Group 11 Proposal:

An Investigation on Petiole Variation in Quaking Aspen

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Introduction

The petiole of a leaf plays an important role in bridging the gap between the leaf blade and tbe stem. Likewise, the length of the petiole significantly influences certain necessary leaf functions. Ulo Niinements, et al. (2003) states that smaller petioles experience a decrease in light harvesting efficiency because "longer petiole lengths lead to larger lamina surface areas near the petiole attachment, thus increasing the area available for photosynthesis." Other past research indicates leaf petiole length is attributed to specific genes: ROT3, ACL2, GAI (Tsukaya, 2005). In contrast, Tsukaya (2005) describes notable influences of the environment on leaf petiole length stating that "the final leaf shape is adjusted based on leaf environment," which influences the petiole length to compensate for the adjusted area. Furthermore, "low light intensity promotes the elongation of leaf petiole size in Arabidopsis" (Tsukaya, 2005). Despite previously established research, there is still a large knowledge gap concerning whether characteristic leaf petiole variation is determined by genetics or the environment. Using *P. tremuloides*, or Quaking Aspen trees, as a model, we can fill in part of this gap with more information regarding petiole lengths.

In this investigation we observed the variation between trees, clones, and ploidy by comparing ratios between leaf area and petiole length in Quaking Aspen trees in order to determine to what extent environment and genetics influence petiole expression. Quaking Aspen serve as an appropriate experimental unit since they exhibit varying characteristics between clones and ploidy which are all easily qualitatively and quantitatively observable such as leaf size, curvature, asymmetry, petiole length, leaf serration and venation. These trees are ideal for genotyping due to their high tendency to grow in large clusters of genetically identical clones.

Furthermore, the fact that Quaking Aspen reproduce asexually indicates that there will be many trees belonging to the same clone within close proximity to one another (Grant & Mitton, 2010). By obtaining a ratio of petiole length to leaf area, we were able to evaluate the proportion of variation between and within each individual clone. In essence, this research investigation was significant in heightening our understanding of the phenotypic impact between genetics and the environment.

Methods

Data for this research was acquired by collecting approximately twenty-five to thirty-five leaves from eight separate trees in Big Cottonwood Canyon, Utah. Each leaf was removed from its branch and taped onto sheets of paper to be scanned into "leaf sheets". An open-source image processing software known as ImageJ was used to obtain width and length measurements in millimeters for each leaf. These measurements were then compiled into an Excel spreadsheet. One leaf from each of the eight aspen trees was genotyped using the Peak Scanner program which analyzes the gel electrophoresis samples in order to determine the clone and ploidy of each tree. After evaluating the tree clone and ploidy data from every tree studied this year and last year, leaves from the six clones which were comprised of the most trees were selected for further use in our research.

Petiole lengths of the selected leaves were measured in millimeters with ImageJ. Afterwards, an Excel data sheet was compiled with the length, width, and petiole length measurements for all of the leaves and then sorted based on which clone, tree, and ploidy each leaf belonged to. Leaf area values were computed by multiplying leaf length by leaf width measurements. Although this rectangular approximation method yielded leaf area values that

were greater than the true area of each leaf, the leaves maintained the same area proportion relative to each other. The petiole length to leaf area ratio was computed by dividing the petiole length by the leaf area.

Following the image-processing procedure, statistical analysis was performed on our data in order to determine leaf petiole variance. An Analysis of Variance (ANOVA) was generated using an open-source statistics language program known as R. From the results produced after running the ANOVA, a numerical r-squared value regarding the proportion of petiole variation with respect to clonality was obtained. Through the ANOVA, we can also determine the proportion of petiole variation with respect to tree and ploidy. We were able to calculate the approximate amount of petiole length variation that can be attributed to genetic factors by observing the variation of petiole length measurements among both individual and multiple trees based off of ploidy and clonality. Finally, this observation of variance was repeated among trees belonging to one clone and multiple trees belonging to the same clone in order to determine the amount of influence that environmental factors have on petiole length variation.

Conclusion

Overall, the research conducted in this experiment provides evidence showing genetic and environmental influences on leaf petiole length. Results from our ANOVA script revealed that ploidy had the most significant influence on leaf petiole length variation, then clones (taking out ploidy), and finally individual trees (taking out ploidy and clones). The variation between clones and ploidy suggests that 49% of petiole length can be attributed to genetic factors. However, the obvious variation between trees suggests that the other 51% of leaf petiole variation may be attributed to environmental factors.

In addition, our results showed that triploid clones had a lower average area to petiole length ratio compared to that of diploid clones. This may be explained biologically by the allocation of volume in the leaf petiole. Because we measured the length of the petiole instead of the volume, the triploids may have a different volume compared to the diploids, thus providing more scientific insight into the variation between triploid and diploid clones.

Altogether, these results may be used for future investigations related to photosynthesis, growth, or other "fitness factors" pertaining to Quaking Aspen trees. Certainly, a larger sample size would improve the validity of our results; nevertheless, our data provides valuable information with regards to the petiole length variation between and among clones. Our data motivates other scientific research such as if the variation is caused by mass allocation or if there is an actual variation with regards to volume. As discussed above, leaf petioles are pivotal to the function of the leaf since they increase photosynthesis and decrease sun radiation. With our analysis, we have furthered our understanding of how petiole variation can impact these functions. Outcomes from this study indicate that 49% of leaf petiole variation can be attributed to genetics while 51% can be attributed to environmental influences. Therefore, we can conclude that both genetics and the environment equally impact the outcome of petiole length growth.

References

Grant, M., & Mitton, J. (2010). Case Study: The Glorious, Golden, and Gigantic Quaking Aspen. Retrieved from http://www.nature.com/scitable/knowledge/library /case-study-the-glorious-golden-and-gigantic-13261308?refnf

Niinemets, U., Afas, N., Cescatti, A., Pellis, A., & Ceulemans, R. (2004). Petiole length and biomass investment in support modify light interception efficiency in dense poplar plantations. *Tree Physiology, 24*(2), 141-154. Retrieved from http://treephys.oxfordjournals.org/content/24/2/141.full.pdf html

Tsukaya, H., Kozuka, T., & Kim, G. (2002). Genetic Control of Petiole Length in Arabidopsis thaliana. *Plant and Cell Physiology,* 1221-1228. Retrieved from http://pcp.oxfordjournals.org/content/43/10/1221.full.pdf

Tsukaya, H. (2005). Leaf shape: Genetic controls and environmental factors. *The International Journal of Developmental Biology,* 547-555.

Figure 1

Area-to-Petiole-Length Ratio vs. Ploidy

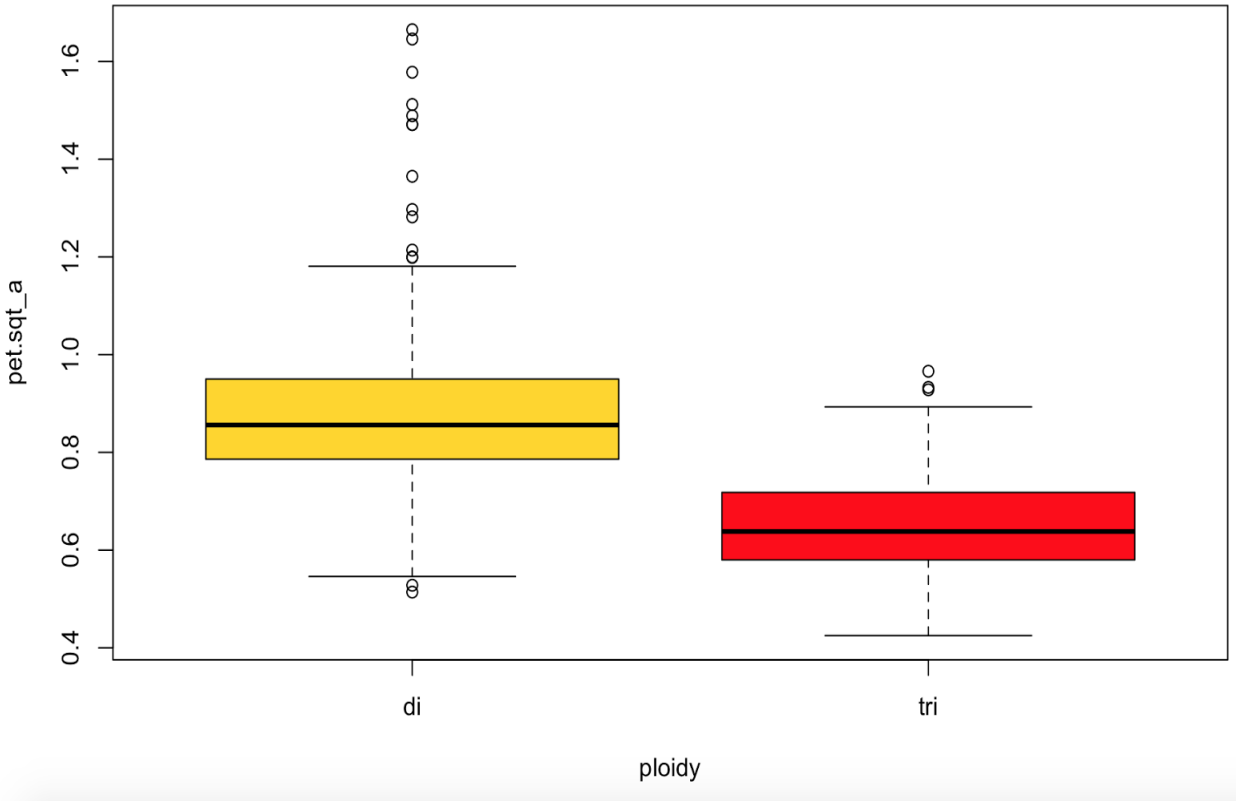

Figure 1. Triploids have a lower petiole ratio and contain less outliers than diploids. They also contain a slightly lower variation in ratio values than diploids.

Figure 2

Area-to-Petiole-Length Ratio vs. Clonality

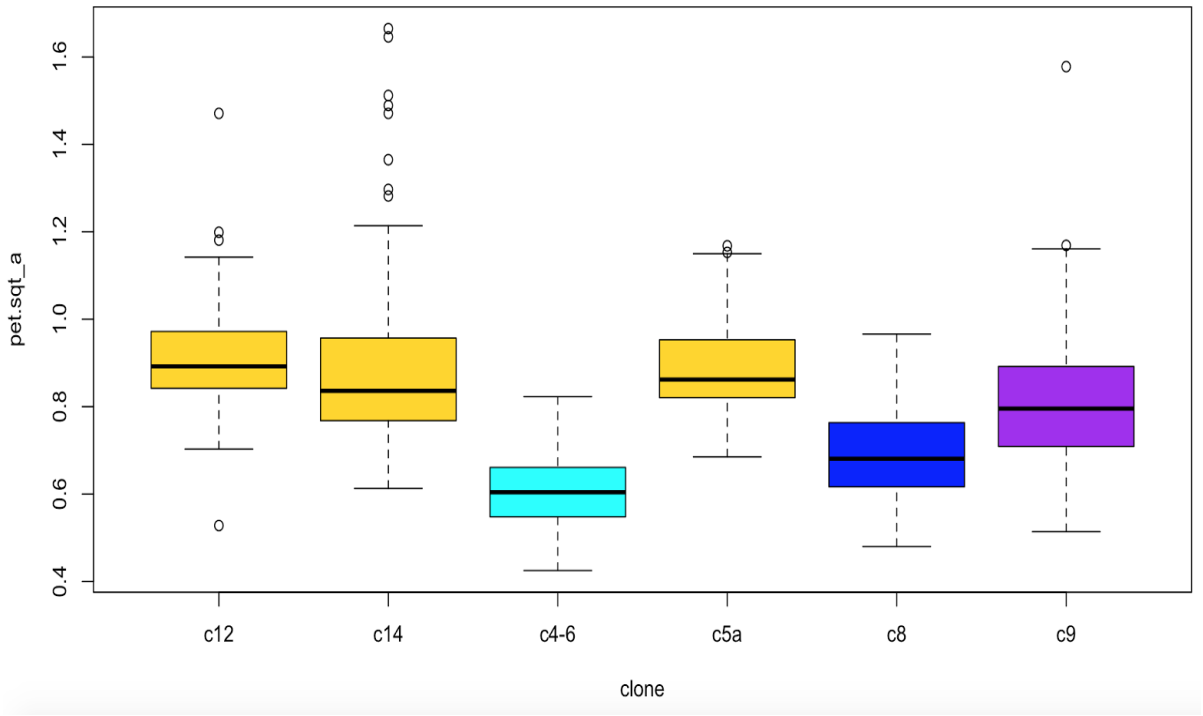

Figure 2. The triploid clones, represented by the light blue and dark blue boxes, have a lower average ratio compared to the diploid clones, which are represented by the yellow and purple boxes.