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The Effect of Drought Stress on the Morphology and Anatomy of *Codiaeum variegatum* (L.) Rumph. ex A. Juss. and *Excoecaria cochinchinensis* Lour

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Abstract: Climate change causes drought in many places, including tropical areas, which affects ornamental plants both in morphology and anatomy. *Codiaeum variegatum* (L.) Rumph. ex A.Juss. and *Excoeceria cochinchinensis* Lour have low transpiration rates and allow them to be resistant to drought. This study aims to observe the morphology and anatomy of *C. variaegatum* and *E. cochinchinensis* leaves due to drought stress. The experimental design was a factorial completely randomized design (2x2) with 3 replications. The main factors are two types of plants and two conditions namely control and drought. The combination was the interaction between the two main factors. Observations included descriptions of leaf blades and edges every week for four weeks. Stomata changes, description of stomata and leaf anatomical tissue were counted and measured using a microscope connected to optilab. Observation results showed changes in the appearance of leaf blades and leaf rolling. Stomata and SD varied as well as the thickness of leaf tissue which was differentiated in both thickness and shape. The conclusion of this research was that *C. variaegatum* was a drought tolerant plant and *E. cochinchinensis* was low tolerant. Both plants can aprropriately be used to green the area with water constrain as well as ornamental plants.

Keywords: *Codiaeum variegatum*; *Excoeceria cochinchinensis*; Drought; Leaf Morphology; Leaf Anatomy.

Introduction

Climate change causes drought, including tropical areas. This drought affects the appearance of ornamental plants used to decorate home gardens and/or greenery arranged in cities and even to reduce certain air pollutants. Hsieh et al. (2018) and Rahman et al. (2020) said planting plants in urban areas aims to reduce warming. However, this plant can experience wilting if it experiences drought, which can affect the beauty of the plant (Toscano et al., 2019) and internally affect changes in the anatomical structure of leaf cells (Boughanch et al., 2014). Like most types of plants, ornamental plants need enough water for their growth. Water is an important factor for development. Lack of water can reduce the morphological performance of plants, such as in terms of size, number and color of flowers, even the yellowish color of the leaves followed by wilting (Alvarez & Blanco, 2013).

Da Costa and Daningsih (2022); Ningsih and Daningsih (2022); Daningsih et al. (2022) shows transpiration as a reflection of different water losses

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between plant species. Dicotyledonous plants have differentiated leaf tissue anatomy (Rahman, 2023; personal communication). All plants have sponges and palisades but the number of palisade layers varies. The sponge and palisade layers experience changes in thickness when transpiration occurs.

Water loss causes changes in leaf thickness (Da Costa & Daningsih, 2022). Rahman, (personal communication) (2023) added that this change in thickness is a result of changes in the structure of the palisades and sponges. However, the presence of cell tissue structures such as a thick epidermis can reduce water loss, for example in *C. variaegatum* plants. A small loss of water can help plants survive when there is a large lack of water. Meanwhile, *Salvia splendens* leaves are not affected by water shortage stress (Burnett et al., 2005). This shows that each type of plant develops adaptations to water shortages (Boughlleb et al., 2014).

Taiz et al. (2018) said that water particles are contained in the mesophyll. This is also supported by Kutlu et al. (2009) and Kapchina-Totevaa et al. (2014) who showed a reduction in shape and a decrease in the number of layers in the mesophyll when the water content in the leaves was reduced. Palisade mesophyll tissue contains chlorophyll so that water use also supports photosynthesis (Cutler et al., 2007). On the other hand, Canny et al. (2012) and Xing et al. (2022)found that the part of the mesophyll that contains water is sponge. Da Costa and Daningsih, (2022) stated that both palisade and sponges have a role in transpiration which is indicated by the transpiration rate. The results of research by Rahman (2023) showed that of the six types of dicotyledonous plants tested, the *C. variaegatum* and *E. cochinchinensis* had more than one palisade layer and a low transpiration rate. Both types of plants have the potential to adapt to drought. Therefore, this study aimed to determine the response of the morphology and anatomy of *C. variaegatum* and *E. cochinchinensis* leaves to drought stress. Climate change has an impact in many condition (Seeda et al., 2022) including water availability and high temperature, the external factors influencing transpiration and photosynthesis (Qaderi et al., 2019). Ornamental plant adapting tu limited water availibility and high temperature are needed especially for greening urban area as well as giving pleasant to its surrounding. Therefore, this study aimed to determine the response of the morphology and anatomy of C. variaegatum and E. cochinchinensis leaves to drought stress.

Method

This research was an experimental metod with a research design using a Factorial Completely Randomized Design (CRD). The main treatments were two types of ornamental plants, namely *Excoeceria cochinchinensis* Lour and *Codiaeum variegatum* (L.) Rumph. ex A. Juss. and two conditions, namely control and drought stress. The combination treatment was the interaction of plant type and drought stress, with three replications.

The plants used were selected base on similar general morphological characteristics, namely having almost the same height and number of leaves. The test plants were then planted in pots containing sand and burnt soil in a ratio of 1:2.

Plants were watered every day and given sufficient NPK fertilizer for four weeks. After that, drought stress treatment was applied for four weeks. Controls were given watering of 100 ml every day and stress was not watered for four weeks. The work flow can be seen in Figure 1.

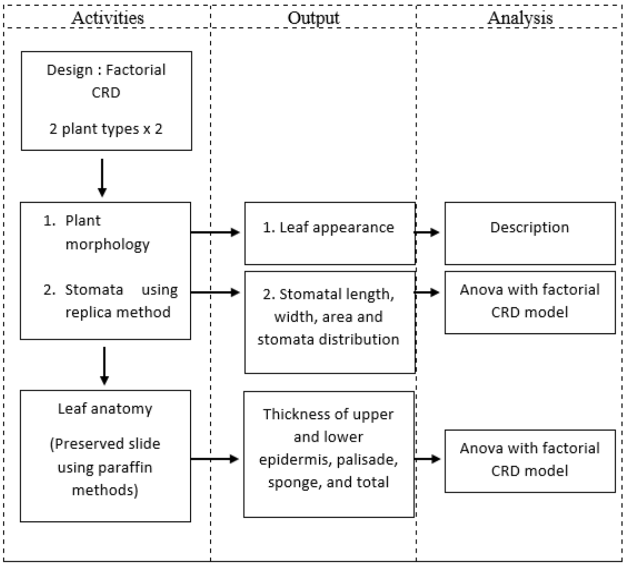


Figure 1. Description of research activities, procedure observation and data analysis.

Observation of the Morphology of C. variaegatum Leaves and E. cochinchinensis

The characters observed were the appearance of the leaves, the shape and edges of the leaf blades (Modification of Radford et al., 1974)

Observation and Measurement of Stomata Using the Replica Method

Samples were taken from the seventh or sixth leaf (the lowest leaf of each plant after observing its morphology). Leaves that have been cleaned from dirt and dried were smeared with clear nail polish and clear insulation was attached to the nail polish on the leaves both on the top and bottom surfaces. The insulation was pulled out and attached to the slide and labeled.

The number of stomata cells and epidermal cells was observed under a microscope connected to an optilab with a magnification of 10x10. Stomata length and width measurements were carried out with a magnification of 10x100. Stomata area was calculated using the formula referring to Meidner & Mainsfield in Avci and Aygün (2014) as follows:

$$\text{Stomata area} = \pi \times a \times b \quad (1)$$

Keterangan:
π = 3.14
a = Lenght of stomata
b = Width of stomata

Stomata Distribution Ratio (SD) was calculated using the Formula 2.

$$\mathbf{SD} = \frac{A}{A+B} \text{ x } 100\% \quad (2)$$

Keterangan:
SD = Stomata Distribution
A = The number of stomata cells per unit area
B = The number of epidermal cells per unit area

Making Preserved Preparations

Making leaf anatomical preparations was carried out at the Biology Education Laboratory and Morphology, Anatomy and Cytology Laboratory, Deputy for Research and Innovation Infrastructure - BRIN using the paraffin method. Leaves were fixed in FAA solution consisting of concentrated formaldehyde: glacial acetic acid: 70% ethanol (5:5:90) for 24 hours. Leaf samples were dehydrated and clarified using Johansen I-VII serial solutions (Johansen, 1940).

Leaf samples were embedded in paraffin and soaked in Giford's solution for one week. The paraffin block was then sectioned using a rotary microtome to a thickness of 14-16 μm. The next process was coloring using a combination solution of Neo Clear - Ethanol as a clearing agent, as well as safranin 2% and fast green 1% as dye. The preparations were then observed using an Olympus BX53 microscope connected to an Olympus DP-22 camera. Samples were observed from the first treatment and combination treatments of different plant types.

Mophological Analysis

Descriptions of leaf appearance, shape and edges are illustrated on a weekly basis.

Stomata Analysis

Stomata length, width and area as well as the stomata distribution ratio (SD) were analyzed using Factorial RAL ANOVA model at α = 0.05 and followed by LSD if the treatment was significant.

Leaf Anatomy Analysis

The leaf anatomy of *C. variaegatum* and *E. cochinchinensis* plants was described between the main and combination treatments. The number and size of the epidermis and mesophyll layers were analyzed using Factorial RAL model ANOVA at α = 0.05 and followed by the LSD test if the treatment was significant.

Result and Discussion

Deskripsi Tampilan Morfologi Daun Puring (C. variegatum) dan Sambang Darah (E. cochinchinensis)

The condition of the plants, especially *C. variaegatum* and *E. cochinchinensis* leaves, was observed for four weeks (Table 1) before taking samples to observe leaf anatomy. Observation results showed that in the second week the *E. cochinchinensis* appeared wilt under drought stress conditions (Table 1). In the third and fourth weeks the *C. variaegatum* and *E. cochinchinensis* leaves looked fresh under control conditions. However, under drought stress conditions, the leaves of *C. variaegatum* and *E. cochinchinensis* appear wilted. Further observation showed that several tips and edges of *E. cochinchinensis* leaves appeared to be shriveled.

According to Hidayati et al. (2017), drought affects morphology such as stems, leaves and flowers. In leaves, visible morphological changes include changes in color, leaf size, the appearance of leaves such as curling and even wilting (A'yuningsih, 2017). In this study, *E. cochinchinensis* and *C. variaegatum* were exposed to drought stress. Dryness of water in the soil causes plants not to absorb enough water and results in irreplaceable water loss during transpiration.

When observing the leaves (Table 1), both *C. variaegatum* and *E. cochinchinensis* looked wilted. Air with high temperatures due to long dry seasons and no rain makes plants more exposed when drought stress is treated. Plants responded differently to ongoing drought, both in morphology, anatomy and physiology. Plant rolling or 8-9% wrinkling can occur in plants when exposed to drought.

Wrinkling of *E. cochinchinensis* leaves (Table 1) was an attempt by the plant to reduce leaf area and reduce transpiration. In this way, water loss through the leaves can be minimized. Some plants experience leaf wrinkling due to the presence of fan cells. Fan cells are useful when there is a water shortage. The number of fan cells increases and causes the leaves to curl, thereby reducing the leaf area, which results in a decreased transpiration rate (Ai & Lenak, 2014)

Table 1. Morphological appearance of the leaves of the *C. variaegatum* and *E. cochinchinensis* plants

Week	*Condition*		*Week*	*Condition*	
	Control	Drought Stress		Control	Drought Stress
1			2		
	In control and stress conditions, *C. variaegatum* plants looked fresh			In control and stress conditions, *C. variaegatum* plants looked fresh	
					
	Under control and stress conditions, *E. cochinchinensis* plants looked fresh			In control conditions, *E. cochinchinensis* plants looked fresh, whereas in stress conditions, *E. cochinchinensis* looked wilted	
3			4		
	In control conditions, *C. variaegatum* plants looked fresh, whereas in stress conditions, *C. variaegatum* plants looked somewhat wilted			In control conditions, *C. variaegatum* plants looked fresh, while in stress conditions, *C. variaegatum* plants looked wilted	
					
	In control and stress conditions, *E. cochinchinensis* plants looked fresh but in stress conditions some of the leaves shriveled			Under control and stress conditions, *E. cochinchinensis* plants looked fresh, but under stress conditions, some of the leaves shriveled	

Stomata

Stomata are pores on the upper and/or lower surfaces of leaves. Most stomata are on the lower surface to reduce the rate of transpiration and exposure to sunlight. Stomata are stimulated by light to open, allowing them to take in CO_2 and release water vapor and O_2 as a result of photosynthesis and transpiration (Campbell & Reece, 2008). Stomata have different shapes and the shape of the stomata is a leaf characteristic to differentiate the genus or species (Sarjani et al., 2017). The shape of the stomata on the *C. variaegatum* and *E. cochinchinensis* is oval or round. Both are dicotyledonous ornamental plants with relatively low transpiration rates (Rahman, 2023; personal communication).

Stomatal characteristics measured from stomatal pores were length, width, area of the stomatal pores, number of stomatal and epidermal cells which were used to calculate the stomatal distribution (SD) or known as the Stomatal Index (SI) (Tables 2-5). Measurements and calculations of stomatal characteristics (length, width and area of stomatal pores) were carried out from the first to the fourth week. In the first week, the plant type factor had a significant effect on all parameters (Table 2). However, only the number of stomata cells was different due to different condition factors, as well as the area of the stomata pores and the SD ratio (stomatal distribution) which were influenced by the combination of factors. This difference is a genetic trait related to each plant. According to Nerva et al., (2023), stomata length and width are traits related to genetics.

C. variaegatum plants have a larger stomatal pore area (60.21 µm) than *E. cochinchinensis* (30.45 µm). However, the number of stomata on *E. cochinchinensis* leaves (203.77) reached 2.3 times more than the number of stomata on *C. variaegatum* (88.77). The number of *E. cochinchinensis* epidermal cells (1285.06) was 4.91 times more than the *C. variaegatum* epidermal cells (261.39) with a higher SD ratio in *C. variaegatum* than *E. cochinchinensis*. With a large stomatal pore area and a large SD ratio, the ability of the stomata to take up CO_2 for photosynthesis also increases and is driven by a large SD ratio which allows adequate water availability in plant parts when facing drought (Sakiroh & Aunillah, 2020).

Table 2. Results of factorial CRD analysis of variance: length and width stomatal pores, area of stomatal pores, number of stomata and epidermis cells, and stomata distribution ratio in the first week.

Treatment	Average (µm)					
	Lenght	Width	Area	Number		SD Ratio
				Stomata	Epidermis	
Plant	*	*	*	*	*	*
C. variaegatum	9.65^{a}	4.62^{a}	147.38^{a}	88.77^{b}	261.39^{b}	25.49^{a}
E. cochinchinensis	7.96^{b}	2.60^{b}	68.61^{b}	203.77^{a}	1285.06^{a}	13.69^{b}
Condition	ns	ns	ns	*	ns	ns
Control	9.29	3.61	115.69	132.05^{b}	723.33	20.00
Drought Stress	8.33	3.61	100.30	160.50^{a}	823.11	19.18
Combinaton of Plant Types and Conditions	ns	ns	*	ns	ns	*
C. variaegatum-Control	10.47	5.03	174.51^{a}	80.66	217.33	26.94^{a}
C. variaegatum-Drought Stress	8.84	4.21	120.25^{b}	96.88	305.44	24.04^{b}
E. cochinchinensis-Control	8.10	2.19	56.86^{c}	183.44	1229.33	13.06^{c}
E. cochinchinensis-Drought Stress	7.82	3.01	80.34^{c}	224.11	1340.77	14.32^{c}

Note: *significant at α = 0.05. ns = non-significant. Different letters behind the average in one column indicated significant differences when tested with LSD at α = 0.05.

In the second week, all parameters were significantly influenced by plant type (Table 3). Condition factors influenced all factors except stomatal pore width and SD ratio. Meanwhile, the combination of plant types and conditions did not influence all parameters.

Table 3. Results of factorial CRD analysis of variance: length and width stomatal pores, area of stomatal pores, number of stomata and epidermis cells, and stomata distribution ratio in the second week.

Treatment	Average (µm)					
	Lenght	Width	Area	Number		SD Ratio
				Stomata	Epidermis	
Plant	*	*	*	*	*	*
C. variaegatum	11.17^{a}	5.15^{a}	60.21^{a}	98.50^{b}	280.06^{b}	26.43^{a}
E. cochinchinensis	9.66^{b}	3.04^{b}	30.45^{b}	228.83^{a}	1288.00^{a}	15.11^{b}
Condition	*	ns	*	*	*	ns
Control	11.13^{a}	4.46	52.63^{a}	153.77^{b}	733.00^{b}	21.60
Drought Stress	9.69^{b}	3.73	38.04^{b}	173.55^{a}	835.06^{a}	19.94
Combinaton of Plant Types and Conditions	ns	ns	ns	ns	ns	ns
C. variaegatum-Control	11.95	5.69	71.03	92.88	240.22	28.28
C. variaegatum-Drought Stress	10.38	4.61	49.40	104.11	319.88	24.58
E. cochinchinensis-Control	10.31	3.24	34.23	214.66	1225.77	14.91
E. cochinchinensis-Drought Stress	9.00	2.85	26.67	243.00	1350.22	15.30

Note: *significant at α = 0.05. ns = non-significant. Different letters behind the average in one column indicated significant differences when tested with LSD at α = 0.05.

In the third week, all parameters were significantly influenced by plant type (Table 4). *C. variaegatum* had a greater area, length and width of stomata than *E. cochinchinensis* stomata. However, the number of stomata was significantly more on *E. cochinchinensis* leaves than the number of stomata on *C. variaegatum*. *C. variaegatum* had more epidermal cells and this meant that its SD ratio was also higher than the SD ratio of *C. variaegatum* (Table 4). The number of stomata was significantly influenced by stress conditions. There were significantly more stomata in the control condition compared to the number of stomata in the stress condition. Likewise, the SD ratio in the control condition was higher than in the stress condition.

Table 4. Results of factorial CRD analysis of variance: length and width stomatal pores, area of stomatal pores, number of stomata and epidermis cells, and stomata distribution ratio in the third week.

Treatment	Average (µm)					
	Lenght	Width	Area	Number		SD Ratio
				Stomata	Epidermis	
Plant	*	*	*	*	*	*
C. variaegatum	12.01ᵃ	5.71ᵃ	222.49ᵃ	91.00ᵇ	270.39ᵇ	25.21ᵃ
E. cochinchinensis	9.71ᵇ	2.55ᵇ	80.78ᵇ	193.61ᵃ	1200.11ᵃ	13.94ᵇ
Condition	ns	ns	ns	*	ns	*
Control	10.52	4.00	142.76	154.61ᵃ	740.33	20.61ᵃ
Drought Stress	11.20	4.26	160.51	130.00ᵃ	730.17	18.53ᵇ
Combinaton of Plant Types and Conditions	ns	ns	ns	ns	ns	ns
C. variaegatum-Control	11.68	5.65	214.80	100.11	278.44	26.52
C. variaegatum-Drought Stress	12.34	5.77	230.17	81.88	262.33	23.90
E. cochinchinensis-Control	9.36	2.36	70.71	209.11	1202.22	14.71
E. cochinchinensis-Drought Stress	10.06	2.75	90.85	178.11	1198.00	13.16

Note: *significant at α = 0.05. ns = non-significant. Different letters behind the average in one column indicated significant differences when tested with LSD at α = 0.05.

The effect of plant type remained visible in the fourth week (Table 5). All parameters of the *C. variaegatum* were much greater than the *E. cochinchinensis* except for the number of stomata which is less than the *E. cochinchinensis*. Condition factors in the fourth week influenced the length and width of the stomata and the number of stomata. *C. variaegatum* has a significantly more length and larger area of stomata than *C. variaegatum*. However, the number of stomata on *C. variaegatum* has significantly less than on *E. cochinchinensis*. The combination factors had a significant effect on the number of stomata and DS ratio (Table 5).

Table 5. Results of factorial CRD analysis of variance: length and width stomatal pores, area of stomatal pores, number of stomata and epidermis cells, and stomata distribution ratio in the fourth week.

Treatment	Average (µm)					
	Lenght	Width	Area	Number		SD Ratio
				Stomata	Epidermis	
Plant	*	*	*	*	*	*
C. variaegatum	11.16ᵃ	5.15ᵃ	60.21ᵃ	93.50ᵇ	279.28ᵇ	25.15ᵃ
E. cochinchinensis	9.65ᵇ	3.04ᵇ	30.45ᵇ	212.50ᵃ	1146.17ᵃ	15.63ᵇ
Condition	*	ns	*	*	ns	ns
Control	11.13ᵃ	4.46	52.63ᵃ	143.27ᵇ	729.72	19.70
Drought Stress	9.69ᵇ	3.73	38.04ᵇ	162.72ᵃ	695.72	21.08
Combinaton of Plant Types and Conditions	ns	ns	ns	*	ns	*
C. variaegatum-Control	11.95	5.69	71.03	93.33ᶜ	276.33	25.39ᵃ
C. variaegatum-Drought Stress	10.38	4.61	49.40	93.66ᶜ	282.22	24.92ᵃ
E. cochinchinensis-Control	10.31	3.24	34.23	193.22ᵇ	1183.11	14.02ᶜ
E. cochinchinensis-Drought Stress	9.00	2.85	26.67	231.77ᵃ	1109.22	17.23ᵇ

Note: *significant at α = 0.05. ns = non-significant. Different letters behind the average in one column indicated significant differences when tested with LSD at α = 0.05.

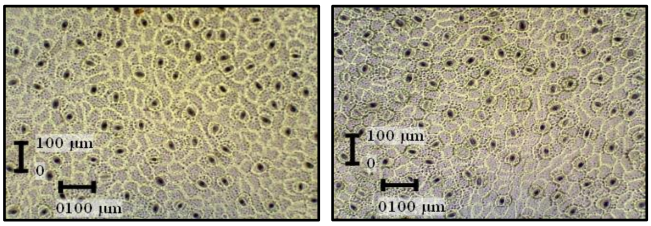
The number of stomata and epidermal cells produced different transpiration rates. This difference occurred from the first week to the fourth week. Rahman (personal communication) (2023), shows that both species (*C. variaegatum* and *E. cochinchinensis*) have low transpiration rates that were not significantly different. Reducing stomatal pores and the number of stomata per area in line with efforts to reduce the transpiration rate. According to Ai and Lenak (2014), rolling of leaf morphology when exposed to drought stress often occurs in plants and leaf area decreases causing changes in the number of stomata per area. Observation of the number of stomata and epidermal cells for 4 weeks showed that the number of stomata per area increased when faced with drought stress in both *C. variaegatum* and *E. cochinchinensis*.

Stomata distribution can influence the exchange of substances in the form of gases such as Pb which are air pollutants. Sulistiana and Setijorini (2016) showed that the higher the SD, the Pb content also increases without disrupting metabolism, so *C. variaegatum* is categorized as an ornamental plant which can also help reduce air pollution due to Pb. The distribution of stomata on *C. variaegatum* ranges from 25.21% - 26.94%. The ratio SD of

C. variaegatum was greater than SD of *E. cochinchinensis* which was only 13.94% - 24.04% and almost half SD of *C. variaegatum*. A high SD indicates the plant's ability to adapt to drought conditions (Rochman and Hamida, 2017).

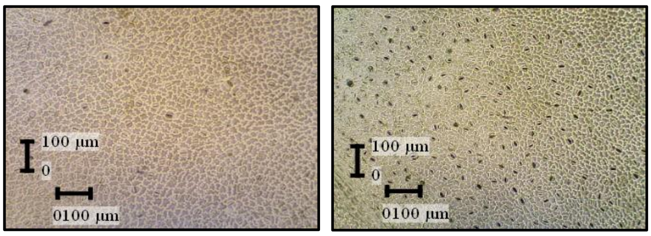
The large number of stomata with a smaller size than the *E. cochinchinensis* stomata (Table 2 – 5) has an impact on the transpiration rate. According to Haryanti (2010) the distribution of stomata is closely related to the speed of transpiration in leaves. The more stomata, the more pores, so the faster the transpiration. However, if the stomata pores are too close together, then evaporation from one pore will inhibit evaporation from nearby pores. This was proven by Rahman (2023) who showed that the transpiration rate of *E. cochinchinensis* was in the low category.

The number of stomata cells and epidermal cells for four weeks can be seen in Figure 2 and Figure 3 with a magnification size of 10x10.



a b

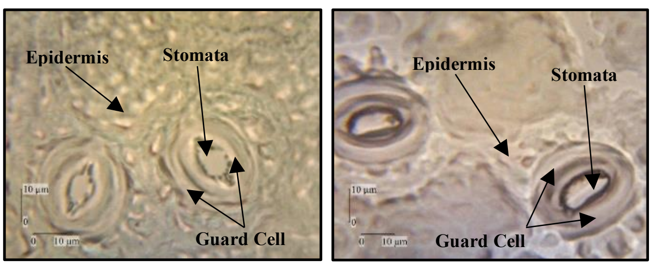
Figure 2. Stomatal and epidermal cells of *C. variaegatum*; (a) control condition; (b) drought stress conditions.



a b

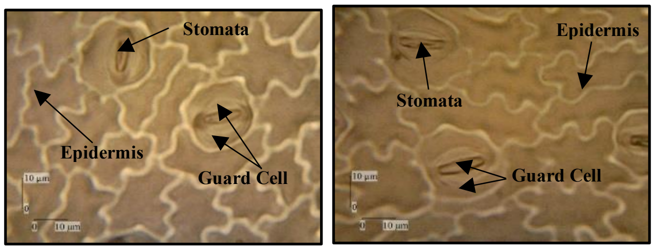
Figure 3. Stomatal and epidermal cells of the *E. cochinchinensis*; (a) control condition; (b) drought stress conditions.

The length and width of *C. variaegatum* and *E. cochinchinensis* stomata under control and drought can be seen on Figure 4 and Figure 5.



a b

Figure 4. Length and width of *C. variaegatum* stomata pores; (a) control condition; (b) drought stress conditions.



a b

Figure 5. Length and width of *E. cochinchinensis* stomata pores; (a) control condition; (b) drought stress conditions.

Leaf Tissue Thickness

Drought stress causes the rate of transpiration to increase and water loss in the leaves resulting in thinning of leaf thickness (Da Costa and Daningsih, 2022; Febriyani et al., (2023); Rahman (2023)). *C. variaegatum* and *E. cochinchinensis* leaves have low transpiration rates and both plants have 2 layers of palisade tissue (Rahman, 2023; personal communication). According to Rindyastuti and Hapsari (2017), one of the 3 characters that influence the dry tropical climate is sponge tissue in addition to the stomatal index or distribution of stomata in woody plants which is an ecophysiological adaptation to the dry tropical climate.

In this study, the thickness of *C. variaegatum* and *E. cochinchinensis* leaf tissue was measured based on the thickness of the upper epidermis (UE), palisade (PL), sponge (SP), lower epidermis (LE) and total thickness (TT) of the leaves for four weeks. In measuring the thickness of anatomical tissue, *C. variaegatum* leaves showed that all UE, PL, SP, LE and TT tissues were higher than all anatomical tissues of *E. cochinchinensis* leaves (Table 6 – 9). Thicker UE and LE tissue can reduce transpiration rates (Rahman, 2023; personal communication).

In the second week of drought stress with high and dry air temperatures, the conditions had affected appearance of the plants, especially the wilted leaves on the *E. cochinchinensis* while the *C. variaegatum* leaves were fresh (Table 1). In the second week of leaf anatomical tissue thickness, apart from the differences between the two plants, sponge tissue was also significantly higher in the control compared to the drought stress conditions (Table 7). This shows that sponge tissue experiences water loss as shown by Rahman (2023) and Rindyastuti and Hapsari (2017). The thickness of the sponge tissue also occurred in the fourth week after the air temperature returned to high and there was no rain (Table 8 – 9).

In the first week, *C. variaegatum* had significantly thicker UE, PL, SP, LE and TT thicknesses compared to all leaf tissue thicknesses in *E. cochinchinensis*. UE is also influenced by condition and combination factors (Table 6).

Table 6. Results of factorial CRD analysis of variance in leaf anatomical thickness in the first week

Treatment	Average (µm)				
	UE	PL	SP	LE	TT
Plant	*	*	*	*	*
C. variaegatum	25.60	51.66	227.69	17.66	322.61
E. cochinchinensis	10.75	40.71	116.97	9.40	177.26
Condition	*	NS	NS	NS	*
Control	19.30	47.87	177.46	13.27	257.42
Drought Stress	17.04	44.50	167.69	13.78	242.45
Combinaton of Plant Types and Conditions	*	NS	NS	NS	NS
C. variaegatum-Control	28.61	52.26	234.22	17.31	332.41
C. variaegatum-Drought Stress	22.61	51.05	221.16	18.00	312.80
E. cochinchinensis-Control	10.00	43.87	120.71	9.56	182.42
E. cochinchinensis-Drought Stress	11.50	37.95	113.24	9.24	172.10

Note: UE = Upper Epidermis, PL = Palisade, SP = Sponge, LE = Lower Epidermis, TT = Total Thickness

In the second week, the thickness of UE, PL, SP, LE and TT of *C. variaegatum* was significantly thicker than all the thickness of UE, PL, SP, LE and TT of *E. cochinchinensis*. Furthermore, the thickness of SP and TT in *C. variaegatum* was significantly thicker than *E. cochinchinensis*, and the thickness of SP and TT was influenced by condition factors and combination factors (Table 7).

Table 7. Results of factorial CRD analysis of variance in leaf anatomical thickness in the second week

Treatment	Average (µm)				
	UE	PL	SP	LE	TT
Plant	*	*	*	*	*
C. variaegatum	24.46	55.14	218.15	16.01	312.49
E. cochinchinensis	11.97	32.97	87.17	8.32	141.99
Condition	NS	NS	*	NS	*
Control	18.68	44.57	171.87	12.41	248.66
Drought Stress	17.74	43.54	133.45	11.92	205.82
Combinaton of Plant Types and Conditions	NS	NS	*	NS	*
C. variaegatum-Control	25.45	55.52	251.12	16.52	348.65
C. variaegatum-Drought Stress	23.46	54.77	185.19	15.49	276.33
E. cochinchinensis-Control	11.92	33.63	92.61	8.36	148.68
E. cochinchinensis-Drought Stress	12.03	32.31	81.72	8.28	135.31

Note: UE = Upper Epidermis, PL = Palisade, SP = Sponge, LE = Lower Epidermis, TT = Total Thickness

In the third week, the thickness of UE, PL, SP, LE and TT were influenced by plant type factors with TT also influenced by condition factors (Table 8).

Table 8. Results of factorial CRD analysis of variance in leaf anatomical thickness in the third week

Treatment	Average (µm)				
	UE	PL	SP	LE	TT
Plant	*	*	*	*	*
C. variaegatum	25.18	57.32	271.07	17.45	371.38
E. cochinchinensis	11.58	29.84	104.52	8.74	153.41
Condition	NS	NS	NS	NS	*
Control	18.36	45.11	197.62	12.96	274.42
Drought Stress	18.39	42.04	177.98	13.23	250.36
Combinaton of Plant Types and Conditions	NS	NS	NS	NS	NS
C. variaegatum-Control	24.67	59.56	281.83	16.64	383.42
C. variaegatum-Drought Stress	25.68	55.07	260.32	18.26	359.34
E. cochinchinensis-Control	12.05	30.66	113.42	9.29	165.43
E. cochinchinensis-Drought Stress	11.10	29.01	95.63	8.19	141.38

Note: UE = Upper Epidermis, PL = Palisade, SP = Sponge, LE = Lower Epidermis, TT = Total Thickness

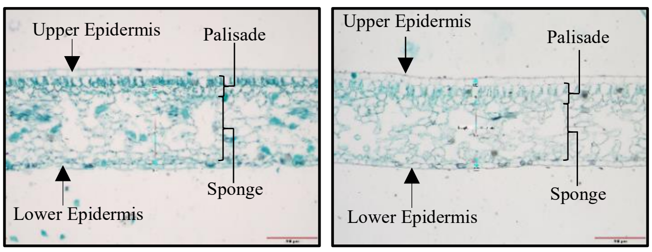
In the fourth week, the UE, PL, SP, LE and TT of the *C. variaegatum* were significantly thicker than the *E. cochinchinensis*. Only the SP thickness of the TT was influenced by condition factors (Table 9).

Tabel 9. Results of factorial CRD analysis of variance in leaf anatomical thickness in the fourth week

Treatment	Average (µm)				
	UE	PL	SP	LE	TT
Plant	*	*	*	*	*
C. variaegatum	25.11	59.13	221.75	17.21	323.22
E. cochinchinensis	10.83	29.36	97.68	8.58	146.64
Condition	NS	NS	*	NS	*
Control	18.42	44.42	171.11	13.43	247.40
Drought Stress	17.53	44.07	148.21	12.63	222.46
Combinaton of Plant Types and Conditions	NS	NS	NS	*	*
C. variaegatum-Control	25.15	59.16	228.24	16.28	328.84
C. variaegatum-Drought Stress	25.07	59.11	215.25	18.15	317.59
E. cochinchinensis-Control	11.68	29.68	113.98	10.59	165.95
E. cochinchinensis-Drought Stress	9.99	29.03	81.18	7.12	127.32

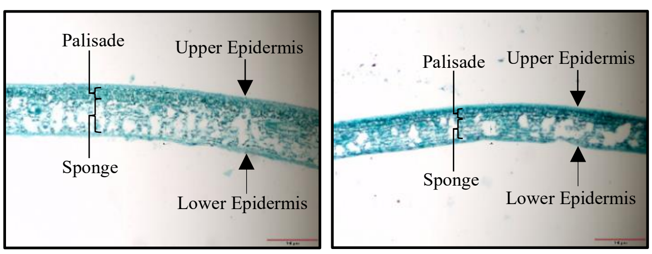
Note: UE = Upper Epidermis, PL = Palisade, SP = Sponge, LE = Lower Epidermis, TT = Total Thickness

Thicker UE and LE tissue can reduce transpiration rates (Rahman, 2023; personal communication). The thickness of epidermal tissue can reduce transpiration because epidermal tissue generally has stronger cell walls. According to Papuangan et al., (2014), on the adaxial (top) part, there is a thick cuticle layer that covers the stomata, thereby blocking the transpiration process and preventing water loss. However, in *C. variaegatum* and *E. cochinchinensis* there is no cuticle layer (Figures 6 and 7). Observations of leaf tissue preparations showed that in the second and third weeks the thickness of SP and TT was influenced by stress conditions.



a b

Figure 6. Thickness of *C. variaegatum* leaf tissue under drought stress; (a) control condition; (b) drought stress conditions.



a b

Figure 7. Thickness of *E. cochinchinensis* leaf tissue under drought stress; (a) control condition; (b) drought stress conditions.

The high and low levels of sponge tissue in response to environmental conditions are the plant's effort to retain water because of its ability to store and release water vapor when the plant is exposed to wet or dry conditions around it. Canny et al. (2012) and Xing et al. (2022) found that the part of the mesophyll that contains water is sponge. In contrast to dicot ornamental plants, the palisade tissue in monocots determines the total thinning of leaf thickness (Febriyani et al., 2023) Palisade tissue is also known to have plastic properties by changing the shape or number of tissue layers to adapt to environmental changes. The palisade layers can be vertical and horizontal without reducing the number of layers. Yano and Terashima (2004) stated that the palisade layers can lengthen and thin depending on the surrounding conditions. In Arabidopsis the change from vertical to horizontal palisades is an attempt by the plant to get more light so that photosynthesis can be made more efficient (Gotoh et al., 2018).

In Rahman's (2023) research, layer reduction did not occur in the *C. variaegatum* and *E. cochinchinensis* after transpiration but the shape and size decreased in the palisade. When the *E. cochinchinensis* and *C. variaegatum* fins were exposed to drought stress, the palisade tissue in both controls and stress had 2 layers. In the second week, the *C. variaegatum* and *E. cochinchinensis* fins began to respond to dryness. In *C. variaegatum* , the upper palisade tissue was upright while the lower palisade was horizontal under stress conditions (Figure 4). Meanwhile, the *E. cochinchinensis* palisade layer under stress conditions remained 2 layers with an upright shape and small size. Only in the fourth week, the palisade layer below the *E. cochinchinensis* is horizontal and small. Changes in layers, shape and size show the plasticity of the palisade layers to avoid high transpiration rates and the need for ground water can be minimized for photosynthesis (Febriyani et al., 2023).

According to Sari (2019) radiation using gamma rays has been proven to cause genetic variations that can be seen from changes both at the tissue level and at the cellular level. Drought-tolerant plants develop a number of strategies related to physiological processes. The drought resistance mechanism is divided into three categories, namely escape, avoidance and tolerance (Mitra, 2001).

Escape is a plant that completes its life cycle, flowering and fruiting before encountering drought stress. Avoidance is the ability of plants to keep the water potential of the plant body high, namely by optimizing the root system to absorb relatively large amounts of water and maintain the water content in the tissues (Pugnaire & Haase, 1996). Tolerance includes the ability of a plant species to remain alive and perform functions even though it experiences drought stress (Mitra, 2001).

By observing the characteristics of the *C. variaegatum* and *E. cochinchinensis* , both morphology and anatomy, the *E. cochinchinensis* is in the low tolerance category and the *C. variaegatum* is in the tolerant category to drought stress.

Conclusion

Two dicotyledonous ornamental plants, namely *C. variaegatum* and *E. cochinchinensis* , were able to survive when exposed to drought stress for 4 weeks. *C. variaegatum* had the appearance of slightly wilted leaves but with greater length, width and stomatal area. The number of stomata decreased and the SD increased but with the thickness of the UE and LE being greater than the *E. cochinchinensis*, as well as the change in the two layers of palisade stands to one layer upright and one layer horizontally during the fourth week, making *C. variaegatum* a plant that is tolerant of drought stress. On the other hand, *E. cochinchinensis* had a leaf blade morphology that wrinkles at the leaf tips in the fourth week and looks wilted in the fourth week. The length and width of the stomata decreased and their number increased, resulting in the stomata closing each other and retaining water much better so that transpiration is low even though the thickness of the leaves was thinner than the thickness of *C. variaegatum* leaves. However, the differentiated leaf tissue showed smaller changes in the palisade layer in the fourth week. This ability of *E. cochinchinensis* shows its resistance to drought. Therefore, *E. cochinchinensis* vines can be categorized as low tolerance plants.

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Conflicts of Interest

The author declare no conflict of interest in this research

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