

The effect of light intensity on the stomatal density of lavender, *Lavandula angustifolia*



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ABSTRACT

The first aim of this investigation was to find whether there is a significant correlation between the stomatal density of lavender plants and the light intensity under which they are grown. The second aim of the investigation was to find out whether the initial height of the plant influences its stomatal density. Cuttings were taken from lavender plants to ensure that all the plants were genetically identical and that the only changes occurring in the stomatal density would be due to environmental conditions. Four cuttings were short (3 cm initial height) and four were tall (6 cm initial height). The cuttings were put under compact fluorescent light bulbs with four different power ratings (8, 11, 14, and 20 W). One short and one tall cutting were put under each of the four light bulbs for 28 days in order to grow them. Both the short and the tall plants showed a positive correlation between their stomatal densities and the light intensity. The correlation was statistically significant at a 0.025 significance level according to the Pearson product-moment correlation test. The short and the tall plants grown under the same light intensity did not show any statistically significant difference between their stomatal densities.

Introduction

Stomata are tiny pores found on the epidermis of the leaf, surrounded by guard cells.^[1] Their main function is gas exchange^[1] for photosynthesis and respiration. The development of stomata on the leaves of a plant is determined by interaction between different genes and environmental factors. A few studies have been conducted in order to establish a relationship between stomatal densities and given environmental factors. Research has shown that stomatal densities are controlled by environmental conditions during leaf development, but are fixed after the leaf matures.^[2]

The article “The influence of light on stomatal density of a tomato” by A. P. Gay and R. G. Hurd describes their findings that plants grown under high light

intensity have more stomata per 1 mm² than plants grown under low light intensity.^[3]

The purpose of my investigation is to determine whether there is a correlation between the light intensity and the stomatal density on lavender leaves and whether the initial height of the plants influences the stomatal densities. The hypothesis is that an increase in the light intensity will lead to an increase in the stomatal density of the lavender leaf.

Materials and Methods

Materials

- Four plant pots with soil
- Rooting hormone
- Lavender plant

- Sterilized scissors
- Clear plastic bags
- Four fluorescent lamps of power ratings 8, 11, 14, and 20 W
- Wooden box with four separate sections; the box dimensions are 0.5 m height by 1.0 m width
- Microscope
- Cover slips and microscope slides
- Nail polish and cellophane tape
- White correction fluid
- Cellophane tape

Methods

1. Take cuttings from a single lavender plant. Use sterilized scissors and cut the branches of the lavender plant at an angle of 45°.
2. Take eight cuttings in a way that four of them should be with an initial height of 3 cm (short plants) and four of them with an initial height of 6 cm (tall plants).
3. Dip the cut end of the cutting in a root hormone and place it in a pot containing soil. Place one short and one tall plant in a single flowerpot. Put a clear plastic bag, with a few holes in it, over the pot. Place the four flowerpots near the window, so that they are exposed to sunlight.
4. Water the plants every day, but not directly in the pot. Put the water in a small tray below the pot.
5. Keep the plants for 2 weeks before they develop roots. You can check whether roots have been developed by gently pulling the plant. Resistance when pulling indicates that roots have developed.
6. After the two initial weeks have passed, remove the plastic bags from the plant pots.
7. Place the flowerpots in a wooden box with four separate sections. Each of the four sections should be illuminated by a fluorescent light bulb of different power [Figure 1].
8. Leave only four old leaves on each plant and put a tiny correction fluid dot on them to mark them.
9. Switch the lights on for an average of 12 h each day. Water the plants with the same volume of water each time. Keep the plants under the lights for 28 days or until new leaves develop.
10. Collect all the new leaves formed under the light bulbs.
11. Paint a thick patch (few millimeters) of nail polish on the lower epidermis of each leaf.
12. Allow the nail polish to dry completely.^[4]
13. Tape a piece of clean cellophane tape to the dried nail polish patch.^[4]
14. Gently peel the nail polish patch from the leaf by pulling on a corner of the tape and “peeling” the

polish off the leaf. This is the leaf impression you will examine.^[4]

15. Put the leaf impression on a clean microscope slide and put a cover slip over the leaf impression.
16. Put the prepared slide on a lit microscope stage.
17. Observe the leaf impression under low magnification.
18. Adjust the light, the fine and coarse focus until a clear image of the leaf can be seen.
19. Change to a higher magnification of about 640×; stomata should appear, looking like tiny pores on the leaf [Figure 2].
20. Count the stomata present in the field view at 640× magnification for short and tall plants grown at the same light intensity and different light intensities.
21. Record your results in a table.
22. You require 10 readings for each short and tall plant grown under the same light intensity. This will ensure you have enough data when performing the Mann–Whitney test to look for significant difference in stomatal density between short and tall plants grown under the same light intensity.

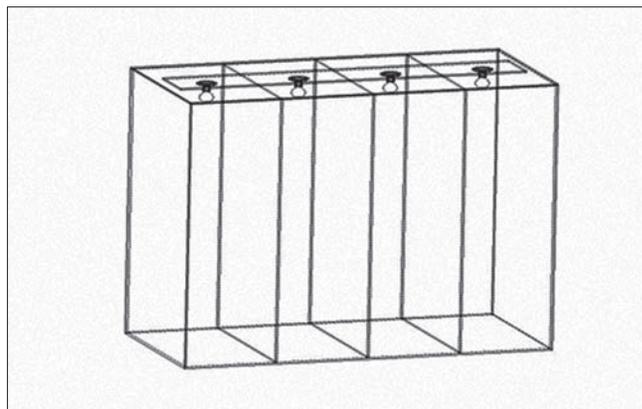


Figure 1: The wooden box with the separations and the light bulbs

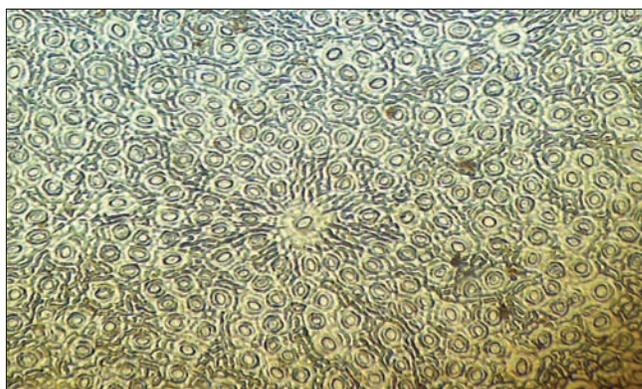


Figure 2: Nail polish impression of stomata (http://www.google.co.uk/imgres?imgurl=http://upload.wikimedia.org/wikipedia/en/d/de/Nail_polish_impression_of_stomata.jpg)

23. Plot a graph of light intensity on the x-axis against stomatal density on the y-axis for the short plants and tall plants separately.
24. To calculate the stomatal density, which is number of stomata in a given area (e.g. 1 mm²), you must first find the microscope field view.
25. To find the field view, place a clear plastic ruler under low magnification (e.g. 40×).
26. Count the millimeter spaces you can see at that magnification;^[5] this is the diameter of the field view at 40' magnification.
27. To calculate field view at 640' magnification, use the formula:^[5]

$$\frac{\text{high power field of view}}{\text{low power field of view}} = \frac{\text{low power magnification}}{\text{high power magnification}}$$

28. The field view under high magnification gives you the diameter of the field view.

To calculate the area, use the formula:

$$\text{Area} = \frac{\pi d^2}{4}, \text{ where "d" is the diameter of the field view.}$$

In the investigation, there are variables that should be kept as controls because otherwise they may affect the investigation. The volume of water that the plants receive and how often they are watered can easily be controlled. Other confounding variables such as carbon dioxide concentration can be controlled in laboratory conditions only. However, in the experiment I conducted, carbon dioxide concentration would not be expected to vary a great deal.

Results

Using the described method, the following results were collected [Table 1] and underwent statistical analysis (below).

Pearson's correlation coefficient^[6]

- 0.978 for short plants
- 0.979 for tall plants

Mann–Whitney U test^[7]

- 8 W/m², U = 44.0
- 11 W/m², U = 48.0
- 14 W/m², U = 41.5
- 20 W/m², U = 51.5

Discussion

In order to establish a correlation between the independent and the dependent variables, Pearson product-moment correlation was used for both the short and the tall plants. The null hypothesis is that there is no statistically significant correlation between the light intensity and the stomatal density on plants. To reject the null hypothesis, the calculated value for Pearson's correlation coefficient must be greater than the critical value. The critical value for four sets of data at a 0.025 significant level is 0.9500.^[6] The values for the Pearson's correlation coefficient for the tall and the short plants are both greater than the critical value; therefore, the null hypothesis can be rejected at a 0.025 significant level. Therefore, there is a correlation between the stomatal density and the light intensity. The correlation is positive as can be seen from Figures 3a and b.

When trying to explain the correlation, it is important to consider what stomata are in the first place and what their most important functions are. Stomata are tiny pores^[1] found on the epidermis of the plants and their main role is gas exchange between the leaf and the environment. Although stomatal development is essentially controlled by different genes, the environment also has a significant effect on stomatal development.^[8] Using plants that are clones in the investigation means that they all have the same genetic material and any changes in stomatal density on their leaves should be due to environmental

Table 1: Results showing stomatal densities for both short and tall lavenders under different light intensities

Light intensity (W/m ²)	Initial height (cm)	Stomata under 640×										Average stomata number	Stomatal density in 1 mm ² area
		6	7	7	9	9	8	8	7	8	7		
8	Short	6	7	7	9	9	8	8	7	8	7	7.6	275
	Tall	8	6	8	7	9	7	8	9	7	9	7.8	283
11	Short	13	14	11	12	12	10	11	11	10	11	11.5	417
	Tall	10	11	13	12	11	11	12	11	12	11	11.4	413
14	Short	15	14	15	16	15	16	14	15	16	14	15.0	544
	Tall	14	15	15	16	16	17	15	16	15	14	15.3	554
20	Short	19	18	19	18	17	20	19	18	18	18	18.4	667
	Tall	18	18	19	18	19	18	17	22	19	19	18.7	678

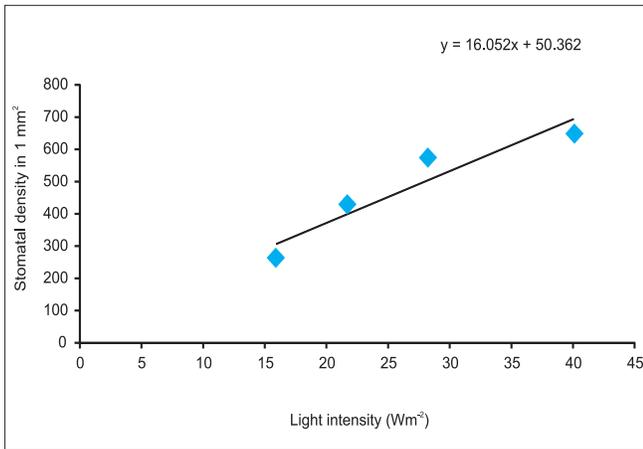


Figure 3a: A graph showing stomatal density against light intensity for the short plants

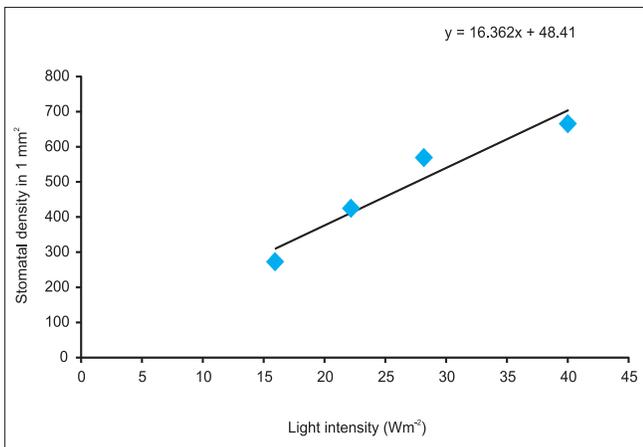


Figure 3b: A graph showing stomatal density against light intensity for the tall plants

factors.^[9] Both light intensity and carbon dioxide concentration have been shown to influence the frequency at which stomata develop on the leaves of plants.^[8] Plants can respond to changes in environmental conditions by changing their stomatal frequency. Recent research has shown that signals from older leaves can influence the development of stomata on the younger leaves.^[10] In that way, if the environmental conditions to which the older leaves are exposed change, then the younger leaves can increase or decrease their stomatal density; this physiological adaptation can help the plant cope with the changing environment.

Why is the increased light intensity leading to increased stomatal density? Photosynthesis is the process by which plants synthesize glucose from carbon dioxide and water. The energy of the reaction is supplied by the sunlight. However, there are two main stages in photosynthesis – light-dependent

and light-independent stages.^[11] The light-dependent stage depends on the light because the energy from the light is used to split water in the process of photolysis and excite electrons in the chlorophyll.^[11] The products from the light-dependent stage are ATP and the electron acceptor – reduced NADP.^[11] The products from the light-dependent stage are fed into the light-independent stage of photosynthesis, the Calvin cycle.^[11] Carbon dioxide is fixed in the light-independent stage and converted to glucose; in the Calvin cycle, the products of the light-dependent stage are needed. So, more ATP and reduced NADP will result in an increased rate of carbon fixation. If the rate of carbon fixation increases, the rate at which carbon dioxide diffuses in and out of the leaf will increase.

The light intensity is simply the energy per second per unit area carried by the incident light and it is proportional to the number of photons per second carried by the incident light.^[12] Higher light intensity means more photons per second resulting in more electrons per second that would be excited during the light-dependent stage of photosynthesis, and more ATP and reduced NADP are produced. Therefore, increasing the light intensity will increase the overall rate of photosynthesis. The rate of gas exchange will increase as a result.

Coming back to the main function of the stomata, increasing the rate of gas exchange may lead to increased stomatal density on the epidermis of the leaf. The adaptation leads to higher carbon dioxide assimilation as the results of recent studies have shown.^[2]

However, the energy of the incident light arriving per second is also proportional to the wavelength of the light. Therefore, the light intensity depends on the light wavelength. Plants have combinations of chlorophyll pigments^[11] that absorb sunlight from the visible spectrum. The light of wavelengths 400–500 nm and 650–700 nm^[11] is absorbed the most. These are blue and red light, respectively. Lavender grows well under compact fluorescent light bulbs.^[13] By placing colored filters in front of the light bulbs, it can be established which color of light is most suitable for growing lavender and whether the color of light affects the stomatal density.

To determine whether there is a statistically significant difference between the stomatal densities on the tall and short plants grown under the same light intensity,

the Mann–Whitney U test is used. The null hypothesis is that there is no statistically significant difference between the stomatal densities of the tall and the short plants grown under the same light intensity. The null hypothesis may be rejected if the calculated value of U is equal to or smaller than the critical value. The critical value for U for 10 sets of data is 16.^[7] Looking back at the results section, all the calculated values of U are bigger than the critical value, so the null hypothesis is accepted. The initial height did not seem to influence the stomatal development in my investigation.

Conclusion

Stomata are tiny pores found on the epidermis of the leaf and they are important for gas exchange between the plants and the environment. Their development is determined both by genes and the environmental conditions. The investigation showed a positive correlation, which was statistically significant at 0.025 level between the stomatal density on lavender plants and the light intensity. The initial height of the plants did not seem to affect the stomatal density and there was no statistically significant difference between the stomatal density on the short and the tall plants grown under the same light intensity.

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About the Author

Yoana Petrova finished college this summer and completed an A Level in Biology, Chemistry, and Physics. During her second year, she did an extended project and this is what her article is based upon. Her plans for the future are to study Biochemistry at university and become a researcher or lecturer in biochemistry/chemistry or biology. At the moment, she is on her gap year and is working as a pharmacy assistant. Her job allows her to learn about the different medicines which is very interesting and could be useful in her future career. She does many activities in her free time such as rock climbing, ice skating, snowboarding, skiing, cycling, jogging, and reading books.