PLS100BL : Lab 6
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Phytochrome Functionality in Seed and Seedling Photomorphogenesis

Aditya Nirgun 999723427

Introduction and Background

Sunlight is the primary source of energy for every photosynthetic organism on the planet - each one having adapted accordingly to be able to receive and rely on this condition in predictable and unpredictable environments. The dynamic response of plants to light conditions is essential for their survival and drives strong selection for processes that perform precise acts of photomorphogenesis. An observation of plant mechanisms in controlled lighting conditions should reveal a sensitive response mediated by plant hormones. With this in mind, this experiment proceeded to demonstrate how the absence of light promotes auxin-induced growth in the form of hypocotyl elongation in pea seedlings. It is expected that the failure of such a hormonal reaction to obtain light results in etiolation, a behavior forged in hopes of growing away from shade conditions and into any available light.

From the planetary conditions of day length to the topology of terrain to the shading canopy of a forest biome, light availability is spectacularly complex to sessile forms yet enforces powerful direction over plant survival and selection. Among many other things, light exposure influences the timing of several stages in the plant's life. Germination and flowering respond to photoperiodism in the case of many plant species. The biochemical machinery within the plant that enables this functionality is a coupling between the light sensing ability of phytochromes and plant hormone production. The photomorphogenic effect of this system has been demonstrated with lettuce seeds in experiments past. According to the literature, phytochrome photoreceptors have two interconvertible forms. On form receives red light in the area of 667 nm wavelength and the other receives far-red light beyond 667 nm. This experiment investigates how the effect of exposure to each or both of these two levels of light can lead to different results in germination in seeds. The literature also indicates that phytohormones associated with the phytochrome response include gibberellin, which can be administered as a supplementary treatment to provide comparison.

Methods

Eleven pea seeds were planted in each of nine pots and maintained in the dark until signs of seedlings above the surface were visible. Everyday for ten days one plant was removed from darkness and placed in light. With this procedure, all seedlings had the same maturity, the only

difference being the amount of light each received. At the end of this two week period we observed the differences between the dark- and light-grown seedlings for indications of photomorphogenesis. The seedlings were then removed and photographed.
The other trials for this experiment involve subjecting lettuce seed to different exposures of red (R, 660 nm) or far-red (FR, 730 nm) light followed by darkness. Three replicates of 25 seeds of Grand Rapids lettuce were imbibed in water for about two hours and then exposed to each of the set of light or hormone conditions. After two days of dark storage following treatment they were retrieved and inspected for germination totals. Germination rates in percentages for the three replicates of 25 seeds were averaged for each treatment to produce a table of results.

Results

Growth and Etiolation Response with Light Treatment

Figure 1. A photograph of each trial pot of 11 pea seeds varying light exposure in days (from the most light days on the left to the longest dark period on the right). This observation was made at the end of 10 days of treatments with all pea plants at the same maturity.

Germination of Lettuce Seeds

The results of the seed germination tests were compiled and reported on as evidence for the phytochrome function as it changes in red light versus far-red light. Gibberellin treatment was also included for comparison.

Treatment #	Treatment Description	Mean % Germination
1	Dark	0
2	Light	92

3	R 15 min	53
4	R + FR	0
5	R + FR + R	56
6	FR 30 min	0
7	R 30 min	62
8	GA in Dark	64

Figure 2. This table presents the eight treatments of light, dark, and hormone that the seeds experienced along with their resulting germination rate in percent for each treatment.

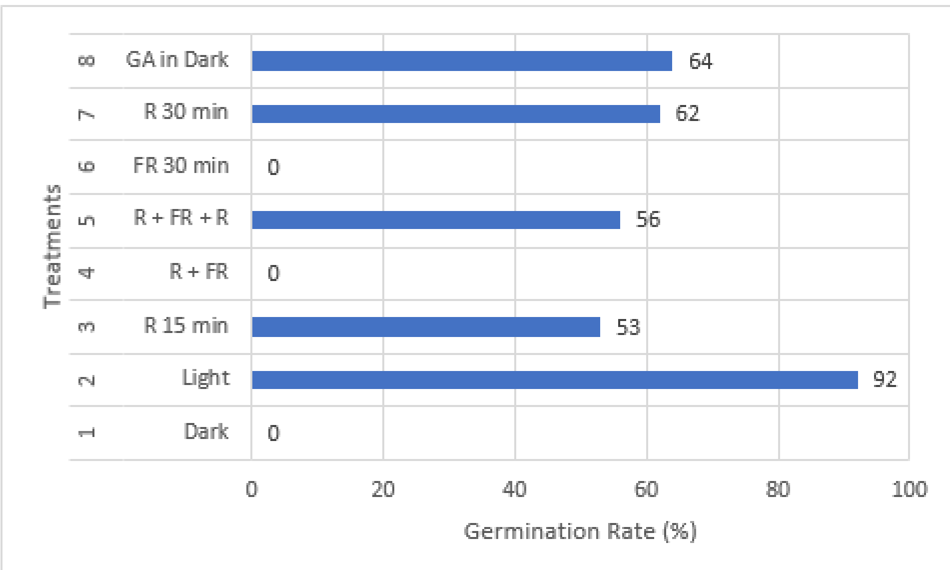

Figure 3. The bar graph above displays the germination rate in percent for each treatment.

Discussion

The pea seedlings demonstrated photomorphogenesis as a clear and consistent trend in the result. The light treatments produced clear visual trends in height, leaf area, color and structure. Large green leaves and short hypocotyls characterized the results of the first four treatments where the seedling trials were consecutively introduced to light. These plants appeared healthier the earlier they were introduced to light conditions. We suspect this is due in part because the photoreceptor response of phytohormone induced growth becomes energetically expensive over time. Therefore when it is minimised in the presence of light, that allocated energy becomes useful in productive vegetative growth. Beginning with the fifth day of dark treatments, hypocotyl elongation is noticeable and increases rapidly among longer dark treatments. At this point, leaf size is beginning to shrink and leaf hue is not as chlorophyll heavy. These are the onset signs of etiolation and they increase drastically onward from day 7. According to the literature, etiolated seedlings and early seedlings sequester precursors for chlorophyll and carotenoid production inside plastids. These plastids only differentiate into chloroplasts with fully formed chlorophyll and carotenoids after irradiation in light, hence the trend in color variation we observed. By the final day of darkness, the remaining seedlings had yet been unable to form functioning photosynthetic systems - their leaf size remained small and their color was devoid of any indication of chlorophyll pigment. The lack of chlorophyll coupled with the energy sink of hypocotyl elongation proves to be a stress upon the seedling that is unsustainable. This behavior from an evolutionary perspective describes a timeless gambit played by seedlings emerging in the shade. The importance of sunlight availability proves to be profound as the plant risks its survival by forgoing energy metabolism installation in favor of desperately growing out of shade conditions. The fixture of this behavior as a heritable trait in many plant species is a testament to the success of this approach. For the purposes of crop production, the photomorphic response could be utilized to produce elongated hypocotyls for ease in harvest of sprouts, as in the case of mung bean, alfalfa, and soy. The photomorphic effect also informs of the benefit of proper lighting conditions in vegetable production. Further studies in this area could involve testing the maximum survival period of etiolated seedlings in dark conditions before they can be revived under light conditions. The parameters surrounding the photomorphic response might vary with amount of phytochrome per unit area or a number of downstream factors. One of these factors could be the target for improvement in a plant breeding project for a plant species that may suffer from stand overlapping and shadowing.

The lettuce seed experiment presented the highest germination response in light treatment and lit conditions after treatment with a rate of 92%. Dark treatment followed by dark storage led to 0% of seeds germinating. This is to be expected as the absence of light contributes to an extended seed dormancy period. In other words, light seems to be one of the conditions that trigger or facilitate germination. Light however is comprised of a spectrum of color and energy that interacts with photoreceptors uniquely across its span. This experiment focuses on the ability of the phytochrome photoreceptor to transform and perform different functions based on the wavelength of light it receives. Our results demonstrated this clearly when we tested the variants of red (R) and far-red (FR) light treatments. Red light reproduced a portion of the germination rate we observed under white light and was slightly more effective with longer exposure time. However, treatments with far-red light as the final light exposure consistently resulted in a germination rate of 0%. This can be explained by a germination suppression effect induced by far-red light activation of the phytochrome complex. FR is not absorbed by chlorophyll and is abundant (relative to red light) in shaded canopy. FR also penetrates into the soil deeper than red light. This allows for FR and R light ratio to be an indicator for seeds on the status of light availability in the immediate environment and on the depth of their seeding in soil. This means seeds can sense the light quality when in shaded canopy or when buried too deeply. With the information from the phytochromes, they can remain dormant until conditions improve which happens to be a strategy essential to any seed's survival. Along with that function, the seed must also initiate germination with the detection of higher R light levels than FR light levels. This ability was also demonstrated in the results of our experiment. A treatment of R, then FR, then R seemed to successfully activate, deactivate, and reactivate the phytochromes responsible for inducing germination. Since the academic literature provides evidence that gibberellin plays a part in this photoreceptor-phytohormone pathway, one treatment included gibberellin application in total darkness. Under the same light conditions as treatment 1, the seeds experienced the increase in germination rate from 0% to the highest levels since the full light treatment. With this kind of effect, gibberellin could be an overwhelming factor in the germination response that lies downstream from the photoreceptor.

References

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