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DIFFERENT LIGHTING CONDITIONS AFFECT THE GERMINATION AND SEEDLING DEVELOPMENT OF TWO VARIETIES OF SHISO

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ABSTRACT

Shiso (*Perilla frutescens* L.) is an Asian plant that contains properties that allow it to be used in both cooking and medicine. The present study set out to understand how different lighting conditions affect the germination and development of shiso seedlings of the purple and green varieties. Germination stabilization occurred 15 days after sowing (DAS), accounting for the environment with 70% shading 84 germinations of the purple variety and 34 germinations of the green variety. While, in the full sunshine treatment, germinations were much lower, however, the seedlings did not remain viable for monitoring the development of seedlings in full sunshine. After stabilization of germination, the following were evaluated: specific leaf area, diameter of the neck, height and number of leaves and but after 35 DAS there was a decrease in the production of biomass by both varieties.

Keywords: *Perilla frutescens* L., shiso, full sunshine, lighting conditions

INTRODUCTION

Perilla frutescens L., is an annual plant of Asian origin (DHYANI et al., 2019) commonly used in oriental cuisine, has been cultivated in Brazil in recent years, mainly in places close to Japanese colonies, such as São Paulo (BRENNER, 1993). Its usages are diverse, being consumed around the world as food for human and poultry consumption; as fuel oil, drying oil or food oil; its leaves as food dyes; or in medicinal methods (BRENNER, 1993; LU et al., 2017).

Also known as shiso, the seeds of *P. frutescens* have from 31 to 51% of a drying oil that can be applied as paints, inks for printers, coating for waterproofing fabrics, fuels, cooking oil, among others

(JAMIESON, 1943). Recently, it was discovered, through research, that the shiso oil has benefits when applied to control the cigarette beetle, an insect that damages several stored products, including a special one, with great added value, the tobacco (HORI, 2004).

The attention to the medicinal properties of shiso started because of its pharmacological effects as antioxidant, antibacterial, anti-atherosclerotic and anticarcinogenic (ŽEKONIS, 2008). Due to this, its seeds have been used over the years in kampo medicine, a traditional Japanese medicine known for the application of techniques that seek the combination of herbs and acupuncture for the treatment of its patients (LEVI et al., 2013).

The presence of the fatty acid α-linolenic acid in shiso seed oil positively influences the prevention of cardiovascular diseases by acting to reduce blood cholesterol levels (GALLI & MARANGONI, 2006). Research on rats exposed to diabetes mellitus has found that drinking shiso tea is effective in preventing diabetes (KISHI et al., 2010). Another research conducted in and out of hospitals in Japan has also shown some effectiveness in the use of shiso in the treatment of allergic rhinitis (YONEKURA et al., 2016).

Thus, due to its numerous applications, the shiso has a great economic potential, however, in Brazil, researches are still incipient regarding the ideal growing conditions, its growth and development, as in tropical climate. Thus, the present study set out to understand how different light conditions affect the germination and development of shiso seedlings of the purple and green varieties.

MATERIALS AND METHODS

For the experiment, seeds of shiso (*Perilla frutescens* L.) of the purple and green varieties were used. The experiment was developed in a greenhouse (70% shade) and in full sunshine, both on the Gragoatá campus of Universidade Federal Fluminense (UFF), located in Niterói, Rio de Janeiro State, Brazil, with the geographic coordinates (22º 54’00” S; 43º 08’ 00” W; altitude of 8 meters) and, according to the Köppen classification, the region has an Aw climate, meaning tropical climate with dry winter and rainy summer, with a mean annual temperature of 23ºC and a mean annual precipitation of 1200 mm.

First the germination percentage was obtained and then the seedlings development was monitored until transplanting. For germination, two germination trays were used per environment (70% shading and full sunshine), one for each variety, for a total of four trays. Each styrofoam tray contained

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200 cells (10 x 20), containing one seed per cell and the substrate used was Geolia® "Terra Vegetal", keeping the substrate irrigated at field capacity.

The germination percentage was measured daily until the fifteenth day after sowing, when germination stabilized. The germination percentage was calculated by the formula proposed in the Rules for Seed Analysis (BRASIL, 2009) and the germination speed index (GSI) according to the equation (EQ(1)) proposed by Maguire (1962) adapted from Moraes et al. (2012).

$$GSI = (G1/N1) + (G2/N2) + \ldots + (Gn/Nn)$$

EQ(1) Where:

G1, G2, Gn= number of germinated seeds on the first, second and last count

N1, N2, Nn= days after sowing on the first, second and last count.

After stabilization of germination (15 days after sowing) five plants were randomly selected per treatment for weekly monitoring of growth parameters for five weeks. The following were analyzed: (i). specific leaf area (length x width) (cm); (ii). neck diameter (mm); (iii). height (cm) and; (iv). number of leaves (units). For the analysis of specific leaf area and neck diameter a pachymeter was used. A ruler was used to measure the height and the leaf counting was done manually. These analyses started at 21 days after sowing (DAS), ending at 49 days after sowing.

An IRRIPLUS® weather station, model E5000, was used to collect climatological data. From the weather station the following data were obtained: maximum and minimum temperature (ºC), relative humidity (%) and total daily radiation ($MJ.m^{-2}. day^{-1}$) for the period from November to January. The data obtained were recorded and treated in EXCEL software.

The adopted design was a randomized block design with two varieties (purple and green) x 5 days with 5 repetitions (for growth parameters), and the data were submitted to analysis of variance (ANOVA) with Tukey's test at 5% and Student's t test at 5%, using the Sisvar software.

RESULTS AND DISCUSSION

The first germinations were observed in the environment with 70% shade conditions and for the purple variety, which already showed 6 germinations at 4 days after sowing (DAS) (Figure 1). The next day, at 5 DAS, germination also occurred in the green variety Germination in the full sunshine treatment started at 12 DAS, with 12 germinated seedlings for the purple variety and two for the green variety.

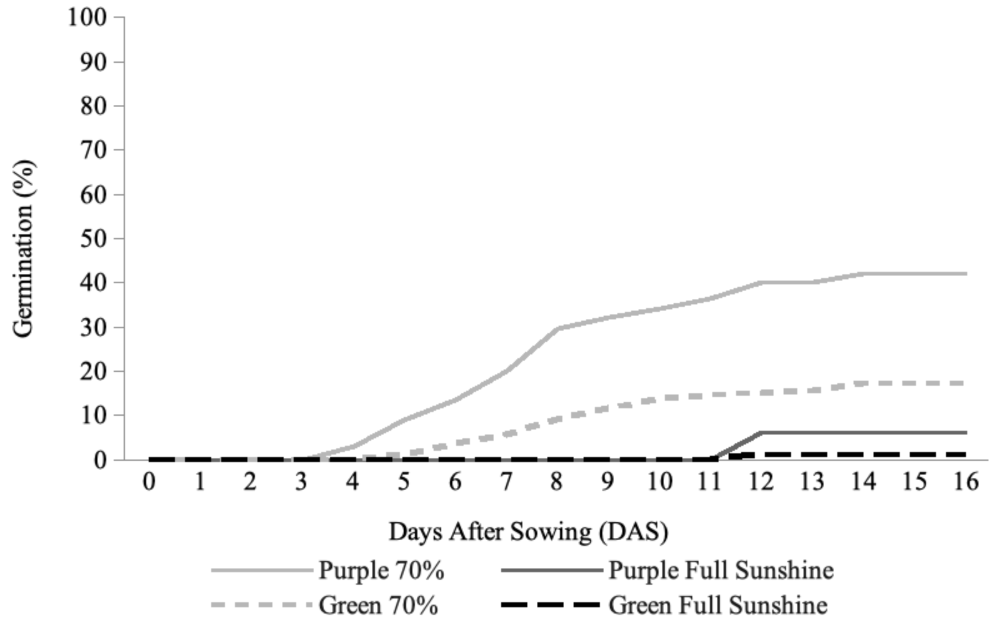

Figure 1. Germination percentage of *Perilla frutescens* varieties in different growing environment.

The low percentage of germination in the full sunshine treatment was probably due to the environmental conditions to which it was subjected, since, according to Mendes and Carvalho (2015), abiotic factors such as water and light have high relevance in the germination process. The seeds from this treatment germinated late, and later, when they germinated, they were already wilted, indicating less acclimation of the seedlings of this species to full sunshine environments when under tropical conditions.

The delayed beginning germination in full sunshine was greater for the green variety when compared to the purple variety, which also showed a lower germination percentage when comparing the same environment between the varieties. At 70% shading, the purple variety showed higher germination, outperforming the green variety by 59%. In full sunshine, the purple variety also outperformed the green variety by a rate of 83%. When comparing the same variety under different luminosities, the purple variety obtained 86% higher germination at 70% shading while the green variety reached a percentage of 94% at 70% shading when compared to full sun (Table 1). Thus, with germination stabilization, 15 days after sowing, 84 germinations of the purple variety and 34 germinations of the green variety were observed for the 70% shading treatment, representing 42% and 17% respectively. While, in the full

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sunshine treatment, germinations were much lower, totaling 12 germinations for the purple variety (6%) and two for the green variety (1%).

Tabela 1. Germinations sum of green and purple varieties for the different growing environments.

	Full sunshine	70% shading
Purple shiso	12*	84
Green shiso	2	34

*Germinations sum for each treatment

Regarding the Germination Speed Index (GSI), analyzing the two treatments separately, it can be seen that the seeds of the green variety are more affected when grown in full sunshine than under 70% shading, as well as the seeds of the purple variety (Table 2). Furthermore, in the full sunshine treatment there was no statistical difference between the two varieties. However, there was a statistical difference between the purple and green varieties in the 70% shading treatment, with the seeds of purple variety showing a significant effect to this treatment.

Table 2. Germination Speed Index (GSI) of *Perilla frutescens* varieties in different growing environments.

	Full sunshine	70% shading
Purple shiso	5,66 Ba*	70,60 Aa
Green shiso	0,94 Ba	25,92 Ab

*Capital letters compare means among shading treatments, in the row, and lowercase letters compare means among varieties, in the column. Averages followed by equal letters do not differ statistically using the Tukey test ($P \leq 0{,}05$).

The low Germination Speed Index (GSI) values for the seeds in the full sunshine treatment can be explained by climatological data, because the seeds were more exposed to abiotic stresses such as the high variation in the level of solar radiation, in addition to direct exposure to precipitation. Thus, the seeds in the greenhouse showed a better response because these parameters were better controlled due to the 70% shading (Figure 2). Choi and Yang (2005) observed higher leaf production and neck diameter in greenhouse-grown Perilla Frutescens seeds. Fonseca (2006) also found difficulties in germination for *Pseudopiptadenia psilostachya* seed in full sunshine because of similar factors.

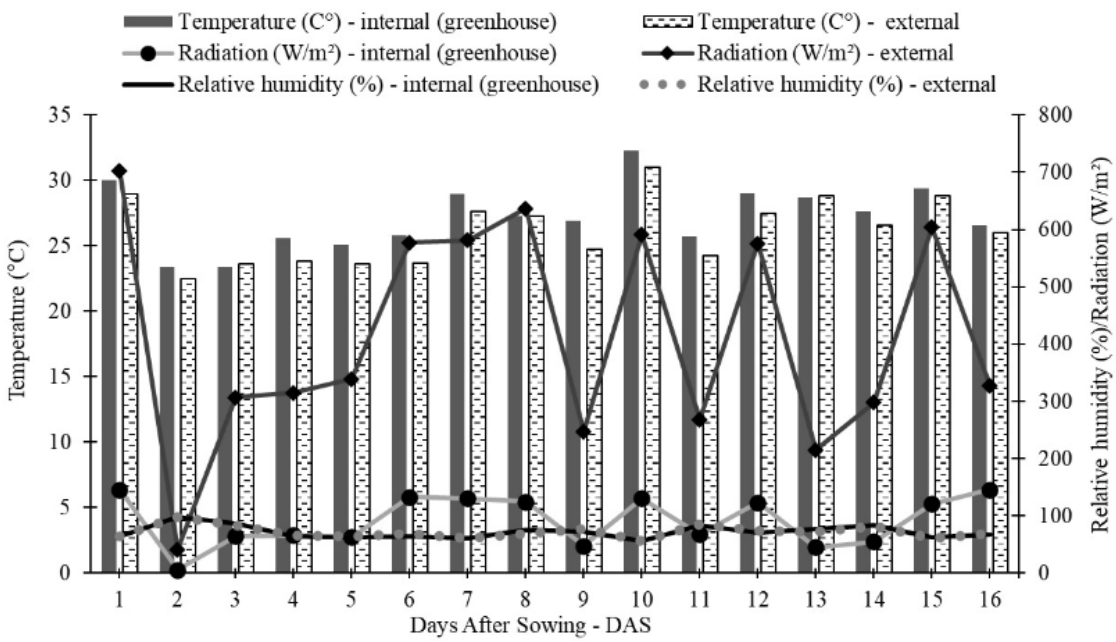

Figure 2. Climatological data outside and inside the greenhouse with 70% shading, during the germination period.

Regarding temperature and relative humidity (RH), the variations were not significantly different. However, from the radiation averages observed during the experimental period and verifying that the 70% shading treatment received 78% less solar radiation when compared to the full sunshine treatment, it is understood that the species is favored when exposed to a lower light regime.

This result indicates that the germination of shiso seeds is dependent on climatic conditions, especially exposure to radiation, plating time, temperature and light. Bhatt et al., (2022), also concluded that temperature and light conditions imposed on *P. frutescens* seeds influence their germination, in their experiment a higher germination rate was observed in seeds exposed to good light conditions and temperatures between 10 and 20ºC. One way to get around these climatic oscillations is by planting in greenhouses, where these characteristics can be better controlled, a conclusion that, through experiments, Lee and Yang (2006) also reached.

Even the germination rate of seeds can be increased by characteristics such as humidity and temperature or even artificial treatments (MASUMOTO, 2010). The germination of shiso seed also

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depends on how its storage has been carried out, since they can die in less than a year if stored at room temperature (CHO et al., 1986).

In relation to the climatological data for the development of the seedlings until they were ready for transplanting, a high variation in the level of solar radiation was also observed, and the use of the 70% shading greenhouse provided a more adequate environment for this level of radiation (Figure 3). Furthermore, it is understood that its development was also favored by the temperature and relative humidity (RH) averages provided during the period.

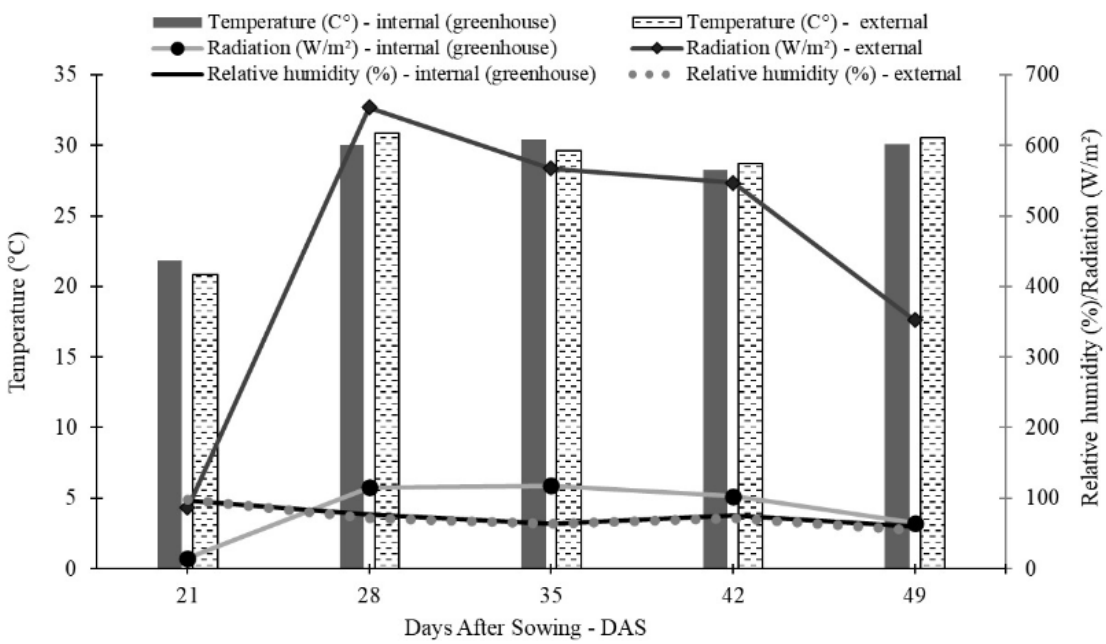

Figura 3. Climatological data (external and internal) in the greenhouse during the period of analysis of growth parameters.

Regarding the monitoring of seedling growth parameters in relation to days after sowing, these are represented in Figure 4 and were only analyzed in seedlings grown in the 70% shading environment, because in full sunshine, the seedlings had not survived. With regard to the specific leaf area (SLA), there was no statistical difference between the varieties, regardless of the days analyzed (Figura 4A). The period that recorded the highest SLA for both varieties was at 35 days after sowing (DAS). However, comparing the first analysis (21 DAS), with the last (49 DAS), there was a decrease for both the purple and green varieties, with a decrease of 73,21% for the purple variety and 69,08% for the green variety.

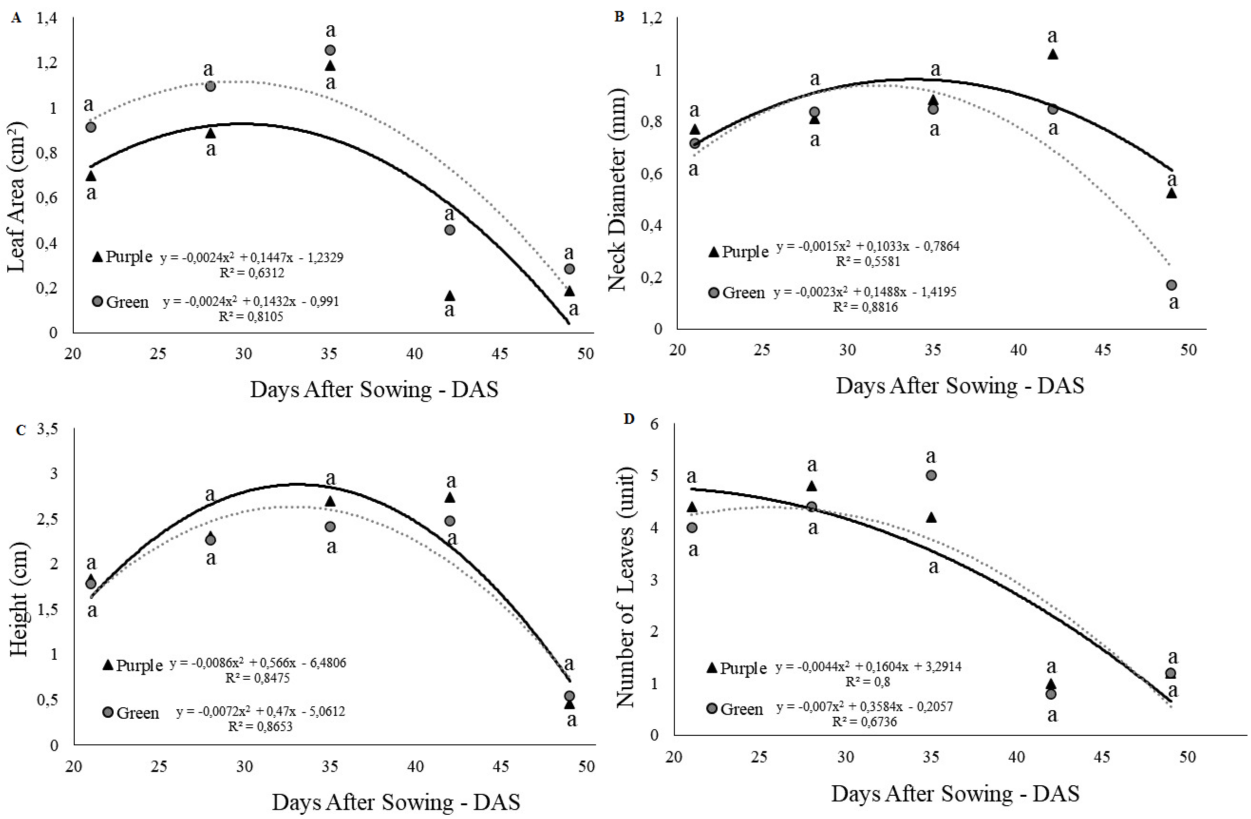

Figure 4. Growth parameters of two varieties (purple and green) of *Perilla frutescens* in relation to days after sowing. (A) Specific leaf area (cm^2); (B) Neck diameter (mm); (C) Height (cm); (D) Number of leaves (unit).

Regarding the neck diameter, there was also no statistical difference between varieties, regardless of the days analyzed, with the highest values for both varieties at 42 DAS. However, comparing the first day of analysis (21 DAS) with the last (49 DAS), a decrease of 31.12% in neck diameter was observed for the purple variety and 76.25% for the green variety (Figure 4B).

For the height, there was also no statistical difference on any of the days analyzed, between the shiso varieties (Figure 4C). However, at 42 days after sowing (DAS) the greatest heights were evident in both varieties, with the purple variety obtaining a maximum of 2,74 cm, while the green variety of 2,48 cm, representing an increase of 49,73% and 39,33%, respectively, in relation to the first day of analysis (21 DAS).

Regarding the number of leaves, the two varieties of shiso showed no statistical difference between them, for any of the days analyzed (Figure 4D). For the purple variety the greatest number of leaves was observed at 28 DAS, while the green variety showed the greatest number of leaves at 35

DAS. From the first analysis (21 DAS) to the last analysis (49 DAS), there was a 72,72% and 70% decrease in the number of leaves for the purple and green varieties, respectively (Figure 4D).

In the four growth parameters considered in the experiment, specific leaf area, neck diameter, height and number of leaves (Figures 4A, B, C and D), there was an initial decrease in the increment of biomass from 35 DAS, for both varieties, and at the end the seedlings were almost leafless. This decrease can be explained by the need to transplant the seedlings before this period, because with the rapid germination of the seeds and the development of the first leaflets, the volume of substrate in the cell tray becomes insufficient, making it necessary to transplant them to larger vessels, seeking better development of the root system, which was also verified by Reghin et al. (2007) in which small cells promote physical stress on the seedling preventing better development of the root system and the aerial part.

CONCLUSION

The purple variety of the species *Perilla frutescens* presents a higher germination percentage, when compared to the green variety, as long as it is cultivated in an environment with 70% shading. However, the volume of substrate in the germination cell promotes an impediment to the further development of both varieties, making it necessary to transplant them before 35 days after sowing, because after this period there is a great loss of biomass, which promotes a decrease in the vigor of the seedlings.

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