



Association of Agronomic, Physiological Traits and Biomass of a Diverse Set of Sugarcane Genotypes under Varied Early Season Droughts

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ABSTRACT

Background: Recently, multipurpose and biomass canes have become important in breeding. However, biomass sampling is limited in early generation selection. The surrogate traits that represent biomass performance need to be established, especially using non-destructive sampling. Therefore, the objective of this study was to determine the correlation between agronomic and physiological traits and the biomass of a diverse set of sugarcane genotypes under different drought durations.

Methods: The experiment was conducted under field conditions and arranged in a split plot in a randomized complete block design with four replications. The main plot was represented by three drought durations: no water stress, short-term drought and long-term drought and the subplot consisted of six sugarcane genotypes. Samples were collected at 3, 6, 8 and 12 months after transplanting to determine biomass and nondestructive agronomic and physiological traits. A simple correlation was used to determine the relationship between biomass and physiological and agronomic traits.

Result: In both field capacity and long drought conditions, 3 months after transplanting, there was a positive correlation between canopy height and biomass and between green leaf number and biomass. The non-destructive leaf area index was a trait that contributed to biomass at 6 months after transplanting under non-water stress conditions. At 8 months after transplanting, a positive relationship between canopy height and biomass was found under short drought and long drought conditions and green leaf number was also related to biomass under field capacity conditions. Biomass at the harvesting stage contributed to canopy width in sugarcane under field capacity conditions. Non-destructive traits in this experiment, such as canopy height and green leaf number, could be used as indirect measurements to reflect the biomass performance under field capacity and long drought conditions at the tillering and physiological maturity phases. For the elongation phase, the non-destructive leaf area index was an altered characteristic that indirectly determined biomass. This information will be useful as an alternative measurement to indicate biomass in the breeding program for drought resistance at the early growth stage.

Key words: Inter-specific hybrid, Leaf area index, Non-destructive, Rainfed, Water deficit.

INTRODUCTION

Sugarcane is native to tropical regions and is generally grown in South American, Asian and Southeast Asian countries, including Thailand. The most common product made from sugarcane and exported from this region is sugar. Brazil is the top exporter of sugar in the world, followed by Thailand. At present, approximately 20% (2.5-2.7 million tonnes year⁻¹) of Thailand's sugar production is used domestically and the remaining 80% (7-11 million tonnes year⁻¹) is exported (Khumla *et al.*, 2022). During the late rainy season, in Thailand's production system, sugarcane is germinated utilizing stored soil moisture (Set-Tow *et al.*, 2020). After germination, sugarcane often faces a water deficit at the initial stage for 2-4 months (Khonghintasong *et al.*, 2021), varying from year to year depending on each rainy season. Therefore, sugarcane crops in this production system may encounter both short-and long-term early season drought. Nevertheless, the water deficit can significantly impact sugarcane growth and yield, which decreases biomass yield by up to 50% (Khonghintasong *et al.*, 2021). In addition, drought decreases stem height, stalk diameter and leaf area (Jangpromma *et al.*, 2012). A

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sugarcane cultivar that obtains drought resistance during the early development stage can alleviate this obstacle (Khonghintasong *et al.*, 2018). Interspecific hybridization is a strategy for improving a new clone with drought resistance in a current breeding program. Wild sugarcane, namely *Saccharum spontaneum*, is a good genetic source

that is prone to drought (Luo *et al.*, 2014). Interspecific hybridization approach enables the maintenance of high sugar and biomass genes from *S. officinarum*, which supports the genetic improvement process, as well as an increase in stress resistance from *S. spontaneum* (Luo *et al.*, 2014; Yu *et al.*, 2018). In this context, many countries, such as Brazil, Australia, Japan and Thailand, have breeding germplasm that has been developed from crossing *Saccharum* hybrids and *S. spontaneum* (Khumla *et al.*, 2022; Terajima *et al.*, 2022).

The biomass parameter is important and breeders pay attention to multipurpose sugarcane (Jangpromma *et al.*, 2012; Knoll *et al.*, 2021). The selection criterion in early generation selection requires non-destructive collection, but biomass and some morphological and growth traits require destructive plant sampling from the field. Therefore, the non-destructive traits that reflect biomass must be determined with the limitations of sampling at the early breeding stage. Physiological parameters, such as canopy size, leaf shape and size, leaf number and leaf distribution, are all directly related to light interception, which is consequently associated with photosynthesis (Luo *et al.*, 2014). In addition, drought during the early growth stage affects canopy development (Geetha and Tayade, 2015). Canopy development, new leaf production and photosynthesis decrease when the plant is subjected to drought stress (Inman-Bamber, 2004). Consequently, crop yield is disturbed by the photosynthetic rate and the canopy light interception rate, which are determined by the leaf area index (LAI) (Luo *et al.*, 2014). The maximum amount of canopy light absorption is achieved for better photosynthetic efficiency and possible yield improvement (Zhao *et al.*, 2002).

However, knowledge of non-destructive sampling, such as canopy size and LAI, which are related to dry mass production in different inter-specific hybrid clones and various commercial sugarcane genotypes under varied early season drought durations, has been limited. Therefore, the objective of this study was to determine the association between agronomic and physiological traits and the biomass of a diverse set of sugarcane genotypes under different drought durations. This information could aid in identification using non-destructive sampling, which is useful for selection processes in drought resistance breeding programs.

MATERIALS AND METHODS

This experiment was conducted under field conditions from November 2020 to December 2021 at the Khon Kaen Field Crops Research Center of Tha Phra, Khon Kaen Province, Thailand. A split plot design in a randomized complete block with four replications was used. The main plots were the three water irrigation treatments: 1) without water stress (Control the soil moisture content at field capacity (FC)); 2) withholding water from 4-6 months after transplanting (MAT) (short drought duration; SD); and 3) withholding water from 2-6 months after transplanting (long drought duration; LD). The subplot includes six diverse varieties, as four inter-specific hybridized sugarcane clones and two commercial sugarcane varieties (Table 1). The subplot was 12 m in width and 13 m long, with 0.5×1.5 m spacing between plants and rows, totaling 72 plots.

A sugarcane set was planted into a plastic bag containing soil and filter cake at a ratio of 2:1. Uniform seedlings were selected and transplanted to the field experiment 30 days after planting. The Fertilizers were applied at rates of 137.5 kg N ha⁻¹, 31.25 kg P ha⁻¹ and 100 kg K ha⁻¹ at 1 MAT and nitrogen and potassium were applied at a rate of 31.25 kg ha⁻¹ at 5 MAT. Weeds were controlled using an application of herbicide (Chloroacetanilide) at pre-emergence and hand weeding when necessary. The sugarcane borer was controlled as necessary by the application of carbosulfan at a rate of 2.5 liters ha⁻¹.

Since planting and up until 1 MAT, water was irrigated to all subplots using a mini-sprinkler system operating at field capacity and a drip irrigation system for maintaining the soil moisture content for FC treatment from planting to harvest. The SD period was withholding water from 4-6 MAT and the LD period was withholding water from 2-6 MAT. After 6 MAT, water was applied to all subplots and the soil water level was controlled at FC until the harvesting stage. The amount of water was calculated based on the daily crop water requirement (ET_{crop}) using the equation described by Khonghintaing *et al.* (2021).

Weather and soil data

Weather data were recorded daily for minimum and maximum temperature (°C), rainfall (mm), relative humidity (%), solar radiation and evapotranspiration (mm). Soil was

Table 1: Six sugarcane clones/varieties, four inter-specific hybridized sugarcane clones (*Saccharum spontaneum* × commercial varieties) and two commercial sugarcane varieties.

Variety	Parents	Source	Remark
F03-362 (F ₁)	88-2-401 × ThS98-178+ThS98-264	KKFCRC	High biomass, low sugar yield
KK09-0358 (BC ₁)	95-2-317 × F03-381 (F ₁)	KKFCRC	High cane yield, medium sugar yield
KK09-0939 (BC ₂)	BC04-251 (BC ₁) × UT4	KKFCRC	High cane yield and sugar yield
TPJ04-768 (BC ₁)	94-2-128 × F03-331 (F ₁)	KKFCRC	High cane yield, medium sugar yield
KK3	85-2-352 × K84-200	KKFCRC	Check (Moderately tolerant to drought) High cane yield, High sugar content
UT12	SP80 × UT3	SPFCRC	Check (Susceptible to drought) High cane yield

KKFCRC = Khon Kaen Field Crops Research Center.

SPFCRC = Suphan Buri Field Crops Research Center.

randomly collected before the experiment to ascertain its chemical and physical characteristics at depths of 0-30 cm and 30-60 cm. The soil at the experimental site is sandy loam texture. Soil pH at 0-30 cm and 30-60 cm soil depths ranged from 5.35-5.83. The soil organic matter and total nitrogen ranges from 0.13-0.29% and 46.04-51.56 mg kg⁻¹, respectively. Exchangeable potassium (44.54-52.81 mg kg⁻¹), available phosphorus (52.83-54.73 mg kg⁻¹) and bulk density was 1.46-1.63 g cm⁻³. The soil moisture content (%) was measured using the gravimetric method at a depth of 0-30 cm and 30-60 cm before planting and at 1, 2, 3, 4, 5, 7, 9, 11 and 12 MAT. The soil moisture content was determined from a soil core (Core size 4.5 cm diameter and 5 cm height) in all 72 subplots. The wet soil was weighed, oven-dried at 105°C for 72 h or until a consistent weight and then the dried soil was weighed. The soil moisture content was calculated as follows:

Soil moisture content (%) =

$$\frac{\text{Soil wet weight} - \text{Soil dry weight}}{\text{Dry weight}} \times 100$$

Non-destructive plant data

The canopy characteristics of each plot were collected at 3, 6, 8 and 12 MAT. Canopy height (CH) was measured from the second fully expanded leaf from the top of the main stem to the last lowest green leaf. Canopy width (CW) was measured as the diameter of the canopy in the widest position. Data on canopy traits were measured from four plants in each plot and the mean of each plot was calculated.

The LAI was measured (Non-destructive) using a plant canopy analyzer (LAI 2000; LICOR, Lincoln, NE, USA) following user manual recommendations for row crops between the late morning and early afternoon (LI-COR, 1992; Goncalves *et al.*, 2020; Fattori Junior *et al.*, 2022). The LAI was measured at three locations in each plot 3, 6, 8 and 12 MAT.

Green and dry leaf numbers were counted on the main stem of four plants per subplot. Stem heights were measured from the ground to the last exposed dewlap of the main

stem in each of the four plants recorded at 3, 6, 8 and 12 MAT. Tiller numbers were counted from four plants in each plot 3, 6, 8 and 12 MAT and stalk numbers were measured 6, 8 and 12 MAT. Node numbers were counted and stem diameters were collected 8 and 12 MAT. These parameters were then calculated as the mean per plant.

Destructive plant data

The destructive LAI was measured from four plants in each subplot and the leaves were used to measure the leaf area using a leaf area meter (LI-3100, Inc. Lincoln, Nebraska, USA). The leaf samples were then oven-dried at 70°C for 72 h or until the dry weight was constant and the dry weights were determined. The LAI was calculated as the ratio of leaf area (cm²) and ground area (cm²). At 3, 6, 8 and 12 MAT, four plants from each plot's harvest area were divided into leaves and stalks. The fresh weight of each plant part was then recorded and a sample of more than 20% of each part was oven-dried at 70°C for 72 hours to determine its dry weight. The dry weight of the leaves and stalks were summarized and converted into the biomass using the fresh weight and then re-calculated into dry matter per plant.

The measured data were subjected to ANOVA, according to a split-plot in a randomized complete block design using the software package Statistix 10 (Analytical Software, Tallahassee, FL, USA). A simple correlation was used to determine the relationships between physiological traits, agronomic traits and biomass. The genotypes by water irrigation treatments interaction for non-destructive variables were significant (data not shown) and then data for each water irrigation treatment were analyzed relationship separately.

RESULTS AND DISCUSSION

Weather condition

During the experimental period from November 2020 to November 2021, the total rainfall was 1207.2 mm. For LD periods, the total amount of rainfall was 410.6 mm and for SD periods, the total amount of rainfall was 364.1 mm. The

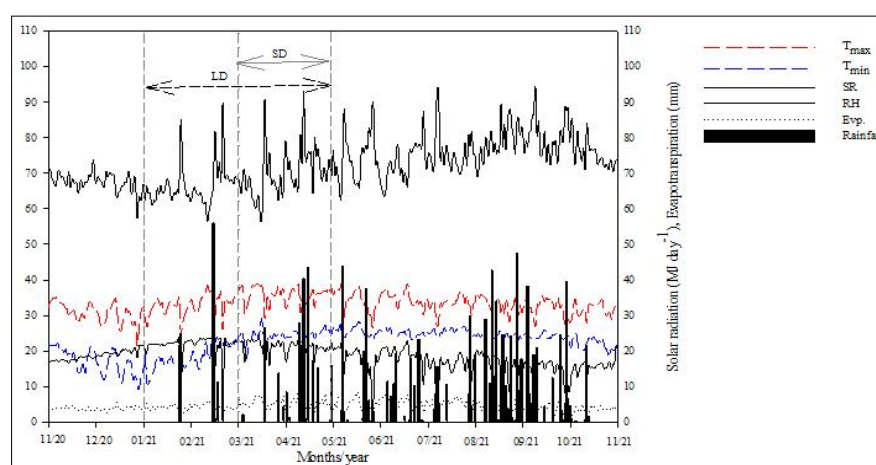


Fig 1: Rainfall (mm), maximum temperature (°C), minimum temperature (°C), relative humidity (%), evapotranspiration (mm) and solar radiation (MJ day⁻¹) throughout the experimental period.

maximum and minimum air temperatures during the experimental period were 33.16 and 22.13°C, respectively. The mean daily pan evaporation was 4.55 mm and the daily relative humidity ranged from 56.5 to 94.5%. The average solar radiation was 20.84 MJ day⁻¹ (Fig 1). For rainfall, sugarcane can survive normal variation around a mean of 1200 mm and optimum cane growth is achieved at temperatures between 24 and 30°C. and temperatures above 38°C reduce the rate of photosynthesis and increase respiration; however, cane variety, irrigation and cultural practices can modify this influence (Andhra Pradesh, 1999). Therefore, the weather data in this study showed adequate normal growth for sugarcane growing in tropical regions.

Soil moisture content

For field capacity treatment, water was regularly supplied to adequately maintain the soil moisture content at the FC level, deviating from FC level by less than 1% (Ranged from 12.56

to 12.67%) (Fig 2A and Fig 2B). Despite the natural rainfall throughout the dry season period of 9.57 mm, the soil moisture content of the LD period was considerably lower from 3-5 MAT at both soil depths, being 3.90 and 3.43% in March and April, respectively, at 0-30 cm soil depth and 5.07 and 4.63%, respectively, at 30-60 cm soil depth (Fig 2A and Fig 2B). Moreover, for SD treatment, the reduction in soil moisture after water withholding from 4-5 MAT was 4.68 and 3.67% at 0-30 cm soil depth in March and April, respectively. However, the rainfall from April to May increased and the soil moisture content of both early drought stress treatments increased 6 MAT (Fig 2A and Fig 2B). Evidently, the soil moisture content in this study differed among the three irrigation treatments, indicating adequate water control and the crop grown under different early drought stress. Under rainfed conditions, sugarcane may be exposed to short droughts and then recover from them (Jangpromma *et al.*, 2012). Both stem and leaf growth were more negatively impacted by water

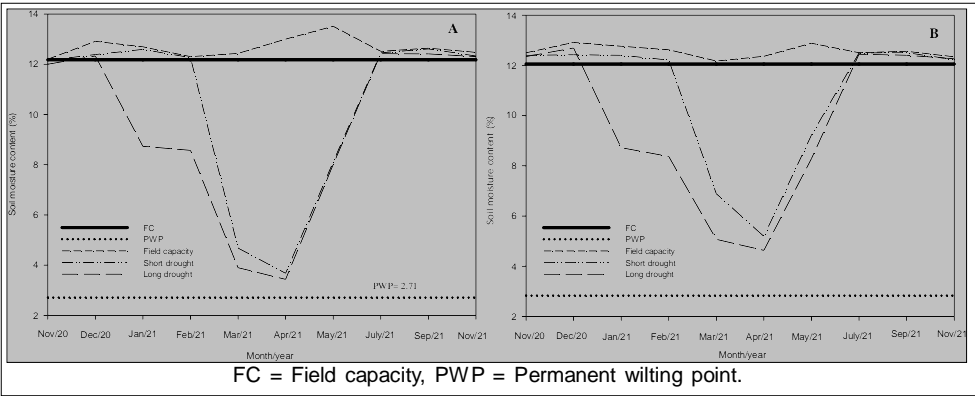


Fig 2: Soil moisture content (%) at 0-30 cm (A) and 30-60 cm (B) during the crop growth period of sugarcane grown under three water irrigation treatments: 1) without water stress (Control the soil moisture content at field capacity (FC) level), 2) short drought period (SD) and 3) long drought period (SD).

Table 2: Correlation coefficients (r) (n = 72) of physiological traits; leaf area index (Non-destructive), leaf area index (destructive), leaf area and agronomic traits; Canopy height, canopy wide, tiller number, leaf green number, leaf dry number, plant height, stalk number, node number, stem diameter and brix at 3, 6, 8 and 12 months after transplanting (MAT).

	Biomass			
	3 MAT	6 MAT	8 MAT	12 MAT
Canopy height	0.31 ^{ns}	0.11 ^{ns}	0.51 [*]	0.06 ^{ns}
Canopy width	0.17 ^{ns}	0.21 ^{ns}	-0.01 ^{ns}	0.23 ^{ns}
Leaf area	0.18 ^{ns}	-0.01 ^{ns}	0.32 ^{ns}	-0.03 ^{ns}
Leaf area index (Nondestructive)	0.18 ^{ns}	0.08 ^{ns}	0.42 ^{ns}	0.08 ^{ns}
Leaf area index (Destructive)	0.18 ^{ns}	-0.01 ^{ns}	0.32 ^{ns}	-0.03 ^{ns}
Number of tillers	0.49 [*]	0.13 ^{ns}	0.18 ^{ns}	0.09 ^{ns}
Number of green leaves	0.48 [*]	0.11 ^{ns}	0.25 ^{ns}	-0.14 ^{ns}
Number of dry leaves	0.43 ^{ns}	0.29 ^{ns}	0.26 ^{ns}	0.11 ^{ns}
Stem height	0.29 ^{ns}	0.28 ^{ns}	0.07 ^{ns}	0.19 ^{ns}
Number of stalks		0.27 ^{ns}	0.34 ^{ns}	0.19 ^{ns}
Number of nodes		0.26 ^{ns}	-0.05 ^{ns}	0.12 ^{ns}
Stem diameter			-0.19 ^{ns}	-0.01 ^{ns}

^{ns} = Non-significant difference, ^{*}Significant at a p<0.05 probability level.

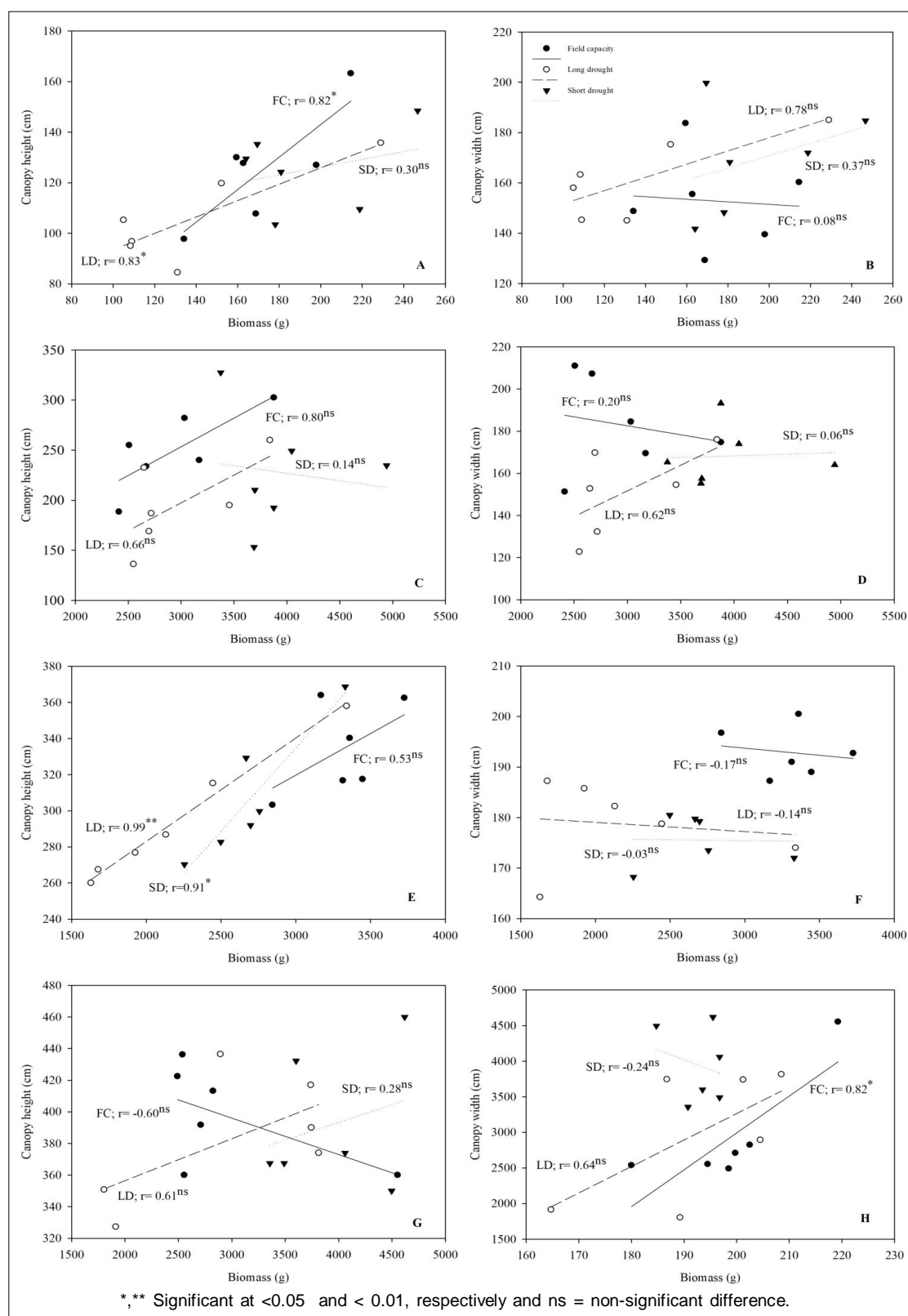


Fig 3: Correlation coefficients between means of biomass and canopy height at 3 months after transplant (MAT) (A), 6 MAT (C), 8 MAT (E) and 12 MAT (G) and correlation coefficients between biomass and canopy width at 3 MAT (B), 6 MAT (D), 8 MAT (F) and 12 MAT (H) of 6 sugarcane genotypes in each water regime.

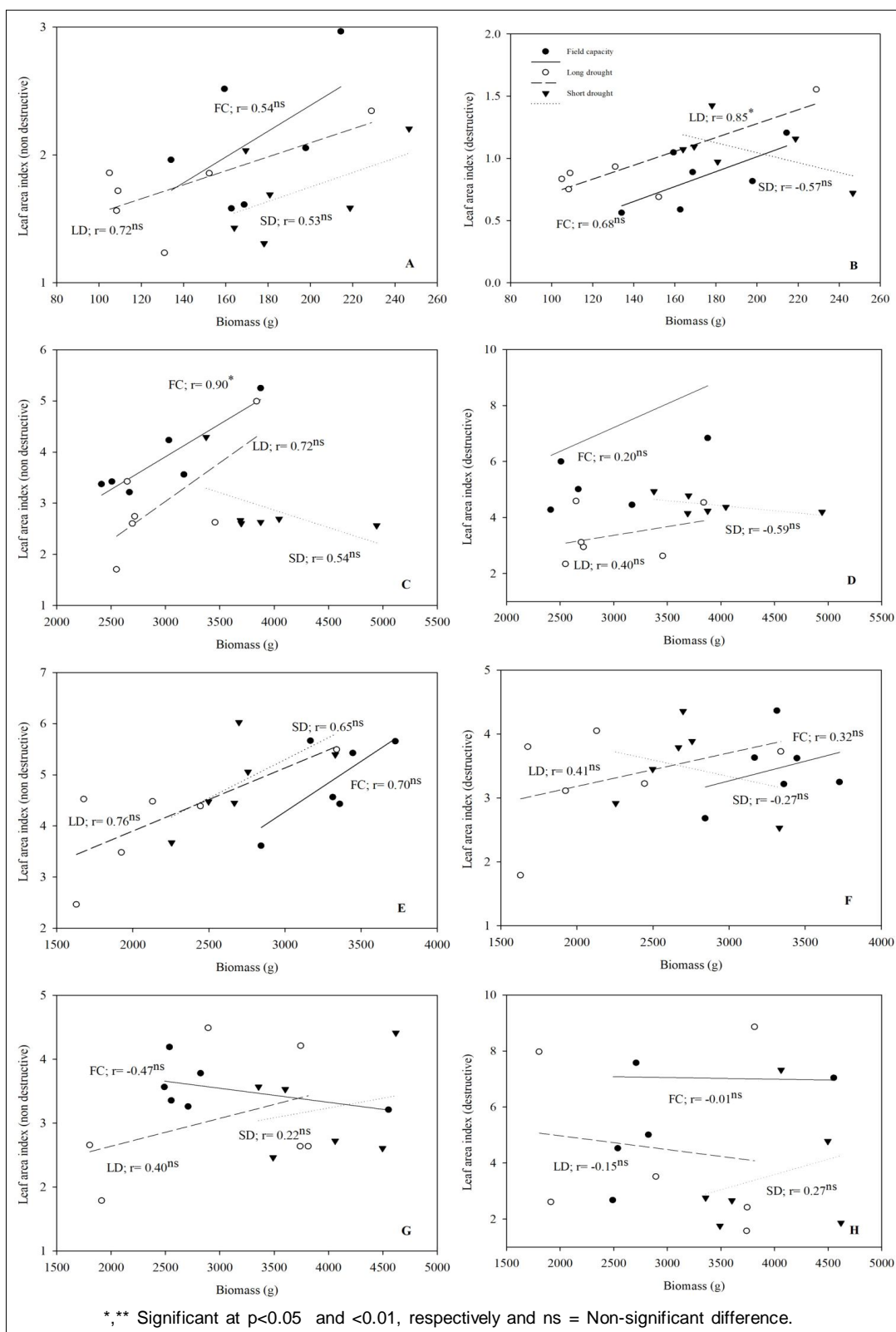


Fig 4: Correlation coefficients between means of biomass and canopy leaf area index (Non-destructive) at 3 months after transplant (MAT) (A), 6 MAT (C), 8 MAT (E) and 12 MAT (G) and correlation coefficients between biomass and leaf area index (destructive) at 3 MAT (B), 6 MAT (D), 8 MAT (F) and 12 MAT (H) of 6 sugarcane genotypes in each water regime.

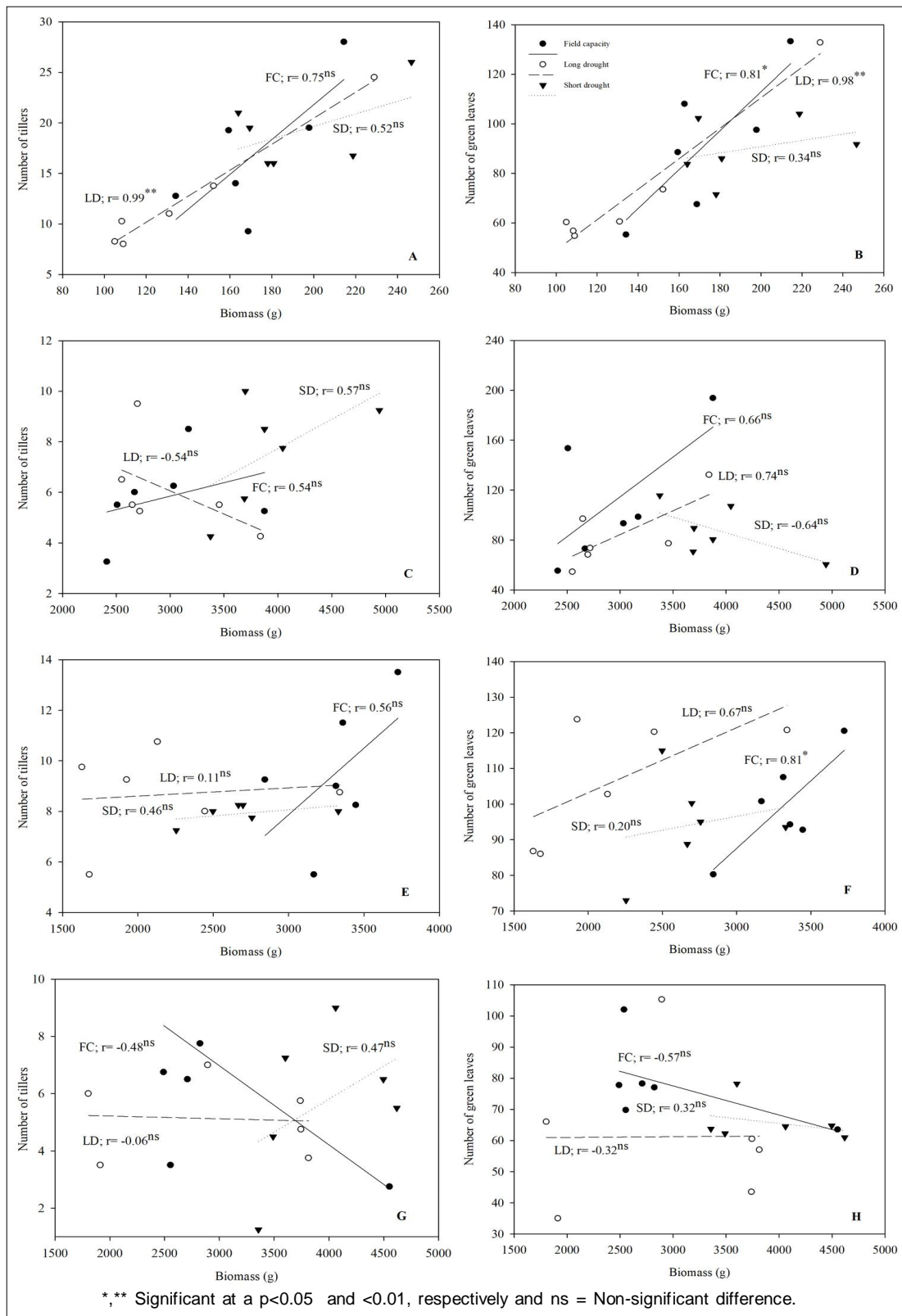


Fig 5: Correlation coefficients between means of biomass and number of tillers at 3 months after transplant (MAT) (A), 6 MAT (C), 8 MAT (E) and 12 MAT (G) and correlation coefficients between biomass and the number of green leaves at 3 MAT (B), 6 MAT (D), 8 MAT (F) and 12 MAT (H) of 6 sugarcane genotypes in each water regime.

stress than other organs throughout the tillering and stem elongation phases of sugarcane development (Inman-Bamber and Smith, 2005; Lakshmanan and Robinson, 2014).

Correlation between agronomic traits, physiological traits and biomass

In terms of the correlation between agronomic traits, physiological traits and biomass in the three irrigation treatments, a correlation between biomass and canopy height existed at 8 MAT. The relationship between the number of tillers and the number of green leaves was found with biomass at 3 MAT. However, the correlation value of these traits and biomass was rather low; thus, the association might need to be analyzed separately. Canopy height at 3, 6, 10 and 12 MAT, canopy width, leaf area, LAI (Non-destructive), LAI (Destructive), tiller numbers, green and dry leaf numbers, stalk numbers, node numbers, plant height and stem diameter were not significant in terms of relationship at 3, 6, 8, or 12 MAT (Table 2).

Correlation coefficients between biomass and canopy height at 3 MAT (Fig 3A) and canopy width at 12 MAT (Fig 3H) were significantly positive under FC conditions. For LD, canopy height was positively correlated with biomass at 3 and 8 MAT (Fig 3A and Fig 3E). However, the relationship between biomass showed no significant correlations for canopy height at 6 and 12 MAT (Fig 3C and 3G), and canopy width at 3 6 and 8 MAT (Fig 3B; Fig 3D and 3F) in all irrigation treatments. A previous study reported that canopy size and biomass showed a positive correlation in commercial sugarcane cultivars during the early rainy season (Pringgani *et al.*, 2023). In addition, as a calibration variable, canopy height can be used to adjust indirect LAI results and enhance agreement with direct LAI observations (Gonçalves *et al.*, 2020). The distribution and absorption of light by the leaf with different layers are directly influenced by the structure of the sugarcane canopy (Luo *et al.*, 2014). The diverse meteorological conditions, including temperature, relative humidity, solar radiation and rainfall, likely resulted in differences in biomass. These climatic conditions are involved with canopy growth and development since they eventually constitute the source of light capture for the photosynthetic process, as well as subsequent assimilate partitioning and assimilate accumulation processes (Odubanjo *et al.*, 2011).

The correlation coefficients between LAI (non-destructive) and biomass were significantly positive in FC at 6 MAT (Fig 4C). The relationship between destructive LAI and biomass was also positively correlated at 3 MAT (Fig 4B) in LD. Simple correlation coefficients between biomass showed no significant correlations for LAI (non-destructive) at 3, 8, and 12 MAT (Fig 4A; Fig 4E and Fig 4G) and LAI (destructive) at 6, 8, and 12 MAT (Fig 4D; Fig 4F and Fig 4H) under three irrigation treatments. This study agreed with a previous study, even though it was conducted on commercial sugarcane. Gonçalves *et al.* (2020) reported that LAI, which directly and indirectly determines the coefficient of determination, ranged from 0.46 to 0.88. The

photosynthetic rate of crops is influenced by the canopy light interception rate, which is determined by the LAI (Luo *et al.*, 2014). Cultivars that have different LAIs also generate significant levels of biomass and the leaf number parameter has a closer correlation with LAI than the leaf size (Pringgani *et al.*, 2023).

A positive correlation between green leaf number and biomass existed in FC at 3 MAT and 8 MAT (Fig 5B and Fig 5F). Tiller numbers, green leaf number and biomass showed a positive relationship at 3 MAT under LD treatment (Fig 5A). Nevertheless, biomass was not correlated with tillers number at 6, 8, and 12 MAT (Fig 5C; Fig 5E and Fig 5G) and with green leaf number at 6 and 12 MAT (Fig 5D and Fig 5H) in all treatments. In addition, chlorophyll content and the biomass performance had a positive relationship, with biomass being related to chlorophyll content (Silva *et al.*, 2008; Liu *et al.*, 2019). The amount of chlorophyll in a sugarcane leaf plays a significant role in the photosynthetic process, which strongly correlates with crop production (Silva *et al.*, 2014). High tiller numbers imply rapid growth, which is the foundation of excellent productivity. The tiller production peaked two to three months after planting and then started to decline depending on the genotype, tiller class, planting date and cultural conditions (Vasantha *et al.*, 2012). The tiller number was decreased by drought stress Gomathi *et al.* (2020); however, the shoot population with stems and leaves was closely related to plant dry matter.

CONCLUSION

This is the first report detailing the determination of the correlation between non-destructive traits in agronomy and physiology and biomass of a diverse set of sugarcane genotypes under different drought durations, especially inter-specific hybrid sugarcane. At the early developmental stage, the correlation between non-destructive parameters such as canopy height and green leaf numbers and biomass existed under FC and LD and found the relationship between tiller numbers and biomass under LD. At 6 MAT of this study, non-destructive LAI was a parameter that could be used as an indirect parameter of biomass under a full water regime. In the water recovery stage during the rainy season, 8 MAT, canopy height reflected the biomass performance of sugarcane that experienced SD and LD stress. The green leaf number was also correlated with biomass under FC treatment. Under full irrigation, canopy width and biomass were correlated at the final harvest period 12 MAT but not with other parameters. In general, canopy height and green leaf number were alternative parameters to use as indirect determinations of sugarcane biomass with non-destructive sampling; however, the recommendation needs to be considered in varied drought conditions.

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Conflict of interest: None.

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