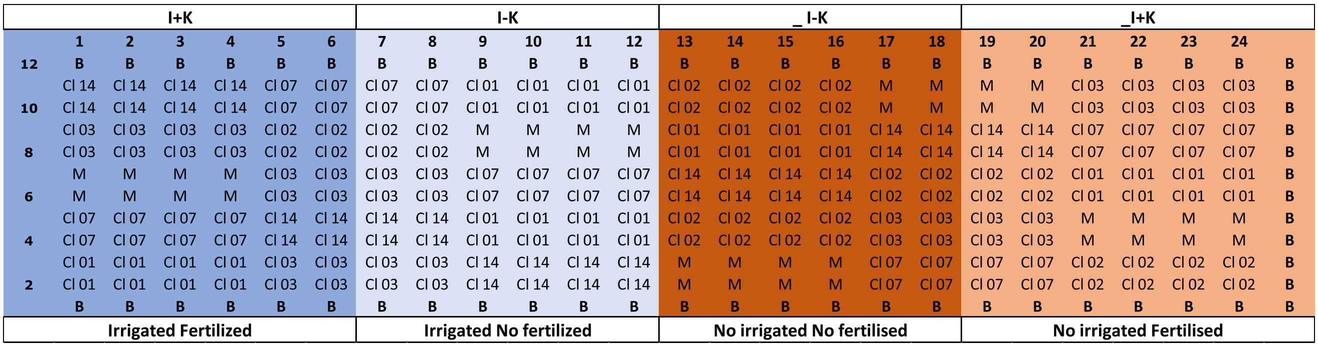


Fig. A3. The the effect of irrigation, measurement period (dry vs. wet) and their interaction on cocoa (A) leaf area index changes, (B) Percentage of light intercepted (%) by the crown, (C) water stress index and (D) monthly litterfall (Kg ha^{-1}) in the irrigated treatment (Irrig) and the non-irrigated treatment (No-Irrig). Error bars represent standard errors. Tukey PostHoc letters represent significant interaction effect between irrigation, period (Table A). Different letters above the bars indicate a significant difference.



I+K							I-K						_ I-K						_I+K						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
12	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	Cl 14	Cl 14	Cl 14	Cl 14	Cl 07	Cl 07	Cl 07	Cl 07	Cl 01	Cl 01	Cl 01	Cl 01	Cl 02	Cl 02	Cl 02	Cl 02	M	M	M	M	Cl 03	Cl 03	Cl 03	Cl 03	B
10	Cl 14	Cl 14	Cl 14	Cl 14	Cl 07	Cl 07	Cl 07	Cl 07	Cl 01	Cl 01	Cl 01	Cl 01	Cl 02	Cl 02	Cl 02	Cl 02	M	M	M	M	Cl 03	Cl 03	Cl 03	Cl 03	B
	Cl 03	Cl 03	Cl 03	Cl 03	Cl 02	Cl 02	Cl 02	Cl 02	M	M	M	M	Cl 01	Cl 01	Cl 01	Cl 01	Cl 14	Cl 14	Cl 14	Cl 14	Cl 07	Cl 07	Cl 07	Cl 07	B
8	Cl 03	Cl 03	Cl 03	Cl 03	Cl 02	Cl 02	Cl 02	Cl 02	M	M	M	M	Cl 01	Cl 01	Cl 01	Cl 01	Cl 14	Cl 14	Cl 14	Cl 14	Cl 07	Cl 07	Cl 07	Cl 07	B
	M	M	M	M	Cl 03	Cl 03	Cl 03	Cl 03	Cl 07	Cl 07	Cl 07	Cl 07	Cl 14	Cl 14	Cl 14	Cl 14	Cl 02	Cl 02	Cl 02	Cl 02	Cl 01	Cl 01	Cl 01	Cl 01	B
6	M	M	M	M	Cl 03	Cl 03	Cl 03	Cl 03	Cl 07	Cl 07	Cl 07	Cl 07	Cl 14	Cl 14	Cl 14	Cl 14	Cl 02	Cl 02	Cl 02	Cl 02	Cl 01	Cl 01	Cl 01	Cl 01	B
	Cl 07	Cl 07	Cl 07	Cl 07	Cl 14	Cl 14	Cl 14	Cl 14	Cl 01	Cl 01	Cl 01	Cl 01	Cl 02	Cl 02	Cl 02	Cl 02	Cl 03	Cl 03	Cl 03	Cl 03	M	M	M	M	B
4	Cl 07	Cl 07	Cl 07	Cl 07	Cl 14	Cl 14	Cl 14	Cl 14	Cl 01	Cl 01	Cl 01	Cl 01	Cl 02	Cl 02	Cl 02	Cl 02	Cl 03	Cl 03	Cl 03	Cl 03	M	M	M	M	B
	Cl 01	Cl 01	Cl 01	Cl 01	Cl 03	Cl 03	Cl 03	Cl 03	Cl 14	Cl 14	Cl 14	Cl 14	M	M	M	M	Cl 07	Cl 07	Cl 07	Cl 07	Cl 02	Cl 02	Cl 02	Cl 02	B
2	Cl 01	Cl 01	Cl 01	Cl 01	Cl 03	Cl 03	Cl 03	Cl 03	Cl 14	Cl 14	Cl 14	Cl 14	M	M	M	M	Cl 07	Cl 07	Cl 07	Cl 07	Cl 02	Cl 02	Cl 02	Cl 02	B
	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Irrigated Fertilized							Irrigated No fertilized						No irrigated No fertilised						No irrigated Fertilised						

Fig. A4. The layout of the experiment showing how the different genotypes were incorporated into the experiment.

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