The Effect of Gibberellin A3 on Wild and Dwarf *Brassica rapa* As Studied under Laboratory Conditions

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Biological Sciences 155L
29 November 2000

ABSTRACT

The effect of Gibberellin A3 on wild and dwarf *Brassica rapa* was studied under laboratory conditions in order to gain a better understanding of plant hormones, most specifically what the effect of gibberellin is on these plants. The hypotheses tested were 1) that the dwarf growth form is the result of the chemical regulator being present in low concentrations endogenously, 2) that there are physiological receptors functional in the dwarf plants, 3) that topically applied chemical regulator does not fully rescue the wild type phenotype in treated dwarf plants, 4) that there are dosage dependent and upper threshold responses present in the physiological responses of the plants, and 5) that the pooled class data do not show that the endogenous gibberellin concentration in the wild type plants is at an upper threshold. In carrying out the experiment 32 Styrofoam wells were prepared over a water reservoir system that operated on the basis of capillary action. Half of the plants were dwarf and half were wild. After one week of growth, gibberellin A3 was applied in dosages of two, twenty, 200, and 500 micrograms, and the mean heights were taken at the end of the second week. There were mass replications of the experiment to ensure the viability of the results. Statistical ANOVA and Tukey's'MCT tests were performed to determine the significance of the results. The results showed that the wild type plants had a much higher mean height than the dwarf plants and that the

gibberellin did spur growth in both varieties of plant. All the hypotheses were supported, with the third hypothesis being the most robustly supported. In conclusion, the experiment showed that gibberellin spurs stem elongation and growth to a point, but once an upper threshold is reached, it inhibits growth, and the plant will eventually return to its normal growth rate.

INTRODUCTION

In the study of physiology, one of the major topics covered is the endocrine system. It is very commonplace to know at least something about the animal endocrine system as people must be concerned with the function of their bodies. Of primary knowledge are usually the various sex hormones and the effects of adrenaline, known as epinephrine in biological terms. However, animals are not the only organisms that utilize hormones in carrying out their existence. Plants also rely on hormones to function, and a lack of certain hormones can often signal certain death. Among the most common plant hormones are auxin, which is very important in root formation, leaf abscission, apical dominance, and fruit development, cytokinins, which are important in everything from promotion of plant cell division to delaying leaf senescence, ethylene, a hormone which aids in leaf abscission and inhibits elongation among other functions, and abscisic acid, which is commonly referred to as the stress hormone (Purves et al. 1998). And finally, there is gibberellin, the hormone of concentration for my experiment. This hormone is essential to plant growth as it spurs stem elongation and fruit growth. Although only one gibberellin, gibberellin A1, controls stem elongation, others such as A3, the gibberellin in my experiment, are intermediates in the production of A1 (Purves et al. 1998). Thus, in

order to better understand the effects of Gibberellin on dwarf and wild type plants, this study was carried out.

In choosing the system and organisms that were utilized, many factors were considered. First of all, because Gibberellin A3 is available commercially, it was chosen as the hormone of choice. Second, in choosing *Brassica rapa* as the organism on which to test the Gibberellin, time of the experiment was the major consideration. Because I had only approximately one month in which to generate my results, the *B. rapa* were chosen because it takes them two weeks to grow to adult and four weeks to grow to seed (Olsen et al. 2000). Finally, the watering reservoirs and planting chambers that I chose were used for their ease of usage and efficiency in watering.

During the course of my study, the following hypotheses concerning *B. rapa* and gibberellin A1 were tested. First, the dwarf form is a result of the chemical regulator (gibberellin) being in low concentration endogenously. Second, the physiological receptors are functional in the dwarf (rosette type) plants. Third, topically applied chemical regulator does not come close to rescuing the wild type phenotype in treated dwarf plants. Fourth, the results will indicate that physiological responses by the *B. rapa* in the experiment include dosage dependence of the gibberellin and an upper threshold of the gibberellin, as well. Finally, the data will show that the endogenous gibberellin concentration in the wild type plants is not at an upper threshold. All of these hypotheses were be tested through the application of gibberellin A3 to wild and dwarf *B. rapa* plants.

This study, it must be known, was not undertaken without credible scientific precedent. On several occasions the effect of gibberellin has been studied. Some notable examples include Cosgrove and Sovonick-Dunford (1989), Brian and Hemming(1955),

Moore (1967), and Sachs et al. (1959). Furthermore, Bernard O. Phinney of UCLA, in 1956, tested the effect of gibberellin on certain dwarf strains of corn and found a very astounding flux in corn growth (Purves et al. 2000).

MATERIALS AND METHODS

The plant organism studied in this experiment was the *Brassica* rapa, a plant within the mustard family (Olsen et al. 2000). Although the family itself is very complex, genetic manipulation of its various members was done to obtain the strands used in the experiment, strands which grow to adult in two weeks and seed in four weeks (Olsen et al. 2000). Then, within these strands, specific wild type and dwarf-type (plants growing close to the ground) plants have been bred (Olsen et al. 2000). Thus, the miracle of genetic engineering in many ways makes this lab possible. These plants were purchased from Carolina Biological Supply.

In carrying out this experiment, a planting system was arranged in which a water system reservoir was filled with tap water, and a cloth mat was used to draw the water into wicks that extended from the bottom of a Styrofoam growing chamber into the reservoir. To prevent algal growth in the water, a copper sulfate tablet was placed in the reservoir water. The thirty-two chambers of the Styrofoam were filled with dirt and then sixteen cells were allotted for the dwarf plants and the remaining sixteen went to the wild type plants. Within each subset of sixteen then, there were eight cells allotted for the control plants and eight cells allotted for the treated plants. Three seeds of one variety were put into each depression. After the seeds were planted, the dirt was watered gently until water dripped from each wick tip. In the week following the planting, seeds were watered, and seedlings were thinned so that only one plant was in each cell.

After one week, gibberellin A3, purchased from Sigma pharmaceuticals, was applied in various microgram dosages of two, twenty, 200, and 500 micrograms to the treated organisms by placing the solution on two leaves of each plant. The watering and thinning was continued during week two. During the entire course of the experiment, constant twenty-four hour fluorescent light at a strength of 80 watts was maintained at a height of 5 centimeters from the top of the plants. After three weeks, the experiment was terminated, and the plants were measured for their height. For more detailed information on the set-up and procedure of this experiment, see James (1989) and Olsen et al. (2000).

In order to determine which pairs of mean heights were not significantly different and also test my hypotheses, ANOVA and Tukey's MCT results were calculated (Sokal and Rohlf 1987). Means connected by unbroken lines were not significantly different.

Finally, it must be noted that there were numerous replications of this experiment as it was performed by twenty-five different lab groups. Thus, the results of the tests are statistically viable.

RESULTS

Dwarf Type *Brassica rapa*

Among the dwarf type plants, there were 141 controls (DC), thirty-five treated organisms (DT) having two micrograms of gibberellin applied, forty-six DT having twenty micrograms applied, thirty-six DT having 200 micrograms applied, and thirty DT having 500 micrograms applied. Figure 1 shows these numbers for sample size above the bars indicating the mean height compared to gibberellin dosage. As Figure 1 shows, the DC have a mean height of .86 cm. For the DT having two micrograms of gibberellin applied, the mean height rose to 2.34 cm, and this height continued to increase as the DT

having twenty micrograms of gibberellin applied had its mean height increase to 4.00 cm. However, this trend did not hold for the DT-200 and DT-500 as their mean heights decreased to 3.18 cm and 2.54 cm, respectively.

Wild Type *Brassica rapa*

Among the wild type plants, there were 146 controls (WC), thirty-eight organisms treated (WT) with two micrograms of gibberellin, forty-eight WT with twenty micrograms of gibberellin, thirty-eight WT with 200 micrograms of gibberellin, and thirty-two WT with 500 micrograms of gibberellin applied. The respective mean heights for the wild type *B. rapa* starting with the WC and ending with the WT-500 were 8.70 cm, 9.33 cm, 11.15 cm, 10.40 cm, and 10.38 cm. These specific wild type results are graphically illustrated in Figure 1.

Results of Statistical Tests

The ANOVA and Tukey's MCT statistical tests were run on the data. The p-value for the ANOVA test was less than 0.0001($P<0.0001$), indicating that some mean heights are statistically different from one another. This is shown on the top horizontal axis of Figure 1. Then, a Tukey's MCT test was run on the data. The color-coated bold lines below the graph in Figure 1 show the results of the Tukey's MCT test. The lines connect means that do not differ significantly from each other ($P>0.05$). Therefore, the mean heights of the DT-20 and DT-200 are not significantly different, and the mean heights for the DT-200 and DT-500 are likewise not significantly different. For the wild type plants, the mean heights of WC and WT-2 are not significantly different, and the mean heights of WT-20, WT-200, and WT-500 are also not significantly different. All Tukey's MCT results just discussed are illustrated in Figure 1.

DISCUSSION

In comparing my results with my hypotheses, it is very apparent that all of my hypotheses were correct. Statistically speaking, I reject the null hypothesis in each case in order to accept my favored hypothesis. For my first hypotheses which claims that the dwarf form is the result of the gibberellin being in low concentration endogenously, I first turn to Purves et al. (1998) in which it discusses how dwarfed plants are simply those plants that have had a genetic mutation, thus causing them not to produce normal amounts of gibberellin. It is also very important to note that gibberellin promotes seed germination (Purves et al. 2000). If the dwarf plants did not have gibberellin, they would not be able to grow. Thus, because my dwarf controls did grow to a mean height of .86 cm, this substantiates the claim that the gibberellin is present in dwarfs, but simply in low concentration.

In some ways, my second hypothesis, concerning the function of physiological receptors in the dwarf (rosette type) plants, lends itself to the first. Indeed the receptors of hormone are very present in the dwarf plants based solely on fact that the dwarf controls do grow. However, when the gibberellin A3 was added to the rosetta types, the mean height increased above the mean height of the control in all cases, thus showing that gibberellin was received by the plant.

Of my five tested hypotheses, the third, that topically applied chemical regulator in the form of gibberellin would not fully rescue the wild type phenotype in treated dwarf plants, is the most robustly proven. Based on the Tukey's MCT test, we see that none of the wild Tukey lines cross over into the dwarf region, thus indicating that the mean heights are significantly different between the two variations of *B. rapa*. Furthermore, as

Figure 1 shows, in the presence of gibberellin the tallest the dwarf ever gets is a mean height of 4 cm. This is more than 4 cm below the mean height of the wild control plants, further showing the robustness of this result.

My fourth hypothesis, which asserts there are dosage dependent and upper threshold physiological responses present in the *B. rapa*, is very nicely proven. However, for me, this was also the most unexpected finding. First of all, because in both the dwarf and wild type organisms, after twenty micrograms of gibberellin were applied, the mean heights were significantly greater than those of the control, this shows that substantial growth as a result of gibberellin was at least in part dependent on the dosage level. This was not surprising. However, the sense of an upper threshold did surprise me. In both the wild and dwarf organisms, the mean height dropped from the twenty microgram dosage to the 200 microgram dosage. Furthermore, it dropped again, and significantly even in the dwarf plants from the twenty microgram level, when the 500 microgram dosage was applied. Thus, somewhere between the twenty and 200 microgram level of gibberellin, an upper threshold is reached, and after this point, the gibberellin actually inhibits growth rather than spurring it. This was very unexpected for me.

Finally, for my fifth hypothesis, the pooled data do not suggest that the endogenous gibberellin concentration in the wild type plants is at an upper threshold because the Tukey's MCT test illustrates that there is a significant difference in mean height between the wild control and the treated wild *B. rapa*. Because significant growth occurs, the endogenous gibberellin is not at an upper threshold.

In order to ensure the validity of my results I would propose doing the experiment again, both with the *B. rapa* and with other types of plants, testing the effects of

gibberellin on a wide range of plant species. Also, I would more carefully monitor the care of the plants during the growth stages, making sure that water was given daily. In some instances, a daily treatment was neglected, and this could have affected the results.

In comparing my results with the given references, it becomes very evident that my conclusions concerning gibberellin matched those of the journal articles. In Moore (1967), it is reported that by the time treated seedlings were approximately two weeks old, they failed to respond to the applied gibberellin. Then, Brain and Hemming (1955) report that compared to untreated plants, the growth rate of gibberellin treated plants increases for a time but then eventually falls. A similar result is seen in Sachs et al. (1959) as they report an increase in the mitotic activity just below the apical meristem in the first twenty-four hours after the gibberellin was applied, but then no evidence of elongation 72 hours after the application. Finally, in Cosgrove and Sovonick-Dunford (1989), it is seen that gibberellin reverses the effects of cell wall reduction and growth retardation. Thus, all of these various findings support my main idea that gibberellin spurs stem elongation and growth to a point, but after an upper threshold is reached, the gibberellin becomes an inhibitor to growth.

ACKNOWLEDGEMENTS

In carrying out this experimental research, many people made my work and efforts possible. First of all, my biology professors, Dr. Olsen, Dr. Lodge, and Dr. Duman, challenged my colleagues and I to further our knowledge of science through this study, and indeed they did just that. But in challenging us, they also were always there to answer any questions we had, and for this we are eternally grateful. Also, the teaching assistants of lab section 1, Stacy Pluger, Jeff Luebbert, Emily Contreras, Stacy Leicht,

and Allison Troy were always there for us, and they managed to keep us sane throughout this entire semester of work. Of course, the one and only Fungi lab bench, which will one day rule the world, was awesome, and all of you, you know who you are, will be in my ad infinite memory.

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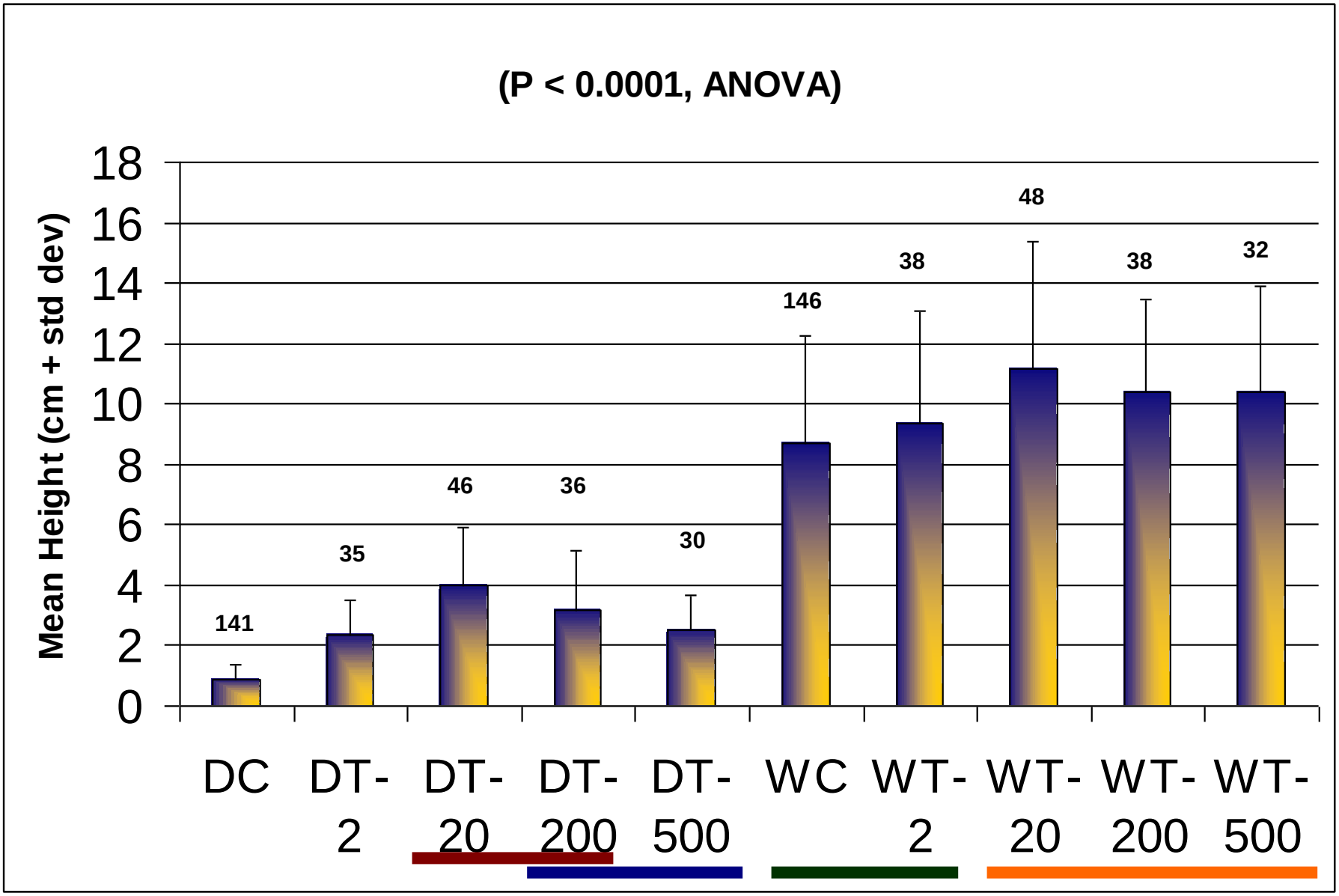

Figure 1- Graphic presentation of data from *Brassica rapa* endocrinology experiment. Gibberellin hormone was applied in micrograms to all treated samples of dwarf and wild *Brassica rapa* species. This is represented by DT (dwarf treated) and WT (wild treated). DC (dwarf control) and WC (wild control) represent the controlled organisms, respectively. The numbers above each mean height bar indicate the sample size, and the error bars show one standard deviation above the mean. The ANOVA test was significant ($P<0.0001$), indicating that some means are significantly different from one another. Tukey's MCT results are shown by the unbroken lines below the numbers. The lines connect means that do not differ significantly from each other ($P>0.05$). Means not connected by unbroken lines are significantly different ($P<0.05$). The various colors of the lines are used for clarity.