2023 Selection Camp Practical Student Code:

Candidate ID, Score, **2023 , 34th** International Biology Olympiad National Team Selection Camp

Test Room **4**

Plant Group Practical Questions

Note: Operation time is limited, please make good use of the time waiting for the experiment to take effect.

This experiment will utilize wild-type Col-0 treated with darkness and far-red light, as well as mutants a and b.

Using Arabica mustard as the material, this study investigated the effects of light treatment on plant growth and gene expression.

I. Materials and Experimental Equipment:

| A. Experimental plant materials: | | | quantity |
|---|---|---|---|
| 1. Arabian mustard (wild-type Col-0, mutant a, and mutant b) grow in darkness and far-red light (light intensity... Seedlings aged 4 days in 15 μmol/m2 s. | | | 25 shares each /per person |
| B. Experimental Equipment: | Quantity B: Experimental equipment: | | quantity |
| 1. Small grinding rod | 6 15. Timers | | 1 unit |
| 2. Tweezers (with fine pointed tips) | 1. 16. Computer | | 1 unit |
| 3. 1.5 ml microcentrifuge tube (Eppendorf tube) | 8 17. Oscillators (common) | | 3 units |
| 4. One 1000 μl and one 200 μl micropipettes each; | 18. Centrifuge (shared) | | 2 units |
| 5. One box each of 1000 μl and 200 μl micropipette tips; | 19. Spectrophotometer (for public use) | | 3 units |
| 6. Centrifuge tube rack | 1 glove for 20. | | 1 pair |
| 7. Spectrophotometric colorimetric solution tank | 1 piece of 21. Explosion-proof clip | | 6 |
| 8. Waste liquid cup | 1 | | |
| 9. Oil-based ballpoint pen | 1 piece | | |
| 10. Dissecting microscope | 1 unit of C drug solution: | | quantity |
| 11. 2mm graph paper and 9cm petri dishes (It can be placed under the petri dish to measure the length of the primary roots) | Group 1. Extract (containing Methanol) | 1% HCl) | 2 ml |
| 12. 1 foot (15 centimeters) | 1. Chloroform | | 1 tube |
| 13. Spectrophotometer Cuvette (0.6 ml) | 6. Distilled water | | 2 ml |
| 14. Microbalance | 1 unit of 4. Liquid nitrogen (thermos) | | Some |

## II. Experimental Methods, Results and Discussion:

Experimental methods:

A student planted wild-type Col-0, as well as mutants a and b, of Arabian mustard seedlings under far-red light (15 Å) and 15 Å.

Anthocyanin extraction was performed using the following experimental steps: 1. Fifteen seedlings (wild-type Col-0, mutant a, and mutant b) were grown in darkness and far-red light for 4 days .

The processed materials were placed into 1.5 ml centrifuge tubes, and their fresh weight was measured and recorded.

Centrifuge tubes must be clearly labeled with a pen .

As shown in the table below:

| The following numbers are marked: Exam Number-1, Exam Number-2, Exam Number-3, Exam Number-4, Exam Number-5, Exam Number-6. | | | | | | |
|---|---|---|---|---|---|---|
| Material | Dark Group Col-0 | Dark Group mutant a | Dark Group Mutant b | Far-infrared light Col-0 | Far-infrared light mutant a | Far-infrared light Mutant b |

2. Pour liquid nitrogen into the lid of the thermos flask, and use tweezers to place the 1.5ml centrifuge tube from the previous step into the liquid nitrogen.

Soak for a few seconds (be careful not to exceed the centrifuge tube lid), and after the seedling tissue is completely frozen and the liquid nitrogen has evaporated, pour in a small amount of water.

The seedling tissue was ground into powder using a grinding rod, and then 200 µl of extraction solution was added. The mixture was then placed in a shaker and thoroughly mixed for 10 minutes.

Note: Please complete **6** small centrifuge tubes at once. After clamping with explosion-proof clips, place the tubes on a shaker to mix thoroughly **for 10** minutes.

3. Add 200 µl each of chloroform and sterile water to each of the 6 tubes and mix thoroughly.

4. Take the 6 small centrifuge tubes to the common area and have the teaching assistant centrifuge them at 13000 rpm. $^{the}$C. Centrifuge for 5 minutes.

5. Pipette 400 µl of the supernatant into a Cuvette spectrophotometer and take it to the public spectrophotometer. Ask the teaching assistant for assistance.

To aid in operation, measure OD530 and OD657 nm respectively. Place the colorimetric tubes into the spectrophotometric bath according to the markings on the colorimetric tube box.

6. Calculate the anthocyanin content using the formula:

[ OD530 – 0.25OD657] / seedling fresh weight [unit: µg / FW g]

Fresh Weight Record Table **(5 points)** Unit: grams (g)

| | Dark far-red light | |
|---|---|---|
| Wild-type Col-0 | | |
| mutant a | | |
| Mutant b | | |

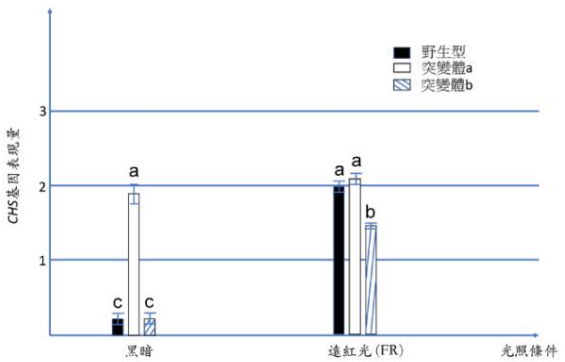
Anthocyanin content (5 **points)**

| | Dark far-red light | |
|---|---|---|
| Wild-type Col-0 | | |
| mutant a | | |
| Mutant b | | |

OD value record sheet pasting area

In addition, a student used the above materials to measure the expression level of the anthocyanin biosynthesis gene *CHS* (Chalcone Synthase).

The detection process is shown in the image below:




The different lowercase letters above the bar chart in the left image

The letters represent the expression of *the CHS* gene between samples .

Significant differences exist for those that are identical, while no differences exist for those that are identical.

Results and Discussion: Based on the above experiments and related results figures, answer the following questions:

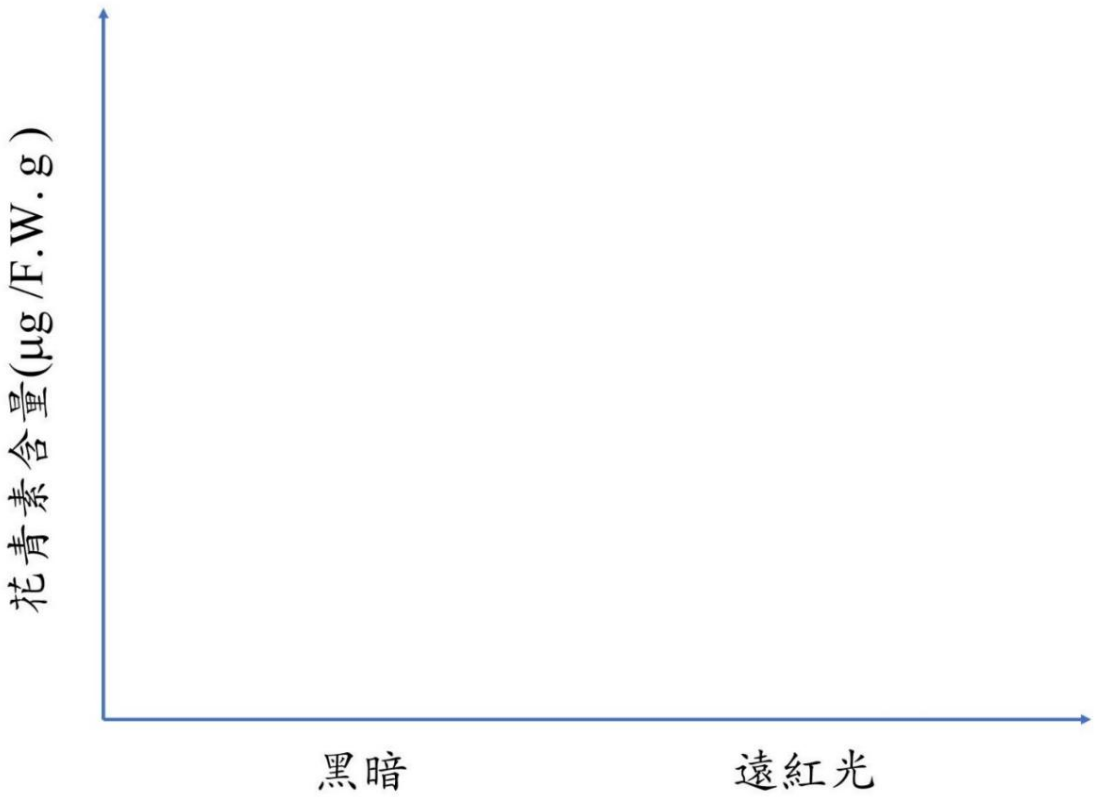
1. Using a dissecting microscope, graph paper, and ruler, observe and attempt to depict the phenotypes (such as root length, hypocotyl length, cotyledon closure, anthocyanin distribution or content) of wild-type Col-0 and mutants a and b Arabica mustard seedlings after four days of cultivation in darkness and far-red light. **(10 points)** (1). In darkness

| | | |
|---|---|---|
| | | |

(2). In far-red light

| | | |
|---|---|---|
| | | |

2. Please draw the anthocyanin content of wild-type Col-0, and mutants a and b, Arabica mustard seedlings after four days of cultivation in darkness and far-red light, respectively, in the diagram below. **(20 points)**




3. Based on the results of *the CHS* gene expression in wild-type Col-0 and mutants, explain how genes A and B regulate the expression of the *CHS* gene. **(10 points)**

4. Explain what the *CHS* gene is? What is its relationship with anthocyanins? (10 **points)**

5. Based on the phenotypic and anthocyanin content of wild-type Col-0, and mutants a and b, in darkness and far-red light, and their correlation with *CHS* gene expression, explain how genes A and B regulate photomorphogenesis in seedlings (positive or negative regulation?). Furthermore, explain why mutant a exhibits particularly high *CHS* gene expression in darkness. **(20 points)**

6. Besides light affecting anthocyanin accumulation, list two other hormones that can influence anthocyanin accumulation. (10 **points)**

7. Assume that gene C is a positive regulator of photomorphogenesis. However, after a mutant of this gene was grown in darkness and far-red light for 4 days, the anthocyanin content was measured, and the results are shown in the figure below.

Based on these results, explain the possible mechanism by which gene C regulates anthocyanin production in darkness. (10 **points)**

explain: