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Plant Hormone Lab Module: Assessing Different Factors to Create the Most Effective Lab

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Abstract

The research project focused on evaluating 3 different factors in creating a plant hormone lab module for Principles of Biology II lab. The main hormone of focus was brassinosteroid. In the model plant *Arabidopsis thaliana*, brassinosteroids promote cell elongation, cell division, root growth, and seed germination. Three goals were established to create the most efficient lab module possible. First, mutant (*bes1,bri1*) root length was assessed without addition of brassinolide (BL), a synthetic brassinosteroid. Then, ½ MS and ½ MS + 20% sucrose plates were compared to see which media produced the greatest difference in root growth between the mutant lines. The effect of brassinosteroids in the root growth assay was also assessed in this project with two different application methods: before germination and after germination. The results showed that *bri1* seeds had the longest roots and *bes1* seeds had shorter roots compared to the wildtype, as expected. Second, plates that contain 20% sucrose provided the best conditions for observing differences in root growth. Finally, application of BL was shown to be better after germination, but the most suitable solvent still needed to be determined. With a better development of the application of BL and more seed production from *bri1* plants, the lab module could be ready for students to learn from it.

Introduction

The purpose of this research project was to develop a lab module for Principles of Biology II students. This module would introduce students to the effects of hormones on plant growth patterns. If incorporated, it would allow students to use a hands-on approach and formulate their own hypothesis about how one class of plant hormones, brassinosteroids, will affect root growth. While attempting to create the module, the focus had to be slightly shifted because one of the mutant lines would not produce enough seeds to perform the growth assays. Thus instead of creating of module that could be used in Biology II labs, the goal of this project was to evaluate 3 different variables in making the experiment as proficient as possible. The first goal of the project was to assess the root growth of *bes1* and *bri1* mutant plants compared to wildtype plants without brassinolide (BL), a synthetic brassinosteroid. The second goal was to find a media that produced the greatest difference in root lengths between the plant lines. To this end, I compared ½ MS solid media versus ½ MS + 20% sucrose solid media. The final goal was to find the best way to apply the hormone, BL.

Arabidopsis thaliana is the genetic model plant that will be used in this lab module. It has been studied for over 70 years to understand plant biology (Meinke et al. 1998). Due to this, many hormone interactions in *Arabidopsis thaliana* have been characterized, which made the species attractive to use in this research project. *Arabidopsis thaliana* plants are also fairly cheap to grow and maintain which makes it amenable for a course lab module.

Initially, cytokinin was the hormone chosen for the lab module. However, the specific cytokinin-signaling mutants needed for the module were unavailable, so the focus was shifted to

brassinosteroid-signaling mutants in order to build on what a previous research student started. Brassinosteroids play critical roles in plant biology, including cell elongation, cell division, root growth, and seed germination (Tang et al. 2016). Brassinosteroids have a unique and complex signaling response pathway. First, BRI1 and BAK1 kinases form a heterodimer in the presence of brassinosteroid (Zhu & Wang, 2013). The heterodimer recruits the BIN2 protein out of the nucleus and deactivates it. Once this occurs, BES1 and BZR1 are dephosphorylated and translocate into the nucleus where they turn on brassinolide-response genes, which promote cell elongation and root growth (Belkhadir et al. 2006).

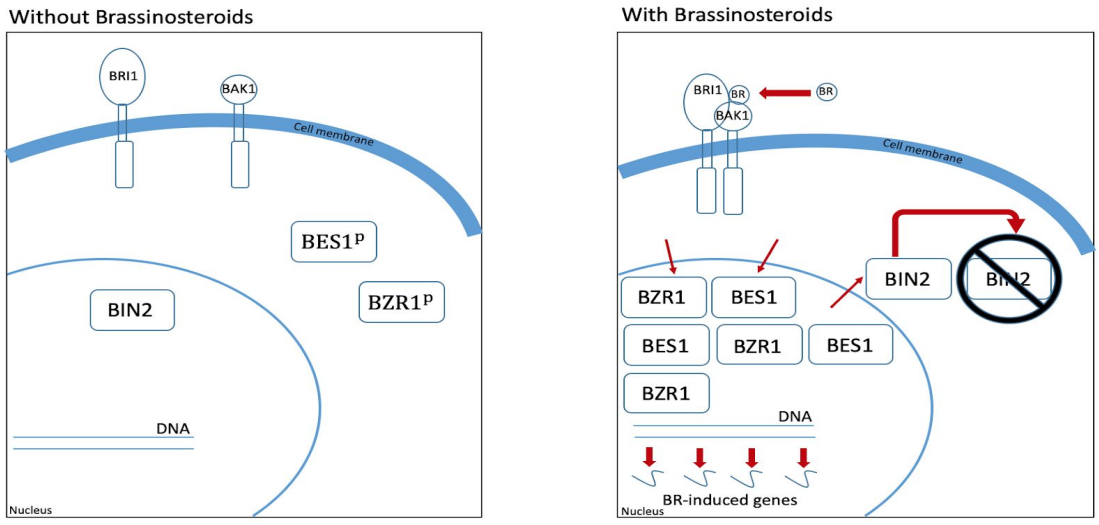


Figure 1: The brassinosteroid signaling response pathway (Snyder 2019)

Methods

First, *Arabidopsis thaliana* seeds were grown on soil for bulk seed collection. The plants were grown in Shrank Hall, University of Akron. When the seeds became available, preliminary growth assays were completed. Each assay was different with changes to the types of solid media or if BL was added. The first types of plates made were ½ MS + phytagar plates. These plates were filled with media composed of 4.31 g/L Murashige and Skoog (MS) salt mixture,

0.50 g/L 2(N-morpholino) ethanesulfonic acid, and 5g/L of phytagar. The liquid media was sterilized for 20 minutes at 121 °C and poured into petri dishes and cooled. Before the seeds were plated, they were surface sterilized using 50% bleach and 0.1% TWEEN detergent. They were placed onto the upper surface of the agar with a pipette. The seeds were stratified in the dark at 4°C for 4 days. Plants were then vertically grown at room temperature for 5 days and analyzed for root growth. The sucrose plates differed by adding 20 mL of 20% sucrose solution (final 1% w/v) after the liquid agar solution was autoclaved. After the 5th day, pictures were taken of each plate. The root lengths were measured using ImageJ. Bars graphs were created with the means of each root length, and standard error bars were added to show the standard deviation of the sample size.

There were three lines of seeds used during the experiment. The two mutant lines used were *bri1_188B5* and *bes1-D*. Two different mutant lines were used to examine how each of them grow with no treatment and with the addition of BL. The wildtype line examined was WT-PST99998 (*Arabidopsis thaliana)*.

The first mutant seed line, *bri1_188B5,* is insensitive to brassinosteroids (Tang et al. 2016). It is able to synthesize the hormone but unable to respond to it. The insensitivity is caused by a defect in the signaling transduction response pathway. In this mutant the Bri1 receptor cannot be activated upon brassinosteroid binding (Zhu & Wang, 2013). Due to this inhibition, BZR1 and its homolog BZR2/BES1 remain in the cytoplasm and brassinosteroid-response genes remain silent. (Zhu & Wang, 2013).

Figure 2: Growth of Bri1 plants done in Shrank Labs over 4 months

Because *bri1* mutants are insensitive to brassinosteroid, I expected no change between the growth of the roots with and without the application of BL. In order to collect enough seeds for growth assays, I grew each line on soil until termination of flowering. *bri1_188B5* plants were able to flower but did not produce as many seeds as expected. *bri1_188B* plants should have very small roots compared to the wildtype (TAIR).

The second mutant seed line, *bes1-D,* has a constitutive signaling response (Yin et al. 2002). These plants will always exhibit a brassinosteroid growth response with or without the hormone available. This is caused by the suppression of the BIN2 kinase, which causes constitutive dephosphorylation of the activators BZR1 and BES1 and activation of brassinosteroid-response genes. When growing *bes1-D* plants on soil, the plants were able to flower and produce an abundance of seeds. The *bes1-D* mutant line should have longer roots compared to the wildtype.

Figure 3: Growth of Bes1 plants done in Shrank Labs over 4 months

Application of Brassinolide (BL): 5mg of BL was dissolved in 15 mL of 100% ethanol to create a 20 mM working solution of BL. In the first application, a total of 50 uL of BL was micro-pipetted onto the agar plates before the plates were set in light for growth. In the second application, a total of 50 uL of 20 mM BL was micro-pipetted onto the agar plates after the plates sat in the light for three days. This was done to determine

Figure 4: The application of BL was done with a 16uL micropipette. The pipette was used parallel to the 3 regions of seeds to apply about 50 mL of BL.

the effect of BL on the seedling root growth after germination began. 100% EtOH was used as a control.

Results

The first goal of the project was to assess the root growth of *bes1* and *bri1* mutant plants compared to wildtype plants without BL treatment. The *bri1* seeds had the longest roots of all of the seeds. They had an average root length of 11.06 mm after 5 days. The *bes1* seeds had the shortest roots with an average of 7.69 mm. The wildtype seeds grew an intermediate size when compared to the mutants and had an average length of 9.74 mm (Figure 5 and 6). After performing an anova test, it is determined that there is no significant difference in wild type between both mutants. However, the *bri1* plants had significantly longer roots than *bes1* plants.

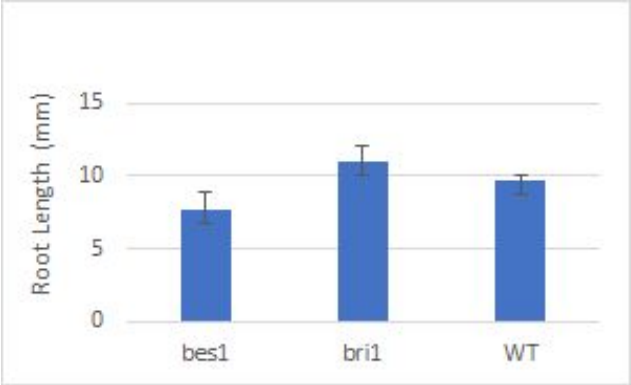


Figure 5: The mean root length in *bes1, bri1, and WT* are compared after five days of growth. P= 0.15 between all three of the groups. P = 0.029 between *bri1* and *bes1*. Standard error bars are added to show the standard deviation of the sample size (N=4).

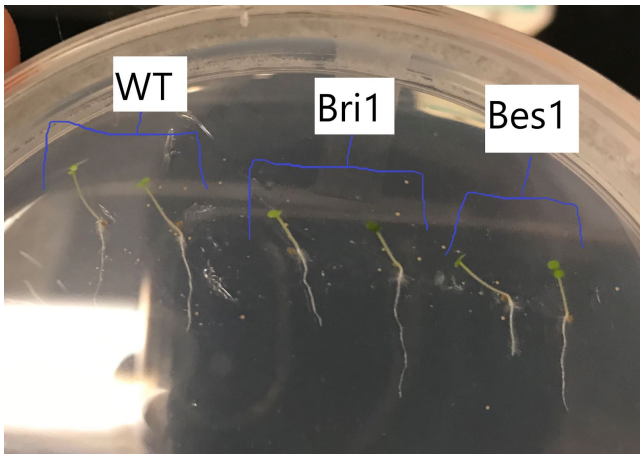
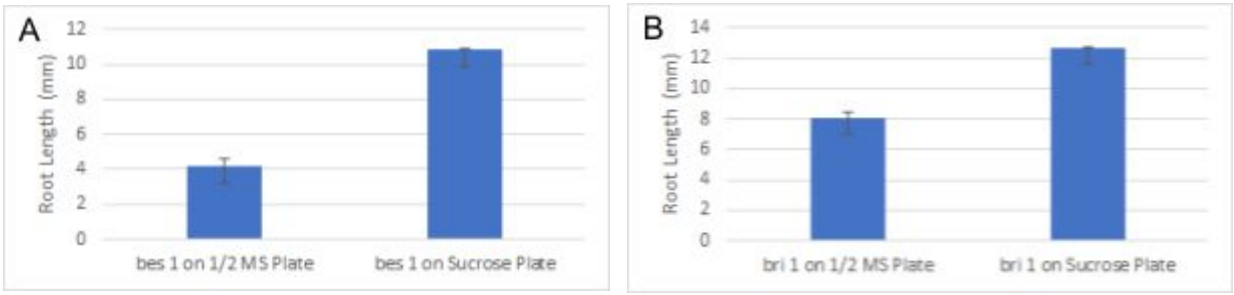


Figure 6: The root length of each line of seeds was calculated after being grown on agar plates. For this specific plate; 2 seeds of each line was plated.

The second goal was to determine the best media for the growth assay, such that the different lines had the greatest root length difference. To this end, I grew seedlings on ½ MS media with or without 20% sucrose. In *bes1* plants, there was an average difference in the means of 6.63 mm (Figure 7a). In *bri1* plants, there was an average difference in the means of 4.59 mm (Figure 7b). In both instances, the mutant plants grew significantly better on plates that contained sucrose. The average difference between the mutants on ½ MS plates was 3.85 mm and on sucrose plates was 1.81 mm (Figure 7).



Figures 7a and 7b: The means root length was calculated for *bes1*(a) *and bri1*(b) on plates made with ½ MS and plates made with sucrose. The root lengths are measured after 5 days of growth. P= 0.0037 for *bes1* plates and 0.0075 for *bri1* plates. Standard error bars are added to show the standard deviation of the sample size (N=3).

The final goal was to find the best way to apply the hormone, BL, to the seedlings. The application of BL was done in two different ways. When BL was applied prior to germination, no seeds germinated in either of the plant lines. When applied post-germination, 4 out of 9 of the seeds germinated ; 1 *bes1*, 2 *bri1*, and 1 *WT*. In a control group, 100% ethanol was applied to the seedlings because it was used as a solvent in the BL solution. When applied prior to germination, 0 % of the seeds germinated . When added after germination, 55% of seeds germinated.

Type of Plate	Total seeds per plate	# of seeds that germinated	% of seeds with germination
BL before germination	6	0	0%
BL after germination	9	4	44.4 %
EtOH before germination	6	0	0%
EtOH after germination	9	5	55.5 %

Table 1: The methods of applying BL to plates are compared. 0 % of seeds germinated in application of BL before germination while 44.4 % of seeds germinated when adding BL after germination.

Discussion

This experiment was designed at first to create a lab module for Biology II lab. With the plants not producing seeds the way I anticipated, I am going to discuss the results in a way to show the best conditions of a hypothetical lab situation. When assessing the growth of *bri1, bes1, and WT* seedlings without hormone treatment, the seeds germinated differently from the expected from published reports (Yin et al. 2002). The *bri1* roots were the longest after 5 days growth while the *bes1* seeds were the shortest of the lines (Figure 5). The *bes1* roots should have been longer than *bri1* roots. More growth assays with a different batch of seeds needs to be performed to figure out why this was the case. When looking at the difference in ½ MS plates and sucrose plates, it was clear that the sucrose plates enabled the seeds to grow longer roots (Figure 7). The sucrose clearly permitted the seeds to germinate and grow more quickly and should be used to grow seeds on agar plates. However, the difference between the lines was lower. For a future experiment, I would try using 10% sucrose instead. Also, there is an increased chance of microbial contamination on sucrose plates, so the sterilization technique needs to be carefully followed. Finally, the application of BL was assessed. Adding the BL after germination is clearly the way to do it. There was not enough time to fully infer the best way for application, but I believe there were a couple of factors that may have affected the application. First, using 100% ethanol in the solution may have inhibited the germination and/or growth of the seeds (Kato-Noguchi 2001). To combat this, I would have looked for a different solvent to dissolve BL in or dissolved BL in a more aqueous solution of ethanol. Second, the

amount of BL added could have affected the growth of the seeds. With more time I would have experimented with different concentrations of BL.

For future work, it would be important to focus on perfecting the application of BL and improving the method for growing *bri1* plants to increase the amount of seeds produced. With more time, both of these variables could be corrected and the lab module would be ready for future placement in Biology II classes. Once both of the variables are corrected, the module should be tested on a smaller group of students. If it works out, it can be integrated into the Lab II module.

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