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COMPARISONS OF ANATOMICAL TRAITS IN DIFFERENT LEAF POSITIONS OF DIVERSE SETS OF SUGARCANE GENOTYPES

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SUMMARY

Research on appropriate leaf positions in diverse sugarcane genotypes is crucial due to the significance of leaf anatomical characteristics in determining plant adaptability. This study aimed to compare the anatomical traits among the varied leaf positions within a tiller and tillers under normal conditions. A randomized complete block design (RCBD) setup used four replications. Four commercial canes, two wild types, and three F_1 interspecific hybrids underwent examination on leaf thickness (LT), cuticle thickness percentage (CT%), vertical length bulliform cell percentage (VBC%), and stomatal crypt depth percentage (SCD%) across 1st to 5th leaf positions on main, first, and second tillers. The 1st to 5th leaf positions had no differences when compared within the tiller in commercial and wild cultivars for LT, CT%, VBC%, and SCD% traits, and F_1 hybrids demonstrated no variation in CT% and VBC% traits. The LT, SCD, and CT of commercial canes had a high proportion, and VBC had a slender shape and a large size. Inversely, the wild type had a low LT but high SCD and CT and a circular shape with a small size VBC. Leaf anatomy in the F_1 hybrid resembled the wild type, and leaf positions 1st to 3rd were not different among tillers, but the 4th and 5th leaf positions differed. Therefore, anatomical trait collection should continue among 1st to 3rd leaf positions for all sugarcane types. Moreover, the 1st to 3rd leaf positions within the 1st and 2nd tillers can represent the anatomical performance of the main tiller in commercial cane cultivars.

Keywords: Leaf position, leaf divergence, bulliform cells, stomatal crypt depth, leaf sequences

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Key findings: The 1st to 3rd leaf positions for all sugarcane types can help to investigate drought resistance traits in the leaf anatomy. There were no differences among tiller sequences of 1st to 3rd leaf positions in commercial cane cultivars.

INTRODUCTION

In tropical and subtropical regions, sugarcane is vital in the economy as a food and bioenergy source (Moore *et al*., 2013). The easy processing locally can produce added-value products as a raw resource for the sugar, energy, and surfactant industries, contributing to sustainable economic growth and food security (Taratima *et al.*, 2019; Singh *et al.*, 2020). Most sugarcane production occurs under rainfed conditions, and its development and yield depend on the amount and distribution of precipitation (Taratima *et al.*, 2019; Khonghintaisong *et al.*, 2021). In this context, drought in sugarcane production is a serious problem that affects growth and yields up to 60% (Robertson *et al.*, 1999). A strategy to alleviate the drought problem is to develop drought-resistant cultivars derived from interspecific hybridization between commercial and wild types.

Saccharum spontaneum L. is a wild species of sugarcane classified into the Andropogoneae tribe of the Poaceae family (Guo, 1987). In sugarcane breeding programs, it often serves as a male parent for extending the genetic base of parents and improving cultivar tolerance to adverse environments (Liu *et al.*, 2015). Past efforts have evolved to breed and enhance sugarcane varieties for drought-tolerant potential and high yields. Hybrids between commercial and wild sugarcane in the same genus (*S. spontaneum*) have developed (interspecific hybrids) with the foremost goal of obtaining drought-tolerant sugarcane cultivars (Paterson *et al.*, 2012). The *S. spontaneum* has anatomical traits that help sugarcane species resist drought. For instance, small bulliform cells, high stomatal crypt depth, and high cuticle thickness are anatomical characteristics linked to drought resistance (Taratima *et al.*, 2019; Jumkudling *et al.*, 2022).

Anatomical characteristics earlier identified proved important for studying crop acclimation to environmental stress, morphology, physiology, plant development, and plant genetics (Rae *et al.*, 2013). For example, drought, flood, or fluctuations in the quality and intensity of the radiation falling on the leaves can all cause changes in vascular tissues, thickness in mesophyll, epidermis, and cuticle, and stomatal density (Gardoni *et al.*, 2007; Castro *et al.*, 2009; Pincelli and Silva, 2012; Ribeiro *et al.*, 2012; Figueiredo *et al.*, 2015). The anatomical structure of a plant can help crops that tolerate an unsuitable environment; it has representations from tolerant varieties (Artschwager and Brandes, 1958). The ability of sugarcane to resist drought has shown correlations with leaf thickness related to an increase in midrib cuticle thickness, bulliform cell expansion, stomatal density increase, stomatal size decline, and upper and lower cuticle thickening, but in drought-susceptible cultivars, the lower epidermal cuticle thickness decreases under severe drought conditions (Taratima *et al.*, 2020; Jumkudling *et al.*, 2022). Many previous reports have shown a relationship between anatomical structure and a plant's ability to tolerate drought (Nawazish *et al.*, 2006; Wang *et al.*, 2006; Zhang *et al.*, 2015; Taratima *et al.*, 2019, 2021; Jumkudling *et al.*, 2022). However, previous investigations collected samples at a varied leaf position, measuring the 1st to 3rd leaf positions (from the top visible dewlap), and some reports have sampled different tillers (main, 1st, and 2nd tillers), which is less due to destructive sampling.

However, there has been no evidence of an appropriate leaf position in anatomy for investigation under normal conditions. This study hypothesized that the 1st to 5th leaf positions and among tillers within a set of genotypes (commercial cane, wild type, and F_1

interspecific hybrid) do not differ under normal conditions. The use of anatomy variation among individual leaf positions related to previous research was inexistent in diverse leaf positions and tiller sequences. Therefore, the research purpose sought to investigate anatomical variations in separate leaf positions within a tiller and among tillers in a diverse set of sugarcane genotypes under normal conditions. This information would provide the most appropriate leaf position and tiller for designing further anatomical collections for research with production and breeding aspects.

MATERIALS AND METHODS

Experimental design and cultural practices:

The experiment progressed in field conditions at the Agronomy Research Station, Khon Kaen University, Thailand (16° 28′ N, 102° 48′ E, 200 masl) from July 7, 2021 to March 31, 2022. A randomized complete block design (RCBD) setup had four replications. Nine sugarcane genotype samples included UT5, UT16, KK07-599, and F152 from commercial cane (*Saccharum* spp. hybrid), ThS98-91 and ThS98-94 were wild types (*S. spontaneum*), and F4-19, F2-15, and F6-13 were F_1 hybrids from interspecific hybridization between commercial cane (*Saccharum* spp. hybrid) and wild type (*S. spontaneum*).

Sugarcane seedlings' propagation began by planting a sugarcane set in a plastic bag. At 45 days after planting (DAP), selected uniform seedlings continued transplanting in the field. The soil characteristics were of the Yasothon series (fine-loamy, siliceous, isohypothermic, and oxic paleustults). The planting plot preparation comprised digging planting holes with 1.5 m between rows and 0.5 m between plants. Before planting, fertilizer application transpired (47 kg N, 47 kg P_2O_5, and 47 kg K_2O ha^{-1}). A second fertilizer application (47 kg N, 47 kg P_2O_5, and 47 kg K_2O ha^{-1}) followed three months after planting (MAP). A drip irrigation system provided supplementary water for a uniform seedling stand at the formative growth stage until six MAP. The crop water requirement has daily calculations for keeping a normal condition, with a total crop water requirement from transplanting to six MAP was 461.68 mm. The total rainfall throughout the experiment was 591.54 mm; thus, the water regime depended on the daily crop water requirement and rainfall. At the earliest stage, weed removal engaged a small, multipurpose soil tillage machine. When the sugarcane was about four months old, human labor helped to weed until the harvesting period. Throughout the experiment, no significant outbreaks of either diseases or insects occurred, not requiring chemical pesticide use.

Leaf anatomy studies

The first, second, third, fourth, and fifth leaves of the main tiller, the 1st, and the 2nd tiller held separate collections at six MAP (during the grand growth phase of sugarcane) (Figure 1) in each replication. The leaf length measurements ensued. Each leaf sample was cut in the middle (10 cm) (Taratima *et al.*, 2021), with the specimens immediately soaked in 100 ml of 70% ethyl alcohol for 48 h to maintain and stabilize the cells for anatomical studies. Leaf areas, such as the collected middle, continued dissection into small pieces by free-hand cross-sectioning of the tissue samples, made as thin as possible. Subsequently, the tissue's placement on the slide received dye with 1% (w/v) Safranin O for about 1 min. Mounting the slide with distilled water went on for later anatomical study to create a slide culture.

The quantitative anatomical characteristics of the leaf included leaf thickness (LT), cuticle thickness percentage (CT%), bulliform cell vertical length percentage (VBC%), and stomatal crypt depth percentage (SCD%). The traits' proportion (percent) attained calculation with leaf thickness. The formula used was, according to Jumkudling *et al.* (2022), as follows:

$$\text{cuticle thickness (\%)} = \frac{\text{cuticle thickness } (\mu m)}{\text{leaf thickness } (\mu m)} \times 100$$

$$\text{bulliform cells vertical length (\%)} = \frac{\text{bulliform cells vertical length } (\mu m)}{\text{leaf thickness } (\mu m)} \times 100$$

$$\text{stomatal crypt depth (\%)} = \frac{\text{stomatal crypt depth } (\mu m)}{\text{leaf thickness } (\mu m)} \times 100$$

Anatomical features assessment and recording used a light compound microscope (Olympus BH-2) and a Zeiss 540214-0000004 with the MB2004 configuration AxioVision (MB2004 configuration-AV) programme having a magnification of 10×.

Statistical analysis

The measured data sustained analysis of variance (ANOVA) following the RCBD. Mean comparisons among leaf positions within individual tillers in each genotype materialized employing the least significant difference (LSD) test at the 95% confidence level ($P \leq 0.05$) using the Statistix 10 software program and standard error (SE). In addition, the comparison of individual leaf positions among tillers ran separately for each genotype by LSD.

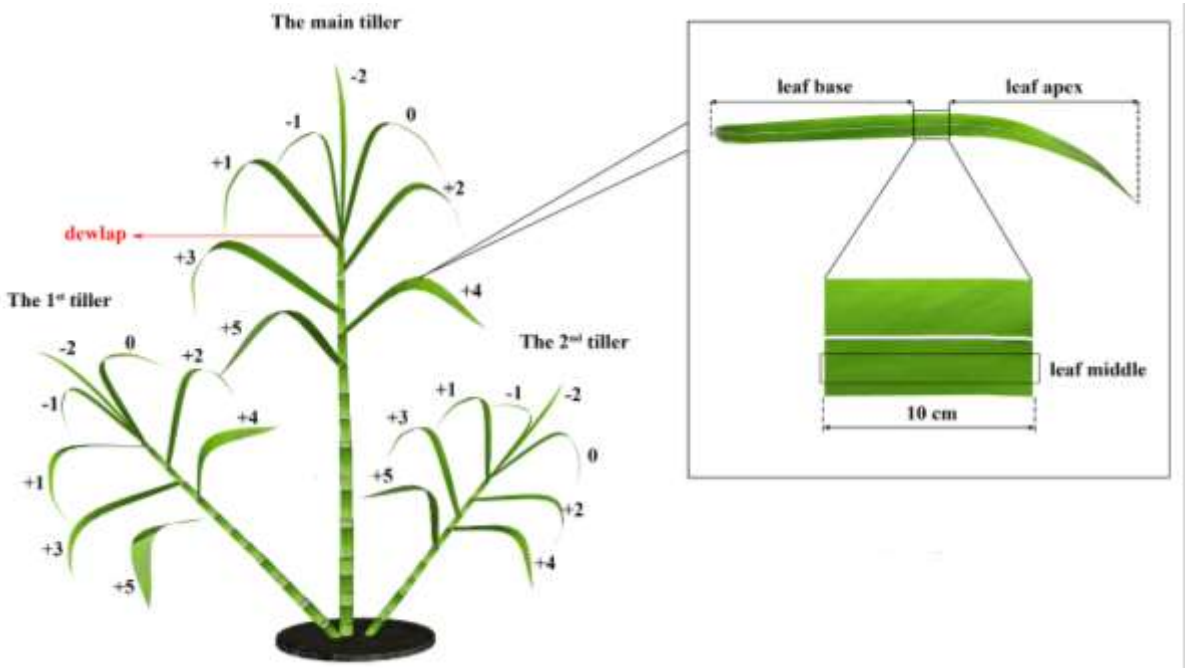

Figure 1. Leaf measurement position in each tiller of each sugarcane genotype. Sugarcane leaves are numbered from top to bottom, starting with the uppermost leaf showing a visible dewlap designated as leaf +1.

RESULTS

Comparison of leaf anatomy in commercial cane (*Saccharum* hybrid spp.)

The LT, CT%, VBC%, and SCD% were not significant among the leaf positions of each tiller for four commercial cane genotypes, namely, UT5, UT16, KK07-599, and F152 (Figure 2). The CT%, VBC%, and SCD% did not differ significantly among tillers for the leaf positions in the commercial cane cultivars (Table 1). However, when examined among tillers, the KK07-599 and F152 genotypes showed different LT at the 4th leaf positions and 2nd leaf position sequentially (Table 1). Therefore, all leaf positions and any tillers in commercial cultivars could generally serve as representative samples.

Table 1. Assessment of leaf anatomical features of nine sugarcane genotypes: UT5, UT16, KK07-599, and F152 from commercial cane (*Saccharum* spp. hybrid), ThS98-91 and ThS98-94 from wild type (*S. spontaneum*), and F4-19, F2-15, and F6-13 as F_1 hybrids from interspecific hybridization, by comparing the positions of leaf among tillers.

Characters	the 1st leaf					the 2nd leaf					the 3rd leaf					the 4th leaf					the 5th leaf				
	T1	T2	T3	CV%	F-test	T1	T2	T3	CV%	F-test	T1	T2	T3	CV%	F-test	T1	T2	T3	CV%	F-test	T1	T2	T3	CV%	F-test
UT5																									
Leaf thickness	174.99	181.34	180.05	4.14	ns	178.25	187.19	188.27	4.83	ns	183.35	180.90	186.62	7.27	ns	172.29	170.83	192.73	7.31	ns	174.85	175.68	187.04	5.29	ns
Cuticle thickness (%)	2.74	2.73	2.55	15.63	ns	2.50	2.49	2.44	17.21	ns	2.64	2.62	2.73	7.92	ns	2.76	2.67	2.58	14.92	ns	2.90	2.59	2.58	22.85	ns
Bulliform cells (%)	25.68	25.91	26.74	15.76	ns	26.59	25.48	27.69	12.16	ns	27.96	25.42	25.72	12.66	ns	28.32	27.67	26.84	8.09	ns	28.51	26.10	27.54	9.61	ns
Stomatal crypt depth (%)	5.23	4.70	4.43	13.20	ns	5.02	4.44	4.09	21.11	ns	4.59	4.75	4.39	16.08	ns	4.79	4.47	4.50	19.81	ns	4.64	4.40	4.36	7.36	ns
UT16																									
Leaf thickness (µm)	172.16	169.77	172.62	6.97	ns	177.21	173.63	170.87	8.07	ns	177.20	180.37	175.08	9.88	ns	176.17	185.99	182.57	5.56	ns	187.37	189.34	172.82	9.62	ns
Cuticle thickness (%)	3.04	2.78	2.96	19.93	ns	2.82	2.88	2.76	10.28	ns	2.73	2.69	3.05	18.27	ns	3.07	2.78	2.90	10.02	ns	2.78	3.0425	3.07	8.21	ns
Bulliform cells (%)	31.54	31.03	32.31	9.85	ns	30.01	29.71	30.71	13.02	ns	30.12	31.41	30.42	11.24	ns	31.18	29.73	30.08	10.32	ns	29.78	29.61	31.59	14.6	ns
Stomatal crypt depth (%)	4.58	4.85	5.13	19.87	ns	4.45	5.30	4.88	18.31	ns	4.82	5.15	4.61	16.89	ns	4.30	5.00	4.99	9.42	ns	5.04	4.83	5.23	19.58	ns
KK07-599																									
Leaf thickness (µm)	189.24	187.77	186.68	4.18	ns	179.73	188.57	179.43	3.81	ns	185.54	184.25	186.16	11.08	ns	171.07b	175.31b	183.55a	2.41	*	180.86	175.92	174.32	5.26	ns
Cuticle thickness (%)	2.53	2.50	2.71	13.90	ns	2.84	2.49	2.83	10.31	ns	2.62	2.66	2.55	18.17	ns	2.98	2.79	3.05	19.30	ns	2.91	3.09	3.19	9.05	ns
Bulliform cells (%)	24.40	24.26	22.00	18.26	ns	25.22	22.22	22.81	9.24	ns	22.16	23.88	22.08	12.44	ns	22.34	25.28	24.90	21.14	ns	21.82	22.68	20.20	14.77	ns
Stomatal crypt depth (%)	5.20	4.48	5.12	28.27	ns	4.99	4.97	4.11	14.82	ns	4.92	4.08	3.53	28.37	ns	4.49	4.07	4.01	12.79	ns	4.35 ab	4.84 a	4.16 b	8.46	ns
F152																									
Leaf thickness (µm)	158.83	163.34	162.63	9.33	ns	156.25b	161.59b	176.18a	4.46	*	166.86	168.89	161.36	6.51	ns	157.63	150.37	158.44	8.09	ns	167.02	163.4	173.42	7.17	ns
Cuticle thickness (%)	2.98	2.72	2.77	13.89	ns	2.81	2.77	2.85	8.37	ns	3.00	2.73	2.85	8.84	ns	2.72	2.98	2.71	12.89	ns	2.84	3.15	2.81	10.56	ns
Bulliform cells (%)	29.58	27.47	30.52	10.46	ns	28.76	27.677	27.338	15.76	ns	27.18	28.84	27.05	13.43	ns	26.33	29.93	26.77	12.38	ns	25.94	27.80	25.93	13.07	ns
Stomatal crypt depth (%)	5.18	5.46	4.50	25.88	ns	4.48	5.40	4.34	18.08	ns	5.36	4.54	4.59	16.75	ns	4.93	5.01	4.07	20.97	ns	4.87	5.28	4.23	13.59	ns
ThS98-91																									
Leaf thickness (µm)	134.59	125.4	136.72	6.16	ns	132.93	130.14	139.18	11.56	ns	140.94	139.23	130.77	8.63	ns	134.27	139.42	128.74	5.49	ns	146.3 a	146.95a	122.85b	8.18	*
Cuticle thickness (%)	2.75	3.19	2.89	12.65	ns	3.04	2.69	3.04	18.45	ns	2.68	2.80	3.12	13.96	ns	3.09	2.84	2.77	12.61	ns	2.20	2.92	2.47	16.90	ns
Bulliform cells (%)	27.02	24.84	22.10	18.41	ns	19.63	23.27	22.49	16.78	ns	21.69	23.23	22.60	14.59	ns	29.96	24.23	25.19	15.80	ns	23.95	21.61	24.24	12.00	ns
Stomatal crypt depth (%)	11.84	12.63	12.46	7.74	ns	11.07	12.57	11.07	11.03	ns	11.77	13.25	11.34	8.84	ns	12.49	10.63	12.60	10.16	ns	12.29	10.35	14.34	14.03	ns
ThS98-94																									
Leaf thickness (µm)	134.16	127.9	136.35	4.48	ns	129.6	132.64	139.18	6.84	ns	132.6	135.06	130.77	2.97	ns	127.88	135.33	131.24	6.54	ns	141.3	132.02	127.85	4.76	ns
Cuticle thickness (%)	2.91	3.05	2.88	9.86	ns	3.11	2.69	3.04	18.13	ns	2.82	2.89	3.12	13.67	ns	3.10	2.92	2.71	12.42	ns	2.73 b	3.22 a	2.75 b	4.48	*
Bulliform cells (%)	25.91	25.42	21.97	15.12	ns	23.49	22.83	23.31	18.77	ns	22.89	23.82	22.60	13.30	ns	26.66	24.96	24.60	15.13	ns	24.76	23.85	23.35	10.28	ns
Stomatal crypt depth (%)	11.84	12.39	12.46	10.27	ns	11.30	12.31	11.30	10.49	ns	12.40	12.83	12.07	8.78	ns	12.45	11.55	12.34	5.17	ns	11.94	12.39	12.05	7.99	ns
F4-19																									
Leaf thickness (µm)	128.09	135.66	134.67	11.5	ns	138.59	134.05	133.32	6.05	ns	137.33	135.29	126.19	6.33	ns	138.78	132.22	145.25	8.93	ns	132.04	138.13	147.34	11.86	ns
Cuticle thickness (%)	3.14	3.43	2.90	11.19	ns	3.50	3.01	3.35	18.65	ns	3.60	3.06	3.51	14.14	ns	3.10ab	3.59 a	2.85 b	11.74	ns	3.47 a	3.34 a	2.78 b	9.54	*
Bulliform cells (%)	32.47	25.83	28.63	17.39	ns	27.16	27.74	27.58	12.41	ns	28.42	27.07	29.67	18.03	ns	26.76	27.78	26.87	13.70	ns	27.09	27.69	26.83	14.24	ns
Stomatal crypt depth (%)	5.77	6.18	5.81	19.31	ns	5.10	6.12	5.93	14.15	ns	5.62	5.51	5.57	9.70	ns	5.94 a	5.62 ab	4.37 b	15.68	ns	5.77	5.81	4.98	10.79	ns
F2-15																									
Leaf thickness (µm)	147.08	151.1	137.81	13.01	ns	147.32	153.65	150.56	6.58	ns	156.15	146.96	136.46	6.53	ns	154.6	156.58	146.52	4.22	ns	158.23	142.46	152.91	10.65	ns
Cuticle thickness (%)	3.15	2.99	3.22	9.65	ns	3.24	3.19	2.83	13.29	ns	2.94	2.92	2.89	5.10	ns	2.94	2.86	3.20	11.46	ns	2.84	2.93	3.16	15.28	ns
Bulliform cells (%)	26.11	29.49	28.94	11.42	ns	29.92	27.16	26.11	12.33	ns	29.44a	23.12b	29.45a	10.23	*	23.76b	26.40ab	30.89a	10.71	*	25.59	27.01	26.94	19.14	ns
Stomatal crypt depth (%)	8.91	9.64	11.11	19.78	ns	10.10	10.99	9.74	9.77	ns	9.47	9.38	10.73	23.19	ns	8.81	10.91	9.58	13.87	ns	9.61	10.91	11.18	15.75	ns
F6-13																									
Leaf thickness (µm)	156.96	147.61	151.06	4.55	ns	149.34	141.9	145.04	4.23	ns	148.06	148.18	154.61	8.48	ns	156.97	151.15	148.88	6.90	ns	156.29	149.75	145.01	6.79	ns
Cuticle thickness (%)	3.18	3.09	3.34	9.78	ns	3.3	3.83	3.28	8.76	ns	3.36	2.97	2.83	9.30	ns	3.02	3.30	3.39	14.71	ns	3.80	3.25	3.08	20.69	ns
Bulliform cells (%)	26.66	24.59	27.32	12.72	ns	28.38	24.99	29.10	17.65	ns	28.26	26.80	27.30	17.99	ns	27.661	25.079	26.73	17.17	ns	25.18	23.46	26.60	19.00	ns
Stomatal crypt depth (%)	10.99	11.82	11.82	17.15	ns	9.47	12.04	12.21	12.08	ns	12.52	12.21	10.40	12.84	ns	12.63	12.40	10.59	20.73	ns	12.05	12.57	11.70	11.36	ns

ns Nonsignificant, * Significant difference at *P < 0.05* ** Significant difference at *P < 0.01*; T1-the main tiller; T2-the 1st tiller; T3-the 2nd tiller.
CV%; Coefficient of variation indicated the diversity of segregation in each trait and calculated by (SD/mean) × 100.

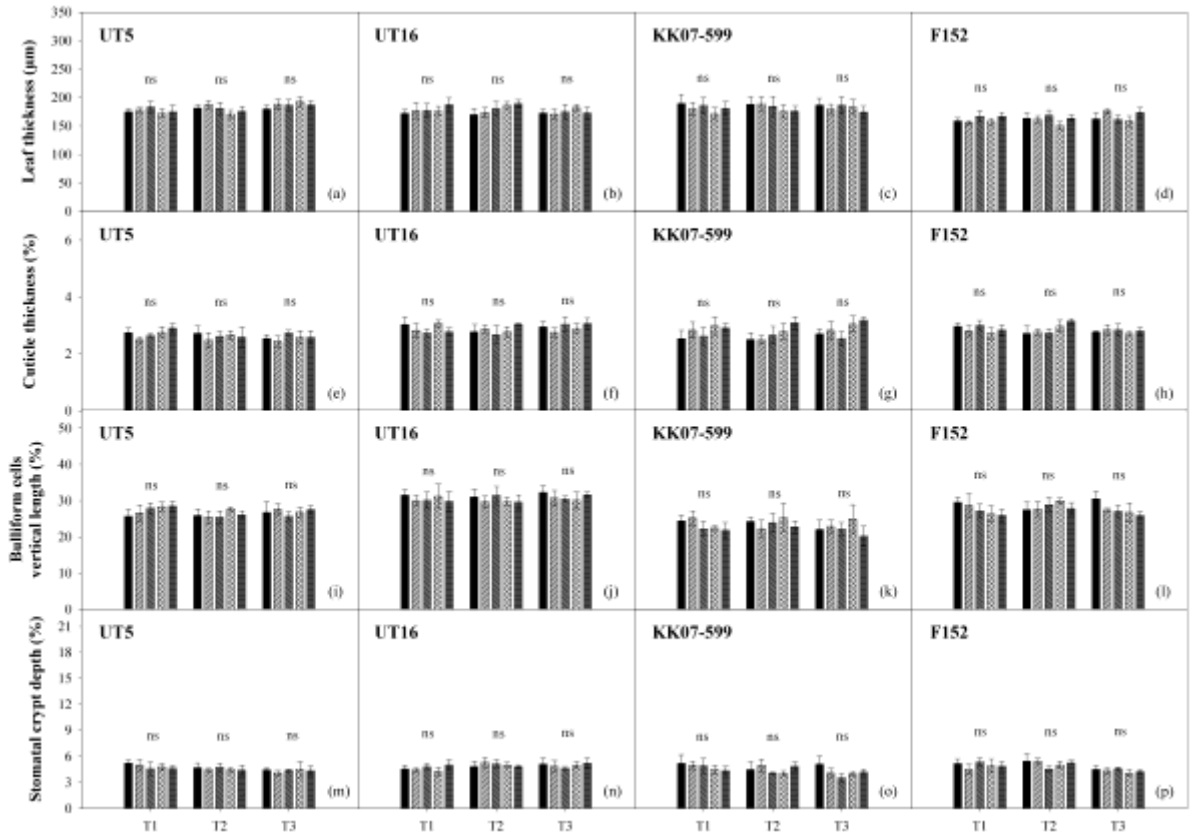

Figure 2(a-p). Leaf thickness (µm), the percentage of cuticle thickness, the percentage of bulliform cells vertical length, and stomatal crypt depth of each leaf positions (the 1st leaf [L1], the 2nd leaf [L2], the 3rd leaf [L3], the 4th leaf [L4], and the 5th leaf [L5]) in the main tiller (T1), the 1st tiller (T2), and the 2nd tiller (T3) of four commercial cane cultivars (*Saccharum* hybrid spp.), such as, UT5, UT16, KK07-599, and F152.

Comparison of the leaf anatomy in wild type (*S. spontaneum*)

This study found that LT, CT%, VBC%, and SCD% were not significant for any of the leaf positions within the tiller in the set of wild genotypes (Figure 3). When comparing tillers, both ThS98-91 and ThS98-94 cultivars had non-differentiated VBC% and SCD% (Table 1). However, significant differences were apparent for the ThS98-91 and ThS98-94 traits in LT and CT%, respectively, at the position of the 5th leaf (Table 1).

Comparison of the leaf anatomy in F_1 interspecific hybrid

The F_1 hybrid sugarcane cultivars, F4-19, F2-15, and F6-13 did not exhibit any differences in LT and CT%, including VBC% in the leaf position within the individual tiller (Figure 4). However, there were differences in the F4-19 cultivar LT and SCD% in the 2nd tiller (Figure 4[a], [j]). Likewise, no differences in LT and the SCD% appeared among tillers of the F4-19 (Table 1), whereas the F4-19 cultivar showed a variance in the CT% and the position of the 5th

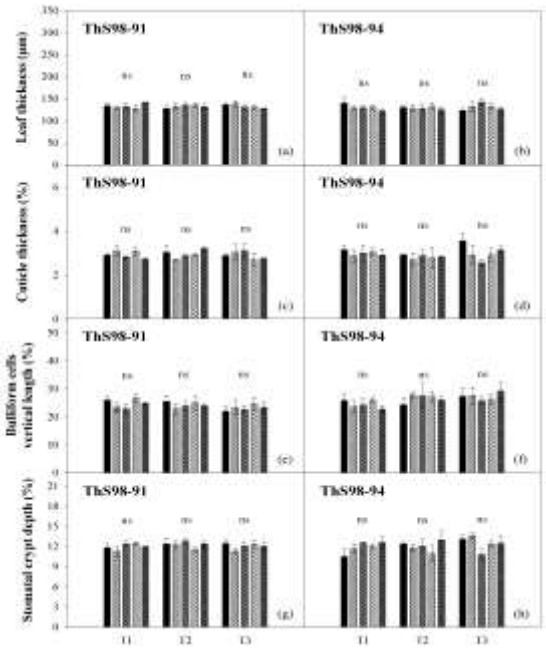

Figure 3(a-h). Leaf thickness (µm), the percentage of cuticle thickness, the percentage of bulliform cells vertical length, and stomatal crypt depth of each leaf positions (the 1st leaf [L1], the 2nd leaf ([L2], the 3rd leaf [L3], the 4th leaf [L4], and the 5th leaf [L5]) in the main tiller (T1), the 1st tiller (T2), and the 2nd tiller (T3) of two wild sugarcane cultivars (*S. spontaneum*) via ThS98-91 and ThS98-94.

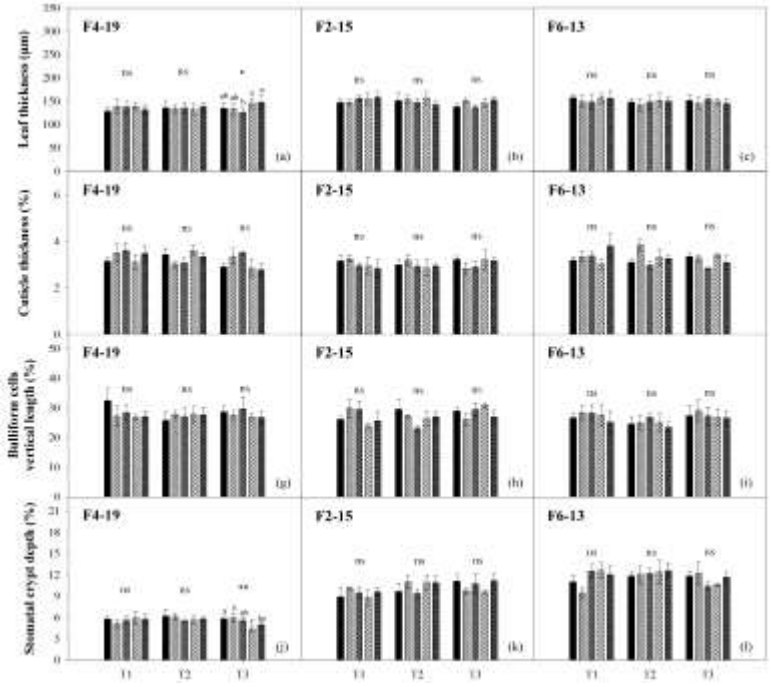

Figure 4(a-l). Leaf thickness (µm), the percentage of cuticle thickness, the percentage of bulliform cells vertical length, and stomatal crypt depth of each leaf positions (the 1st leaf [L1], the 2nd leaf [L2], the 3rd leaf [L3], the 4th leaf [L4], and the 5th leaf [L5]) in the main tiller (T1), the 1st tiller (T2), and the 2nd tiller (T3) of three interspecific hybridization via F4-19, F2-15, and F6-13.

leaf in the 2nd tiller when compared with the main and the 1st tillers. Furthermore, the VBC% trait was not significant for all F_1 hybrid cultivars when compared with leaf positions among tillers except F2-15, which demonstrated a noteworthy distinction in the location of the 3rd leaf (Table 1).

Overall, from this study, it seems likely that all the collected leaf anatomical characteristics involved with drought resistance verified beneficial in any leaf sequences, from 1st to 5th leaf samples for all studied genotypes. In addition, for commercial and wild sugarcane, any leaf sequences within the main, 1st, and 2nd tillers could be collected. However, leaf anatomical characteristics of the F_1 hybrids derived from interspecific hybridization showed differences in LT and SCD% in the 2nd tiller. Therefore, for all sugarcane species used in this study, the leaf anatomical characteristics related to drought resistance could be obtainable at any leaf position within the tiller, such as, the chief and 1st tillers. Additionally, if there was any destruction in the central stalk, the recommendation to use the 1st or 2nd leaf positions within the 1st or 2nd tillers as a representative sample of the main tiller for CT%, VBC%, and SCD% is possible.

Leaf anatomical characteristics of diverse sets of sugarcane genotype

The commercial cane cultivars had a high leaf thickness but a low percentage of stomatal crypt depth, cuticle thickness, and a slender shape and bigger size of bulliform cells at the center when compared with the subsidiary bulliform cells (Figure 5), while the wild type had a relatively small leaf thickness value, the bulliform cells exhibiting a round shape, and the sizes of the border cells were equal to those in the center (Figure 6). However, the wild type had a relatively high percentage of stomatal crypt depth and cuticle thickness (Figure 6). For the qualitative leaf sample characteristics, the stomatal crypt depths of wild and commercial cane were different, as the wild type had a high stomatal crypt depth, but an unclear crypt showed in commercial stakes (Figures 5 and 6). Moreover, these traits in F_1 interspecific hybrids existed between commercial cane and wild type (Figure 7). Still, the anatomical features associated with drought resistance in different leaf positions and tillers likely occurred within individual genotypes (Figures 5–7).

DISCUSSION

Understanding the diversity of leaf anatomy as a systematic characterization is essential to comprehending the dynamics influencing diverse conditions (Chatterjee *et al.,* 2016). Differing anatomical characteristics may evolve with differences in the genetic backgrounds of sugarcane grown under water-stress conditions (Taratima *et al.,* 2020*,* 2021). Leaf anatomy for this study, whether commercial cane, wild-type, and F_1 interspecific hybrids growing under normal conditions, varied markedly between diverse sets (Fig. 5–7), and leaf position within genotype was not different (Fig 2–4). Leaf morphology participates in the cell elongation process (Weigel, 2012).

In this study, the anatomy among various leaf positions within each genotype showed no general variation. The average size of the elongation cells was relatively steady throughout the cell growth and division phases (Ferjani *et al.,* 2007; Namwongsa *et al*., 2019). Cell elongation and expansion work together to determine the final leaf size, and there are interconnections between cell growth and cell division processes (Tsukaya, 2008; Songsri *et al*., 2019). Previous studies involved the structural and compositional differences of the cuticle between tender leaf and fully expanded leaf in *Camellia sinensis* at the 1st to 5th positions and revealed that the thickness of the epicuticular wax layer was similar in different leaf positions or on different sides of the same leaf (Zhu *et al.,* 2018; Mangrio *et al*., 2022). However, the 2nd leaf in *Camellia sinensis* is more similar to the 1st leaf than the 3rd or 5th in terms of specific anatomical characteristics (Zhu *et al.,* 2018).

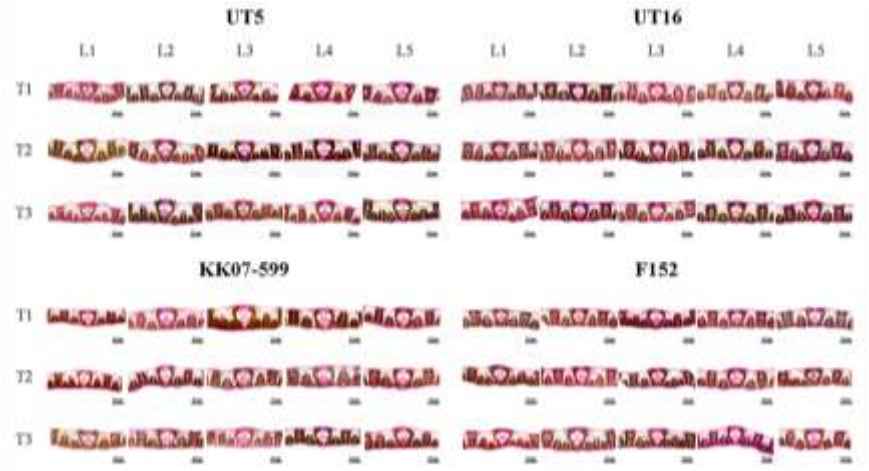

Figure 5. Leaf transverse section of sugarcane genotypes, commercial cane (*Saccharum* hybrid spp.) (UT5, UT16, KK07-599, and F152), comparing the appearance of stomatal crypt depth (SCD) and bulliform cells (BC) at six months after planting under field conditions.

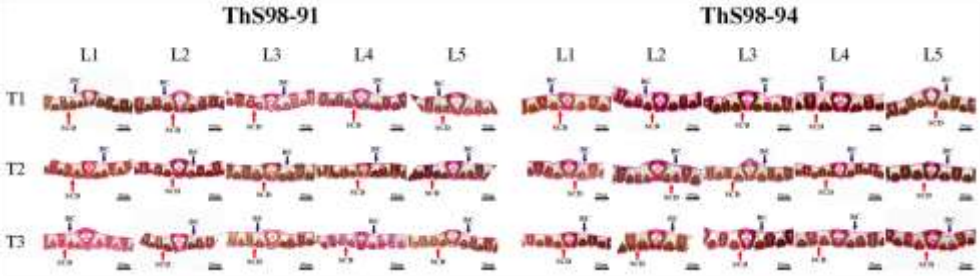

Figure 6. Leaf transverse section of sugarcane genotypes: ThS98-91 and ThS98-94 were wild type (*S. spontaneum*), comparing the appearance of stomatal crypt depth (SCD) and bulliform cells (BC) at six months after planting under field conditions.

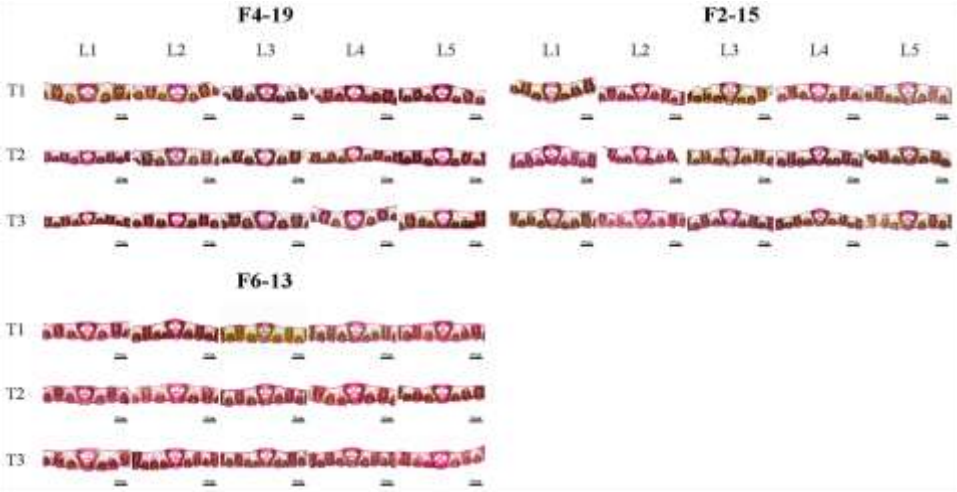

Figure 7. Leaf transverse section of sugarcane genotypes: F4-19, F2-15, and F6-13 were interspecific hybridization between commercial cane (*Saccharum* hybrid spp.) and wild type (*S. spontaneum*), comparing the appearance of stomatal crypt depth (SCD) and bulliform cells (BC) at six months after planting under field conditions.

In our study, there were different leaf anatomical traits among tiller sequences in *S. spontaneum* but not among commercial types. The species *S. spontaneum* has a very complex model with numerous secondary, tertiary, quaternary, and even higher-order shoots, whereas *S. officinarum* has the simplest tillering model, which can generally be a representation from the main shoot, three secondary shoots, and three tertiary shoots (Moore, 1987). Hence, wild species tend to have an indeterminate tillering form, while commercial species display a definite shape.

The diverse sets of genotypes in this study showed visible difference in anatomical features (Fig. 5–7). It was useful in breeding programs to select the parent cultivars for creating a new clone with drought tolerance characteristics. The anatomical characteristics of the leaf, including cuticle thickness, bulliform cell, and stomatal crypt depth, attained labels as drought resistance traits (Taratima *et al.,* 2019). Plants acquire protection from biotic and abiotic stresses by the extracellular hydrophobic layer known as the cuticle, which covers the outer epidermal surface of leaves (Chen *et al.,* 2021). In this relevant study, the cuticle thickness of the commercial cane was lower than that of the wild type, while the F_1 hybrids had a cuticle thickness between the commercial cane and the wild type. Cuticles are the primary targets of evolutionary adaptations to varying environmental conditions because of their key roles in controlling CO_2 influx and water efflux (Šantrůček, 2022).

Bulliform cells prevent water loss in monocots (Zhang *et al.,* 2015). The size of bulliform cells in commercial cane was greater than that of a wild type, while F_1 hybrids had an intermediate size between the commercial pole and wild type (Figs. 5–7). Leaf rolling gains stimulation from a change in water status in bulliform cells (Zheng *et al.,* 2003; Wang *et al.,* 2019). Under normal conditions, the leaf area of commercial cane varieties with bulliform cells expands, and the cuticle thickness is thin (Zheng *et al.*, 2015). The ability of plants to thrive in dry, high-irradiance settings has correlations with a quantitative trait, namely leaf thickness (Coneva and Chitwood, 2018). For this study, the commercial cane had thicker leaves than the wild type, while the F_1 hybrids had intermediate leaf thickness (Figs. 5–7).

Stomata are vital to controlling gas exchange and water movement (Cutler *et al.,* 2008). In addition, stomata have crypts and crypt trichomes for adaptation to aridity (Roth-Nebelsick *et al.,* 2009). In this study, wild species and F_1 interspecific hybrids had stomatal crypt depth, which did not show in commercial canes (Figs. 5–7). Likewise, in a previous report by Jumkudling *et al.* (2022), the stomatal crypt depths of wild and commercial cane differed, as the wild type had a high stomatal crypt depth, while an unclear crypt appeared in commercial ones. Crypts reduce transpiration by less than 15% compared with non-encrypted stomata in the leaves of *Banksia ilicifolia* (Roth-Nebelsick *et al.,* 2009). Therefore, the interspecific crossing is an alternative to improve drought-resistant sugarcane genotypes in breeding programs.

The leaf thicknesses, cuticle thickness, bulliform cell vertical length, and stomatal crypt depth in this study among wild, commercial, and F_1 hybrid sugarcane cultivars varied in each genotype**.** The change in leaf shape depends on the leaf position (Tsukaya, 2005). This research agreed with a previous report by Jumkudling *et al.* (2022), who reported that wild sugarcane species have practically round bulliform cells, and the sizes of border cells are equal to the center, while the commercial cane cultivars have a slender shape and a bigger size at the middle when compared with the subsidiary bulliform cells. The precise control of cell proliferation is a prime aspect of leaf morphogenesis, and the modification or manipulation of this process may lead to leaves of different sizes and shapes and changes in the organ margins and curvature (Rodriguez *et al.,* 2013). The heritable variation that underpins phenotypic differences between ecotypes is crucial for evolutionary divergence and diversification (Manier *et al.,* 2007; Richards *et al.,* 2019)**.** In the F_1 clones in this study, bulliform cell shape and stomatal crypt depth proved diverse; some clones were similar to commercial canes, while some were similar to wild types. F_1 interspecific

hybrids have revealed many variations in anatomy inherited from the parents, making the expression of F_1 anatomy specific to each genotype (Jumkudling *et al.,* 2022).

CONCLUSIONS

The measurement of all leaf anatomical characteristics could be a collection from any leaf sequence from the 1st to the 5th leaf sample for all studied genotypes. Moreover, measurements from commercial and wild sugarcane genotypes are collectible on any leaf sequence within the main, 1st, and 2nd tillers, whereas the F_1 hybrids derived from interspecific hybridization could gain collection only from the central and the 1st tiller. Therefore, for all sugarcane species used in this study, the leaf anatomical characteristics involved with drought resistance should be notable in any leaf positions from the 1st to the 5th leaf sample within a tiller, such as, the core and 1st tiller.

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