Optimal Nitrogen and Light Regimes to Grow a Medicinal Herb, *Calendula officinalis*

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Background

Calendula officinalis (pot marigold) is a perennial plant belonging to the family Asteraceae and is native to the Mediterranean region. They grow up to between 30 and 50 cm high with flower heads measuring up to 5 cm in diameter, which are relatively larger than other species in the genus. The flower consists of active compounds such as oleanolic acid, and are shown to exhibit medicinal properties such as anti-inflammatory, antiviral, inhibition of existing tumor cells as well as protection against adverse effects of chemotherapy and radiation therapy.

However, very little has been understood about the optimal indoor growth requirements of the plant. The aim of the project is to evaluate the responses of vegetative growth and flower production of Calendula under the interaction of differential nitrogen fertilizer and light intensity.

Materials and Methods

Experimental Setup and Treatment:

- Eight different treatments were imposed to grow 32 Calendula plants in the greenhouse.
- Sixteen of them were grown under 100% light and the remaining 16 under 50% light.
- Under each light condition, four plants were grown as the replicates for each nitrogen treatment. The concentrations of nitrogen fertilizer (NH_4NO_3) were as follows: control (distilled water), 0.01M, 0.05M and 0.10M.
- Each plant received 100mL of treatment solution, for a total of 7 weeks.

Image 1: Set up of C. officinalis under 100 % Light (Top) and 50% Light (Bottom) in the greenhouse

Measurements:

- The height of the plant was measured as the difference in length between the highest point on the plant and the level of the soil. The total number of leaves for each plant was counted manually.
- Biochemical quantification of total chlorophylls and carotenoids was done by selecting a random new leaf from each plant and approximately 0.1g of the leaf tissue was incubated in DMSO in the dark for 2 days. Using a spectrophotometer, extracts were read at 470nm, 645nm, and 663 nm.

Statistical Analysis:

- For statistical validity, the plants were randomized every week after treatment based on the output of a python script that I implemented using uniform distribution number generator.

Image 2: Output of the script that was used to randomize pots

Computational Analysis:

- BLAST tool from NCBI was used to find homologous sequences for RbCL gene in *C. officinalis*. The sequences from the search were downloaded. A bootstrapping analysis was conducted using the "one-click" tool in Phylogeny.fr to build a phylogenetic tree for the characterization of the RbCL gene.

Image 3: RT-PCR machine

Gene Expression Analysis:

- Total RNA was extracted using an RNeasy Plant Mini Kit. The extraction was quantified using a Nanodrop machine. cDNA was synthesized from the extracted RNA using iScript cDNA Synthesis Kit.
- PCR was run on the cDNA and the products were separated by 1% agarose gel electrophoresis.

Results and Discussion

Physiological:

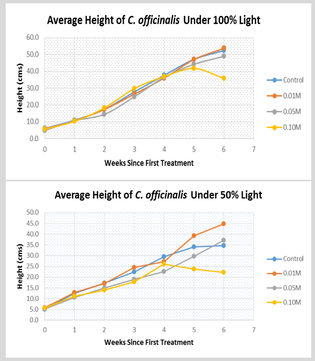


Figure 1: Increases in the height of *C. officinalis*

- Calendula plants grew well under all treatments in the first four weeks as manifested by a steady increase in height.
- Under 100% light, no differences were observed in the height among the control plants, 0.01 M and 0.05 M nitrogen-treated plants; however, plants treated with 0.10M nitrogen stopped growing since the fifth week of treatment.
- Under 50% light, the differences in height were observed as the treatment prolonged. Plants treated with 0.01M nitrogen grew the best, while plants treated with 0.1 M grew the poorest.

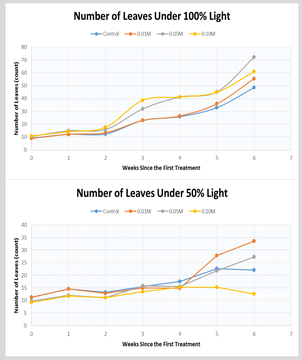


Figure 2: Changes in the total number of leaves on *C. officinalis*

- The increases in the number of leaves were more pronounced among different treatments.
- Under 100% light, the plants treated with 0.10M and 0.05M nitrogen had higher number of leaves compared to plants treated with 0.01M nitrogen and control.
- Under 50% light, the plants treated with 0.01M nitrogen had more leaves towards the end of the treatment compared to other treatments. On the contrary, the plants treated with 0.10M had the lowest number of leaves.

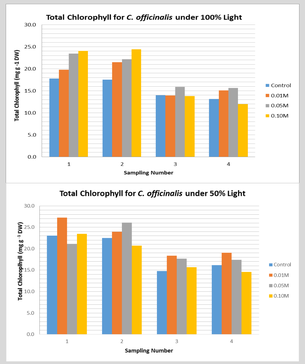


Figure 3: Changes in the total chlorophyll (chlorophyll A and B) contents in the leaves of *C. officinalis*

- Nitrogen-treated plants had more chlorophyll compared to the control for both 100% and 50% light during the first two sampling dates (2 and 4 weeks of treatment).
- Under 100% light, plants treated with 0.10M nitrogen had more chlorophyll until the second sampling (4 weeks). The amount of chlorophyll decreased in all plants as the treatments prolonged (6 and 8 weeks), with the biggest decrease in plants treated with 0.1 M nitrogen
- Under 50% light, plants treated with 0.01M and 0.05M nitrogen had consistently higher chlorophyll contents than the control.
- Under 100% light, the carotenoid content was higher for nitrogen-treated plants during the first two sampling dates (2 and 4 weeks of treatment).
- Under 50% light, plants treated with 0.01 M and 0.05 M nitrogen maintained more carotenoids at the end of treatments.

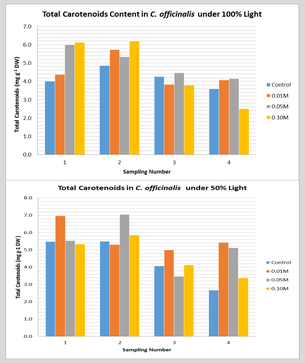
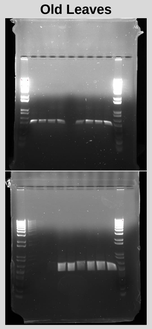


Figure 4: Changes in the total number of buds and flower heads

Results and Discussion

Molecular:



- *RbCL* gene was expressed under all the treatments in the old leaves. However, stronger bands were seen for plants under 50% light (right) compared to those grown under 100% light (left).
- *RbCL* gene was not expressed in the new leaves of control and 0.01M nitrogen treated plants under 100% light (left), but in all of the plants grown under 50% light.

Image 4: Transcript levels of *RbCL* gene under 100% and 50% light

- The expression of a flowering time (*FT*) gene was detected in the new leaves.

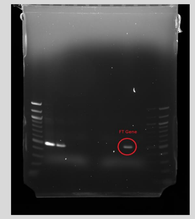


Image 5: Expressed *FT* gene in new leaves.

Computational:

- A phylogenetic tree was constructed to compare the sequence of *RbCL* gene in *C. officinalis* with other species in the same genus and family.

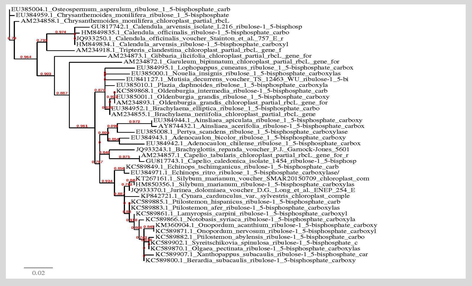


Image 6: Bootstrapping analysis of *RbCL* gene in *C. officinalis*

Future Work

- Improve primers for the *FT* gene using Multiple Sequence Alignment (MSA) and characterize its gene expression profile in *C. officinalis* using molecular techniques.
- Use Real Time-qPCR to quantitatively determine the expression level of the RbCl gene in different tissues.
- Examine expression of other genes in the hormonal regulation pathways and other genes in nitrogen metabolism and sexual reproduction.
- Quantify the accumulation of active ingredients used for medicinal and cosmetic purposes, using HPLC and spectrophotometric methods.

Acknowledgments

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