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Article

The impacts of varying types of light on the growth of five *Arabidopsis* varieties

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SUMMARY

***Arabidopsis* is known as the "fruit fly of plants." It is small and easy to grow, has a short life cycle, and has a small, easy-to-manipulate genome. Using Arabidopsis, we tested the effects of varied light conditions on the plant growth of mutants with dysfunctional light pathways. We tested five different strains: wild type, a phytochrome A mutant (phyA), a phytochrome B mutant (phyB), a phyA/phyB double mutant, and a DET1-1 mutant. With these mutants, we investigated how varied wavelengths and exposure of light affect the growth of the mutants. We found that the phyA mutant, the phyB mutant, and the double mutant all grew well in red light, with high germination rates and the largest average plant size. The phyB mutant grew the best under blue light, with the highest germination rate and the second largest average plant size. Under natural light, every strain grew relatively well, with high germination rates and consistent sizes. Although the DET1-1 mutant had a lower average size compared to the phyA and phyB mutations, it had the highest germination percentage, making it the most successful under no-light conditions.**

INTRODUCTION

Just like animals, plants have developed many mechanisms to respond to the surrounding environment. They have systems of receptors that receive stimuli and activate pathways to create responses. One of these systems is the phytochrome system. Plants rely on photoreceptors to mediate responses to light stimuli. Phytochromes are types of photoreceptors that are sensitive to red light, a main component of natural light, and far-red light, a lower wavelength of red light often produced after light is filtered through the leaves of other plants. Red light activates phytochromes while far-red light (FR) de-activates them. When phytochromes are active, growth is induced, and when inactive, growth is slowed (1). Furthermore, activated phytochromes trigger germination; thus, plants only germinate when exposed to red light. Research has already been done on the effects of phytochrome A and B mutations on *Arabidopsis*. One study found that phyA had a germination defect in FR while the phyB mutant had a germination defect in the dark; however, they also found that the effects of the phyA mutation could be suppressed by the phyB mutation (2). Both the phyB and the phyA mutants grew well under red light but were underdeveloped under FR. In contrast, the phyA phyB double mutant showed underdevelopment in red light but better development in FR, especially in the cotyledons (embryonic leaves) (3). Another study has also found the *det1* gene in *Arabidopsis* to allow the plants to grow like a light-grown plant in the absence of light (4). We wanted to see how these mutants would behave under more varied light conditions, not just under the types of light for which they have mutations. Thus, we tested these mutants not only under red light and natural light, but also under no light and blue light to see how different wavelengths would affect development in plants with nonfunctional phytochromes.

Phytochrome A and B have overlapping but different functions. PhyA is much more sensitive to FR and is responsible for germination (initial growth from seed) and de-etiolation (the greening of plants through the development of chloroplasts). Under the shade of other plants, light is often filtered of red and blue light leaving FR which is lower on the spectrum. FR triggers a light pathway through phyA stimulating germination and de-etiolation, which is an important stage towards plant maturation. PhyA also inhibits responses for avoiding shade, like the elongation of the hypocotyl (stem). When exposed to high levels of red light, phyA degrades. Under shade, plants will accelerate their growth in attempt to outcompete competitors, but excessive elongation growth can inhibit plants from establishing maturity, causing abortion. With a high FR to red light ratio, phyA inhibits elongation while promoting germination and de-etiolation. The null phyA mutation would lead to unchecked etiolation, which includes long hypocotyls and undeveloped leaves (5). PhyB also regulates de-etiolation but in a different manner. Under red light, phyB is activated, suppressing shade avoidance responses. When red light is reduced, the phyB becomes inactive which in turn stimulates shade avoidance. Thus, the phyB mutation would prevent the plant from suppressing excessive elongation (6). The DET1-1 mutant is slightly different because the *det1* gene acts as a transcriptional repressor for genes that are expressed by light stimulus transduction pathways. A nonfunctional *det1* gene would prevent the mediation of plant development in response to light, causing the plant to grow regardless of stimuli— including in the dark (4).

Based on previous research, we hypothesized that the DET1-1 mutant would grow best in the dark since it would grow regardless of stimuli from the lack of light. We also hypothesized that the phyA and phyB mutations would grow best in the red light because the pathway suppressing shade

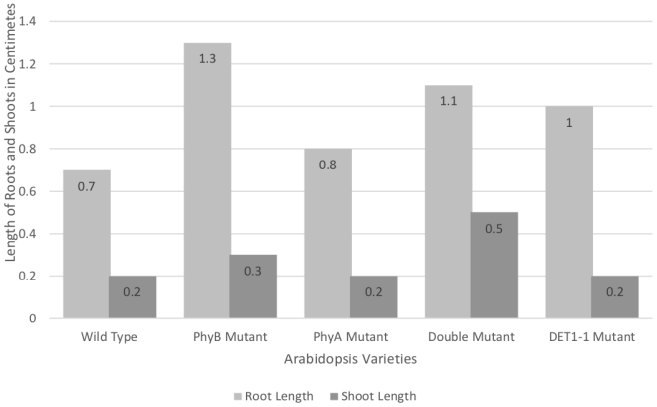

Figure 1: The mean length of five Arabidopsis varieties' roots and shoots in 24-hour light. The graph shows that every variety was successful at producing both shoots and roots in our control experiment. The phyB mutant had the greatest mean root growth at 1.4 cm, and the double mutant had the greatest mean shoot length at 0.5 cm.

avoidance responses, de-etiolation, and elongation under light would be blocked, and the double mutant would grow best under the blue light because with both pathways for red light blocked, the plant would be forced to rely on blue light to initiate a growth response.

RESULTS

We tested how mutant varieties of *Arabidopsis* respond to different colors and amounts of light. The tests were run on five varieties of *Arabidopsis*: wild-type; CS6213, which had a mutation in phyB; CS6219, which had a mutation in phyA; CS6224, which had mutations in both phytochrome A and B; and CS6158, which had a *det1* gene mutation that encouraged growth in the dark. We prepared and plated four agar plates under similar conditions to minimize experimental error; we prepared the same agar solutions on the same day, and we allowed it to sit for the same amount of time before parafilming each plate. Our independent variables were the four different light conditions under which we ran our experiments, and the dependent variable was the resulting growth of each *Arabidopsis* variety. We determined growth success by looking at the average of the total lengths of each plant, including the roots and shoots, and germination ratio under each condition. Plants with longer average lengths and higher germination ratios were considered more successful. We consider germination ratios to be more important than the lengths when determining growth success, so we would consider a variant with a high germination rate and smaller lengths to be more successful than a variant with low germination but longer lengths.

Our baseline showed that all five varieties grew comparably well in 24 hours of direct light. They all had a germination percentage ranging from 75% to 100% and average lengths ranging from 0.9-1.6 cm (**Figure 1**). Based on the parameters gathered from the baseline experiment, we ran a positive control trial in natural light to test each variety's ability to undergo daily functions in a normal growth environment. We also ran trials in no light to test the DET1-1 mutant, and trials in both red and blue light to test the phytochrome mutations. We measured the root and shoot length of each plant after one week and calculated the successful germination percentage.

Under natural sunlight, there were no statistically significant

		NATURAL (SUN)LIGHT		NO LIGHT		RED LIGHT		BLUE LIGHT	
TYPE OF SEED	Root/ Shoot	Mean±SD (cm)	Stan. Err.	Mean±SD (cm)	Stan. Err.	Mean±SD (cm)	Stan. Err.	Mean±SD (cm)	Stan. Err.
WILD TYPE (N=6)	Roots	0.9±0.40	0.07	0.0±0.0	0.00	0.5±0.06	0.02	0.5±0.06	0.02
	Shoots	0.2±0.12	0.02	0.0±0.0	0.00	0.6±0.15	0.05	0.2±0.06	0.02
PHYB MUTANT (N=6)	Roots	0.9±0.18	0.04	0.7±0.00	0.00	0.9±0.13	0.03	1.0±0.24	0.04
	Shoots	0.4±0.05	0.01	1.1±0.00	0.00	1.1±0.13	0.03	0.3±0.12	0.02
PHYA MUTANT (N=6)	Roots	0.6±0.29	0.06	0.4±0.00	0.00	0.6±0.22	0.04	0.7±0.24	0.06
	Shoots	0.2±0.07	0.01	1.2±0.00	0.00	0.6±0.21	0.03	0.2±0.21	0.05
DOUBLE MUTANT (N=6)	Roots	0.4±0.19	0.05	0.0±0.00	0.00	0.6±0.10	0.03	1.4±0.14	0.07
	Shoots	0.4±0.40	0.10	0.0±0.00	0.00	1.5±0.32	0.11	0.6±0.07	0.04
DET1-1 MUTANT (N=6)	Roots	1.0±0.37	0.06	0.7±0.19	0.08	0.8±0.14	0.02	0.9±0.31	0.05
	Shoots	0.1±0.00	0.00	0.4±0.32	0.14	0.1±0.04	0.01	0.1±0.06	0.01

Table 1: The mean root and shoot length with germination percentage of each Arabidopsis variety in four light conditions. Each plate includes an Arabidopsis wild type and four mutant varieties, and all plates were set up identically. The table shows the average root and shoot lengths of each variety, as well as the germination percentage. All plates were placed at the same angle to get qualitative data about phototropism. The mean, standard error, and standard deviation were calculated separately for each variety in each condition. The no-light data for all varieties except the DET1-1 mutant are slightly misleading, because only one plant germinated in each condition, so the mean of the results is only based on one test.

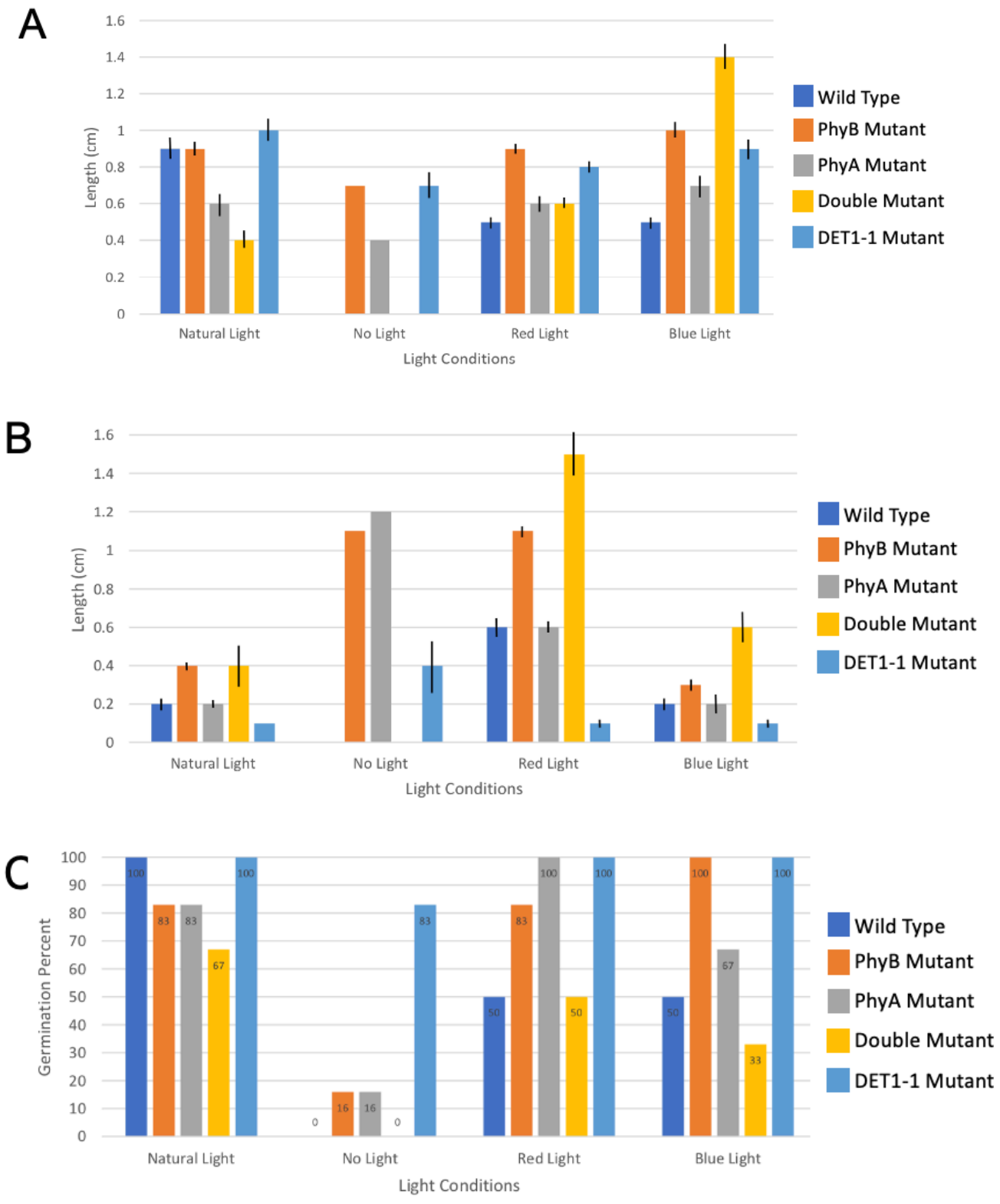

Figure 2: The mean root and shoot lengths of five Arabidopsis varieties grown in sunlight, no light, and red and blue light after one week. A) Mean shoot lengths in all light conditions. **B)** Mean root lengths in all light conditions. The graphs are organized by light condition, so each graph compares the root or shoot lengths of the five varieties under each of the four light conditions. The error bars were created using the standard error values calculated separately for each variety in each condition, as shown in Table 1. **C)** Germination percentage of five Arabidopsis varieties' grown in sunlight, no light, red light, and blue light. In cases where the majority of the seeds did not germinate, the numerical result is based on very few trials and is not a true average.

differences in the shoot lengths of the five varieties. However, the phyA mutant and double mutant had significantly less root growth compared to the others, averaging 0.6 cm and 0.4 cm respectively. Overall, the wild type, phyB, and double mutant all grew well, with high germination percentages from 83%-100% and average lengths from 1.1 cm - 1.3 cm long (**Table 1**). We interpreted overlapping error bars as not showing a statistically significant difference and non-overlapping error bars as suggesting a possible statistical significant between treatments.

Under no light, the phyB mutant, phyA mutant, and DET1-1 mutant were the only mutants to experience any growth. While the phyA mutants had an average length of 1.6 cm and the phyB mutants 1.8 cm, only 16% of the phyA mutants and phyB mutants germinated, while 83% of DET1-1 mutants did so. Thus, although the DET1-1 mutant had a smaller average length of 1.1cm, under no light the DET1-1 mutant grew the best. (**Figure 2**).

In the plate grown with red light, all of the varieties had statistically similar roots lengths of 0.5 cm to 0.9 cm except for the wild type and the phyA mutant which were less statistically significant compared to the phyB mutant. Furthermore, the phyB mutant and double mutant had the greatest shoot lengths, 1.1 cm and 1.5 cm respectively, while the DET1-1 mutant had the shortest shoot growth at only 0.1 cm (**Figure 2**). Overall, the phyB mutant grew best under red light, with an 83% germination rate and an average length of 2.0 cm, and the double mutant grew next best with an average length of 2.1 cm and a 50% germination rate.

The plate grown under blue light produced similar results to the plate grown under red light, with the phyB mutant growing most successfully with an average length of 1.3 cm and a 100% germination rate. The double mutant had the longest length of 2.0 cm, but had a very low germination rate of 30%. The wild type showed diminished growth with 50% germination and an average length of 0.7 cm. According to our data, varying amounts and types of light results in unique growth patterns amongst the five *Arabidopsis* varieties.

DISCUSSION

Our data supports the hypothesis that the DET1-1 mutant grows best in the dark and that there would be variation between the positive control and the sunlight test due to the plants following their normal growth cycle rather than a 24-hour day, which was mimicked by the constant light source in the control. The data also supports our hypothesis that under blue light, the double mutant grows most successfully, since with both red light growth-inducing pathways blocked, the plant relies solely on blue light for energy and growth. However, the data refuted our hypothesis for the tests under red light. In the red light, we expected both of the mutants with one functioning phytochrome, the phyA mutant and the phyB mutant, to grow the best. Contrary to our expectations, the double mutant missing both phytochromes grew the best, along with the phyB mutant instead of the phyA mutant. For the red light, we predicted the double mutant to grow the worst because it has mutations in both phytochromes, which are the pigments that plants use to capture red light. A normal functioning phyA would degrade under red light, inhibiting de-etiolation and plant elongation, while a normal phyB would promote de-etiolation under red light, suppressing shade avoidance responses (5). Mutations in the phytochromes block these light pathways, but their functions cancel each other out. With a dysfunctional phyB pathway, de-etiolation would be less active and shade avoidance would be more active; however, the phyA mutation would leave de-etiolation and elongation unchecked. Thus, the double mutant would be able to de-etiolate and have excessive elongation (6). This is reflected by the increased growth of the double mutant under red light and blue light. Thus, the double mutant was able to grow best under red light due to unregulated elongation. The phyB mutant would also grow well in red and blue light since the pathway suppressing shade avoidance responses under light would be blocked, allowing it to grow longer. The results of the DET1-1 mutant supports the idea that the mutation blocks the *det1* gene, which regulates the transcription of light-mediated pathways for plant development. With a nonfunctional gene, the plant will develop regardless of light stimuli. Therefore, the DET1-1 mutant was able to grow in the dark (4).

Other researchers came to similar conclusions. In a study conducted by Peng Liu and Robert Sharrock, they found that the phytochromes A and B have slightly different functions that are not directly involved in the same pathway, explaining how our double phytochrome mutant could still grow and capture light instead of having the light-capturing pathway shut down (1). They also observed extended growth in their phyA mutants, matching the longer shoots and roots found in the phyB mutant throughout the experiment (1). The increased growth found with a phyA mutation could be further explored, because the mutation enables growth in shady areas, opening new environments to sustain agriculture.

Since our experiment had many steps that took place over the course of a few weeks, there was a lot of room for small errors to build up in our data. The media created was not measured when it was split into the two plates, so one plate may have more agar than others. This difference in nutrients is one of the small inconsistencies in the experiment. Secondly, due to our schedules, it was not always possible to record the data exactly a week after. Instead, the data was collected after a week and one day or a week and two days. In order to finish our data collection on time, we only let the plates sit in the refrigerator for a 72-hour germination period during the follow-up test, compared to the germination period of a week that was used in the baseline. This factor may contribute to the lower germination rates that we observed in the follow-up. Another possible bias is the effect of ambient light on the tests using blue and red light. In the red light test, the plate was placed in a box near the windows, with an opening on the side facing the classroom. The placement of the box near the

window may have given the plants some ambient sunlight, as well as some ambient light from the classroom, which may have skewed the data. In the test with the blue light, the lamp was placed in a closet which was dark most of the time, but the light was occasionally turned on, and the plant received some ambient light.

In further investigations, we would explore how a mutant containing mutations in both the *det1* gene and the phytochromes would grow in situations with reduced or no light. This mutant could be compared to plants known to grow well in the shade, to investigate if plants have naturally evolved these mutations in order to grow in new environments.

MATERIALS AND METHODS

The *Arabidopsis* plants were grown in 0.8% agarose plates. The environment was created by mixing a solution of 0.8 grams of agarose with 100 ml of water, which was then boiled by placing the flask in the microwave while it was "sealed" by a paper towel stopper. The flask is placed at room temperature until cool (up to 24 hours) to kill any bacteria spores that may have entered the solution. This boiling and cooling process is repeated two more times to remove all spores. Although a 24 hour cooling period between the boiling steps is preferred, the same result was also reached by boiling the solution twice in one day, allowing the solution to cool completely between boils.

The plants were grown in flat plates, but the plates were gridded on the bottom. This allowed each row to hold six seeds of each type, as long as the seed was placed in the center of one of the grid boxes in that row. To prepare the plates, the agar solution was split equally between two plates. The plates, sealed with parafilm, were placed in the refrigerator to set overnight. This process was completed twice since four plates were needed for the experiment.

The following strains were obtained for this experiment from the Arabidopsis Biological Resource Center at Ohio State University: Stock #CS39005 (wild type), Stock #CS6213 (phyB mutant), Stock #CS6219 (phyA mutant), Stock #CS6224 (double phytochrome mutant), and Stock #CS6158 (DET1-1 mutant). After the plates were set, the rows were labeled from top to bottom with the stock numbers in the order listed above, skipping the first gridded row on the plate, which was labeled as X for clarity. The plates were removed from the refrigerator, and the parafilm was removed to plate the seeds. The seed canisters were opened inside small petri dishes to catch overflow seeds. Using a toothpick, one seed was picked up and placed in one of the gridded boxes in the corresponding row for its seed type. The seed type containers were sealed into the petri dishes when each of the six grid spots had been filled for that seed type. This process was repeated for all seed types. The plates were sealed and put back into the refrigerator for a one-week germination period.

After the germination period, the plates were placed into their experimental environments. The plates were oriented so that they were upright, with the light source above the X row. The no-light environment was created by wrapping the plate in tinfoil. This plate and the plate for natural light were placed adjacent to the same window following the above orientation. Finally, one environment with a blue lamp and one with a red lamp in which the light source is only the colored light were created. One plate was placed in each environment.

Every week, the length of the roots and shoots was measured on each plant. Pictures were taken of the model plant for that test, which was chosen on week one and labeled with a dot in the grid box on the bottom of the plate. The pictures were taken under a microscope. In addition, the number of germinated seeds in each row was recorded.

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