

Article

Dynamic Recovery of Canopy and Growth Traits in Diverse Sugarcane Genotypes Following Early-Season Drought

Pornthiwa Khwanthaworn ¹, Jidapa Khonghintaiong ², Patcharin Songsri ^{1,2}  and Nakorn Jongrunklang ^{1,2,*} 

¹ Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand; pornthiwa.k@kku.ac.th (P.K.); patcharinso@kku.ac.th (P.S.)

² Northeast Thailand Cane and Sugar Research Center, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand; jidapa.k.h.thais@gmail.com

* Correspondence: nuntjo@kku.ac.th; Tel.: +66-982-255-598

Abstract

Sugarcane biomass and yield decline under drought, and interspecific hybridization may improve tolerance, but how non-destructive traits such as canopy size and leaf area index (LAI) respond across genotypes under varying drought duration and recovery remains unclear, limiting their use in breeding selection. This study evaluated non-destructive traits in six sugarcane genotypes under field capacity (FC), short-duration drought (SD), and long-duration drought (LD) using a split-plot RCBD with four replications. Canopy height, canopy width, LAI, green leaf number, tiller number, leaf dry weight, and stalk dry weight were measured at 3, 6, and 8 months after transplanting (MAT). By 3 MAT, LD reduced canopy height, LAI (by up to approximately 30% relative to FC), and green leaf number in most genotypes. By 6 MAT, F03-362 showed reduced canopy height, width, green leaf number, and tiller number under drought, with LAI increasing during recovery, whereas KK3 stayed stable in most traits except LAI, which declined under LD. By 8 MAT, canopy height under LD had recovered to match or slightly exceed that under SD in most genotypes, and F03-362 produced the highest biomass of all genotypes under every water regime and, at 6 MAT, showed a proportional biomass decline close to the smallest among all genotypes, although this relative advantage narrowed by 8 MAT, whereas total biomass under SD and LD averaged approximately 34% lower than under FC across genotypes and stages overall. F03-362 and KK3 thus emerge as complementary genotypes for breeding drought-tolerant sugarcane, F03-362 through high absolute biomass potential with a favorable proportional response at 6 MAT, and KK3 through green leaf number stability under short-duration drought, offering practical, repeatedly measurable traits for early selection under limited sample size.

Keywords: interspecific breeding; water deficit; leaf area index; canopy height



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1. Introduction

Sugarcane (*Saccharum* spp.) is of significant global economic importance due to its role in sugar and bioenergy production [1]. Effective water management is crucial for mitigating annual variations in sugarcane yields [2]. Drought frequency and duration within sugarcane production systems are influenced by the length of the rainy season. Compared to rainfed conditions, irrigation can enhance cane yields by up to 80% [3]. Conversely, water shortages can severely impede sugarcane growth, potentially reducing biomass yields by 50% [4]. Dehydration during early developmental stages significantly affects leaf growth and canopy

development [5]. The effects of prolonged water deficits on physiological and biochemical processes have been extensively studied, consistently demonstrating a significant impact on plant growth, development, and overall productivity [4].

In several crops, including sugarcane, preliminary physiological, biochemical, morphological, and developmental changes—such as canopy size and leaf area index (LAI) exhibit high sensitivity to water deficits [5,6]. Under drought conditions, sugarcane often exhibits a reduction in green canopy, thereby affecting LAI and photosynthetic capacity [7]. Additionally, green leaf number, leaf dry weight, and stalk dry weight were reduced under low water potential during the early sugarcane growth stage [8], along with a decline in stalk number [9]. Commercial sugarcane sustained shoot dry weight throughout the recovery phase [8]. Utilizing drought-tolerant genotypes is a practical strategy for maintaining sugarcane yields during drought conditions [10]. Genotypes that demonstrate early drought adaptability can effectively mitigate this challenge [4]. Interspecific hybridization is a viable strategy in breeding programs aimed at enhancing drought resistance [11]. The wild sugarcane variety, *Saccharum spontaneum*, is a valuable genetic resource known for its inherent drought tolerance [6]. This approach enables the retention of important sugar and biomass-related genes from *S. officinarum*, thereby enhancing genetic improvement [12]. Moreover, it boosts stress resistance by introducing genetic material from *S. spontaneum*. This method represents a promising strategy for developing sugarcane genotypes that enhance both sugar and biomass production while increasing resilience to environmental stress. The magnitude and patterns of these responses vary based on sugarcane genotype or species [13]. In the context of sugarcane, specific parameter variations have been used to differentiate between genotypes that respond to drought and those that exhibit drought tolerance [14].

Various methods exist for measuring drought tolerance in sugarcane, some of which are complex and time-consuming to implement [15,16]. Early-generation selection in sugarcane faces the challenge of limited population sampling, necessitating the use of non-destructive data collection methods. Destructive methods, such as direct measurement of root and shoot biomass, generally provide the most precise assessment of physiological drought response, but they require sacrificing the sampled plant and therefore cannot be repeated on the same individual over time, a substantial constraint when only a few plants per genotype are available, as is typical of early-generation breeding populations. Non-destructive methods, in contrast, allow the same plant to be monitored repeatedly across the growth cycle, capturing the trajectory of stress response and recovery, but they generally offer lower measurement precision and can be more subjective, for example, in the visual counting of green leaves, than direct biomass sampling. Given this trade-off between precision and repeatability, non-destructive monitoring is generally favored for early-stage screening in breeding programs, where preserving limited plant numbers across generations takes priority over the higher precision achievable through destructive assessment. However, it is important to acknowledge that certain critical parameters require destructive sampling from the field. Therefore, when evaluating the response of interspecific hybrid varieties to early drought stress, it is essential to focus on non-destructive traits during the initial breeding stages, given the inherent sampling constraints. Physiological parameters, such as canopy size and LAI, are particularly important in this context, as they directly influence light interception and are closely linked to photosynthesis [6]. In addition, water deficits significantly impact sugarcane, resulting in substantial early physiological, morphological, and developmental changes, including photosynthesis and leaf area index [17]. Previous studies have demonstrated that non-destructive traits, such as canopy size and LAI, are correlated with sugarcane biomass yield [18]. However, how these traits change over time across diverse genotypes under varying drought durations and recovery

periods remains poorly documented. In particular, it is not yet known how genotypes with high biomass potential express these traits at different growth stages. Therefore, this study aimed to investigate the changes in non-destructive traits among diverse sugarcane genotypes subjected to short- and long-duration drought during early growth and recovery stages. Understanding the response patterns of these traits over time would benefit sugarcane breeding programs, as the responses of non-destructive traits could serve as selection criteria for identifying drought-tolerant genotypes. This approach would be particularly useful during early breeding stages, when the number of available plant samples is limited.

2. Results

2.1. Variation in Soil Moisture Content and Meteorological Data During the Study Period

This experiment received a total natural rainfall of around 180.0 mm during the dry season, whereas soil moisture content in short drought duration (SD) and long drought duration (LD) treatments from 5 to 6 months after transplanting (MAT) remained low at a depth of 0–30 cm and 30–60 cm, approximately 2.71–5.99% and 3.88–8.04% for the two soil depths, respectively (Figure 1). Furthermore, the lowest soil moisture content in SD and LD decreased by 4.41% and 2.71%, respectively, at a depth of 0–30 cm after water withholding at 5 MAT (Figure 1a); while at a depth of 30–60 cm, it was reduced by 4.40% in LD at 5 MAT and by 3.88% in SD at 6 MAT (Figure 1b). Nevertheless, the soil moisture content of both early drought stress treatments increased at 6 MAT, except for SD at 7 MAT due to the rise in rainfall from April to May. Certainly, the soil moisture content in this study varied among the three irrigation regimes, indicating the successful management of water and the crop's diverse reactions to varying levels of early drought stress. Sugarcane may undergo brief dry spells when grown under rainfed conditions, but it also has the potential to recover.

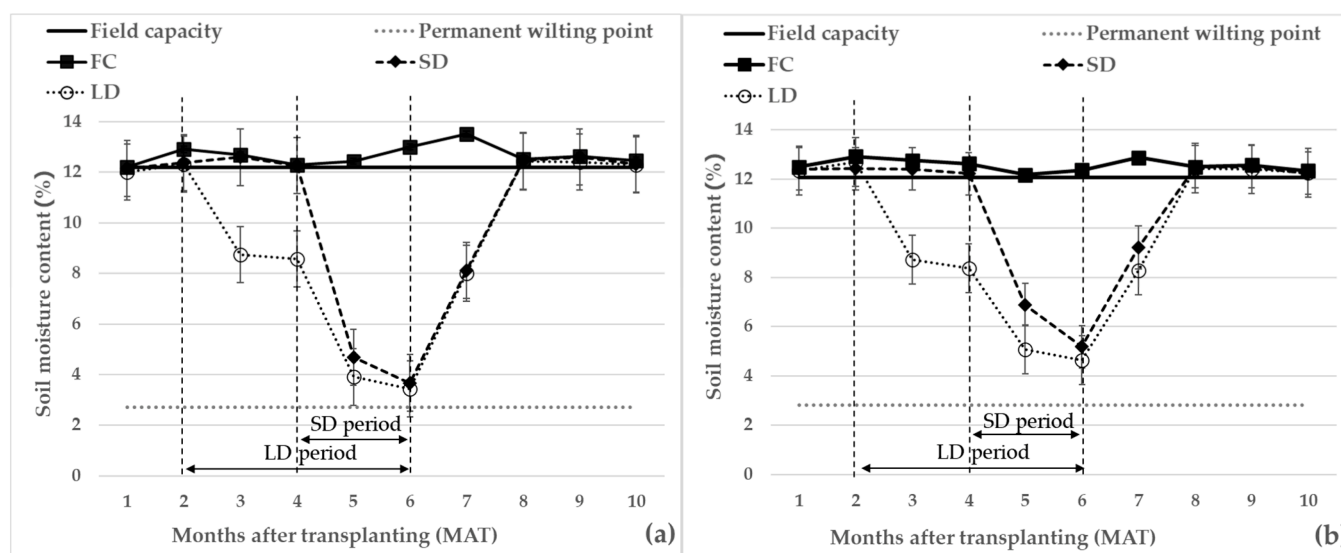


Figure 1. Soil moisture content (%) at depths of 0–30 cm (a) and 30–60 cm (b) was monitored throughout the growth cycle of sugarcane subjected to three distinct water irrigation treatments: (1) without water stress (control the soil moisture content at field capacity (FC) level), (2) short drought period (SD), and (3) long drought period (LD).

The maximum and minimum temperatures ranged from 10.5 to 25.4 °C and 21.4–38.9 °C, respectively; total rainfall during November 2020 to December 2021 was recorded at 1210.77 mm. For LD durations, the total amount of rainfall was 410.6 mm, and

for SD durations, the total amount of rainfall was 364.1 mm. The average solar radiation was $20.84 \text{ MJ day}^{-1}$, and the mean daily pan evaporation was 4.55 mm (Figure 2).

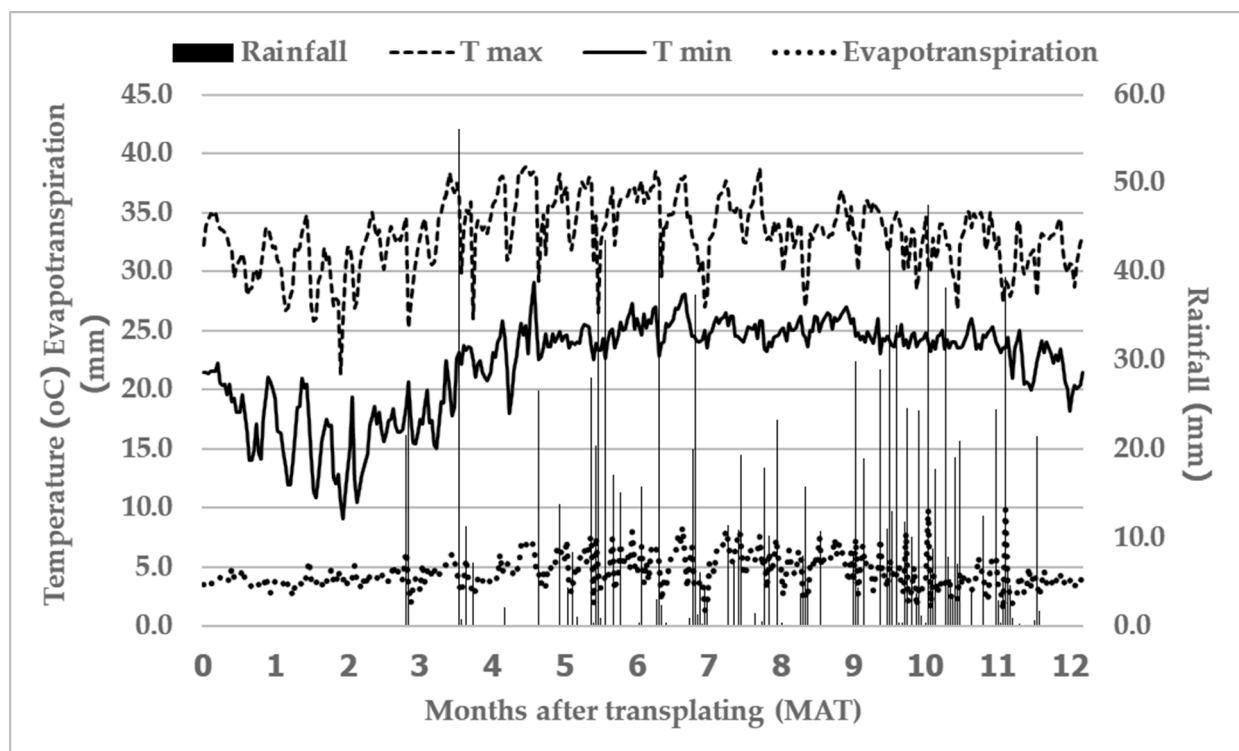


Figure 2. Rainfall (mm), maximum temperature ($^{\circ}\text{C}$), minimum temperature ($^{\circ}\text{C}$), relative humidity (%), evapotranspiration (mm), and solar radiation (MJ day^{-1}) throughout the experimental duration (November 2020 to December 2021).

2.2. The Response of Canopy Traits and LAI

Canopy height responses to water treatment became evident earlier than those of the other canopy traits. At 3 MAT, canopy height and LAI had not yet diverged between the field capacity (FC) and SD treatments, indicating that short-term water deficit had not yet exerted a measurable effect on canopy development at this early stage. Comparisons at 3 MAT were therefore restricted to the FC and LD treatments, which already differed significantly in canopy height across all six sugarcane genotypes (Figure 3), demonstrating that prolonged early-season drought suppresses canopy expansion well before short-duration stress does. By 6 MAT, genotypic differences in drought response became apparent. KK09-0358, TPJ04-768, and KK3 showed no significant differences in canopy height among the three water treatments, suggesting an earlier recovery response or greater physiological buffering against water deficit in these genotypes. The remaining genotypes continued to display significant treatment effects at this stage, consistent with a slower or incomplete recovery trajectory. By 8 MAT, canopy height differed significantly among all three water treatments in every genotype. Notably, LD-treated plants had recovered to surpass SD-treated plants in canopy height in most genotypes, pointing to a compensatory late-season growth response following prolonged early-season stress. F03-362 was the sole exception, with SD height remaining higher than LD at 8 MAT, indicating a genotype-specific constraint on canopy recovery after extended drought exposure. This transient exceedance of SD by LD likely reflects a short-term compensatory or overshoot growth response following partial rehydration or rainfall events within the drought window, rather than a sustained drought-tolerance advantage, and should be interpreted cautiously given that this pattern was not consistent across growth stages or across all genotypes.

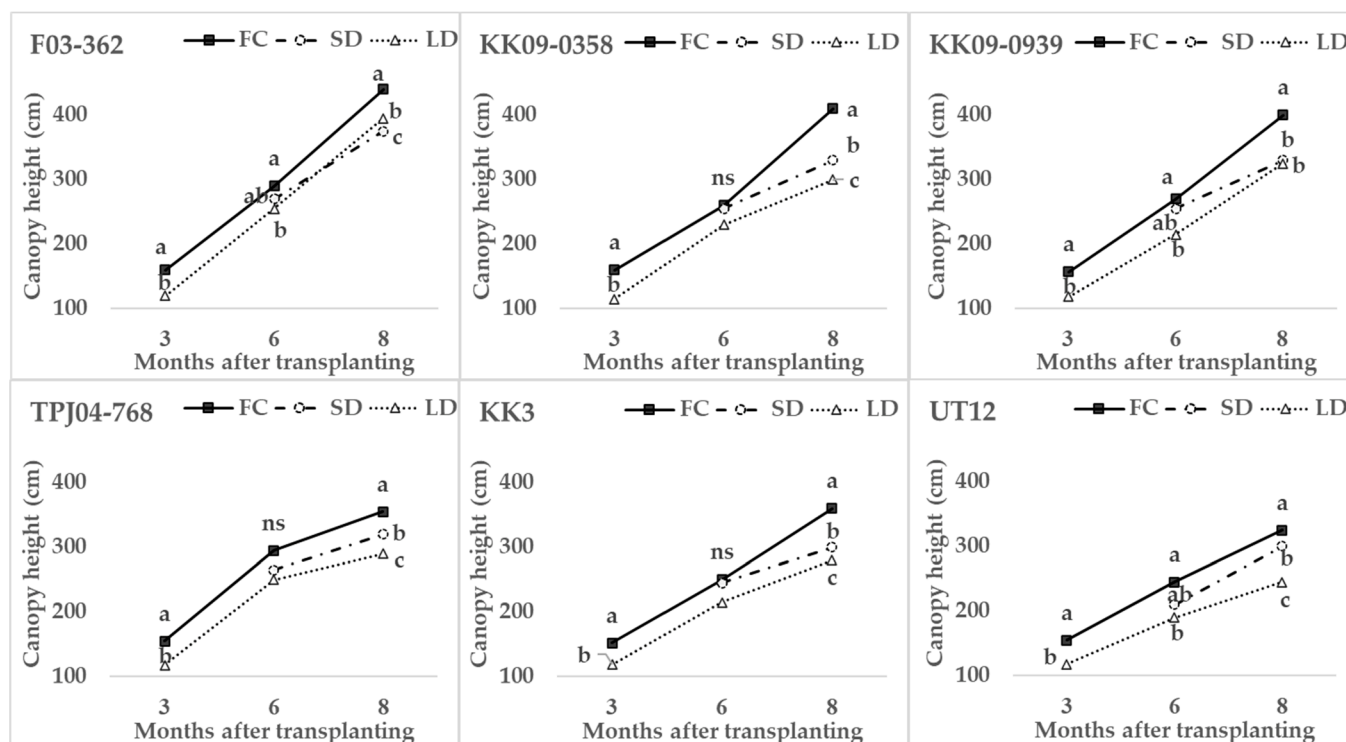


Figure 3. Canopy height (cm) of F03-362, KK09-0358, KK09-0939, TPJ04-768, KK3, and UT12 sugarcane varieties at 3, 6, and 8 months after transplanting (MAT) under different water regimes: field capacity (FC), short drought duration (SD), and long drought duration (LD). Different lowercase letters within the same growth stage indicate significant differences among water treatments according to the LSD test ($p < 0.05$); 'ns' indicates no significant difference.

Canopy width showed a delayed but broadly similar response pattern to canopy height. No significant differences among water treatments were detected for any genotype at 3 MAT (Figure 4), indicating that early-season drought had not yet constrained lateral canopy expansion. By 6 MAT, genotype-specific responses began to emerge. KK09-0358 and KK3 still showed no significant treatment effect. In contrast, the remaining four genotypes exhibited significant differences among treatments. Two genotypes showed an unexpected drought response at this stage. KK09-0939 had greater canopy width under LD than under FC, and TPJ04-768 had greater canopy width under SD than under FC. This pattern suggests a transient compensatory growth response to water deficit in these genetic backgrounds. As with the canopy-height compensation noted above, this transient exceedance of FC likely reflects a short-term overshoot growth response following partial rehydration or intervening rainfall rather than a genuine growth advantage of drought over well-watered conditions, and should be interpreted cautiously given that it appeared at only one growth stage and was not sustained by 8 MAT in either genotype. By 8 MAT, canopy width differed significantly among treatments in all genotypes, with FC consistently producing the greatest width. The relative recovery pattern between the two drought treatments was, however, genotype-dependent. SD and LD did not differ significantly from one another in F03-362, KK09-0939, and KK3, indicating comparable canopy recovery irrespective of stress duration in these genotypes. In contrast, KK09-0358 and TPJ04-768 exhibited greater canopy width under SD than under LD, consistent with more complete recovery following shorter stress exposure, whereas UT12 showed the opposite trend, with lower canopy width under SD than under LD.

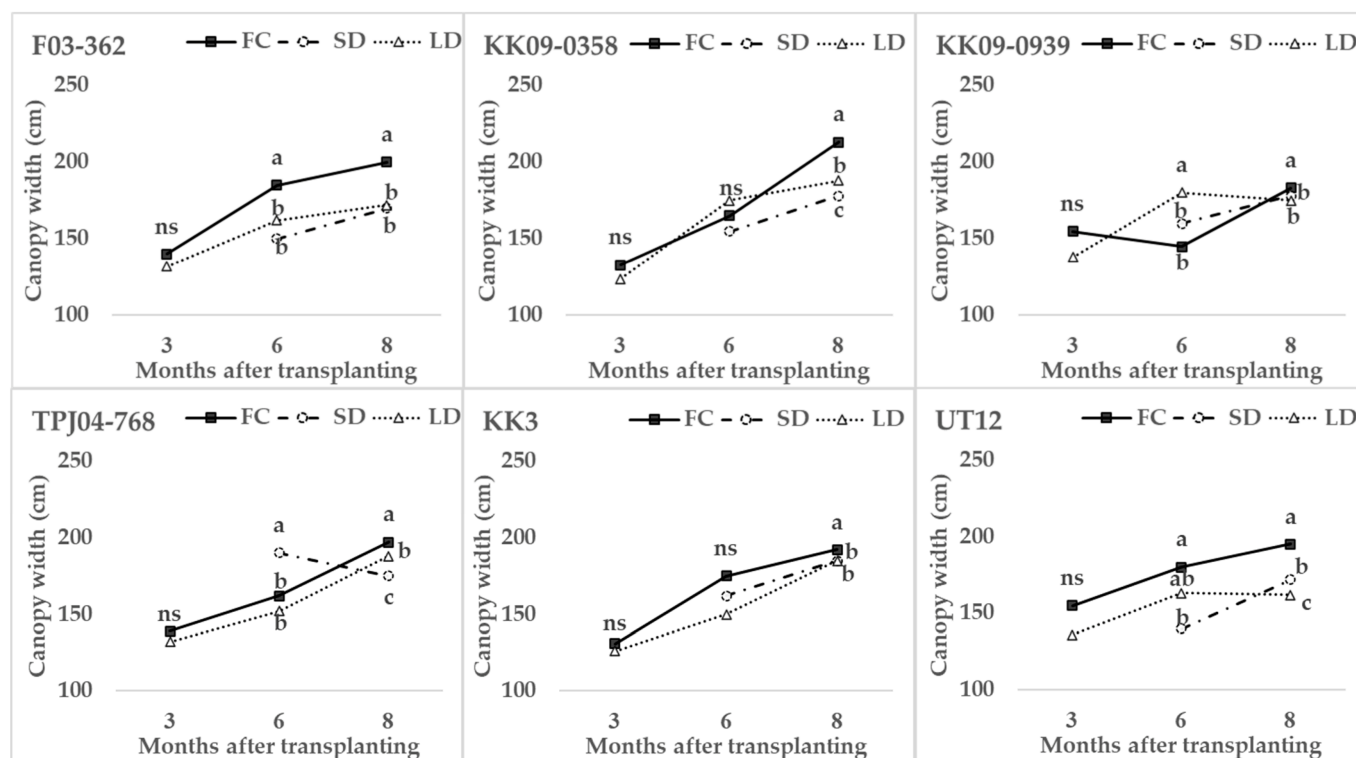


Figure 4. Canopy width (cm) of F03-362, KK09-0358, KK09-0939, TPJ04-768, KK3, and UT12 sugarcane varieties at 3, 6, and 8 months after transplanting (MAT) under different water regimes: field capacity (FC), short drought duration (SD), and long drought duration (LD). Different lowercase letters within the same growth stage indicate significant differences among water treatments according to the LSD test ($p < 0.05$); 'ns' indicates no significant difference.

LAI followed a comparable developmental trajectory to the other canopy traits, with FC consistently associated with the highest values across genotypes and growth stages. At 3 MAT, LAI was generally higher under FC than under LD across the six genotypes, with the exception of TPJ04-768, which showed no significant difference between FC and LD at this stage (Figure 5). By 6 MAT, all six genotypes exhibited significant treatment effects, with FC consistently producing the highest LAI. Genotype-specific recovery patterns among the drought treatments became apparent at this stage. F03-362 showed no difference in LAI between FC and LD, but had significantly lower LAI under SD. However, SD and LD themselves did not differ from one another. KK09-0358, KK09-0939, and TPJ04-768 showed no difference in LAI between SD and LD. KK3 and UT12 maintained comparable LAI under FC and SD. KK3 exhibited lower LAI under LD, although this did not differ significantly from SD. UT12 recorded its lowest LAI under LD relative to the other two treatments. By 8 MAT, F03-362 and KK09-0358 no longer differed significantly in LAI among any of the three water treatments, indicating a convergence in canopy photosynthetic capacity by the late season regardless of prior drought exposure. KK09-0939, TPJ04-768, and UT12 showed no difference in LAI between FC and SD, but LD remained the lowest of the three treatments in these genotypes. KK3 retained significantly higher LAI under FC than under either SD or LD, with SD and LD not differing from one another (Figure 5).

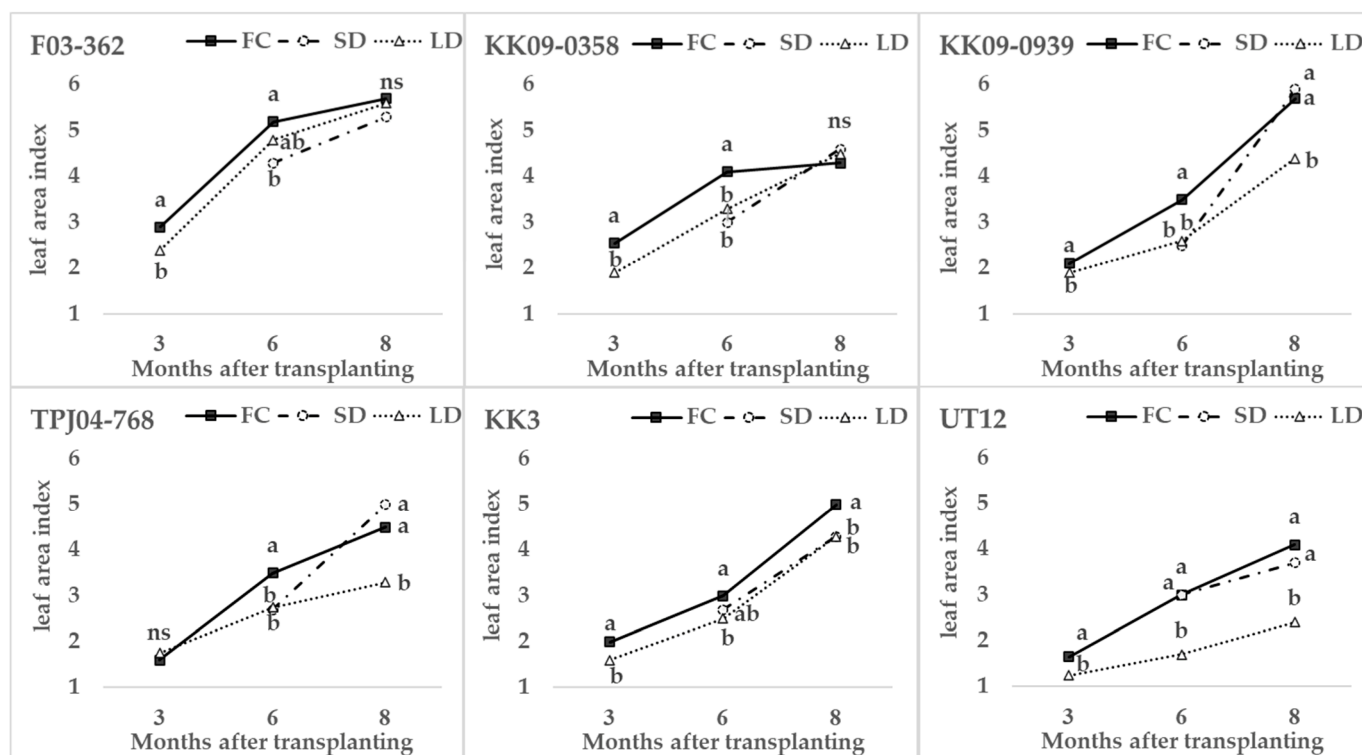


Figure 5. Leaf area index of F03-362, KK09-0358, KK09-0939, TPJ04-768, KK3, and UT12 sugarcane varieties at 3, 6, and 8 months after transplanting (MAT) under different water regimes: field capacity (FC), short drought duration (SD), long drought duration (LD). Different lowercase letters within the same growth stage indicate significant differences among water treatments according to the LSD test ($p < 0.05$); ‘ns’ indicates no significant difference.

2.3. Determination of Growth Responses to Green Leaves Number and Tillers Number

Green leaf number differed significantly among water treatments as early as 3 MAT in five of the six genotypes—F03-362, KK09-0358, KK09-0939, TPJ04-768, and KK3—whereas UT12 showed no significant treatment effect at this stage (Figure 6), indicating that drought-induced leaf senescence emerged earlier in most genotypes than the canopy height, width, or LAI responses described above. By 6 MAT, the two commercial genotypes, KK3 and UT12, still showed no significant differences in green leaf number among water treatments, suggesting a delayed onset of drought-related leaf loss in these cultivars. In the remaining four genotypes (F03-362, KK09-0358, KK09-0939, and TPJ04-768), FC consistently produced a higher green leaf number than either SD or LD, while SD and LD themselves did not differ significantly at this stage (Figure 6). By 8 MAT, F03-362, KK09-0358, KK09-0939, and TPJ04-768 continued to show a higher green leaf number under FC than under either drought treatment, and, notably, SD now exceeded LD in all four genotypes, indicating a genotype-independent recovery advantage associated with shorter stress duration in this trait. Significant differences also emerged among the two commercial genotypes by this stage, although with distinct patterns: in KK3, FC and SD did not differ from one another but were both significantly higher than LD, whereas in UT12, FC significantly exceeded both SD and LD, which did not differ from each other (Figure 6). Overall, these results indicate that prolonged early-season drought accelerates the loss of green leaves across genotypes, with recovery differing both in timing and magnitude depending on genetic background.

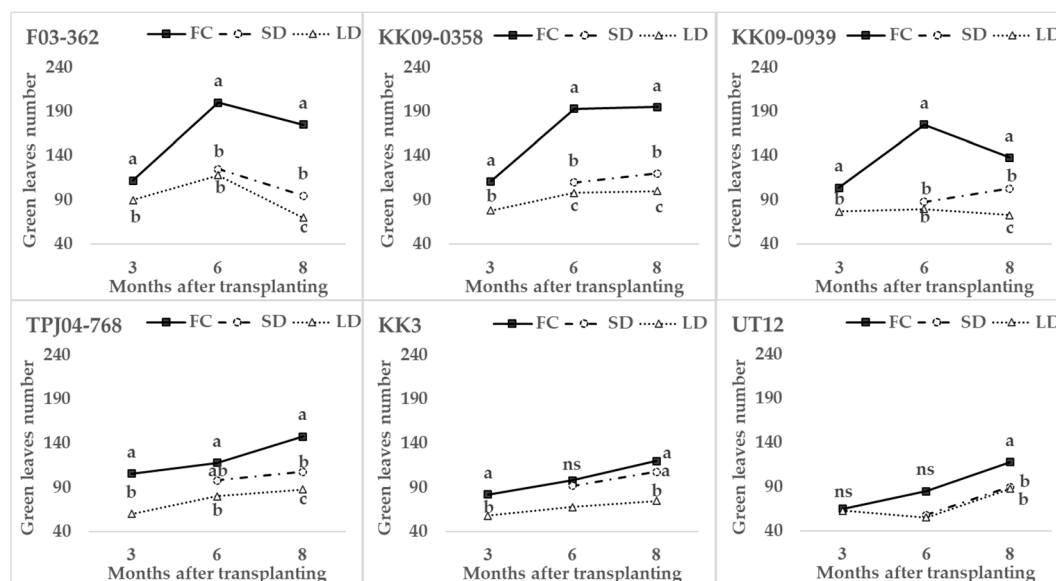


Figure 6. Number of green leaves of F03-362, KK09-0358, KK09-0939, TPJ04-768, KK3, and UT12 sugarcane varieties at 3, 6, and 8 months after transplanting (MAT) under different water regimes: field capacity (FC), short drought duration (SD), and long drought duration (LD). Different lower-case letters within the same growth stage indicate significant differences among water treatments according to the LSD test ($p < 0.05$); 'ns' indicates no significant difference.

Tiller number did not differ significantly among water treatments in any genotype at 3 MAT (Figure 7), indicating that tillering had not yet been affected by early-season drought regardless of breeding background (F1, BC1, BC2, or commercial). At both 6 and 8 MAT, tiller number remained unaffected by water treatment in the BC1, BC2, and commercial genotypes (KK09-0358, KK09-0939, TPJ04-768, KK3, and UT12), suggesting that this trait was comparatively insensitive to drought duration in these genetic backgrounds. The F1 hybrid, F03-362, was the exception: it showed a significant reduction in tiller number under LD relative to FC at both 6 and 8 MAT (Figure 7). The recovery pattern of F03-362 also shifted between growth stages. At 6 MAT, tiller number under SD did not differ significantly from that under LD, both being lower than FC. By 8 MAT, however, SD had recovered to a level statistically comparable to FC, while LD remained significantly lower, resulting in a significantly higher tiller number under SD than under LD. This shift indicates a genotype-specific, stage-dependent recovery in tillering capacity following short-duration drought, which was not observed under prolonged (long-duration) stress.

2.4. Leaf Dry Weight and Stalk Dry Weight of Six Diverse Sugarcane Genotypes

Dry-weight traits, including leaf dry weight and stem dry weight, were examined in different sugarcane genotypes exposed to drought stress conditions. The highest dry weight of all genotypes was found in FC at 3, 6, and 8 MAT (Figure 8). At 3 MAT, the biomass of F03-362 under LD conditions exhibited a consistent ratio between leaf dry weight and stalk dry weight, whereas in KK09-0358, KK09-0939, TPJ04-768, KK3, and UT12, leaf dry weight exceeded stalk dry weight. Additionally, a notable difference was observed between FC conditions and LD conditions across all sugarcane genotypes during tillering stages (Figure 8a). At 6 MAT, the biomass of all sugarcane genotypes under the three water regimes was significantly different, indicating low potential for dry weight in LD for all sugarcane genotypes. F03-362, KK09-0358, KK09-0939, and KK3 showed similarity in the proportions of leaf and stalk dry weight in LD conditions. However, when compared to FC and SD conditions, it becomes apparent that the proportion of stalk dry weight surpasses that of leaf dry weight. For the TPJ04-768 genotype across the three water levels, it becomes

evident that the proportion of stalk dry weight consistently exceeds that of leaf dry weight. This suggests a distinct pattern in this cultivar, regardless of the water regime. Conversely, in the case of the UT12 genotype under SD conditions, the ratio of stem dry weight to leaf dry weight remains the same. Under LD conditions, there is a notable shift, with the proportion of leaf dry weight significantly surpassing that of stem dry weight. However, UT12 under FC conditions showed greater partitioning to stalk dry weight than to leaf dry weight at 6 MAT. This trait was particularly noteworthy given that UT12 was identified as a drought-susceptible genotype (Figure 8b). Following rehydration at 8 MAT, the biomass of all sugarcane genotypes under different water regimes showed a statistically significant difference. All sugarcane genotypes during the rehydration phase exhibited the highest biomass under FC conditions, followed by SD and the lowest under LD, except for KK09-0939, which showed higher biomass under FC than under SD and LD, with no significant difference between SD and LD conditions. F03-362 showed higher biomass than other genotypes under all water conditions at 8 MAT. In the case of F03-362, KK09-0358, KK09-0939, and TPJ04-768 genotypes, a consistent trend emerges across all three water levels, revealing a significantly higher proportion of stalk dry weight compared to leaf dry weight. This suggests a shared trait among these genotypes in prioritizing stalk development over leaf biomass under various water conditions. For the KK3 genotype under SD conditions, a higher proportion of stalk dry weight compared to leaf dry weight is observed, similar to the FC conditions. However, under LD conditions, the proportion of stalk dry weight and leaf dry weight showed no significant difference. This implies that the KK3 genotype may exhibit resilience under SD conditions but faces challenges in maintaining the balance between stem and leaf growth under prolonged drought conditions. In the case of the UT12 genotype under FC conditions, a higher proportion of stalk dry weight is observed compared to leaf dry weight. However, the proportions of stalk and leaf dry weight of UT12 were comparable under SD and LD conditions. This suggests that, despite entering the sugar-accumulation stage and the completion of stem growth, UT12 maintains an equilibrium in the division between leaf formation and stem growth (Figure 8c).

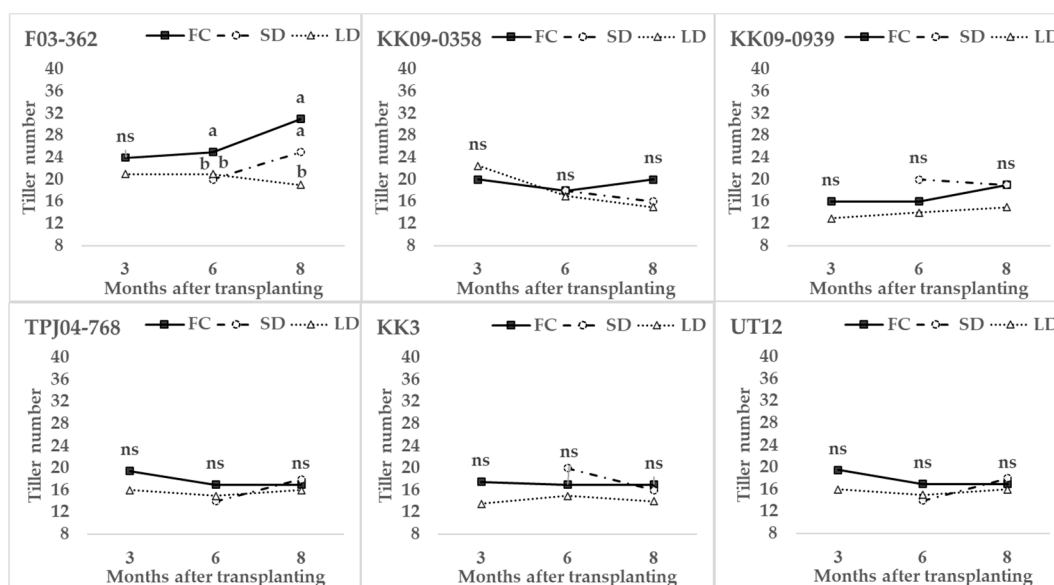


Figure 7. Tiller number of F03-362, KK09-0358, KK09-0939, TPJ04-768, KK3, and UT12 sugarcane varieties at 3, 6, and 8 months after transplanting (MAT) under different water regimes: field capacity (FC), short drought duration (SD), and long drought duration (LD). Different lowercase letters within the same growth stage indicate significant differences among water treatments according to the LSD test ($p < 0.05$); 'ns' indicates no significant difference.

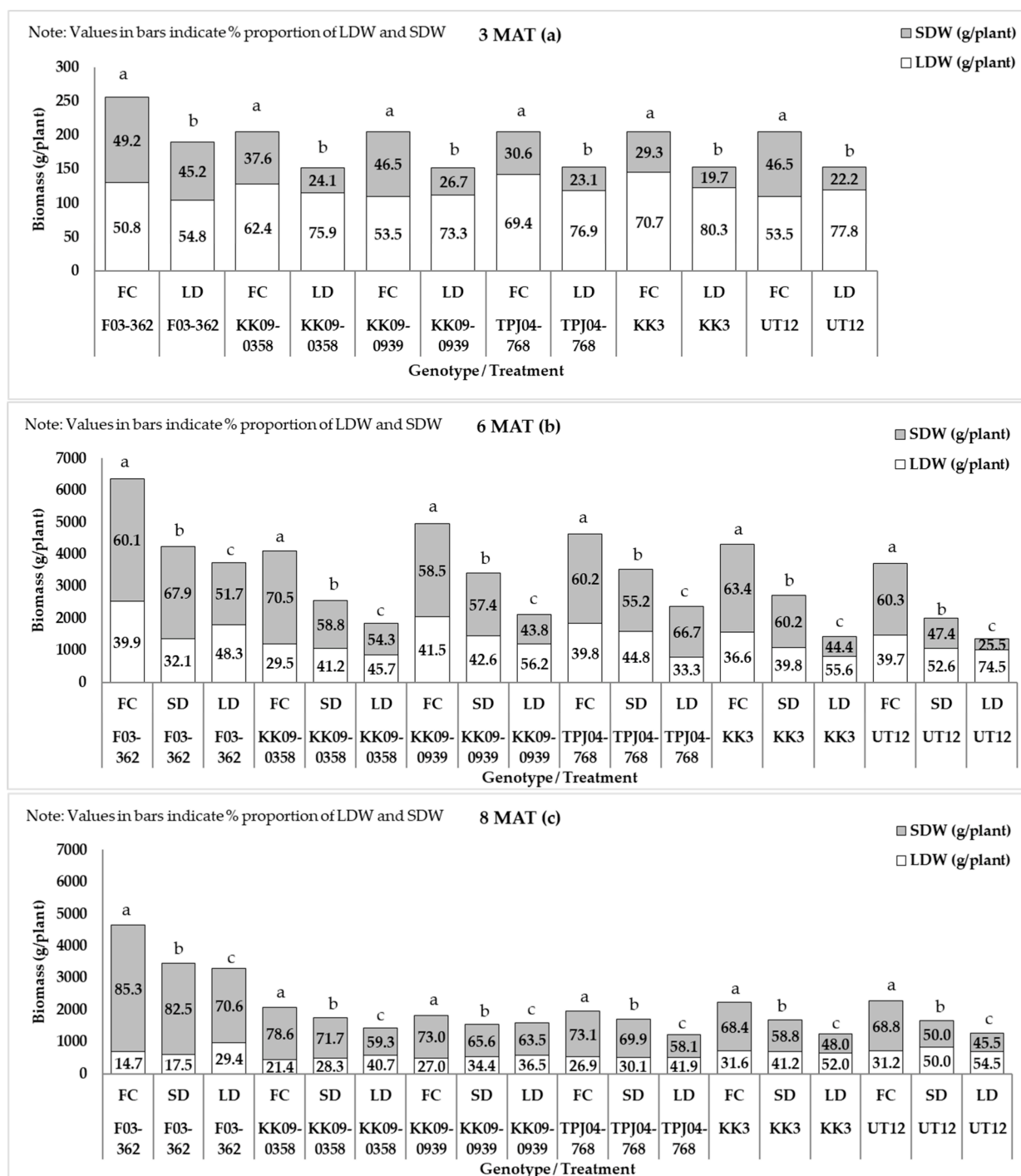


Figure 8. Biomass, leaf dry weight (LDW), and stalk dry weight (SDW) percentage proportions under field capacity (FC), short drought duration (SD), long drought duration (LD) at 3 months after transplanting (MAT) (a), 6 MAT (b), and 8 MAT (c) of six sugarcane varieties (F03-362, KK09-0358, KK09-0939, TPJ04-768, KK3, and UT12). Different lowercase letters within the same growth stage indicate significant differences among water treatments according to the LSD test ($p < 0.05$); 'ns' indicates no significant difference.

Overall, the six sugarcane genotypes showed distinct patterns of biomass accumulation and partitioning under drought stress. F03-362 consistently produced the highest biomass across all water regimes at 8 MAT while maintaining stable stalk-to-leaf partitioning, indicating both strong biomass production and partitioning stability under drought. KK09-0939 showed no further biomass reduction between SD and LD at 8 MAT, suggesting limited

additional loss under prolonged water deficit. KK3 maintained a stalk-to-leaf partitioning pattern comparable to FC under SD, but this balance shifted under LD, suggesting that this genotype tolerates short-term drought better than prolonged water deficit. UT12 showed the most pronounced shifts in stalk-to-leaf partitioning across water regimes and growth stages, consistent with its classification as a drought-susceptible genotype. These shifts in KK3 and UT12 may reflect earlier stage-specific changes in canopy traits that carried over into later biomass partitioning. Together, these results indicate that drought response in sugarcane is better characterized by combining biomass magnitude, partitioning stability, and canopy trait dynamics rather than partitioning ratio alone.

3. Discussion

This study shows that non-destructive canopy and biomass traits track distinct, genotype-specific paths of decline and recovery across an early-season drought cycle in sugarcane. These traits fall into three broad response categories. Certain morphological traits, such as canopy height and green leaf number, respond immediately to water deficit. Others, such as tiller number in most genotypes, barely respond at all. A third group, including canopy width and, in most genotypes, canopy height as well, only reveals compensatory growth once plants are rehydrated. Together, these patterns point to which non-destructive traits are most useful for early screening of drought response across diverse sugarcane breeding material.

Canopy height and green leaf number were the first traits to show a drought effect. By 3 MAT, canopy height differed between FC and LD in all six genotypes (SD had not yet diverged from FC), and green leaf number differed between FC and both drought treatments in five of six genotypes. Drought is known to reduce plant height in sugarcane by 15% to 21% [19] and stalk height by 18% [20], and lower green leaf number and photosynthetic pigment content [8,21], so this early response fits what has been reported before. By 6 MAT, though, both traits settled down in certain genotypes. Canopy height stopped differing by treatment in KK09-0358, TPJ04-768, and KK3. Green leaf number did the same in both commercial genotypes, KK3 and UT12. Then, by 8 MAT, treatment effects showed up again for both traits, but the patterns had shifted. Canopy height recovered more under LD than SD in most genotypes, with F03-362 the one exception, where the pattern ran the other way. The compensatory growth we observed, in which canopy height under LD recovered to match or exceed SD by 8 MAT in most genotypes, is consistent with reports that plants subjected to prior drought can show accelerated growth upon rewatering, partly offsetting earlier biomass loss, with the extent of compensation depending on the severity and duration of the preceding drought [22]. However, because this compensation was inconsistent across genotypes and, in F03-362, ran in the opposite direction (SD height exceeding LD at 8 MAT), it should be interpreted as a genotype-specific and only partial recovery mechanism rather than a general capacity of the species to fully offset early drought damage [22]. Green leaf number told a different story in the two commercial genotypes. In KK3, FC and SD ended up similar, and both stayed above LD. In UT12, FC stood above both SD and LD. This kind of recovery lines up with earlier work showing that height growth does not slow during rewatering, that KK3 tends to grow taller than usual once drought lifts [4], and that canopy height tracks biomass accumulation under long drought and recovery [17]. Green leaf number is mechanistically tied to LAI [23,24]. Both traits first diverged from FC at 3 MAT. However, green leaf number stayed a significant discriminator in all six genotypes through 8 MAT. LAI, in contrast, became non-significant in two genotypes (F03-362, KK09-0358) by 8 MAT. This suggests green leaf number may be the more durable indicator of canopy photosynthetic status across the full growth cycle. This durability, however, comes with trade-offs. Green leaf number is a manual visual count

and therefore inherently subjective, with counting criteria that can vary between observers and over time, whereas LAI is obtained from instrument-based optical measurement and is therefore more objective and standardized, though its readings can be affected by field light conditions, canopy row orientation, and sensor placement. In practical terms, LAI's earlier convergence across treatments may reflect a genuine biological ceiling in canopy light interception rather than a measurement shortcoming, while green leaf number's sustained discriminating power may partly reflect the trait's finer resolution at high canopy density, where LAI saturates but individual leaf counts can still differentiate genotypes. For breeding screening, this suggests a combined approach in which LAI serves as a rapid, objective, high-throughput indicator during early-to-mid growth stages, and green leaf number serves as a lower-throughput but more discriminating confirmatory measure through later growth stages, rather than treating either trait as a stand-alone replacement for the other. Taken together, the early onset, the mid-season lull, and the late re-emergence of treatment effects in both traits show why these traits need to be tracked across the whole season, not just checked once, to get an accurate picture of drought response. The mid-season attenuation of treatment effects at 6 MAT, when canopy height no longer differed significantly among treatments in KK09-0358, TPJ04-768, and KK3, and green leaf number likewise converged in both commercial genotypes, followed by the re-emergence of treatment differences at 8 MAT, parallels the concept of drought stress memory, in which plants that have previously experienced water deficit do not simply track current soil moisture but instead show altered, sometimes delayed, expression of the stress phenotype after the stress period has ended [25]. This pattern implies that a single mid-season measurement could substantially under- or overestimate a genotype's true drought sensitivity, reinforcing the value of repeated, whole-season phenotyping over single-timepoint screening.

Tiller number hardly moved with water treatment, showing no real differences at any stage in the BC1, BC2, or commercial genotypes. This runs somewhat counter to earlier reports of drought lowering tiller number in sugarcane more broadly [1]. Only F03-362, the F1 hybrid, responded at all, and even that response changed over time. SD and LD looked the same at 6 MAT. By 8 MAT, though, tiller number under SD had bounced back to match FC, while LD stayed well below both. This suggests tillering in this genotype is affected by how long the drought lasts, not simply by drought itself. Since most genotypes showed so little response, tiller number is probably not a useful early indicator of drought response in this breeding population, except perhaps in F1 hybrids.

Canopy width and LAI followed a trajectory similar to canopy height, though delayed. Canopy width showed no treatment effect at 3 MAT in any genotype, consistent with earlier findings that low soil water potential reduces shoot mass and canopy size [8]. LAI was highest under FC across most genotypes and growth stages, in agreement with earlier reports that drought reduces LAI in sugarcane [7,8,17], though this pattern converged by 8 MAT in F03-362 and KK09-0358. Both traits also showed unexpected departures from this general pattern. KK09-0939 and TPJ04-768 exhibited transiently greater canopy width under LD and SD, respectively, than under FC by 6 MAT. By 8 MAT, F03-362 and KK09-0358 no longer differed significantly in LAI among water treatments, suggesting that canopy photosynthetic capacity converges by late season regardless of prior drought exposure. This pattern is consistent with reports that LAI continues to increase toward crop maturity even in the presence of leaf senescence [26]. Canopy width has previously shown a weak or non-significant association with biomass under either drought duration [18], whereas higher LAI is more consistently linked to greater biomass accumulation and yield potential [6,27–29]. This suggests that LAI recovery by 8 MAT is a more reliable indicator of restored photosynthetic capacity than canopy width. Canopy width may nonetheless retain

value in commercial genotypes, where canopy size is associated with light interception during the early rainy season [29]. The LAI recovery observed by 8 MAT, particularly in F03-362, is consistent with reports that delayed leaf senescence under stress supports better post-stress performance by preserving photosynthetically active leaf area for longer, allowing more rapid canopy re-expansion once water becomes available [30]. This suggests that the LAI recovery seen here reflects renewed expansion and reduced turnover of existing leaves rather than the production of new tillers, since tiller number in F03-362 remained suppressed under LD at the same growth stage.

Total biomass dropped under both SD and LD relative to FC across every genotype and growth stage, and LD hit harder than SD. This tracks with earlier reports of drought cutting sugarcane yield by 50% to 60% and stalk biomass by as much as 60% under extreme water deficit [31–34], usually put down to reduced light interception, slower plant extension, and lower photosynthetic efficiency under water deficit [21,35,36]. Biomass partitioning between leaf and stem varied considerably across genotypes and growth stages, rather than following a single consistent pattern. By 8 MAT, F03-362, KK09-0358, KK09-0939, and TPJ04-768 all put more biomass into stem than leaf across all three water treatments, pointing to a shared partitioning strategy during maturation that does not depend on water status. KK3 kept that same stem-heavy pattern under FC and SD, but lost it under LD, suggesting prolonged drought throws off its usual leaf-to-stem balance even though its canopy traits hold steady. UT12 went the other way, keeping a balanced leaf-to-stem split under both SD and LD despite favoring stem under FC, which is worth noting given that UT12 is classified as drought-susceptible, and hints that partitioning strategy alone does not tell the whole story on drought tolerance. These genotype-specific patterns support the proposed adaptive mechanism whereby drought-tolerant genotypes reduce both stalk and leaf dry weight while reallocating a greater proportion of assimilates toward shoot biomass during recovery [4], and align with reports that photosynthesis, stomatal conductance, dry-weight partitioning, root dry weight, and green leaf number together shape how shoot biomass holds up under low water potential [8].

F03-362 and KK3 represent two distinct but complementary strategies for managing drought stress. F03-362 produced the greatest biomass among all genotypes at every water treatment by 8 MAT, and, when expressed as a relative reduction index against its own FC baseline (Table S1). The performance of F03-362 was distinguished by both a consistently high biomass potential and a comparatively moderate proportional decline across the study period. At 6 MAT, F03-362 showed the second-smallest average proportional biomass decline (62.8% of FC) among all six genotypes, closely behind TPJ04-768, and by 8 MAT its proportional decline (72.5% of FC) remained below the six-genotype average, even though it no longer ranked among the two smallest. Most notably, by 8 MAT, F03-362 total biomass under both SD (3464.1 g/plant) and LD (3284.3 g/plant) exceeded the FC biomass of every other genotype in this study (the highest was UT12, at 2271.2 g/plant, see Table S1), meaning F03-362 under the most severe drought treatment still outproduced all other genotypes under optimal water conditions. The value of F03-362 as a breeding parent therefore rests on this combination of consistently high absolute biomass potential and a moderate-to-favorable proportional response across stages, rather than on the smallest relative loss at every single timepoint. Its increase in LAI during recovery, rather than canopy size, likely sustained this continued assimilate accumulation. KK3 followed a different route. Canopy height and canopy width in KK3 declined under drought in the same pattern seen across nearly all genotypes and therefore do not distinguish it from the others, but green leaf number under SD remained statistically equivalent to FC through 8 MAT, a stability not observed in any other genotype. This SD-specific stability broke down under LD, where KK3's green leaf number, along with its LAI, declined significantly,

indicating that even in this genotype, tolerance is duration-dependent rather than absolute and is expressed through green leaf number rather than canopy size. F03-362 thus pairs high yield potential with a drought response strategy distinct from that of an established drought-tolerant check such as KK3, which is unsurprising given the complexity of selecting for drought response in a polyploid genome such as sugarcane [37–40].

These contrasting response profiles also point to different breeding environments where each genotype would be expected to add the most value. Because KK3's tolerance is expressed specifically under short-duration drought and does not extend to long-duration drought, it is best suited as a parental background for environments characterized by repeated, short-term drought events interspersed with rainfall or irrigation, rather than for regions experiencing a single continuous long-duration dry season. F03-362, by contrast, combines the highest absolute biomass with a comparatively small proportional biomass loss under both drought durations, indicating that it may be better suited to environments where drought duration is less predictable, including both short repeated events and more prolonged continuous water deficit. The genotype-specific biomass-partitioning patterns described above are consistent with our related work on the same genotype panel, which identified F03-362 as maintaining high biomass and stable resource-use efficiency during drought and recovery, supporting its designation as a drought-tolerant parental line [41]. These contrasting strategies indicate that biomass partitioning, canopy-trait stability, and green-leaf-based tolerance are not necessarily coupled, and that all three dimensions should be considered jointly, alongside the target drought regime, when selecting parental material for breeding. This suggests that the two genotypes embody complementary rather than overlapping strategies, both of which could inform the development of future sugarcane genotypes that combine high yield potential with improved drought tolerance.

Taken together, these patterns have direct implications for breeding practice. Because genotype rankings for canopy height and green leaf number shifted between 3, 6, and 8 MAT, a breeding program relying on a single-timepoint assessment would risk misclassifying at least one genotype's drought response. Non-destructive, repeated monitoring of canopy traits over the growth cycle is increasingly recognized as a practical way to raise the throughput and reliability of drought-tolerance screening in breeding programs handling large numbers of early-generation lines with limited plant numbers [42]. The genotype-specific recovery and partitioning trajectories identified here could therefore support more informative early-generation selection than a single measurement, particularly where population sizes are too small to permit destructive sampling. The practical relevance of these early-season canopy and biomass responses depends on their eventual link to yield, since abiotic stress experienced at any stage of the crop cycle, including the vegetative stage well before harvest, is known to carry forward and affect final yield in many field and horticultural crops through cumulative effects on canopy development, assimilate accumulation, and stress-recovery capacity [43]. This supports treating the early drought responses characterized here as a meaningful proxy for later yield performance, while still requiring direct confirmation through yield measurement at harvest.

4. Materials and Methods

4.1. Plant Materials

Six diverse varieties, as four interspecific hybridization sugarcane clones (*S. spontaneum* × commercial genotypes), namely F03-362 (F1), KK09-0358 (BC1), TPJ04-768 (BC1), and KK09-0939 (BC2), were used in this study, and two commercial sugarcane genotypes, namely KK3 and UT12, were used as moderately drought-tolerant and susceptible genotypes, respectively (Table 1).

Table 1. Six sugarcane clones/genotypes, four interspecific hybridized sugarcane clones (*Saccharum spontaneum* × commercial genotypes) and two commercial sugarcane genotypes.

Genotypes	Parents	Remark
F03–362 (F ₁)	88–2–401 × ThS98–178 + ThS98–264	High biomass and low sugar yield
KK09–0358(BC ₁)	95–2–317 × F03–381 (F ₁)	High cane yield and medium sugar yield
KK09–0939 (BC ₂)	BC04–251 (BC ₁) × UT4	High cane yield and sugar yield
TPJ04–768 (BC ₁)	94–2–128 × F03–331 (F ₁)	High cane yield and medium sugar yield
KK3 (commercial)	85–2–352 × K84–200	Check (moderately tolerant to drought)
UT12 (commercial)	SP80 × UT3	High cane yield and high sugar content
		Check (susceptible to drought)
		High cane yield

4.2. Field Experiments

A split-plot design with four replications was implemented within a randomized complete block design (RCBD). The main plots comprised three water management treatments: (1) maintaining soil moisture at field capacity (FC), (2) withholding irrigation for 4 to 6 MAT (short drought duration; SD), and (3) withholding irrigation for 2 to 6 MAT (long drought duration; LD). The subplots consisted of six diverse genotypes, measuring 12 m in width and 13 m in length, with plants spaced 0.5 m apart in rows and 1.5 m between plants, resulting in a total of 72 individual plots. This study was conducted at the Khon Kaen Field Crops Research Center in Tha Phra station, Khon Kaen province, Thailand (latitude 16°20′ N, longitude 102°49′ E, elevation 166.7 m above sea level) from November 2020 to December 2021.

Sugarcane sets were initially placed in plastic bags containing a soil and filter cake mixture at a 2:1 ratio. After 30 days, well-established seedlings were selected and transplanted into the field experiment. Fertilizers were applied at specific rates: 137.5 kg N ha^{−1}, 31.25 kg P ha^{−1}, and 100 kg K ha^{−1} at 1 MAT, with nitrogen and potassium applied at 31.25 kg ha^{−1} at 5 MAT. Weed control was achieved through the application of pre-emergence herbicides (chloroacetanilide) and manual weeding as necessary. Sugarcane borer management involved the application of carbosulfan at a rate of 2.5 L ha^{−1} as needed.

A mini-sprinkler system irrigated all subplots from planting until 1 MAT, maintaining soil moisture at FC. A drip irrigation system was employed to sustain soil moisture levels for the FC treatment from planting to harvest. The daily crop water requirement (*ET crop*) was calculated using the equations provided by Khonghintaisong [4].

$$ET\ crop = ET_o \times Kc \quad (1)$$

where *ET crop* is the crop water requirement (mm day^{−1}).

ET_o is the evapotranspiration of a reference plant under specified conditions, calculated by the pan evaporation method.

Kc is the crop water requirement coefficient for sugarcane, which varies with genotype and growth stage.

4.3. Soil Moisture Content and Meteorological Data

Soil moisture content (%) was measured using the gravimetric method at depths of 0–30 cm and 30–60 cm at 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 MAT. Measurements were taken from soil cores in all 72 subplots. Wet soil samples were weighed and then dried in an oven at

105 °C for 72 h. The weights of the wet and dry soils were used to calculate soil moisture percentage as follows:

$$\text{Soil moisture content (\%)} = \left[\frac{\text{Wet soil weight} - \text{Dry soil weight}}{\text{Dry soil weight}} \right] \times 100 \quad (2)$$

Daily measurements of maximum and minimum temperature (°C), rainfall (mm), solar radiation, and evapotranspiration (mm) were recorded at the meteorological station of the Khon Kaen Field Crops Research Center in Tha Phra station, Khon Kaen province, Thailand.

4.4. Canopy Traits

Data on canopy characteristics were collected at 3, 6, and 8 MAT. Canopy height was calculated as the distance from the second fully expanded leaf at the top of the main stem to the lowest green leaf. A leaf was classified as green when more than 50% of its lamina area remained green (non-dried, non-yellowed), following standard criteria for classifying photosynthetically functional leaf tissue [44]; this criterion was applied consistently for both canopy-height measurement and green leaf number counting. Canopy width was measured as the diameter of the canopy at its widest point. Four plants from each plot were used for measurements, and the mean values for each plot were determined.

4.5. Leaf Area Index (LAI)

The LAI was measured non-destructively using a plant canopy analyzer (LAI 2000; LI-COR, Inc., Lincoln, NE, USA) during late morning and early afternoon, following the user manual guidelines for row crops [45,46]. Measurements were taken at three locations within each plot at 3, 6, and 8 MAT.

4.6. Growth Traits

Green leaf number and tiller number were collected at 3, 6, and 8 MAT, using the green leaf criterion defined in Section 4.4. Leaf and stalk dry weights were determined at 3, 6, and 8 MAT by drying samples in an 80 °C incubator for 48 h or until constant weight.

4.7. Statistical Analysis

Data were analyzed by ANOVA based on a split-plot design in randomized complete blocks, according to the procedures described by Gomez and Gomez [47], using Statistix 10 (Analytical Software, Tallahassee, FL, USA). The least significant difference (LSD) test was employed for mean comparisons across all parameters. Different lowercase letters within the same growth stage indicate significant differences among water treatments according to the LSD test ($p < 0.05$); 'ns' indicates no significant difference.

5. Conclusions

This study demonstrates that non-destructive canopy and biomass traits reveal dynamic, genotype-specific patterns of decline and recovery across an early-season drought cycle in sugarcane. Canopy height, leaf area index, and green leaf number responded to water deficit as early as three months after transplanting, making them useful early screening indicators. The shifting recovery patterns across the season show that single-timepoint evaluation is insufficient to capture genotype-level drought response. Two genotypes emerged as complementary breeding resources. F03-362, an F1 hybrid, combined the consistently highest absolute biomass potential under every water regime with a drought-responsive canopy partially offset by increased LAI during recovery, and showed a proportional biomass loss close to the smallest among all genotypes specifically at the 6 MAT stage. KK3, a commercial genotype, maintained a stable green leaf number specifically under

short-duration drought, a trait not observed in the other genotypes, although this stability did not extend to long-duration drought. Incorporating both high-yield-associated, drought-responsive and physiologically stable, drought-tolerant backgrounds into future sugarcane crossing programs, guided by repeated non-destructive monitoring, may help combine yield potential with early-season drought resistance. This remains a hypothesis rather than a demonstrated outcome, however, since cane and sugar yield were not directly measured in this study and the observation period (3–8 MAT) ends well before the sugar-accumulation and maturation stages. This study is further limited in that it examined only the seedling-elongation period from 3 to 8 months after transplanting and did not assess later sugar-accumulation or perennial (ratoon) performance, and, as a field-based experiment combining rainfed and water-controlled conditions, it could not fully exclude the interference of natural rainfall on the imposed treatments. Confirming the breeding value of these genotypes will therefore require direct yield assessment carried through to harvest, together with further verification of these screening traits across multiple locations and under perennial cropping conditions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/stresses6030065/s1>. Table S1: Total biomass (leaf dry weight + stalk dry weight) and relative biomass index (FC = 100) for six sugarcane genotypes under field capacity (FC), short-duration drought (SD), and long-duration drought (LD), at 3, 6, and 8 months after transplanting (MAT).

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