

First Investigation of Seasonal Changes in the Ecological and Anatomical Traits of *Launaea sarmentosa* (Willd.) Kuntze (Compositae) on the Andaman Coasts, Thailand

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Abstract

Launaea sarmentosa (Willd.) Kuntze, a creeping herb native to the Andaman coasts of Southern Thailand, faces significant threats from habitat loss and environmental changes following the 2004 tsunami. This study investigated its ecological and anatomical characteristics across 4 coastal sites in Phuket and Phang-Nga Provinces. The parameters were categorized into 4 groups: plant traits (including photosynthetic pigment contents and anatomical features), upper soil layer features (e.g., sand, clay, chloride, magnesium, and pH), lower soil layer features (e.g., sand, silt, nitrogen, phosphorus, calcium, chloride, and magnesium), and climatic variables (air temperature and relative humidity). Sampling was conducted during the rainy season (November 2023) and the dry season (April 2024). Results revealed significant seasonal and site-specific differences in plant traits. Shoot density and below-ground fresh weight were highest during the rainy season and at sites with moderate sunlight. The chlorophyll *a/b* ratio varied significantly between seasons and sites, while the total chlorophyll/carotenoid ratio differed between sites only. Leaf anatomical features also showed seasonal variation, with upper and lower epidermis thickness, mesophyll thickness, and upper epidermal cell area being greater in the dry season, while the lower epidermal cell area remained unchanged. In the upper-layer soil, chloride content was influenced by season, while sand, clay, magnesium, and pH varied significantly between sites. In the lower-layer soil, sand, silt, chloride, and magnesium varied seasonally, while nitrogen, phosphorus, and calcium showed site-specific differences. Climatic factors also showed both seasonal and spatial variability, with cooler temperatures and higher humidity during the rainy season, particularly at moderately sunny sites. In summary, *L. sarmentosa* appears to favor conditions with partial sunlight, moist soils, and clay-rich substrates. These conditions were associated with greater shoot density and biomass. The findings highlight the role of seasonal and site-specific factors and offer useful guidance for conservation and coastal ecosystem management.

Keywords: Climate parameters, Coastal vegetation, Plant anatomy, Seasonal variation, Soil properties

Introduction

Launaea sarmentosa (Willd.) Kuntze (Family Asteraceae) is a coastal, perennial, prostrate creeping herb. This plant is native to Andaman Islands, Bangladesh, Cape Provinces, China Southeast, Hainan, India, Jawa, KwaZulu-Natal, Laccadive Islands, Madagascar, Maldives, Mozambique, Myanmar, Nicobar Islands, Pakistan, South China Sea, Sri Lanka,

Vietnam, Western Australia, and Thailand [1]. In Thailand, it is distributed along the Andaman coast of Southern Provinces including Ranong, Phang-Nga, Krabi, and Phuket [2]. In Phuket and adjacent regions, the plant was formerly well known for its abundance along the coasts within sandy vegetation communities. However, following the devastating tsunami in

December 2004 in Thailand, the plant is now found only in small, scattered populations. Its survival in local areas now largely depends on cultivation efforts by local villagers, as it faces the threat of near extinction in the wild (personal surveys and communication with local residents).

Besides the populations cultivated by the local community, such as those at Mai Khao Beach in Phuket Province and Natai Beach in Phang-Nga Province, small naturally occurring populations of *L. sarmentosa* have been observed, although the plant has not spread its asexual stems into nearby areas. Moreover, our observations revealed a much higher abundance of above-ground shoots during the rainy season, whereas in the dry season, it survives only as scattered individuals within the area. Notably, the shoots reemerge during the following rainy season (preliminary observations). The majority of existing research has concentrated on aspects such as secondary metabolites and antioxidant properties [3], anti-inflammatory potential [4], and the pharmacognostical evaluation of roots [5]. However, critical ecological data on the plant, for example, shoot density, above- and below-ground biomass, and chlorophyll content across rainy and dry seasons in natural populations, remain largely unexplored. In addition, anatomical features in response to changing environmental conditions are not available in the literature.

This study aims to assess the ecological and anatomical characteristics of *Launaea sarmentosa* across coastal sites in southern Thailand, with particular emphasis on the species responses to seasonal variation in soil properties and climatic conditions. The findings contribute to a deeper understanding of the plant ecological adaptations and provide a scientific basis for conservation planning and the sustainable use of this native coastal species.

Materials and methods

Study sites and plant samples

A study was conducted to examine the natural growth of *Launaea sarmentosa* across 4 coastal sites in Southern Thailand including Bor Dan Beach (S1) and Natai Beach (S2) in Phang-Nga Province, as well as Sai Kaew Beach (S3) and Mai Khao Beach (S4) in Phuket Province (**Figures 1(A) - 1(E)**). For conservation purposes, precise GPS coordinates for these sites are not disclosed. In Phang-Nga, the plant grew under the shade of *Casuarina equisetifolia* L. (Casuarinaceae). At Bor Dan Beach, the sampling site was 55 m from the high tide line, with 14,000 lux of sunlight on a sunny day, while at Natai Beach, it was 40 m with 25,400 lux. In Phuket, Sai Kaew Beach was 10 m from the high tide line, with 95,400 lux, and Mai Khao Beach was 30 m with 65,800 lux.

Plant sampling was carried out using a 20×20 cm quadrat (**Figure 1F**). At each study site, all above- and below-ground plant material within the quadrat was carefully collected (**Figure 1G**). We examined 17 features of the plant including (1) shoot density, (2) above-ground fresh weigh, (3) below-ground fresh weigh, (4) above-ground biomass, (5) below-ground biomass, (6) the below-/above-ground biomass ratio, (7) chlorophyll *a* content, (8) chlorophyll *b* content, (9) total chlorophyll content, (10) carotenoid content, (11) ratio of chl *a* to chl *b*, (12) ratio of total chl to carotenoid, (13) leaf upper epidermis thickness, (14) leaf upper epidermal cell area, (15) leaf lower epidermis thickness, (16) leaf lower epidermal cell area, (17) mesophyll thickness. Replication numbers for plant traits varied across sites based on material availability. Features (1) - (12) were measured with 5 replicates, while features (13) - (17) were assessed using 18 replicates (positions). Sampling was conducted during 2 distinct seasons: the rainy season (November 2023) and the dry season (April 2024).

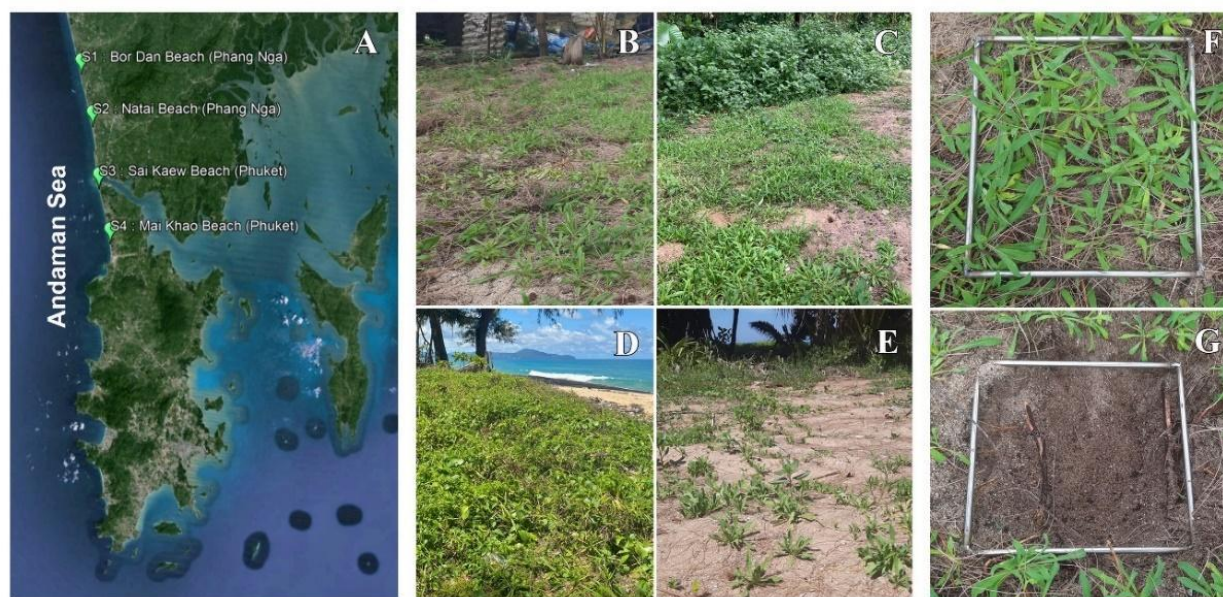


Figure 1 Study sites and plant sampling. (A) Map of the study sites in Phuket Province and parts of Phang-Nga Province where *Launaea sarmentosa* naturally grows; (B) Bor Dan Beach (S1); (C) Natai Beach (S2); (D) Sai Kaew Beach (S3) (a mix vegetation where *L. sarmentosa* scattered grows within); (E) Mai Khao Beach (S4); (F) a 20×20 cm quadrat used for plant samplings; (G) above- and below-ground plant samples were gently collected from the quadrat.

Measurement of environmental parameters

To characterize the aboveground environment of the study sites (climatic variables), we measured air temperature (°C) and relative humidity (%) using data loggers (RC-51H, Elitech). These devices were deployed in each plot and programmed to record data at 15-minute intervals over a five-day period during both the rainy and dry seasons. Sunlight intensity (lux) was recorded using a light meter logger (LX-75SD, Digicon).

Soil samples were collected from the sampling plots at each study site and divided into 2 layers: the upper layer (0 - 15 cm depth) and the lower layer (15 - 30 cm depth). Soil parameters were analyzed separately for each layer and included: (1) sand content (%), (2) silt content (%), (3) clay content (%) (Hydrometer method), (4) soil pH (1:1 soil-to-water suspension method), (5) organic matter (g/kg) (Walkley and Black method), (6) total nitrogen (g/kg) (Kjeldahl method), (7) available phosphorus (mg/kg) (Bray II method), (8) available potassium (mg/kg) (NH₄OAc extraction, pH 7.0), (9) available calcium (mg/kg) (NH₄OAc extraction, pH 7.0), (10) soluble chloride (mg/kg) (1:2 soil-to-water suspension method), and (11) available magnesium (mg/kg) (NH₄OAc extraction, pH 7.0). In total, 22 soil

parameters were evaluated across the 2 layers. Analyses were performed using 3 replicates for each study site. All soil analyses were conducted at the Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand.

Anatomical study of leaf, stem, and root

Five mature rosette shoots were collected from each study site during both the rainy and dry seasons for anatomical analysis. Leaves were separately fixed in FAAII (formaldehyde: glacial acetic acid: 70 % ethyl alcohol; 5:5:90 v/v/v) for 48 h. The fixed samples were rinsed with glacial acetic acid, dehydrated in a graded ethanol-tertiary-butyl alcohol series (50, 70, 85, 95, and 100 %), and embedded in Histoplast PE (Thermo Scientific). The embedded samples were sliced into 10 µm sections with a rotary microtome, dewaxed, and stained with Safranin and Fast Green. Stained permanent slides were prepared using Permount™ Mounting Medium.

Chlorophylls and carotenoid content

The eighth leaves of 5 mature rosette shoots were collected from each study site during both the rainy and dry seasons for the analysis of chlorophyll and

carotenoid content. The leaves were weighed to determine fresh weight (FW), then crushed in 5 mL of cooled 80 % acetone for 1 min 30 s. The mixture was centrifuged at 3,000 rpm for 10 min, and the resulting supernatant was collected into a 15 mL polypropylene (PP) tube. The remaining plant debris was resuspended in 5 mL of cooled 80 % acetone, centrifuged for 10 min, and the supernatant was combined with the previous extract. This process was repeated once more to obtain a total volume of 15 mL of supernatant. The optical density of the supernatant was measured at 480, 510, 645 and 663 nm using an HP 8453E UV-visible spectrophotometer (Agilent, USA). The chlorophyll and carotenoid contents ($\mu\text{g/g}$ FW) were calculated using the formulas provided by Mistra et al. [6] as follows:

$$\text{Chlorophyll } a = [12.7(A_{663}) + 2.63(A_{645})] \times V/1000 \times \text{FW}$$

$$\text{Chlorophyll } b = [22.9(A_{645}) - 4.68(A_{663})] \times V/1000 \times \text{FW}$$

$$\text{Total Chlorophyll} = [20.2(A_{645}) + 8.02(A_{663})] \times V/1000 \times \text{FW}$$

$$\text{Carotenoid} = [7.6(A_{480}) - 2.63(A_{510})] \times V/1000 \times \text{FW}$$

Statistical analysis

All analyses were performed within one of the 5 groups of metrics that were obtained from the samples, namely, (1) plant traits (shoot density, aboveground fresh weigh, below-ground fresh weight, above-ground biomass, below-ground biomass, chlorophyll *a* content, chlorophyll *b* content, total chlorophyll content, carotenoid content, chl *a/b* ratio, total chl/carotenoid ratio), (2) leaf anatomy (leaf upper epidermis thickness, leaf upper epidermal cell area, leaf lower epidermis thickness, leaf lower epidermal cell area, mesophyll thickness), (3) the upper-layer soil parameters, (4) the lower-layer soil parameters, and (5) climatic variables (temperature and humidity).

Pairwise correlations using Pearson's *r* were performed between variables within each group, so that pairs of highly correlated variables could be identified. The correlation tests were conducted using R software v4.3.0 [7] with the psych package v2.3.3 [8]. For each pair of variables within a group with a correlation coefficient (*r*) > 0.8, only one variable was retained to reduce redundancy in the following analyses. The

variables to be retained were selected based on their biological relevance informed by prior knowledge.

Statistical models were then applied to determine whether the retained plant or leaf properties were different between sites and seasons, using Analysis of Variance (ANOVA). Similarly, ANOVA was used to assess whether each of the retained environmental metrics varied significantly between sites and seasons. Each ANOVA for each metric was first performed with interaction between predictors; in cases with no significant interaction, the interaction term was removed from the final model. For models with significant effects of differences between the 4 sites, a post-hoc Honestly Significant Difference Tukey's test was performed. Model fit was tested with the R package performance v0.10.4 [9], to check assumptions of normality and uniformity of residuals, homoscedasticity, effect of influential observations, and multicollinearity. Due to the fact that the same structure of ANOVA was repeated for several metrics, the likelihood of overinflating *p*-values increases; thus, we used a lower threshold of significance for alpha level than the commonly used *p* = 0.05, namely *p* = 0.01.

Given that only 4 sites and 2 seasons were sampled, a direct test of the effects of environmental variables on plant and leaf features would not be feasible. The inference is then based on significantly different environmental variables between the same sites and seasons for which effects were found on plant and leaf features.

The R script used for the analyses and the data files are uploaded as supplementary files (**Supplement 1 - 5**).

Results and discussion

Plant traits of *Launaea sarmentosa*

Among the 12 metrics describing plant traits, 6 groups resulted not correlated with the others, namely shoot density (less than 0.75 with other variables), below-ground fresh weigh (0.86, 0.88, and 0.97 with 3 fresh weigh and biomass variables), carotenoid (higher than 0.97 with other chlorophyll variables), biomass ratio (less than 0.46 with other variables), the chlorophyll *a/b* ratio (less than 0.72 with other variables), and the total chlorophyll/carotenoid ratio (less than 0.27 with the other variables) (**Supplement 6**).

The density of *L. sarmentosa* (shoots/m²) was therefore not correlated to any other plant feature. It exhibited a great variation between the rainy and dry seasons, with significant differences between seasons, between sites, and in their interactions (**Table 1**). Overall, shoot density was higher in the rainy than in the dry season and higher in Natai Beach (S2) compared to all other sites (Tukey HSD: all $p < 0.006$). The magnitude of the differences between seasons was different between sites ($p = 0.0032$ for the interaction term in **Table 1**) (**Figure 2**).

The patterns of above-ground and below-ground fresh weight (g/m²) and biomass (g/m²) were positively and highly correlated: below-ground fresh weight had Pearson's r values ranging from 0.86 to 0.97 with the other 3 variables. Therefore, only one variable from each group, below-ground fresh weight, was selected for the analyses. A significant effect of seasons, sites, and their interaction was found (**Table 1**). Significant differences between sites were observed between Bor Dan Beach (S1) and Sai Kaew Beach (S3) (Tukey HSD: $p = 0.0003$), as well as between Natai Beach (S2) and Sai Kaew Beach (S3) ($p = 0.0003$) (**Figure 2**).

The below-/above-ground biomass ratio was not correlated with any other plant feature. However, it

exhibited significant differences between seasons and sites (**Table 1**). Overall, the ratio was higher in the dry season than in the rainy season and higher at Sai Kaew Beach (S3) compared to other sites (Tukey HSD: p -values ranging from 0.0106 to 0.0015). The magnitude of the seasonal differences varied between sites ($p = 0.0032$ for the interaction term in **Table 1**) (**Figure 2**).

Chlorophyll (a , b and total) and carotenoid contents had Pearson's r higher than 0.97. Only one variable, carotenoid, was therefore selected for the analyses. No significant effect of season and site was found for it (**Table 1**). The chlorophyll a/b ratio varied significantly between seasons and sites: the ratio was higher in the rainy season than in the dry season and higher at Mai Khao Beach (S4) than to Natai Beach (S2) and Sai Kaew Beach (S3) (Tukey HSD: $p = 0.0012$ and $p = 0.0019$, respectively) (**Table 1**) (**Figure 2**). The total chlorophyll/carotenoid ratio varied significantly between sites, not between seasons, but the magnitude of the differences between sites depended on the season. In Sai Kaew Beach (S3) the ratio was significantly lower than in Bor Dan Beach (S1) (Tukey HSD: $p = 0.0002$) and in Natai Beach (S2) ($p = 0.0037$) (**Table 1**) (**Figure 2**).

Table 1 Results of the Analysis of Variance (ANOVA) assessing differences in plant traits (response variables) between seasons and among sites (predictor variables). df = degrees of freedom, F = Fisher's F-statistic, p = p -value.

Response	Predictor	df	F	p
Shoot density	Season	1	21.8	< 0.0001
	Site	3	10.4	< 0.0001
	Season : Site	3	5.6	0.0032
	Residuals	32		
Belowground fresh weigh	Season	1	95.2	< 0.0001
	Site	3	9.8	0.0001
	Season : Site	3	7.7	0.0006
	Residuals	32		
Biomass ratio	Season	1	12.6	0.0012
	Site	3	6.5	0.0014
	Residuals	33		
Carotenoid	Season	1	0.9	0.3601
	Site	3	4.2	0.0130
	Residuals	30		

Response	Predictor	df	F	p
Chlorophyll a/b	Season	1	168.6	< 0.0001
	Site	3	7.5	0.0006
	Residuals	30		
Total chlorophyll/carotenoid	Season	1	4.1	0.0534
	Site	3	8.7	0.0003
	Season : Site	3	8.6	0.0003
	Residuals	27		

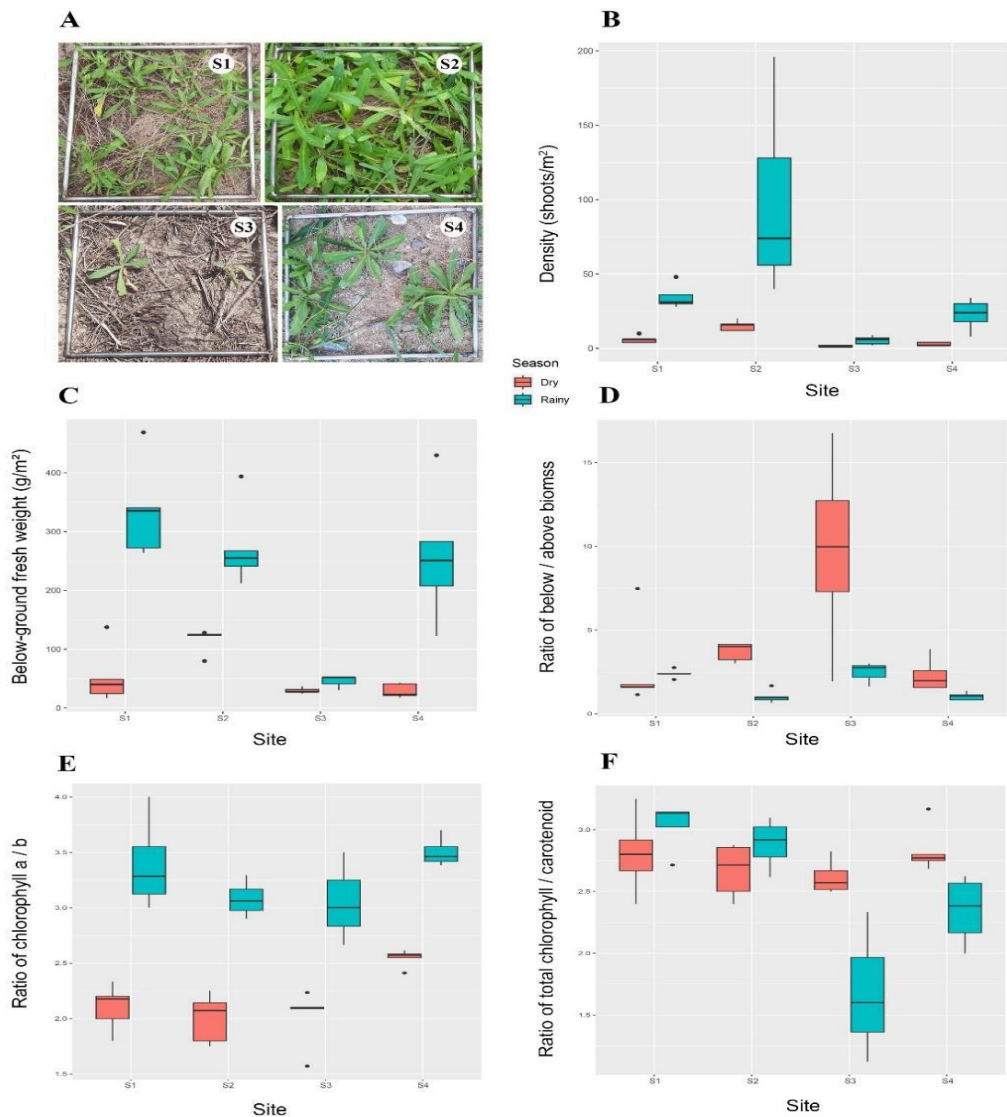


Figure 2 Plant traits of *Launaea sarmentosa* across 4 study sites during the rainy and dry seasons. (A) examples of shoot density in 20×20 cm quadrats; (B) shoot density; (C) below-ground fresh weigh; (D) the below/above biomass ratio; (E) the chlorophyll *a/b* ratio; and (F) the total chlorophyll/carotenoid ratio.

Leaf anatomy

For the set of variables describing leaf anatomic features (Figure 3), all the metrics resulted not

correlated with the others (Supplement 7). Significant effects were observed for both upper and lower epidermis thickness in relation to season, site, and their

interaction (**Table 2**). For the upper epidermis, the dry period exhibited greater thickness than the rainy period. Site-specific differences were attributed to the pairs Bor Dan Beach (S1) - Sai Kaew Beach (S3) (Tukey HSD: $p = 0.008$) and Sai Kaew Beach (S3) - Mai Khao Beach (S4) ($p = 0.0004$). Similarly, the lower epidermis thickness was greater during the dry period compared to the rainy period. Bor Dan Beach (S1) was significantly different from all other sites (Tukey HSD: all $p < 0.001$), indicating the combined effects of seasonal and spatial factors on epidermal thickness.

The upper epidermal cell area differed significantly between seasons but not between sites, with the dry period exhibiting a larger area than the rainy period. In contrast, the lower epidermal cell area showed no significant differences between seasons or sites (**Table 2**).

Mesophyll thickness was significantly affected by differences between seasons, not by sites, but by their interaction (**Table 2**), with the dry period showing higher thickness compared to the rainy period.

Table 2 Results of the Analysis of Variance (ANOVA) assessing differences in leaf anatomical features (response variables) between seasons and among sites (predictor variables). df = degrees of freedom, F = Fisher's F-statistic, p = p -value.

Response	Predictor	df	F	p
Upper epidermis thickness	Season	1	121.4	< 0.0001
	Site	3	7.1	0.0002
	Season : Site	3	24.8	< 0.0001
	Residuals	136		
Mesophyll thickness	Season	1	125.7	< 0.0001
	Site	3	3.4	0.0193
	Season : Site	3	12.9	< 0.0001
	Residuals	136		
Lower epidermis thickness	Season	1	62.7	< 0.0001
	Site	3	9.5	< 0.0001
	Season : Site	3	28.9	< 0.0001
	Residuals	136		
Upper epidermal cell area	Season	1	10.8	0.0013
	Site	3	0.7	0.5784
	Residuals	139		
Lower epidermal cell area	Season	1	0.1	0.8681
	Site	3	1.2	0.3122
	Residuals	139		

Soil parameters

For the set of variables describing the upper-layer soil parameters, 9 metrics resulted not correlated with the others, namely proportion of sand ($r = -0.86$ with proportion of silt), proportion of clay, pH, OM ($r = 0.84$ with N), P, K, Ca, Cl, and Mg (**Supplement 8**). In none of the models a significant interaction between seasons and sites was present (**Table 3**). Only for one metric, Cl, season was a significant predictor, whereas for all other

metrics, site was a significant predictor (**Table 3**). Regarding these variables, mostly Natai Beach (S2) was different from the other sites (sand, clay, pH, Mg), Sai Kaew Beach (S3) is different from Natai Beach (S2) and Mai Khao Beach (S4) for OM, Sai Kaew Beach (S3) was different from Mai Khao Beach (S4) for P, Natai Beach (S2) was different from Sai Kaew Beach (S3) for K, and Mai Khao Beach (S4) was different from the others for Ca (Tukey HSD: all $p < 0.01$).

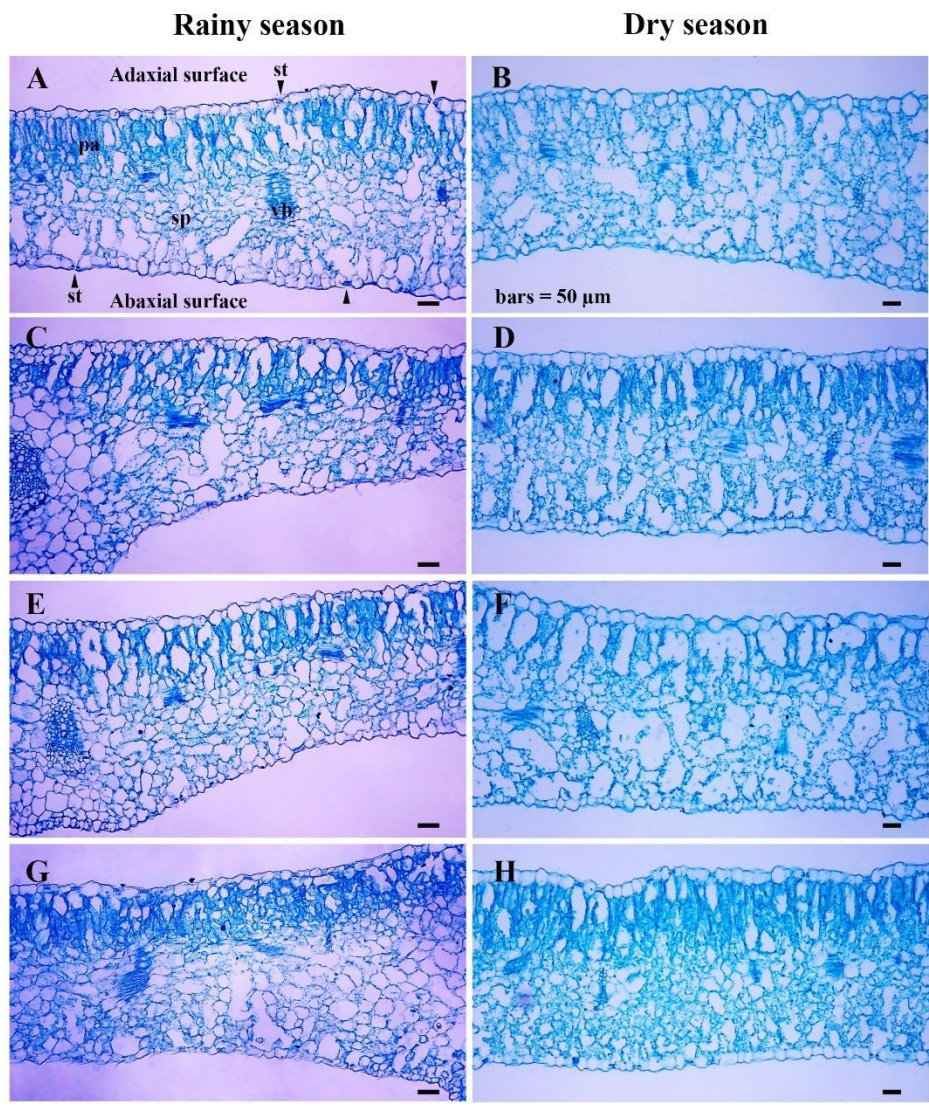


Figure 3 Leaf anatomical features *Launaea sarmentosa* across 4 study sites during the rainy and dry seasons. (A) - (B) Bor Dan Beach (S1); (C) - (D) Natai Beach (S2); (E) - (F): Sai Kaew Beach (S3); (G) - (H): Mai Khao Beach (S4). st: stoma, pa: palisade mesophyll, sp: spongy mesophyll, vb: vascular bundle.

Table 3 Results of the Analysis of Variance (ANOVA) assessing differences in the upper-layer soil parameters (response variables) between seasons and among sites (predictor variables). *df*= degrees of freedom, *F* = Fisher’s F-statistic, *p* = *p*-value.

Response	Predictor	<i>df</i>	<i>F</i>	<i>p</i>
Sand %	Season	1	1.0	0.3360
	Site	3	8.2	0.0011
	Residuals	19		
Clay %	Season	1	0.2	0.6889
	Site	3	5.5	0.0071
	Residuals	19		
pH	Season	1	5.7	0.0269
	Site	3	33.5	< 0.0001

Response	Predictor	df	F	p
OM	Residuals	19		
	Season	1	1.7	0.2074
	Site	3	11.1	0.0002
	Residuals	19		
P	Season	1	1.5	0.2358
	Site	3	6.0	0.0048
	Residuals	19		
	Residuals	19		
K	Season	1	0.5	0.4692
	Site	3	5.9	0.0051
	Residuals	19		
	Residuals	19		
Ca	Season	1	0.8	0.3742
	Site	3	35.5	< 0.0001
	Residuals	19		
	Residuals	19		
Cl	Season	1	13.1	0.0018
	Site	3	2.9	0.0596
	Residuals	19		
	Residuals	19		
Mg	Season	1	8.1	0.0103
	Site	3	8.9	0.0007
	Residuals	19		
	Residuals	19		

For the set of variables describing the lower-layer soil parameters, all the metrics resulted not correlated with the others (**Supplement 9**). In none of the models a significant interaction between seasons and sites was present (**Table 4**). For 4 metrics, namely proportion of clay, pH, OM, and K, no significant differences were found between seasons and sites; for other 4 variables, proportion of sand, proportion of silt, Cl, and Mg,

season was a significant predictor; for the other 3 metrics, N, P, and Ca, site was a significant predictor (**Table 4**). Regarding these 3 variables, Bor Dan Beach (S1) and Mai Khao Beach (S4) were different for N, the pairs Bor Dan Beach (S1) - Sai Kaew Beach (S3) and Natai Beach (S2) - Sai Kaew Beach (S3) were different for P, Mai Khao Beach (S4) was different from the other sites for Ca (Tukey HSD: all $p < 0.01$).

Table 4 Results of the Analysis of Variance (ANOVA) assessing differences in the lower-layer soil parameters (response variables) between seasons and among sites (predictor variables). *df* = degrees of freedom, *F* = Fisher's F-statistic, *p* = *p*-value.

Response	Predictor	df	F	p
Sand %	Season	1	15.8	0.0008
	Site	3	3.8	0.0284
	Residuals	19		
	Residuals	19		
Silt %	Season	1	13.2	0.0018
	Site	3	2.4	0.0963
	Residuals	19		
	Residuals	19		
Clay %	Season	1	0.1	0.9999
	Site	3	1.3	0.3143
	Residuals	19		
	Residuals	19		

Response	Predictor	df	F	p
pH	Season	1	3.7	0.0687
	Site	3	1.6	0.2115
	Residuals	19		
OM	Season	1	0.5	0.4956
	Site	3	2.7	0.0764
	Residuals	19		
N	Season	1	4.7	0.0423
	Site	3	8.8	0.0007
	Residuals	19		
P	Season	1	0.1	0.7660
	Site	3	10.5	0.0003
	Residuals	19		
K	Season	1	5.5	0.0307
	Site	3	3.7	0.0304
	Residuals	19		
Ca	Season	1	0.2	0.6770
	Site	3	20.7	< 0.0001
	Residuals	19		
Cl	Season	1	9.3	0.0066
	Site	3	1.0	0.3993
	Residuals	19		
Mg	Season	1	19.5	0.0003
	Site	3	2.7	0.0771
	Residuals	19		

Climatic variables

During the rainy season, lower average air temperatures (27.8 ± 4.3 °C) coupled with higher relative humidity (81.7 ± 14.6 %) characterized the climate, in contrast to the dry season, which featured higher temperatures (31.4 ± 4.9 °C) and lower relative humidity (75.0 ± 14.6 %). Indeed, a significant difference was found between seasons regarding temperatures (ANOVA: $p < 0.0001$, **Table 5**). Further analysis revealed variations in air temperature among the study sites ($p = 0.0002$, **Table 5**). Regarding air temperature, Bor Dan Beach (S1) and Natai Beach (S2) had comparable values (Tukey HSD: $p = 0.9963$), whereas all other pairs of comparisons revealed

significant differences (all $p < 0.0001$). Bor Dan Beach and Natai Beach exhibited comparatively lower air temperatures, whereas the temperature was higher at Sai Kaew Beach and at Mai Khao Beach. No pattern of relative humidity was quantitatively analysed, given that temperature and relative humidity had a very high correlation ($r = -0.95$, **Supplement 10**). Qualitatively, we can say that in the rainy period, humidity was highest at Natai Beach, followed by Bor Dan Beach, Sai Kaew Beach, and Mai Khao Beach, respectively. However, in the dry period, humidity was highest at both Natai Beach and Bor Dan Beach, with no statistically significant difference between the sites, and followed by Sai Kaew Beach and Mai Khao Beach, respectively.

Table 5 Results of the Analysis of Variance (ANOVA) assessing differences in climate variables (response variable) between seasons and among sites (predictor variables). df = degrees of freedom, F = Fisher's F-statistic, p = p -value.

Response	Predictor	df	F	p
Air temperature	Season	1	121.4	< 0.0001
	Site	3	7.1	0.0002
	Season : Site	3	24.8	< 0.0001
	Residuals	136		

Discussion

Starting with the current knowledge of *Launaea sarmentosa* in Thailand, the Flora of Thailand (Vol. 13, Part 2, 2016) records its distribution in Prachuap Khiri Khan (Hua Hin), Ranong (Ko Kho Khao, Bang Ben, Khlong Nakha), Phang-Nga (Takuapa), Phuket (Khao Phra Thaeo Wildlife Sanctuary), and Krabi (Lanta National Park) [2]. As we have worked in Phuket and Phang-Nga, however, Khao Phra Thaeo Wildlife Sanctuary of Phuket, formally designated as a Non-hunting Area and located in the eastern part of Phuket Island with a large tropical rainforest, has not been its distribution range. Indeed, the plant has been observed growing scattered along the sandy beaches of the western coasts of Phuket, as well as in Phang-Nga and Ranong Provinces. Moreover, there was a report of *L. sarmentosa* from coastal areas on the Gulf of Thailand, in Bangkok Subdistrict, Phetchaburi Province [10]. Therefore, a revision of the distribution of *L. sarmentosa* is needed to better understand the status of its populations, which are currently at risk of extinction in their natural habitats along the Thai coasts.

The present study represents the first investigation of *L. sarmentosa* populations beyond the areas where the plant is commercially cultivated by local communities. We found significant variation in shoot density across different sites and seasons. Spatially, moderately sunny sites (S1, S2) exhibited higher shoot densities compared to those with full sun exposure (S3, S4). Temporally, shoot density during the rainy season was approximately 4–8 times higher than during the dry season. This suggests that *L. sarmentosa* favors moderately sunny areas with moist soil. Our results agree with those of Sorce et al. [11], who reported that Mediterranean coastal dune species such as *Ammophila arenaria* and *Achillea maritima* exhibited reduced photochemical efficiency and increased physiological

stress under intense sunlight and low soil moisture conditions. Similarly, Zunzunegui et al. [12] found that species inhabiting less exposed dune zones with higher soil moisture availability maintained greater growth rates and water-use efficiency, particularly during periods of increased precipitation. This preference is particularly evident in cultivated areas, where the local community provides shade and ensures consistent watering. In such conditions, the plant achieves nearly 100 % ground cover per square meter, regardless of the season (personal observation). Since local communities may eventually abandon the cultivation of *L. sarmentosa* due to fluctuating demand, it is essential to focus on preserving its natural populations. Coastal development, particularly activities like clear-cutting beach vegetation, increases soil exposure and dryness, which threatens the plant's natural habitat. Policymakers should implement sustainable coastal management practices to maintain moderately sunny areas. Additionally, local communities should protect and conserve these natural habitats, allowing *L. sarmentosa* to grow and adapt independently, thereby ensuring the long-term survival of native populations.

Nevertheless, below-ground structures appear to play a more critical role than above-ground parts. Our results showed that the below-/above-ground biomass ratio was higher during the dry season compared to the rainy season (**Figure 2**). In response to drought conditions, many beach plant species tend to prioritize root growth to enhance their ability to access deeper or more limited water sources [13]. This root-focused adaptation is vital for survival, often leading to increased below-ground biomass as energy and resources are redirected from the growth of above-ground structures such as leaves and stems [14]. Such allocation reflects the development of extensive root systems that support more effective water and nutrient

uptake under stressful conditions. Our result agrees with findings in *Salix caprea*, a fast-growing pioneer species, which exhibited a higher root/shoot (R/S) ratio during the early stages of growth and under resource-limited conditions. In that study, the R/S ratio exceeded 1 at the beginning of the growing season and decreased as the growing season progressed, indicating an initial investment in root development to support establishment and resource uptake under fluctuating environmental conditions [15]. Additionally, the ratio was highest at Sai Kaew Beach (S3), the most exposed site among the study areas (**Figure 1**). This suggests that local environmental conditions influence the magnitude of drought-induced changes in below-ground biomass.

We observed amphistomatic stomata, a type of adaptation found in certain plant species. These stomata are present on both the adaxial (upper) and abaxial (lower) surfaces of the leaves, facilitating increased gas exchange and enhancing photosynthetic efficiency. This adaptation is particularly advantageous in environments where optimizing water and nutrient uptake is crucial for survival, as it maximizes the plant's ability to absorb CO₂ and release O₂, even under stressful conditions such as drought or high temperatures [16]. However, other anatomical adaptations, such as sunken stomata and glandular trichomes, were not observed in this study. Regarding anatomical features in response to seasonal changes, the present study revealed significant increases in upper and lower epidermis thickness, upper epidermal cell area, and mesophyll thickness during the dry season. These findings contrast with those of Mendes et al. [17], who reported a decline in leaf area and thickness in newly produced leaves of *Croton blanchetianus* Baill. (Euphorbiaceae) during the dry season. This discrepancy may arise from the fact that *C. blanchetianus* employs different strategies suited to its inland habitat, possibly involving thinner leaves to reduce the surface area exposed to solar radiation and transpiration. This indicates the importance of considering habitat-specific environmental factors when interpreting plant responses to seasonal changes. Our results suggest that *L. sarmentosa* may adopt distinct anatomical adaptations to cope with the combined stresses of drought and coastal conditions. Thicker epidermal layers could improve the plant's ability to retain water within leaf tissues, while increased mesophyll thickness may enhance its photosynthetic

rate due to the presence of stomata on both the adaxial and abaxial surfaces, a feature characteristic of amphistomatic leaves. According to Richetti et al. [18], amphistomatic leaves facilitate greater leaf conductance, thereby improving photosynthesis, particularly in herbaceous and fast-growing plants.

Examining photosynthetic pigments revealed that the chlorophyll *a/b* ratio varied seasonally, with a higher ratio observed in the rainy season than in the dry season. While chlorophyll *a* content was not significantly affected by season, chlorophyll *b* increased during the dry period, resulting in a decreased chlorophyll *a/b* ratio (**Table 1, Supplement 2**). In contrast, this ratio serves as an indicator of photosynthetic efficiency, which tends to increase during the rainy season. These findings agreed with Kim et al. [19], who studied *Vitex rotundifolia* (Lamiaceae) in coastal sand dunes and reported that the chlorophyll *a/b* ratio was higher in dune plant leaves than in potted plants. A lower ratio during the dry season suggests an increase in chlorophyll *b*, potentially reflecting a physiological adaptation to environmental stress. The ratio between total chlorophyll and carotenoids varied significantly across sites but not between seasons. Differences in the chlorophyll/carotenoid ratio among sites suggest site-specific variations in the enhancement or reduction of photoprotection mechanisms. Since carotenoids play a crucial role in enhancing photoprotection, these variations may reflect adaptive responses to differing environmental conditions at each site [20]. This was more evident when considering site-specific variation, with the ratio being higher at Mai Khao Beach (a fully sunny site) compared to Natai Beach and Sai Kaew Beach (moderately sunny sites). Regarding the total chlorophyll/carotenoid ratio, which did not vary seasonally, site-specific differences were observed, with the magnitude of these differences depending on the season (**Table 1**). This was demonstrated at Sai Kaew Beach (the site with the most sun exposure), where the ratio was significantly lower than at other sites. This indicates that carotenoid levels were high in response to the full-sun exposure habitat.

Our study was the first to investigate soil parameters alongside air temperature and relative humidity in the sampling areas. Among the measured soil variables, only soluble chloride (Cl⁻) exhibited clear seasonal variation in both upper and lower soil layers,

with higher concentrations recorded during the dry season compared to the rainy season. This seasonal trend is consistent with the findings of Yan et al. [21], who demonstrated that chloride distribution in coastal saline soils is strongly influenced by proximity to the sea, soil depth, and precipitation patterns. They observed that chloride tends to accumulate in surface soils during dry periods due to reduced leaching and increased atmospheric deposition. The underlying processes are further supported by White and Broadley [22], who explained that in the absence of rainfall, chloride can build up in upper soil layers, while evaporation may draw it upward from deeper soil profiles through capillary movement. Our observations reflect these dynamics, as rainfall during the wet season promotes chloride leaching, resulting in lower concentrations in the surface soil. Although soluble chloride is widely recognized as a key indicator of soil salinity rather than a direct contributor to plant growth, Geilfus (2018) [23] reported that chloride toxicity typically occurs only at substantially higher concentrations than those observed in our study. Specifically, we recorded low chloride levels, with 0.37 mg/kg in the rainy season and 2.82 mg/kg in the dry season (**Supplement 4**), suggesting that chloride is a natural and non-harmful component of the sand dune ecosystem in our study area.

At Natai Beach (S2), where *L. sarmentosa* exhibited the highest density and biomass, the soil, particularly in the upper layer, was characterized by higher available magnesium (Mg), a greater proportion of clay, and a lower pH compared to other sampling sites. Magnesium plays a key role in photosynthetic efficiency and plant growth, as it is essential for chlorophyll synthesis and serves as the central atom in the chlorophyll molecule. It also supports the transport of carbohydrates from source to sink organs, thereby maintaining energy balance and enhancing productivity. This function is supported by Farhat et al. [24], who showed that Mg deficiency disrupts carbohydrate partitioning and suppresses root development. However, we found no significant differences in photosynthetic pigment levels among sites, likely because Mg availability at all sites exceeded the minimum threshold required for chlorophyll production and photosynthetic function. The higher clay content at S2 may also have contributed to better water-holding capacity, which is

advantageous in fast-draining and nutrient-poor sandy soils. This is consistent with Bordoloi et al. [25], who identified clay percentage as a key variable influencing soil water retention, particularly in coarse-textured environments. Additionally, the lower pH observed at this site may enhance the availability of important micronutrients such as manganese (Mn^{2+}), iron (Fe^{2+}), and copper (Cu^{2+}), which are vital for plant metabolic processes. This agrees with the findings of Fageria et al. [26], who emphasized that the solubility and availability of these micronutrients increase under more acidic soil conditions. Overall, these favorable soil characteristics, together with a moderately sunny and humid microclimate, appear to provide optimal conditions for the growth of *L. sarmentosa* at Natai Beach. These results offer new insight into the ecological preferences of this coastal species and support practical conservation strategies for sustaining its natural populations.

Conclusions

This study provides the first detailed investigation into the seasonal and site-specific variations in the ecological and anatomical traits of *Launaea sarmentosa* along the Andaman coasts of Thailand. Our findings highlight the important role of below-ground structures in supporting the plant's survival under fluctuating environmental conditions. These insights not only contribute to the conservation of *L. sarmentosa* but also enhance our understanding of how coastal herbaceous species adapt to dynamic coastal ecosystems. The patterns observed in this species reflect key ecological processes, such as resource allocation and morphological adaptation, which are relevant to many plant species in coastal environments. To ensure the conservation of *L. sarmentosa*, it is essential to preserve its natural habitats and implement sustainable coastal management practices to mitigate the impacts of habitat loss and localized stressors. Future research should focus on long-term population monitoring and experimental studies on environmental stress adaptation to better understand the mechanisms underlying survival and recovery in coastal plant species.

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References

- [1] The World Checklist of Vascular Plants, Available at <https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:113642-3>, accessed March 2024.
- [2] H Koyama, S Bunwong, P Pornpongrungrueng and DJN Hind. *Flora of Thailand*, Vol. 13, Part 2: Compositae (Asteraceae). Royal Forest Department, Bangkok, 2016, p. 349-350.
- [3] LT Them, PTN Dung, PTN Trinh, QT Hung, LNT Vi, NT Tuan, TD Lam, VT Nguyen and LT Dung. Saponin, polyphenol, flavonoid content and α -glucosidase inhibitory activity, antioxidant potential of *Launaea sarmentosa* leaves grown in Ben Tre province, Vietnam. *IOP Conference Series: Materials Science and Engineering* 2019; **542**, 012036.
- [4] QC Nguyen, TD Binh, R Kusunoki, TLA Pham, YDH Nguyen, TT Nguyen, K Kanaori and K Kamei. Effects of *Launaea sarmentosa* extract on lipopolysaccharide-induced inflammation via suppression of NF- κ B/MAPK signaling and Nrf2 activation. *Nutrients* 2020; **12**, 2586.
- [5] Y Salih, CR Harisha, VJ Shukla and R Acharya. Pharmacognostical evaluation of *Launaea sarmentosa* (Willd.) Kuntze root. *International Quarterly Journal of Research in Ayurveda* 2013; **34(1)**, 90-94.
- [6] P Mishra, DD Toppo, N Gupta, D Chakrabarty and R Tuli. Effect of antioxidants and associated changes in antioxidant enzymes in controlling browning and necrosis of proliferating shoots of elite *Jatropha curcas* L. *Biomass Bioenergy* 2010; **34**, 1861-1869.
- [7] R Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2023. Available at: <https://www.R-project.org>, accessed March 2024
- [8] W Revelle. *Psych: Procedures for Psychological, Psychometric, and Personality Research*. R package version 2.3.3. Northwestern University, Evanston, Illinois; 2023. Available at: <https://CRAN.R-project.org/package=psych>, accessed March 2024.
- [9] D Lüdtke. Performance: An R package for assessment, comparison, and testing of statistical models. *Journal of Open Source Software* 2021; **6(60)**, 3139.
- [10] V Prapasanobol. Biological activities and phytochemical screening of *Launaea sarmentosa* root. *Science and Technology Nakhon Sawan Rajabhat University Journal* 2018; **10(11)**, 89-100.
- [11] C Sorce, S Bottega and C Spanò. Seasonal and microclimatic influences on the ecophysiology of Mediterranean coastal dune plants. *Estuarine, Coastal and Shelf Science* 2019; **219**, 317-327.
- [12] M Zunzunegui, MP Esquivias and JB Gallego-Fernández. Spatial and seasonal patterns of water use in Mediterranean coastal dune vegetation. *Plant Soil* 2022; **477**, 807-828.
- [13] D Guasconi, S Manzoni and G Hugelius. Climate-dependent response of root and shoot biomass to drought duration and intensity in grasslands: A meta-analysis. *Science of the Total Environment* 2023; **903**, 166209.
- [14] A Eziz, Z Yan, D Tian, W Han, Z Tang and J Fang. Drought effect on plant biomass allocation: A meta-analysis. *Ecology and Evolution* 2017; **7**, 11002-11010.
- [15] J Dusek and J Kvet. Seasonal dynamics of dry weight, growth rate, and root/shoot ratio in different-aged seedlings of *Salix caprea*. *Biologia, Bratislava* 2006; **61(4)**, 441-447.
- [16] RF Evert. *Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development*. 3rd eds. John Wiley & Sons, New Jersey, 2006.
- [17] KR Mendes, W Batista-Silva, J Dias-Pereira, MPS Pereira, EV Souza, JE Serrão, JAA Granja, EC Pereira, DJ Gallacher, PR Mutti, DTC da Silva, RSS Júnior, GB Costa, BG Bezerra, CMS e Silva and MF Pompelli. Leaf plasticity across wet and dry seasons in *Croton blanchetianus* (Euphorbiaceae) in a tropical dry forest. *Scientific Reports* 2022; **12**, 954.
- [18] E Richetti, AJ Poletto, AC Rodrigues, FMC Oliveira and MG Lusa. Anatomical adaptations of plants within the Southern Brazil coast. *Flora* 2025; **323**, 152661.

- [19] BJ Kim, SH Yim, YS Sim and YS Choo. Photosynthetic characteristics and chlorophyll of *Vitex rotundifolia* in coastal sand dunes. *Journal of Ecology and Environment* 2023; **47**, 10.
- [20] A Kyparissis, P Drilias and Y Manetas. Seasonal fluctuations in photoprotective (xanthophyll cycle) and photoselective (chlorophylls) capacity in eight Mediterranean plant species belonging to two different growth forms. *Australian Journal of Plant Physiology* 2000; **27**, 265-272.
- [21] S Yan, L Zhai, Q Deng, D Pan, S Gao and C Zou. Spatial-temporal dynamics of soil chloride distribution in a coastal saline plain: implication for ocean and climate influences. *Journal of Soils and Sediments* 2017; **18**, 586-598.
- [22] PJ White and MR Broadley. Chloride in soils and its uptake and movement within the plant: A review. *Annals of Botany* 2001; **88(6)**, 967-988.
- [23] CM Geilfus. Chloride: from nutrient to toxicant. *Plant and Cell Physiology* 2018; **59(5)**, 877-886.
- [24] N Farhat, A Elkhouni, W Zorrig, A Smaoui, C Abdelly and M Rabhi. Effects of magnesium deficiency on photosynthesis and carbohydrate partitioning. *Acta Physiologiae Plantarum* 2016; **38**, 145.
- [25] R Bordoloi, B Das, G Yam, PK Pandey and OP Tripathi. Modeling of water holding capacity using readily available soil characteristics. *Agricultural Research* 2019; **8(3)**, 347-355.
- [26] NK Fageria, VC Baligar and RB Clark. Micronutrients in crop production. *Advances in Agronomy* 2002; **77**, 185-268.