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EFFECT OF DIFFERENT LIGHT TYPES AND EXPOSURE DURATIONS ON STOMATAL CHARACTERISTICS IN TWO CULTIVARS OF CARNATION (*Dianthus caryophyllus* L.)

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ABSTRACT:

A study was conducted to examine the effects of different light types, colors, and exposure durations on the morphological and stomatal characteristics of two carnation (*Dianthus caryophyllus*) cultivars, Ormea and Moonlight, cultivated under controlled greenhouse conditions, during the period of (2024 - 2025) at the College of Agricultural Engineering Sciences, University of Duhok, to understanding how Light quality and photoperiod play a crucial role in regulating stomatal density, size, and function, which are vital for key physiological processes such as transpiration and photosynthesis. The results indicate that specific light treatments significantly influence both morphological traits and stomatal parameters. The highest leaf number, leaf length, and leaf area per plant, were measured under 14-hour incandescent lighting (42.872 leaves/plant, 13.050 cm, and 25.801 cm^2), respectively, followed by 14h LED-mix (39.37leaves/plant, 12.020 cm and 24.218cm^2), respectively. Similarly, the longest leaf observed on the Ormea cultivar (12.336 cm) when exposed to 14 hours of incandescent light significantly outperformed other treatments. While the results of the interaction between cultivar and light on stomatal density and number showed that the Moonlight cultivar could achieve higher stomatal densities and stomatal numbers when exposed to 14 hours of incandescent. On the other hand, the Ormea and Moonlight cultivars showed the highest significant increase in stomatal densities (96.482 and 95.587) in the lower epidermis when exposed to 14 hours of incandescent lights. In the Ormea cultivar, growing under incandescent light for 14h caused a notable increase in both stomatal length and width (24.978 and 13.389 µm), followed by stomatal length and width (24.533 and 13.067 µm), respectively, under LED-mix for 14h.

KEYWORDS: Carnation plant. Supplementary lighting, leaf parameters, and stomatal characteristics

1. INTRODUCTION

The carnation plant (*Dianthus caryophyllus* L.) belongs to the Caryophyllaceae family and is among the most widely cultivated cut flowers worldwide. Half-hardy herbaceous perennials, carnation plants grow to a height of 1.0 to 1.5 meters. Another significant cut flower in several countries is the carnation, which is cultivated in different houses. The Caryophyllaceae family comprises over 80 genera and 3,000 species, which are mainly found in the Holarctic (i.e., temperate to arctic regions of North America and Eurasia) (Harbaugh *et al.,* 2010). Commonly referred to as carnations or pinks, Dianthus includes over 300 species recorded (Galbally & Galbally, 1997; Jurgens *et al.,* 2003). Carnation flowers are utilized for landscape borders, bedding, and pot planting flowers. Flowers are additionally beautiful cut flowers. France, Holland, Italy, Colombia, Kenya, Sri Lanka, the Canary Islands, the United States, and Germany are the leading countries that cultivate carnations. However, the countries that import the most carnations include France, the United Kingdom, the Netherlands, Israel, Italy, Spain, Peru, Greece, Mexico, and Ecuador. The vase quality and varied color of the petals make this crop highly profitable and in high demand internationally. This genus is significant due to its pharmacological and aromatic properties and is characterized by polymorphism in morphology, genetics, and hybridization (Hammett & McGeorge, 2002; Lee *et al.,* 2005; Yousef *et al.,* 2024).

Light is a critical environmental factor affecting plant growth and physiology, particularly influencing photosynthesis, stomatal development, and leaf morphology. Red and blue light are the two most effective bands for photosynthesis in plants among the several types of light (Hogewoning *et al.,* 2010). By changing the palisade and spongy mesophyll, the two lights have an impact on photosynthesis (Zheng & Van Labeke, 2017). The blue light enhanced stomatal conductance and photosynthetic efficiency, reduced leaf elongation, and stimulated the growth of chloroplasts and leaf tissue structure. Extended exposure to red light resulted in loosely arranged leaf palisade tissues, thinner grana lamellae, and shorter starch grains. It also prevented net photosynthesis. (Li *et al.,* 2021; Miao *et al.,* 2019). Stomatal density and index tend to increase with rising light intensity (Volenikova & Ticha, 2001; Omar & Mohammed, 2023).

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Additionally, photoperiod has a significant effect on the stomatal density of plants (Casson & Gray, 2008).

AL-Mizory and Hammo (2024) observed that the maximum number of leaves per plant and leaf area were recorded under 14-hour incandescent lighting, while Ouzounis *et al.* (2014) found that red and blue LED combinations increased leaf area in chrysanthemums. Supplementary lighting is the sole effective method for significantly enhancing the daily light integral (DLI) in such environments (Currey *et al.,* 2013). It has also been used to enhance plant growth and development (Wallace and Both, 2016). The morphology and physiology expressions vary in the light spectrum (Haliapas *et al.,* 2008; Ma *et al.,* 2001). Changes in the light can affect the leaf structure, palisade mesophyll cell thickness, and epidermis (Hogewoning *et al.,* 2010; Macedo *et al.,* 2011).

Stomatal characteristics such as density, size, and aperture are influenced not only by light quality and intensity but also by plant species and cultivar-specific genetic factors (Haryanti, 2010; Stefanova *et al.,* 2024; Tambaru, 2013). Sena *et al.* (2024) observed that the light quality (spectral arrangement) and quantity (photoperiod and intensity) influence plant growth and metabolism, and also interact with several factors, including environmental parameters, in defining the plant behavior. The Light Emitting Diode (LED) lights are extensively utilized in the cultivation of several plant species, especially horticultural plants, due to their lower power consumption and higher luminous efficiency compared to conventional fluorescent lights.

The objective of this study is to investigate the effects of different light sources and duration of exposure on the stomatal properties, including density, size, and aperture, of two different carnations (*Dianthus caryophyllus* L.) cultivars. The study specifically aims to comprehend how various light treatments influence the morphology and activity of stomata.

2. MATERIALS AND METHODS

This study was conducted in the greenhouse of the Horticulture Department nursery at the College of Agricultural Engineering Sciences, University of Duhok, Kurdistan Region, Iraq. The lighting treatments began in September and continued for three months. A PolyVinyl Chloride (PVC) frame was installed, divided into four sections using thick black cloth to prevent light contamination between treatments. Four supplementary lighting treatments were applied within the greenhouse as follows:

1. LED mixed light (blue, green, red) for 14 hours per day.
2. LED mixed light (blue, green, red) for 18 hours per day.
3. Incandescent light for 14 hours per day.
4. Incandescent light for 18 hours per day.
5. Natural light (control) under ambient light conditions.

Each lighting treatment was controlled by an automated electric timer, with lights turned on at 6:00 PM daily and maintained for the specified duration. Two carnation (*Dianthus caryophyllus*) cultivars were used:

- **Moonlight** (white flowers)
- **Ormea** (red flowers)

Data Were Recorded on the Following Parameters:

The Olympus microscope was used with a magnification of (10x) for stomatal density, stomatal Index, and stomatal frequency, and (40x) for stomatal sizes and types, subsequently. The microscope was equipped with an ocular and an object micrometer that had been standardised. Microscope slides of stomata were made using the replicate method. A Dino Capture 2.0 camera was used to take the photos of stomata under microscope observation. The sizes of stomata were categorized into length and width. The stomatal observation was from the upper and lower surfaces of the epidermis.

Stomatal Measurements:

Stomatal area (μm²), Stomatal size (μm), Stomatal length (μm), Stomatal width, pore area (μm²) (aperture), pore size (μm), pore length (μm), and pore width (μm). Measured according to the (ImageJ 1.52a) software (Fig. 1) (Edo & Al-Bamarny, 2020).

- Stomatal Density (mm^2) = $\frac{\text{Number of stomata}}{\text{Area (mm2)}}$
- Stomatal frequency (mm^2) = $\frac{\text{Number of stomata}}{\text{Field of view (mm2)}}$
- Stomatal Index % = $\left(\frac{\text{Number of stomata}}{\text{Number of stomata+Number of epidermal cells}}\right) * 100$

Where: Area $= \pi r^2$

Field of view = 0.5 mm^2

The stomatal density of the studied plant was evaluated by generating a nail polish leaf impression Xu and Zhou (2008) on a slide. Observation was done using a light microscope at a 40X objective lens. The area of the field of view was divided by the average number of stomata in a field of view to obtain the stomatal density (mm^2).

•**Morphological traits:** Number of leaves per plant**,** Leaf length (cm)**,** Leaf area (cm^2).

•**Stomatal characteristics:** Stomatal density (mm^2)**,** Stomatal number**,** Stomatal frequency (mm^2)**,** Stomatal index (%), Stomatal area (μm^2), Stomatal size (μm), Stomatal length (μm), Stomatal width (μm), Pore area (μm^2), Pore size (μm), Pore length (μm), and Pore width (μm).

Statistical analysis:

The experiment was conducted using a Randomised Complete Block Design (RCBD) with two factors. Each treatment consisted of three replicates, with four plants in each replication. Collected data were subjected to analysis of variance (ANOVA), and the mean values were assessed by Duncan Test at $P \leq 0.05$ using the program (SAS).

3. RESULTS

Effects of Supplementary Lighting on Leaf Morphological Characteristics in Two Carnation Cultivars:

Variations between the two carnation cultivars led to an increase in the number of leaves per plant, leaf length, and overall leaf area of the plant (Table 1). The Moonlight cultivar exhibited a significantly higher average number of leaves per plant (40.481) and greater leaf area (24.913 cm^2) compared to the Ormea cultivar (34.658 leaves and 21.248 cm^2, respectively). Conversely, the Ormea cultivar had significantly longer leaves, averaging 12.261 cm, compared to 11.317 cm in the Moonlight cultivar.

The maximum leaf number, leaf length, and leaf area per plant were recorded under 14h incandescent (42.872 leaves/plant, 13.050 cm, and 25.801cm2), respectively. The increases were significantly compared with all treatments and followed by 14h LED-mix (39.37 leaves/plant, 12.020 cm, and 24.218 cm2), respectively. They also increase significantly compared with each other and with the control, 18-hour incandescent, and 18-hour LED-mix conditions. The minimum number of leaves and leaf length were recorded at 34.417 leaves/plant and 10.983 cm under natural light. In the same Table, the leaf area under 18h

LED-mix light was recorded at (21.036 cm^2), which was the minimum leaf area recorded.

The interaction between cultivar and light treatment showed that the highest number of leaves, leaf length, and leaf area were achieved when the Moonlight cultivar was exposed to supplementary lighting (14 hours of incandescent light). Specifically, the Moonlight cultivar under 14 hours of incandescent light produced the most significant number of leaves per plant (48.244 leaves) and the largest leaf area (29.188 cm^2), with these results being significantly higher than all other treatments. Additionally, the Ormea cultivar exhibited the longest leaves (12.336 cm) when exposed to 14 hours of incandescent light, and this increase in leaf length was significantly greater than that observed in all other treatments.

Table 1: Effect of different supplementary light sources on leaf parameters of two *Dianthus caryophyllus* (carnation) cultivars.

Treatment		Leaves number	Leaf length (cm)	leaf area (cm^2)
cultivar	Moonlight	40.481[a]	11.317[b]	24.913[a]
	Ormea	34.658[b]	12.261[a]	21.248[b]
light	Natural	34.417[c]	10.983[c]	21.874[b]
	LED-Mix 14h	39.37[b]	12.020[b]	24.218[a]
	Incandescent 14h	42.872[a]	13.050[a]	25.801[a]
	LED-Mix 18h	36.087[c]	11.906[b]	22.473[b]
	Incandescent 18h	35.094[c]	10.988[c]	21.036[b]
Cultivar+ light				
Moonlight	Natural	36.244[cd]	10.502[d]	23.034[cd]
	LED-Mix 14h	42.044[b]	11.536[c]	26.016[b]
	Incandescent 14h	48.244[a]	12.336[b]	29.188[a]
	LED-Mix 18h	38.173[c]	11.394[c]	23.943[c]
	Incandescent 18h	37.700[c]	10.820[cd]	22.383[cd]
Ormea	Natural	32.589[e]	11.464[c]	20.715[de]
	LED-Mix 14h	36.711[c]	12.504[b]	22.419[cd]
	Incandescent 14h	37.500[c]	13.764[a]	22.413[cd]
	LED-Mix 18h	34.000[de]	12.419[b]	21.004[de]
	Incandescent 18h	32.489[e]	11.156[cd]	19.689[e]

- Means followed by the same letters within a column or row are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Effects of Supplementary Lighting on Stomatal Densities and Stomatal Number in Two Carnation Cultivars:

The study revealed that supplementary lighting had a significant influence on stomatal densities and numbers in both carnation cultivars (Table 2). Compared to natural light (control), all supplemental light treatments increased stomatal development. Among the two cultivars, the Moonlight cultivar showed significantly higher averages of stomatal density and number under supplementary lighting reached (86.26 and 13.38) in the lower and upper epidermis, respectively, compared to the Ormea, which had minimum values (77.693 and 10.847) in the lower and upper epidermis, respectively. Whereas the stomatal number per unit area, also the Moonlight cultivar had the best result observed (17.70 and 17.58) in upper and lower epidermis, respectively, and the increase was significant, compared to the Ormea cultivar, which gave the minimum values (14.904 and 14.884b) in lower and upper epidermis, respectively.

Regarding the supplementary light, LED-mix (red: blue: green) and incandescent light in different durations on stomatal densities and numbers in both carnation cultivars examined. Plants grown under 14-h incandescent light give the maximum result (96.034 and 15.750) was observed for stomatal densities in lower and upper epidermis and (18.426 and 18.167) stomatal number in lower and upper epidermis respectively and the increased was significantly, compared to the nature light which give the minimum results reached (73.166 and 8.545) stomatal densities in upper and lower epidermis and (14.667 and 12.944) stomatal number in lower and upper epidermis respectively.

The interaction between cultivar and light treatment revealed that greater stomatal densities and stomatal numbers could be obtained when the Moonlight cultivar was exposed to 14h of incandescent. The highest results of stomatal densities (96.482 and 95.587) in the lower epidermis were obtained from Ormea and Moonlight cultivars when exposed to 14 incandescent lights, and the increase was significantly different from all other treatments.

Table (2): Effect of different supplementary light sources on stomatal density (mm²) and stomatal number in two *Dianthus caryophyllus* (carnation) cultivars.

Treatment		Stomata density (mm²)		Stomata number	
		Lower Surface	Upper Surface	Lower Surface	Upper Surface
cultivar	Moonlight	86.26[a]	13.38[a]	17.70[a]	17.58[a]
	Ormea	77.693[b]	10.847[b]	14.90[4]	14.884[b]
Light	Natural	73.166[c]	8.545[c]	14.667[b]	12.944[b]
	LED-Mix 14h	83.910[b]	11.500[b]	16.517[ab]	16.000[ab]
	Incandescent 14h	96.034[a]	15.750[a]	18.426[a]	18.167[a]
	LED-Mix 18h	80.692[bc]	12.417[b]	15.389[b]	17.656[a]
	Incandescent 18h	76.083[bc]	12.367[b]	16.500[ab]	16.389[ab]
Cultivar+ light					
Moonlight	Natural	79.108[c]	8.590[c]	16.889[abc]	13.889[ab]
	Mix 14	91.705[ab]	13.000[abc]	17.811[ab]	18.444[a]
	Incandescent 14	95.587[a]	17.333[a]	19.000[a]	18.667[a]
	Mix 18	86.624[b]	14.167[ab]	16.778[abc]	18.778[a]
	Incandescent 18	78.283[c]	13.833a[bc]	18.000[ab]	18.111[a]
Ormea	Natural	67.225[d]	8.500[c]	12.444[c]	12.000[b]
	LED-Mix 14h	76.115	10.000[bc]	15.222[abc]	13.556[ab]
	Incandescent 14h	96.482[a]	14.167[ab]	17.852[ab]	17.667[a]
	LED-Mix 18h	74.760[cd]	10.667[bc]	14.000[bc]	16.533[ab]
	Incandescent 18h	73.884[cd]	10.900[bc]	15.000[abc]	14.667[ab]

- Means followed by the same letters within a column or row are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Effects of Supplementary Lighting on Stomata Frequency (Mm2) and Stomatal Index (%) in two Carnation Cultivars:

The study revealed that supplementary lighting had a significant influence on both stomatal frequency (mm²) and stomatal index (%) in the two carnation cultivars examined. Overall, plants grown under supplemental light treatments exhibited higher values than those grown under natural light conditions (Table 3). Among the two cultivars, Moonlight exhibited significantly higher average stomatal frequency (37.91 mm² lower, 38.56 mm² upper) and stomatal index (26.42% lower, 32.20% upper) compared to Ormea (28.151 mm² lower, 28.780 mm² upper; 24.024% and 26.476%, respectively).

The supplementary light treatments, including mixed (red: blue: green) and incandescent light in various durations, were applied to examine their effects on stomatal frequency and stomatal index in both carnation cultivars. Light affecting stomatal frequency experienced during growth and stomatal number in lower and upper epidermis (Figure. 2). Leaves grown under incandescent light give the maximum result observed (38.194 and 39.01 mm²) stomatal frequency in lower and upper epidermis (27.555 and 36.554%) stomatal index in lower and upper leaf epidermis respectively and the increased was significantly, compared to the nature light which give the minimum results reached (28.667 and 26.389mm²) stomatal densities in upper and lower epidermis (24.933 and 25.328%) stomatal index in lower and upper epidermis respectively.

The interaction between supplementary lighting and cultivar significantly influenced both stomata frequency (mm²) and stomatal index (%). Specifically, the response to supplementary lighting varied between the two carnation cultivars. About stomata Frequency the leaves growing under supplementary lighting the incandescent light at 14 h affected significantly and the maximum value (40.333 and 42.667 mm²) in lower and upper leaf epidermis for the Moonlight cultivars which exhibited a significant increase in stomata frequency compared to the control conditions, whereas Ormea cultivars showed a marginal or non-significant change (36.056 and 35.367 mm²) in lower and upper leaf epidermis under the same light. The difference in response indicates a cultivar-dependent effect of lighting on stomatal density (29.657 and 46.180%) in lower and upper leaf epidermis for the Moonlight cultivars which exhibited a significant increase in stomata frequency compared to the control conditions. In contrast, Ormea cultivars showed a marginal or non-significant change (25.453and 26.927mm²) in lower and upper leaf epidermis under the same light, while the upper leaf epidermis are the best from the lower leaf epidermis.

Table (3): Effect of different supplementary light sources on stomatal frequency (mm²) and stomatal index (%) in two *Dianthus caryophyllus* (carnation)

cultivars.**Treatment**		**Stomata frequency (mm²)**		**Stomatal Index (%)**	
		lower	**upper**	**lower**	**upper**
Cultivar	Moonlight	37.91[a]	38.56[a]	26.42a	32.20[a]
	Ormea	28.151[b]	28.780[b]	24.024[b]	26.476[b]
Light	Natural	28.667[c]	26.389[b]	24.933[b]	25.328[b]
	LED-Mix 14h	33.000[abc]	29.677[b]	25.581[ab]	29.441[b]
	Incandescent 14h	38.194[a]	39.017[a]	27.555[a]	36.554[a]
	LED-Mix 18h	30.611[bc]	36.811[a]	23.690[b]	28.666[b]
	Incandescent 18h	34.683[ab]	36.444[a]	24.352[b]	26.701[b]
Cultivar+ light					
Moonlight	Natural	33.778[ab]	30.111[cd]	24.952[b]	26.593[c]
	LED-Mix 14h	40.889[a]	36.889[abc]	26.293[ab]	33.877[b]
	Incandescent 14h	40.333[a]	42.667[a]	29.657[a]	46.180[a]
	LED-Mix 18h	33.889[ab]	40.222[ab]	25.516[ab]	28.142[bc]
	Incandescent 18h	40.667[a]	42.889[a]	25.688[ab]	26.210[c]
Ormea	Natural	23.556[c]	22.667[d]	24.915[b]	24.064[c]
	LED-Mix 14h	25.111[c]	22.464[d]	24.870[b]	25.005[c]
	Incandescent 14h	36.056[a]	35.367[abc]	25.453[ab]	26.927[c]
	LED-Mix 18h	27.333[bc]	33.400[bc]	21.865[b]	29.190[bc]
	Incandescent 18h	28.700[bc]	30.000[cd]	23.016[b]	27.191[c]

- Means followed by the same letters within a column or row are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Effects of supplementary lighting on sources of stomatal area (µm²), stomatal length (µm), stomatal width (µm), and stomatal size (µm²) in two carnation cultivars:

The application of supplementary lighting had a significant influence on stomatal traits in both carnation cultivars studied (Table 4). In the Moonlight cultivar, supplementary lighting increased significantly in stomatal area stomatal (254.10 µm²) (Figure 1-A) compared to the Ormea cultivar, which gave (182.233 µm²). In contrast, the stomatal length (Figure 1-B) and stomatal size did not increase significantly in the Ormea cultivar, with mean values rising from (21.646and 188.242 µm) compared with the Moonlight cultivar, which gave (18.98 and 183.45 µm). Stomatal width followed a similar trend, with cultivar Ormea exhibiting a significant increase from 11.290 µm. Similarly, the Moonlight cultivar exhibited a notable increase in stomatal width from 9.15 µm.

The supplementary light treatments, consisting of an LED mix (red:blue: green) and incandescent light at various durations, influenced stomatal characteristics. Leaves grown under incandescent light exhibited the highest values, with stomatal area reaching 289.306 µm². Correspondingly, stomatal length, stomatal width, and stomatal size were observed at (24.239µm, 11.983µm, and 281.167 µm²), respectively. This increase was significantly higher under 14h incandescent light compared with all treatments, especially with 18h incandescent, which produced the minimum measurements (stomatal area 150.594 µm² and stomatal length 17.989 µm), stomatal width, and stomatal size (9.292 µm and 114.386 µm²), respectively, under natural light conditions.

The statistical analysis revealed a significant interaction effect between cultivar type and supplementary lighting treatment on stomatal parameters. Non-significant increase in stomatal area under supplementary lighting at 14h incandescent was more pronounced in Moonlight cultivar, reaching (339.778 µm²) compared to Ormea cultivar (238.833 µm²). While the minimum stomatal area was observed by Moonlight and Ormea cultivars grown under natural light conditions, reaching (153.967and147.222 µm²). However, regarding stomatal Length and width, the supplementary lighting led to a significant increase in Ormea (24.978 and 13.389 µm) when grown under incandescent light and LED-mix light, at 14 hours, respectively, with stomata lengths of 24.53 and 13.06 cm. Similarly, a significant enhancement was noted in stomata size in both cultivars, Moonlight and Ormea, under incandescent light at 14 hours (322.833 and 257.633 µm², respectively). The lowest values of stomatal characters were observed under natural light conditions and incandescent light at 18 hours for two cultivars.

Table (4): Effect of different supplementary light sources on stomatal area (µm²), stomatal length (µm), stomatal width (µm), and stomatal size (µm²) in two *Dianthus caryophyllus* (carnation) cultivars.

Treatment		Stomatal area (µm²)	Stomatal length (µm)	Stomatal width (µm)	stomata size (µm)
cultivar	Moonlight	254.10^{a}	18.98^{a}	9.15^{b}	183.45^{a}
	Ormea	182.233^{b}	21.646^{a}	11.290^{a}	188.242^{a}
light	Natural	177.722^{bc}	19.353^{bc}	9.292^{b}	114.386^{e}
	LED-Mix 14h	255.389^{ab}	20.672^{b}	11.183^{ab}	199.233^{b}
	Incandescent 14h	289.306^{a}	24.239^{a}	11.983^{a}	281.167^{a}
	LED-Mix 18h	217.828^{abc}	19.306^{bc}	9.189^{b}	176.365^{c}
	Incandescent 18h	150.594^{c}	17.989^{c}	9.456^{b}	158.067^{d}
Cultivar+ light					
Moonlight	Natural	194.333^{cd}	18.800^{ab}	8.477^{b}	146.827
	LED-Mix 14h	308.111^{ab}	16.811^{b}	8.978^{b}	140.833^{f}
	Incandescent 14h	339.778^{a}	23.500^{ab}	10.900^{ab}	322.833^{a}
	LED-Mix 18h	274.322^{abc}	18.556^{ab}	8.556^{b}	151.833^{ef}
	Incandescent 18h	153.967^{d}	17.222^{b}	8.844^{b}	154.900^{ef}
Ormea	Natural	161.111^{cd}	19.906^{ab}	10.107^{b}	81.944^{g}
	LED-Mix 14h	202.667^{a-d}	24.533^{a}	13.389^{a}	257.633^{b}
	Incandescent 14h	238.833^{a-d}	24.978^{a}	13.067^{a}	239.500^{c}
	LED-Mix 18h	161.333^{cd}	20.056^{ab}	9.822^{b}	200.897^{d}
	Incandescent 18h	147.222^{d}	18.756^{ab}	10.068^{b}	161.233^{e}

- Means followed by the same letters within a column or row are not different significantly according to Duncan's Multiple Range Test ($P \leq 0.05$).

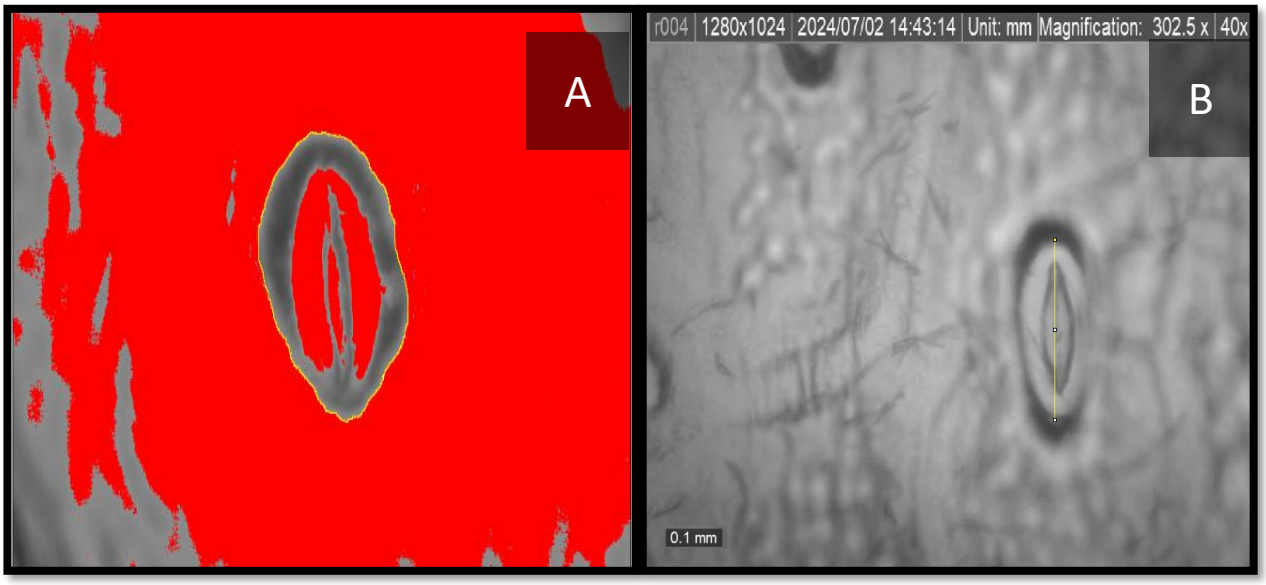

Figure 1: Scanning electron micrographs showing the stomata on carnation (*Dianthus caryophyllus*) leaves via ImageJ 1.52a pro. (A) Stomatal area. (B) Stomatal length. Images captured using a 40× objective lens; field of view = 0.1 mm.

Effects of Supplementary Lighting on Pore Parameter in two Carnation Cultivars:

Table 5 illustrates the effect of cultivars influenced by pore characteristics such as pore length, pore width, pore size, and pore area in both carnation cultivars examined. The highest significant means were recorded by the Ormea cultivar for pore length, pore width, pore size, and pore area, which were (12.600 µm, 5.848 µm, 46.192 µm², and 39.361 µm2), respectively, compared to Moonlight.

Supplementary lighting resulted in a notable and significant increase in pore length, pore width, pore size, and pore area. The highest mean value recorded (13.828 µm, 6.421µm, 48.939 µm, and 47.294 µm^2). Interestingly, pore area peaked at 49.556 µm² under 14h LED-mix light, showing that different light sources influence different stomatal traits. In contrast, the lowest values were consistently recorded under 18-hour LED-mix and incandescent light, with the lowest pore width (3.411 µm) and smallest pore size (34.017 µm²) observed under the 18h incandescent light treatment. These findings indicate that longer light exposure may not necessarily promote better pore development and could even reduce it.

Regarding the effect of the interaction between different lights and cultivars the maximum mean value for pore characteristics such as pore length, pore width and pore size in both carnations were recorded when Ormea and Moonlight cultivar exposed to the 14h incandescent light but Ormea cultivar recorded maximum significant result (15.433, 7.737 and 55.611 µm) for pore length, pore width and pore size parameter respectively under 14h incandescent supplemented light follow by Moonlight cultivar (12.222, 5.106 and 42.267 µm) for the same parameter and same supplemented light. The minimum values recorded by the Moonlight cultivar when exposed to the 18h incandescent light were 9.189 µm for pore length and 3.611 µm for pore width. Also, the pore size recorded the lowest result (24.833 µm) for the same cultivar but when exposed to 18h LED-mix light. Whereas the pore areas recorded the maximum principal value reached (57.111 µm^2) when the Moonlight cultivar was exposed to the 14h incandescent supplemented light, followed by Ormea cultivar, which gave the highest result (46.222 µm^2) when the plant was exposed to 14h Mix supplemented light, and the minimum values
observed for Ormea cultivar under 18h 14h LED-mix light.

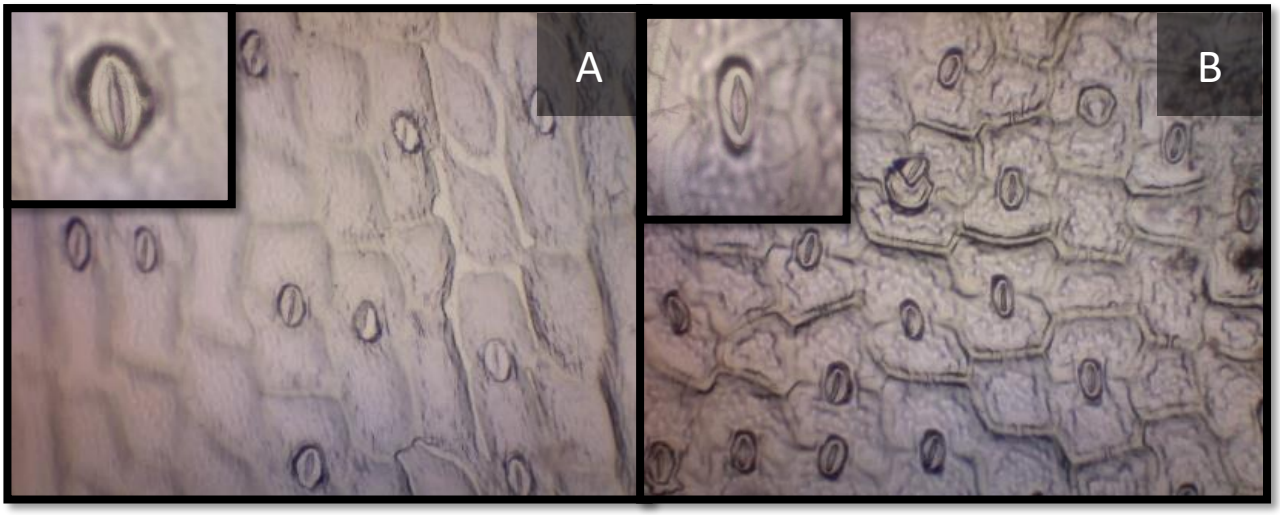

Figure 2: Scanning electron micrographs showing the distribution of stomata on carnation (*Dianthus caryophyllus*) leaves. (A) Stomata on the upper leaf epidermis. (B) Stomata on the lower leaf epidermis. Images captured using a 10× objective lens; field of view = 0.5 mm.

Table (5): Effect of different supplementary light sources on pore length (µm), pore width (µm), pore size (µm²), and pore area (µm²) in two *Dianthus caryophyllus* (carnation) cultivars.

Treatment		Pore length (µm)	Pore Width (µm)	Pore size (µm)	Pore area (µm^2)
Cultivar	Moonlight	10.28^{b}	4.17^{b}	35.51^{b}	47.28^{a}
	Ormea	12.600^{a}	5.848^{a}	46.192^{a}	39.361^{b}
Light	Natural	11.256^{ab}	5.096^{bc}	43.125^{b}	40.435^{a}
	LED-Mix 14h	10.978^{ab}	5.887^{ab}	43.333^{b}	49.556^{a}
	Incandescent 14h	13.828^{a}	6.421^{a}	48.939^{a}	47.294^{a}
	LED-Mix 18h	11.066^{ab}	3.411^{d}	34.833^{c}	39.016^{a}
	Incandescent 18h	10.072^{b}	4.233^{cd}	34.017^{c}	40.298^{a}
Cultivar+ light					
Moonlight	Natural	10.552^{bcd}	4.363^{cde}	40.167^{cd}	41.552^{bc}
	LED-Mix 14h	9.522^{d}	4.834^{bcd}	38.500^{cde}	52.889^{ab}
	Incandescent 14h	12.222^{b}	5.106^{bc}	42.267	57.111^{a}
	LED-Mix 18h	9.911^{cd}	2.944^{f}	24.833^{f}	41.331^{bc}
	Incandescent 18h	9.189^{d}	3.611^{ef}	31.767^{ef}	43.508^{abc}
Ormea	Natural	11.959^{bc}	5.829^{b}	46.083^{bc}	39.318^{bc}
	LED-Mix 14h	12.433^{b}	6.940^{a}	48.167^{b}	46.222^{abc}

	Incandescent 14h	15.433[a]	7.737[a]	55.611[a]	37.478[bc]
	LED-Mix 18h	12.220[b]	3.878[def]	44.833[bc]	36.700[c]
	Incandescent 18h	10.956[bcd]	4.856[bcd]	36.267[de]	37.089[c]

- Means followed by the same letters within a column or row are not different significantly according to Duncan's Multiple Range Test ($P \leq 0.05$).

4. DISCUSSION

In this study, the growth of carnation cultivars under incandescent and mixed LED lighting conditions led to the greatest leaf parameter relative to the other light treatments (Table1). Similar variation in carnations was also observed by Patil (2001), and Shiragur (2002). These variations might be due to the increased the number of leaves and leaf length, which in turn helped in maintaining a higher leaf area, which ultimately might have increased the dry matter production per plant in such superior genotypes (Gurav *et al.,* 2004). in Carnation. Also, the growth of carnation cultivars under incandescent and mixed LED lighting conditions led to the greatest stomatal density relative to the other light treatments (Tables 2 and 3). This finding aligns with previous research indicating that broad-spectrum light, such as white LEDs, can increase stomatal density, stomatal frequency, and stomatal index (Seif *et al.,* 2021; Do Nascimento Vieira *et al.,* 2015). Additionally, studies have shown that plants exposed to broad light spectra generally develop higher stomatal density than those exposed to monochromatic light spectra (Lee *et al.,* 2007; Savvides *et al.,* 2012). The variation in stomatal density among cultivars primarily results from their adaptation to environmental conditions (Xu and Zhou, 2008). In our current study, we observed that species exposed to supplementary light exhibited higher stomatal density, reflecting their adaptation to increased light availability (natural light), In contrast, species adapted to lower light intensities displayed reduced stomatal density. Multiple studies have demonstrated positive correlations between light intensity, stomatal conductance, and photosynthetic performance (Fanourakis *et al.,* 2019; Sakhonwasee *et al.,* 2017; Yang *et al.,* 2020).

Stomatal influenced by many factors like environmental variables, vapor pressure deficit, is also regulated by light intensity. Stomatal morphology depends on the lighting environment (Ghorbanzadeh *et al.,* 2021; Seif *et al.,* 2021). Higher light intensity enhances both stomatal size and pore dimensions. As shown in Table 4, the overall pore area significantly increased with rising growth light intensity. This increase was primarily due to larger individual pore areas per stomatal density remaining relatively unchanged with supplementary lighting during growth (Tables 4 and 5). The expansion in pore size per stoma was at least partly attributable to the larger stomatal size (Tables 4 and 5), since larger stomata tend to have bigger pores (Fanourakis *et al.,* 2014). Additionally, previous studies have also observed an increase in stomatal size with higher irradiance in other plant species (Bell & James, 2000; Lawson, & Matthews (2020). Stomata, the pores on the plant's epidermis, are key innovations of land plants (Bergmann &Vatén 2012; Hetherington & Woodward 2003;). Through regulating their aperture and number, higher plants gain control over carbon uptake and water usage, allowing adaptation to diverse climates and habitats. Drake *et al.,* (2013) demonstrated that leaves with smaller size and a higher density of stomata enhance gas exchange relative to water use, thereby supporting increased photosynthesis. Similarly, Silva *et al.,* (2014) noted that an increase in stomatal number coupled with reduced stomatal size facilitates plant adaptation to arid environments. Drake *et al.,* (2013) and Silva *et al*., (2014) conducted research that aligned in emphasizing the significance of smaller leaf size and a greater number of stomata for enhanced photosynthesis and transpiration. Haryanti (2010) mentioned that the number of stomata could affect the rate of transpiration in the leaves. In addition, Izza and Laily (2015) noted that the number of stomata is closely linked to transpiration activity, as the majority of transpiration occurs through stomata.

CONCLUSION

This study demonstrates that both light source type and exposure duration significantly influence stomatal features in two cultivars of Carnation (*Dianthus caryophyllus* L.). Specifically, incandescent lighting generally promotes favourable stomatal development characterized by optimal density and size compared to LED mixed light. Extended exposure durations, however, induced notable alterations in stomatal morphology and affected negative, which may impact gas exchange efficiency. Additionally, the two cultivars responded distinctly to these environmental factors. These findings suggest that optimizing incandescent lighting regimes and exposure durations can enhance stomatal functioning in carnation cultivation.

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Conflicts of Interest:

The authors declare no conflicts of interest.

Ethical Approval:

This study was conducted in a plant, as it did not involve any experiments or interventions on humans or animals.

Contributions of Authors:

L.S.M.A: Sample collection, Data collection, Write the manuscript. Statistical analysis, read and revise the manuscript
E. N. Y: labour work, leaf anatomy, and read the manuscript
S. A: State key laboratory of tree genetics and breeding.

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