JOURNAL OF
EMERGING INVESTIGATORS

Article

Synthetic auxin's effect on root hair growth and peroxisomes in *Arabidopsis thaliana*

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SUMMARY

Root hairs are long tubular outgrowths of plant's root cells that increase water and solute uptake to nurture plants. Root hairs of *Arabidopsis thaliana* play an important role in studying how auxin affects plants. We investigated the effect of synthetic auxin, naphthalene acetic acid (NAA), on root hair length and peroxisome organelle movement in root hairs. We expected that increasing the concentrations of synthetic auxin in wild-type plants and mutants in class XI myosin, xik and xib would cause an increase in root hair length and linear movement of peroxisomes in the plants. Adding 0.1 µM of NAA to the plant's growth medium resulted in significantly longer root hairs in wild-type and mutant plants compared to other concentrations tested (0 µM, 0.01 µM, and 1µM). Adding 1 µM of NAA to the plants did not produce significantly longer root hairs compared to 0 µM and 0.1 µM. These data suggest that an increase in auxin can help compensate for the observed impacts caused by the mutants, but that there might be a maximum capacity of auxin intake for a plant to receive optimal growth of root hairs. We found no significant trend for peroxisome movement with an increase of NAA in wild-type and mutant types for linear and wiggling velocity.

INTRODUCTION

Root hairs play a key role in obtaining nutrients to nurture the plant and assist with anchorage because they vastly increase the root surface area (1). *Arabidopsis thaliana* root hairs are a useful model to better understand the regulation of plant cell fate and growth in response to environmental signals (1). Hormonal cues can help regulate plant growth, facilitating the plant's response to environmental changes. Hormones like the auxin family affect the activity of proteins involved in gene expression, resulting in changes in root hair growth and peroxisome organelle movement (2).

Root hairs are strongly influenced by external environmental conditions, and root hair regulation is an important mechanism of environmental adaptation of plants. Phytohormones regulate root hairs, and auxins are a well-known phytohormone that massively enhances root hair growth (1). The maximum concentration of the plant hormone auxin is found in the root tip, and the initiation of root hair growth is dependent on auxin (1). Auxins regulate root growth and development by modulating the signaling cascades in auxin-induced lateral formation, which is growth of lateral branches or roots. The rate at which individual parts of the root system develop and grow changes in response to environmental signals (3). Myosin motor proteins couple the hydrolysis of ATP to the active movement of organelles along the actin cytoskeleton (4). The dynamic actin cytoskeleton leads to the regulation of root hair growth by many small vesicles (4). Plants exhibit intracellular dynamics with the help of membrane-bound organelles, like peroxisome, and these helps produce signaling molecules (5*). A. thaliana* has 13 class XI myosin motor proteins (6). Class XI myosins drive rapid organelle movements during cytoplasmic streaming, which is the directional movement of cytoplasm within the cell (4). These myosin proteins maintain cell polarity by moving organelles and vesicles along the actin cytoskeleton. Actin filaments are arranged along the root hair to facilitate the movement of vesicles to the growing tip of the root hair, where they accumulate (4). The accumulation of these organelles and vesicles at the root hair tip, mediated by myosin motor proteins, is crucial for root hair elongation. Specific mutant genes of class XI myosin reduce the length of root hairs and cause defects in cell polarity. Myosin mutants of the genes *XIK* and *XIB* (denoted by *xik* and *xib*, respectively) have been developed to disrupt the elongation of root hairs and organelle movements in root hairs. It has been reported that myosin XIK is required for normal actin dynamics and facilitates growth of root hairs (4). Also, peroxisomes in plants are known to contribute to many metabolic processes, such as cell elongation, and since peroxisomes produce signaling molecules, testing if increasing auxin has an effect is important to this study. Myosin motor proteins additionally help with the rapid movement of peroxisomes, which therefore help facilitate growth (6). These linear movements make cytoplasmic streaming more efficient, therefore, helpful for optimal root hair growth. The linear movements are dependent on actin filaments and myosin motor proteins to help the cytoplasmic streaming. In past research, the *xik* mutant plants have significantly shorter root hairs than wild-type plants because they grow more slowly (4). The impaired actin dynamics help to explain the reduction in root hair and organelle movement in *xik* mutant plants.

Since both auxins and myosin play crucial roles in facilitating root hair growth, it is important to understand their interconnected functions. Numerous studies have explored how myosin interacts with natural auxins in plants. These investigations revealed that the transport of auxins in plants relies on the vesicular cycling of proteins between the plasma membrane and internal compartments (8). Inhibiting myosin activity resulted in a reduction in polar auxins transport and cytoplasmic movements (9). Exploring this interaction stemmed from the understanding that myosin regulates signaling processes, prompting to investigate its potential involvement in auxins transport (9).

https://doi.org/10.59720/23-113

Since myosin mutants *xik* and *xib* in *A. thaliana* plants impair root hair growth and organelle movement, and auxins regulates and enhances root hair length, this experiment investigated to what extent the effect of various concentrations of synthetic auxin could minimize or reverse the defect caused by the mutant proteins. Since the mutant plants create shorter root hairs and exhibit reduced movements in peroxisome organelle movement, the synthetic auxin hormone may help overcome the defects. We hypothesized that increasing concentrations of naphthalene acetic acid (NAA) would increase the growth of root hairs in wild-type and overcome the defects caused by the mutant plants. We also hypothesized that the increasing concentrations of NAA would slow down the peroxisome organelle movement leading to more wiggly movements and less linear direct movements. In this study, we used a solution of NAA in ethanol to make the synthetic auxin. Then, by planting mutant seeds in agar plates induced with various concentrations of NAA, we measured the effect of root hair length and peroxisomes movement of each genotype tested. We showed a maximum capacity of auxin intake for root hair length and no significant findings with peroxisome movement with added NAA. In addition, we showed that auxins could compensate some of the defects on root hair length produced by the myosin mutants. Further studies are needed to support the results of this study.

RESULTS

Root Hair Growth

To examine the effect of synthetic auxin, NAA, on root hair growth, we measured the length of root hairs after the addition of various concentrations of synthetic auxin to wild-type (*TOM*) and mutant (*xik* and *xib*) *A. thaliana*. We allowed the plants to grow for four days to give NAA the time to take effect in the root hairs. We observed the physical characteristics for the root hairs in each plant were generally long or short root hair length growing distal from the root. Some phenotypes observed specifically in *xib* mutants were generally shorter roots and fluffier root hairs (**Figure 1**).

The root hairs were the longest for all plants with NAA concentration of 0.1 µM added. These root hairs were significantly longer than the length of root hairs of other concentrations, including the control group ($p < 0.05$, **Figure 2**). Plants treated with 0 µM, 0.01 µM, and 1 µM NAA were not significantly different for any of the plant types with respect to root hair length. However, it was seen that with 0.01 µM NAA concentration an increase in root hair length was observed in both the wild-type and each mutant types. (**Figure 2**). Although it was expected that an increase of auxin would increase the length of root hairs, an addition of 1 µM NAA to the plants did not make the root hairs longer than the addition of 0.1 µM NAA. Additionally, *A. thaliana* plants grown in either 1 µM or 0.01 µM NAA did not increase as much in root hair length as compared to 0.1 µM NAA and the control (**Figure 2**).

It was unexpected to see the result of *xib* treated with 0 µM NAA being similar or even longer than *TOM* treated with 0 µM (**Figure 2**). *Xik* and *xib* mutant *A. thaliana* plants showed a trend of shorter root hair length than *TOM* with no added synthetic auxin (**Figure 2**). However, as soon as NAA was added, *xib* was longer than *TOM* for all NAA concentrations. *Xib* with 0.1 µM NAA concentration added was significantly longer than *xib* treated with no NAA ($p < 0.05$, **Figure 2**). *Xik* was consistently lower than both *TOM* and *xib* for all NAA concentrations. *Xik* treated with 0.1 µM NAA resulted in significantly longer root hair length than *xik* treated with no NAA ($p < 0.05$, **Figure 2**).

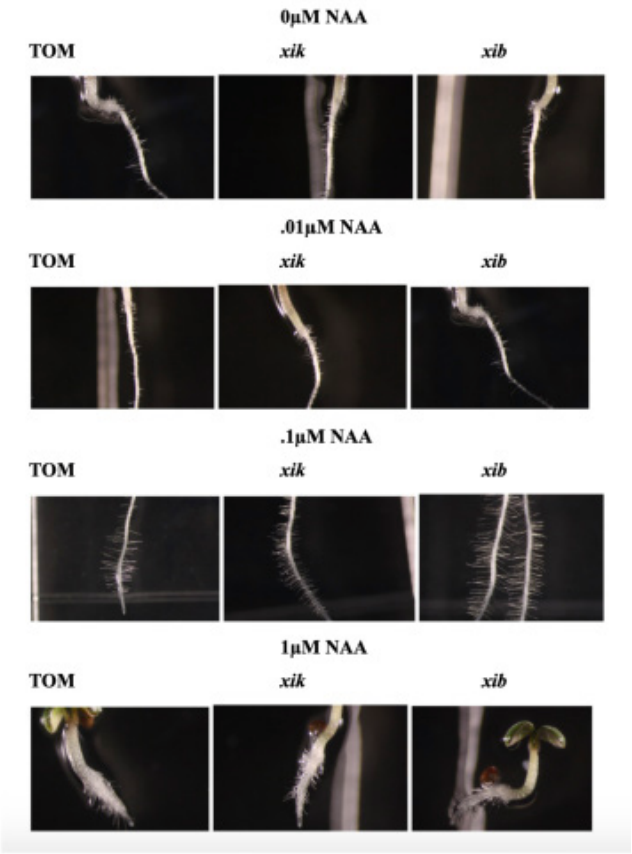

Figure 1. Representative images of changes in root hair growth in response to various NAA concentrations. The root hairs were imaged after four days post-germination. The data from the images were analyzed in Excel. Replicates: 0 µM (TOM: n = 15, *xik*: n = 7, *xib*: n = 7), 0.01 µM (TOM: n= 16, *xik*: n= 7, *xib*: n= 13), 0.1 µM (TOM: n= 19, *xik*: n= 17, *xib*: n= 8), 1 µM (TOM: n= 10, *xik*: n= 9, *xib*: n= 5).

Peroxisome Movement

We took measurements to see the effect of synthetic auxin on peroxisomes in root hairs of wild-type and mutant *A. thaliana*. We took 200 pictures to create a time-lapse of the organelle movement, and measurements and analysis were performed with ImageJ. These time-lapse images revealed that most peroxisome movement was localized to the tips of the root hairs.

Linear movements are the undeviating movements made by the organelle peroxisomes that travel long distances. Linear velocity is measured in µm/s and is the speed of only the linear movements done by the peroxisomes in the root hairs of the types of plants. The data showed that *xik* was the slowest of all three (**Figure 3**). There was not a significant trend seen, however, with auxin concentration and linear velocity speed with all types (**Figure 3**). For *TOM* and *xik*, it was seen that the fastest linear movements for peroxisomes occurred with 0.1 µM NAA concentration. However, for *xib*, the fastest linear movements for peroxisomes occurred with .01 µM NAA concentration, whereas linear movements for *TOM* and *xik* were slowest at this concentration (**Figure 3**).

https://doi.org/10.59720/23-113

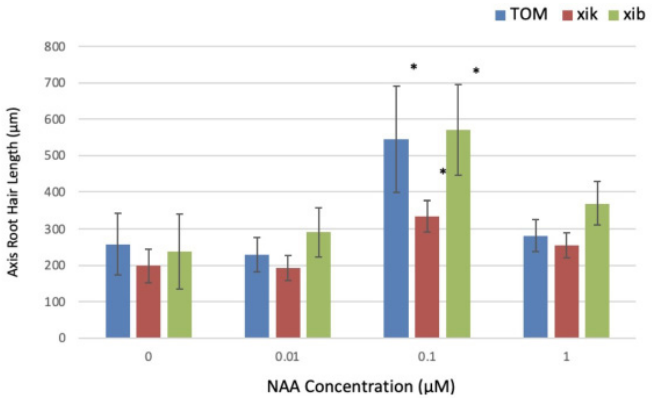

Figure 2. TOM, *xik*, and *xib* treated with 0.1 µM NAA is significantly longer in root hair length compared to their respective plant genotype treated with 0 µM NAA. The sample size amount for the number of plants for each concentration and type measured is respectively: 0 µM (TOM: n = 15, *xik*: n = 7, *xib*: n = 7), 0.01 µM (TOM: n= 16, *xik*: n= 7, *xib*: n= 13), 0.1 µM (TOM: n= 19, *xik*: n= 17, *xib*: n= 8), 1 µM (TOM: n= 10, *xik*: n= 9, *xib*: n= 5). Sample sizes were different due to some seeds failing to germinate or root hairs were unable to be measured. A t-test was used to compare if there was significance between the different concentrations of a plant genotype compared to their respective genotype treated with 0 µM NAA, *p < 0.05.

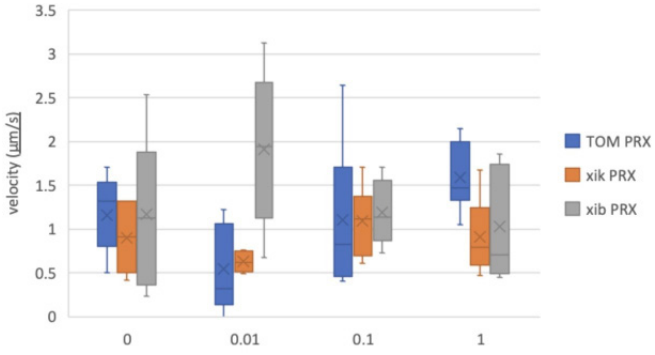

Figure 3. Peroxisome linear velocity does not significantly change with NAA treatment or myosin mutation. Average linear velocity of peroxisomes (PRX) in 75th percentile for wild-type and mutant types of plants grouped by concentrations of NAA. ImageJ was used to analyze 200 time-lapse photos. Then, with FIJI analyzation, data was collected measuring the long linear movements. Only the peroxisome linear velocity (µm/s) in root hairs of plants was considered. Error bars represent the standard deviation from the mean. Replicates: 0 µM (TOM: n = 9, *xik*: n = 6, *xib*: n = 8), 0.01 µM (TOM: n=5, *xik*: n= 4, *xib*: n= 5), 0.1 µM (TOM: n= 10, *xik*: n= 8, *xib*: n= 6), 1 µM (TOM: n= 6, *xik*: n= 6, *xib*: n= 5).

We define wiggle movements as the short, fast linear movements that enable the peroxisomes to look like they are making circular or non-linear movements over a long distance. Wiggle velocity is measured by the mean of all the short linear movements by the peroxisomes. There was a wide range in wiggling velocity seen in the *xik* control. *Xik* was shown to have the highest wiggling velocity movement with 1 NAA concentration, while *xib* has the highest with 0.01 µM NAA concentration. There was no significant trend seen with the wiggling movement of increasing NAA concentrations (**Figure 4**).

DISCUSSION

The mutant *A. thaliana* had shorter root hairs than the wild-type as expected. Previous experiments manipulated the intracellular auxin concentration of developing root hair cells, and they reported a strong positive relationship between auxin concentration and root-hair outgrowth (7). With an increase in synthetic auxin, therefore, we expected to find an increase in root hair length in each genotype because the initiation of root hair growth is dependent on auxin. The root hair length compared to 0 µM of NAA was significantly longer for all genotypes with 0.1 µM of NAA, but was not significantly longer with 1 µM of NAA. With such a high concentration of NAA, the plants may have a maximum capacity of how much auxin they can receive to allow optimal growth for their root hairs. Since auxin is an environmental signal that naturally occurs in plants and can also be obtained by plants, it is important to note that the largest amount of auxin did not produce the longest significant root hairs in this study. The plants may have a maximum intake of auxin due to saturation or toxicity. Adding a large amount of auxin, or any molecule, to a plant can be toxic. In further studies, these qualities can both be tested by seeing the observations of a plant with different levels of high dosage of auxin. An explanation for why the result of *xib* treated with 0 µM NAA was similar to *TOM* treated with 0 µM could have been due to external factors of how well the *XIK* gene was removed out of the seeds or because *xib* is not as an effective mutant compared to *xik*. *Xib* plants for all concentrations of added NAA had root hairs longer than the control for *TOM*. For *xib* treated with 0.1 µM NAA, the root hairs were significantly longer than the control of *TOM*. Also, *xib* treated no NAA is similar in root hair length compared to *TOM* treated with no NAA. These data observations show that this *xib* mutant does not significantly impact root hair length and is more amenable to auxin-mediated repair to the negative phenotypic consequences of the *xib* mutation. *Xik* was lower than both *TOM* and *xib* of the same concentration for all the various concentrations of NAA. This further supports that the *xik* mutant inhibits root hair growth very strongly. In previous experiments manipulating hormone intake, *xik* root hairs grew slower and stopped growth faster than *TOM*, resulting in greater inhibitory consequences for *xik* than wild-type (2). *Xik* treated with 0.1 µM NAA had significantly longer root hairs than the *xik* treated with 0 µM of NAA, supporting the hypothesis that increase in auxin concentration may compensate for the negative impacts on root hair growth caused by the disruption of these genes.

Since 0.1 µM and 1 µM of NAA added to each plant genotype in this study had longer root hairs than the control of each genotype, it is probable that an increase of auxin concentration might compensate for the defects caused by mutants by promoting root hair growth in myosin-independent ways. There could be a maximum concentration of NAA that these plants can obtain that is higher than 0.1 µM but lower than 1 µM.

In previous experiments, peroxisome movement is known to be the most intense in elongated wild-type cells (6). With respect to control for peroxisomes movement, *xik* is the slowest, while *TOM* and *xib* are very similar. This further suggests

https://doi.org/10.59720/23-113

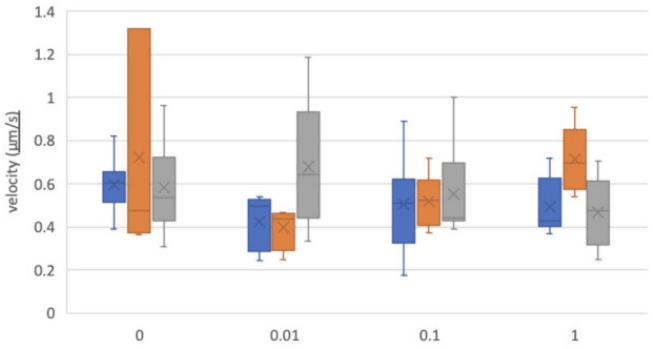

Figure 4. Peroxisome wiggling velocity does not significantly change with NAA treatment or myosin mutation. Wiggling velocity of peroxisomes (PRX) in the 75th percentile for wild-type and mutant types of plants grouped by concentrations of NAA (μM). Wiggle velocity is measured in μm/s and is the speed of only the wiggle movements done by the peroxisomes. ImageJ was used to analyze 200 time-lapse photos. Then, with FIJI analyzation, data was collected measuring the short, non-linear movements. Error bars represent the standard deviation from the mean. Replicates: 0 μM (TOM: n= 9, *xik*: n= 6, *xib*: n= 8), 0.01 μM (TOM: n= 5, *xik*: n= 4, *xib*: n= 5), 0.1 μM (TOM: n= 10, *xik*: n= 8, *xib*: n= 6), 1 μM (TOM: n= 6, *xik*: n= 6, *xib*: n= 5).

that *xik* has a more significant impact on root hair growth compared to *xib*. Since fast peroxisome organelle movement facilitates the growth of root hairs, it is consistent that *xik* would produce slower peroxisomes in the control as it produces shorter root hairs. However, the speed of *xik* is not significantly slower than control of the rest of the types of plants. There was no obvious trend in peroxisomes' linear and wiggling velocity and net displacement with an increase of synthetic auxin. We expected that with an increase in auxin concentration there would be an increase in linear movements for all types. It was also expected that peroxisomes in *xik* would have the slowest linear velocity, and our data confirmed this. In previous experiments, peroxisomes also moved more slowly in *xib* cells than in wild-type cells (6). However, with an increase in auxin concentration, there was no increase or decrease in linear velocity. Each type of plant had varying highest and lowest velocities for linear and wiggling for each concentration. The lack of a clear trend in these data does not support that increasing the auxin concentrations affects the movement of peroxisomes accumulated in root hairs for all types of plants experimented. Such factors in the experiment that may have influenced the lack of trend seen include technical limitations like the tracking algorithms not being sensitive enough to detect the subtle trends in peroxisome movements. External impacts to the dynamic stability of peroxisomes during imaging of the same plants used for analyzing the organelle movement.,

Overall, there was an increase in root hair length with an increase of synthetic auxin in all types. After 1 μM auxin was added, however, there was a decrease in the root hair length, suggesting a maximum capacity of auxin intake. There was no significant trend seen for linear movement of peroxisomes in root hairs for an increase of auxin concentrations.

Studying the role of auxin in mutated root hair plants provides the opportunity to characterize specific characteristics of genes when studying this process. This study is significant in showing how genes are affected by the mutations and how they affect the role in auxin-mediated root hair formation. By understanding the genetic basis of auxin-mediated root hair development in mutants, we can gain more information on how the functional relevance for genes in the context of auxin signaling.

MATERIALS AND METHODS

Synthetic Auxin

To make the synthetic auxin, approximately 17.7 mg of NAA was dissolved in 9500 μL of ethanol. Out of the (approximate) 100 mM stock, A 1mM stock and 0.1 mM stock were made by doing 100-fold and 1000-fold dilutions in H_2O, respectively.

Agar Plates

Growth medium agar plates were made with 4.3 g MS salts (Sigma M5524) and 10 g sucrose with 800 mL of deionized water. KOH was added dropwise (approximately 10-12 drops) to the solution to raise the pH to 6.0, as measured by a pH wand. After the solution reached pH of 6.0, 200 mL of solution was poured into each of the four flasks containing 5 g Phytagel (Sigma). Flasks were covered with tin foil caps, autoclaved on the liquid cycle for about one hour, and then allowed to cool to 55°C in a water bath.

After the flasks cooled to 55°C, each was made to a different concentration of NAA by diluting a NAA stock solution and stirring. The control group flask had 0 μM of NAA. Our experimental plates contained 0 μM, 0.01 μM, 0.1 μM, or 1 μM NAA. After all the solutions were mixed and made, each flask was poured into square sterile Petri dishes. Each petri dish was labeled and then, in the sterilization hood, the plates were left to sit for the night.

Sterilizing Seeds

Three genotypes of *A. thaliana* were used in this experiment: *TOM* 507, *xik* 507 alpha 2, and *xib* t2 507 alpha-2 seeds. Seeds were put into a tube each. Each tube was labeled with its designated name. A solution of 30% bleach, 1:1000 triton x-100, and distilled water was used to sterilize the seeds. For each of the three tubes, 1 mL of the sterilization solution was added. These tubes were then transferred to a rotator for seven minutes. After the rotation, seeds were rinsed in a sterile hood by removing the sterilization solution and adding then removing 1mL of distilled water four times while leaving all seeds in the tube. Then 1mL of water was put into each tube after the sterilization was complete.

Planting Seeds: Single-Seed Method

With a 1000 μL pipette, all the seeds of one genotype were removed from the tube, and seeds settled on the bottom to the tip. They were tapped lightly on the agar to release one seed. Approximately eleven seeds were planted on each row for each plate. Typically, one set of four plates of all concentration groups was plated, and the rest of the agar plates were put in the freezer.

After plating, the Petri dishes were sealed with parafilm and put into a 4°C refrigerator for about one to four days. This allowed the seed to germinate in the agar plates. After, the plates were transferred to the growth chamber at 23°C with continuous light for five days.

https://doi.org/10.59720/23-113

Statistical Analysis: Imaging and Measuring the Root Hairs

After the plants were allowed to grow for five days, the root hairs were prepared for imaging. Using the stereomicroscope Leica MZ16 FA and ImageJ software, the root hairs were each imaged with a certain magnification that was best for the image. This magnification ranged from 10x to 40x. Then, using ImageJ, the images were analyzed by measuring the root length was obtained and put in Excel sheets. The plants measured with the same magnification were measured the same, so there was no difference in the measurements. Once put in Excel, a T-test was used to compare the significance of the different concentrated groups compared to the 0μM NAA control corresponding to the same plant genotype. So, the t-test was run between the 0μM NAA and each non-zero concentration. A p-value of less than 0.05 was statistically significant.

Statistical Analysis: Organelle Movement

After imaging, the same plants were ready to be measured for peroxisome movement. Under the Zeiss Axio Observer Z1 microscope, the 63x/1.4 NA oil immersion objective with a cyan fluorescent protein filter was used to see the peroxisome move. A time-lapse of 200 photos was taken of a designated root hair filled with quality peroxisome movement. Then using ImageJ, the time-lapse videos were analyzed to see the track movements. More analysis occurred in Excel sheets to produce box plot graphs.

Received: May 16, 2022
Accepted: June 26, 2023
Published: May 18, 2024

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