Examination of Induced Polyploidy for Improved Biofuel Traits in Sunflowers

The Center for Initiatives in STEM

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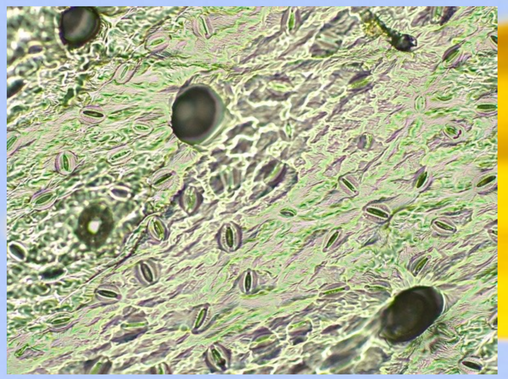
Introduction

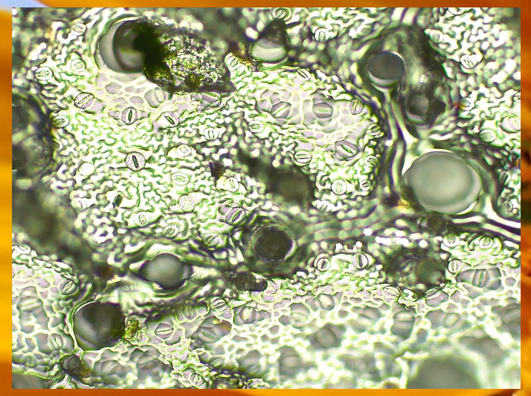
Polyploidy is defined as a heritable condition of passing more than two of the complete set of chromosomes. This is experiment was conducted to induce polyploidy in sunflowers to track any increased biomass traits that can lead to an increase in biofuel production. To induce polyploidy, we used a soaked cotton ball with an aqueous colchicine solution that was placed on the apical meristem for 24 hours after germination. Once germinated, the plants were transfer to larger pots and monitored. Traits that will be measured are rate of stalk density, seed and oil content production, seed, head and leave size, and water flux traits. All the traits are hypothesized to show an extreme increase due to the polyploidy. With increasing sizes and content, it will lead to an increase of biomass and stomata density of the plant.

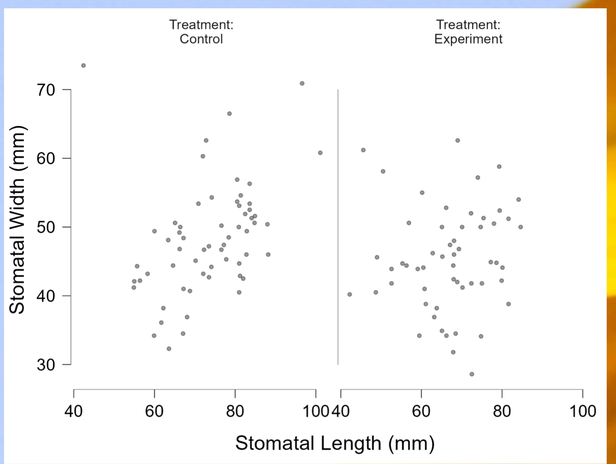
Methods

Once the seedlings matured enough, a solution of 0.15% of colchicine was prepared. With gloves, small cotton balls were soaked in the solution and then place exactly on the apical meristem with forceps (seen in figure 1and 2). The plants were then moved to a hoop house in which they were planted along 12 control sunflowers.. Once the sunflowers were mature enough, stomata analysis was completed. This was done by painting a small square of nail polish on to the underside of the leaf. After the polish has dried, forceps were used to peel off the polish. A photo was taken at both 10x and 20x. The 10x photos were used to count the stomata in a certain area while the 20x photos are used to calculate the stomatal density. The length and width were taken of about 6-10 stomata. This was done for both the experiment and controls which the two are compared so see if the sunflowers were truly polyploid. Figure 3, controlled 20x photo, compared to figure 4, 20x experiment photo, a difference is seen in the width of the stomata. The experiment has a much thicker stoma.

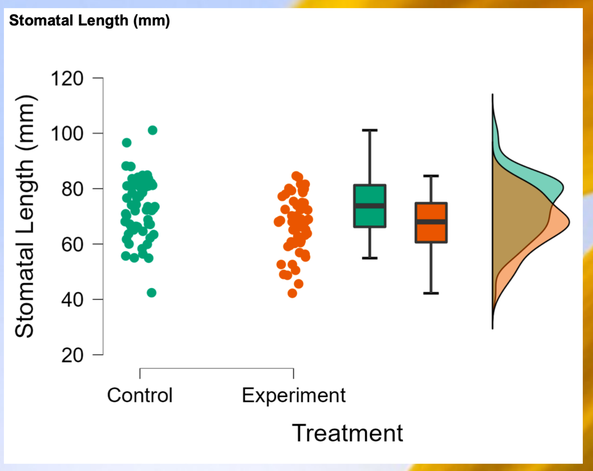


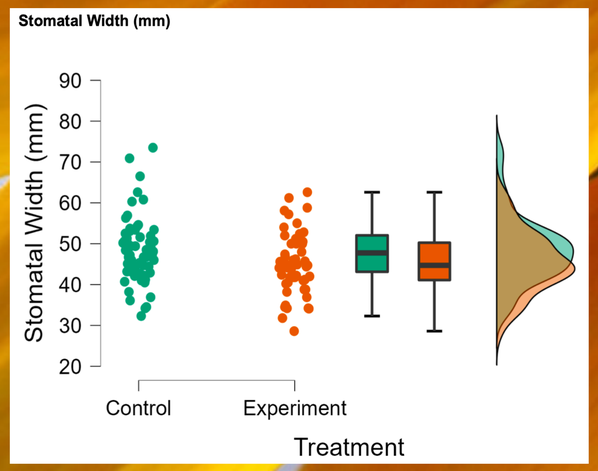
The image on the left is of the peel of control stomata under the microscope at 20x. The image on the right is of the peel of experiment stomata under the microscope at 20x





After stomatal analysis, it appeared that the stomata were smaller in the experimental plants. This is unexpected due to typically it is found that plants which are successful polyploid that their stomata, pollen, and chloroplasts are significantly larger. In the length and width of the stomata are not significant but the pattern of direction is still statistically significant. There is a clear positive relationship in control plants, but this disrupted in the experimental plants.





	t	df	p
Mean Stomatal Length (mm)	1.926	14	0.075
Mean Stomatal Width (mm)	1.853	14	0.085
Mean Stomatal Area (cm2)	2.303	14	0.037

Note. Student's t-test.

Conclusions

It can be concluded that the colchicine likely did not successfully induce polyploidy and instead of seeing a significantly larger stomata and overall traits in the sunflowers, the stomata are showing the effects of colchicine toxicity on the plants which resulted in slightly smaller stomata. This rejects null hypothesis that there was no difference in the two groups since the is a p value less then .5. Although the difference was not what to be expected. This suggests that this form of induction was insufficient to create polyploids in this trail. Therefore, a more complex method such as tissue culture induction on high cellulose line to increase cellulose content. However, sunflowers are still a promising plant species for biofuel productions due to the wasted stalk during harvest. The oil is already used for high-grade food oils and is expected to have properties can be compared to biodiesel specifically from soy. They are an incredibly strong contender for biofuel cropping because they can adapt to many different conditions which allows them to be a more common crop. In a study done on lignocellulosic chemical properties in sunflowers provided that a reduced lignin composition and increase cellulose content will allow for biofuel to be produce from sunflowers. If a polyploid linkage is successfully produced, then the enlarge traits will create even more viability for an increase production of biofuel from the unused stalks.

Future Work

This research steers our polyploid research toward tissue culture for the need to strongly control the process. Seed or meristem soaking does not appear to have clear induction capacity. We are currently getting lignin, starch, cellulose and other biofuel relevant traits so we can perform genome-wide association study to identify candidate genes. Given that, tissue culture induction on the highest cellulose lines to try to increase cellulose content for cellulosic ethanol value of otherwise wasted stalk material.