Effects of Ancymidol on Tall Mutant Brassica Rapa Growth

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This experiment investigates the effects of ancymidol, a gibberellin (GA) inhibitor, on the stem growth of wild-type (WT) and tall mutant Brassica rapa (B. rapa). GAs are plant hormones that promote stem elongation. The tall mutant which overproduces these GAs, was said to show reduced elongation when treated with ancymidol. To test this, both genotypes were divided into control and experimental groups, and developed over thirteen days with treatments on Days 0 and 7 and measurements on Days 0, 4, 7, 10, and 13. WT plants showed no significant response to ancymidol, with final heights nearly identical between groups. However, the tall mutants exhibited a visible effect and those treated were significantly shorter than the control. These results support the hypothesis that ancymidol inhibits growth in GA concentrated plants.

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Introduction

B. rapa, or Wisconsin fast plant, is an upright species of fast-growing plant in the mustard family (Brassicaceae) [1]. It is considered a model organism for study because it has highly responsive hormone signaling pathways and various available mutants [2]. Plant hormones act as chemical messengers that regulate growth, development, and environmental responses. Synthesized in specific tissues, they are transported via the phloem, xylem, or cell-to-cell diffusion to reach various regions of the plant. The hormones then bind to specific receptor proteins and elicit a cellular response. This movement is referred to as a hormone signaling pathway [3].

B. rapa is exceptionally responsive to hormones like GAs, which visibly affect traits such as plant height and flowering time. Through cell expansion, GAs contribute to the elongation of stems and other organs, making them key determinants of a plant's height. Cell expansion is triggered when GAs activate enzymes that break down the cell wall, decreasing the cell's water potential, and allowing the cell to expand [4]. Ancymidol, a growth retardant, inhibits GA production by blocking an enzyme known as monooxygenase, a catalyst in GA biosynthesis [5].

The tall mutant exhibits significantly elongated stems compared to the WT. This is caused by low levels of a GA regulator protein called phytochrome B. Lower levels of this protein are due to the fragmenting of the PHYB gene, disrupting how the gene is expressed [6]. Phytochrome B typically acts as a brake on GA synthesis, but its absence allows the hormone to accumulate unchecked. Ancymidol offers a way to test the relationship between GA and plant height experimentally. This study centers on the hypothesis that the tall mutant treated with ancymidol will have a reduced height, similar to the untreated WT B. Rapa plant. The experimental results of this study provide significant evidence that ancymidol does decrease the height of the tall mutant, and that this result is similar to that of the controlled WT B. rapa plant.

Materials & Methods

A: Organisms

In this experiment, WT and tall mutant strains of B. rapa were used. Two styrofoam quads were prepared for each genotype, four quads in total. Each contained at least eight plants to allow for statistical significance. All plants germinated under identical conditions, and experimental treatments began eight days later.

B: Chemical Treatment

Ancymidol, used at a molar concentration of $4x10^{-4}$ M, was purchased from Sigma Chemical Company (St. Louis, MO). Genotypes were divided into experimental (treated with ancymidol) and control (treated with double-distilled water) groups.

C: Growth Equipment and Setup

Height was measured using 15, 30, and 45 cm rulers. On Day 0, wooden stakes were inserted into the soil of each quad and tied to the stems below the apical bud using sewing thread. No more than two stems were to be tied to each stake. Labels were attached to quads to indicate genotype and treatment group. Height was measured from the base of the soil to the tip of the apical bud; once flowering occurred, measurements extended to the tip of the flower. Micropipettes were used to treat each leaf. All equipment was purchased from Carolina Biological Company (Burlington, NC).

D: Growth Conditions

All quads were placed on a moist felt mat connected to a water reservoir, allowing constant irrigation. Each quad had wicks extending from the bottom, enabling water to move through the felt into the soil. All plants received equal exposure to light and were housed in a communal room to maintain uniform temperature (20ºC). Light duration was constant and was provided by an artificial light source.

E: Timeline

This study lasted 13 days, including two treatment and five measurement days. Treatments occurred on Days 0 and 7, while the height was noted on Days 0, 4, 7, 10 and 13.

F: Data Logging

Measurements were entered into Microsoft Excel. The mean, standard deviation, and standard error of plant height were calculated for each treatment group and time point. Statistical significance was then assessed by the evaluation of the standard error bars. If bars overlapped, there was no significant difference. The procedures followed in this experiment were based on the Laboratory Manual for Organismal Biology [7].

Results

Stem height measurements revealed apparent differences in growth patterns between treatments and genotype groups. On Day 0, heights were comparable; the WT control group began with an average height of 4.02 cm, while the experimental group began at 6.26 cm. Despite this slight difference, the two groups had no statistically significant differences throughout the procedure, as the error bars on the graph overlapped at all points (Figure 1). By Day 13, the WT control and experimental groups reached final heights of 13.30 cm and 13.23 cm. This outcome was unexpected, as we believed that ancymidol would reduce B. rapa plant height. This suggests that WT B. rapa may be less sensitive to GA inhibition than previously thought.

In contrast to the WT, the tall mutant groups displayed a pronounced response to ancymidol. As early as Day 4, a statistically significant difference in height between the treated and control mutant groups emerged, as seen by the separation of the standard error bars in Figure 1. This supported our hypothesis, and from Day 4 on, this trend continued to increase. By Day 13, the untreated mutant group reached an average height of 17.38 cm, while the treated mutant group measured only 13.25 cm, closely resembling the WT groups' final height.

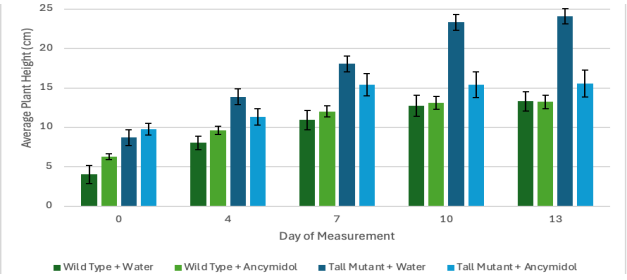

Figure 1: Average B. rapa Height; Treatment of Wild Type and Tall Mutant Plants with Ancymidol and Water

Discussion

The limited effect of the ancymidol on the WT genotype was unexpected. We initially believed that applying ancymidol would inhibit GA biosynthesis by limiting the needed production of the monooxygenase enzyme, thereby reducing stem length. However, the treated WT plants showed little to no difference in growth compared to the control group. This could be attributed to several factors, including already low endogenous GA levels in the WT, hormonal feedback mechanisms, or insufficient ancymidol concentration. A study by John Shive and Hugh Sisler [8] supports the interpretation of WT plants having lower endogenous GA levels. Their study confirms that ancymidol targets GA biosynthesis, but more importantly, suggests that ancymidol is most effective in plants with high endogenous GA activity. This discovery aligns with our findings that the tall mutant, with its elevated endogenous GA levels due to a defective phytochrome B pathway, responded strongly to the treatment. In contrast, the WT with lower levels did not.

The tall mutant's strong response to ancymidol further supported our initial hypothesis that the treatment will reduce stem elongation in the mutants. The tall mutant has an abundance of endogenous GA and depends heavily on GA signaling to maintain its elongated nature. Therefore, adding ancymidol, which inhibits monooxygenase, significantly disrupted this process. As a result, there was a significant reduction in stem growth between the ancymidol treated experimental and untreated control groups. The experimental group ultimately had a final height comparable to that of the WT plants because the ancymidol's regulation of GA biosynthesis reduced the hormone's activity to levels more characteristic of the WT.

Overall, this experiment highlights the utility of hormonal mutants in investigating plant development and chemical regulation. The tall mutant demonstrates how a single mutation can shift hormonal balance and physical outcomes. These findings have broader applications, mainly in agriculture, where understanding hormone regulation can inform selective crop growth, controlled breeding, and the usage of growth inhibitors to manage plant size. Farmers could use tactics such as adding an inhibitor like ancymidol to reduce stem elongation without affecting flowering, or adding a substance similar to GAs to increase crop yield. In future experiments, we could have improved our procedure by testing different concentrations of ancymidol to see varying effects based on dose and if a limiting threshold exists. This study demonstrates that growth regulators such as ancymidol serve as powerful tools for investigating and controlling hormonal function in plant development, particularly when applied to mutant genotypes with altered hormone signaling pathways.

Wild type and tall mutant B. rapa plants were treated with ancymidol, a growth inhibitor, and were measured every 3 to 4 days. The standard error is significant for the tall mutant plants but not for wild type plants. Overall, the ancymidol did not affect the wild type plants, but decreased growth for tall mutant plants compared to the controls.

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