Quantitative Analysis of Growth in Wild-type and Mutant Plants of the Model C4 Grass, *Setaria viridis*

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Abstract

Setaria viridis (green foxtail) is a C4 annual grass species in the same subfamily as maize and sorghum. It is a widespread weed, and its small size, rapid life cycle, large seed production, and sequenced genome make the species a useful model crop for cereal and biofuel grasses (Doust 2004; Doust 2009; Li *et al.* 2010; Rizal *et al.* 2013). We are interested in the genetic regulation of growth and branching in this species and have produced mutants that are affected in these traits. Because growth varies over the lifetime of the plant, we are using measurements at multiple time-points to understand how variation in growth leads to different morphologies at maturity.

Keywords: *Setaria viridis*, leaf lengths, digital analysis, hand measurements, mutant

Introduction

Setaria viridis (green foxtail) is an annual species of grass that is a severe weed in the same subfamily as maize and sorghum. The genetics of *Setaria* are little known, but the small size and genome make the species a useful model crop for cereal and biofuel grasses (Doust 2004). The domesticated form of *S. viridis*, foxtail millet (*S. italica*), is one of the oldest cultivated cereal crops (dating back 5,000 years). It ranks second in the amount of world production of the millets and provides approximately 6,000,000 tons of food annually. This crop holds an important place within agriculture, especially in developing countries. Green foxtail is closely related to various feed, fuel, and bioenergy grasses making it an important model for C4 photosynthesis (Brutnell *et al.* 2010).

S. viridis is an invasive weed with a short life cycle (6-8 weeks) and a large production of seeds with long lasting life (Rizal *et al.* 2013). This weed is easily killed with herbicides but quickly returns due to the rapid growth and large amount of seeds produced (Douglas *et al.* 1985). *S. viridis* plants are hermaphroditic (containing both male and female organs) and pollinate by self-pollination or with the assistance of the wind. This grass is primarily found in temperate zones and has high drought tolerance, in part due to its short life cycle and early maturation. Green foxtail seedlings benefit from high temperatures and light levels, and demonstrate sensitivity to shade (Douglas *et al.* 1985).

In this study, we use Raspberry Pi cameras to digitally take pictures of *S. viridis* grass throughout maturity (roughly 4 weeks) and compare the images to manual measurements taken of leaf growth within wild type *S. viridis* and its two mutants with contrasting phenotypes NMU_009199 and NMU_01004. This will help to better understand how plant architecture develops throughout the life cycle of the plants and to identify when differences begin to take place in development of the wild-type and mutant *S. viridis*.

Methods

S. viridis seeds were grown in Sun Gro Metro-Mix 360 potting soil under identical lab conditions to ensure accurate results. The mutant seeds grown (NMU_01004 and NMU_00919) had either greater or fewer numbers of tillers than wild type *S. viridis* plants. Comparison of the growth patterns of leaf length between wild-type and mutant developing plants were done over a four-week time span (this is the time taken from germination to maturity).

Hand Measurements

Five seeds of NMU_01004 and NMU_00919 were germinated and measurements were taken of each leaf length across a four-week growth period and recorded. Plants were grown under identical light and water treatments.

Digital Measurements

Raspberry Pi cameras were positioned to take photos of each plant daily on set time intervals to collect growth data and record leaf lengths. These images were analyzed with a script written in Python, which used a measurement of incremental changes in

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*=Mentor

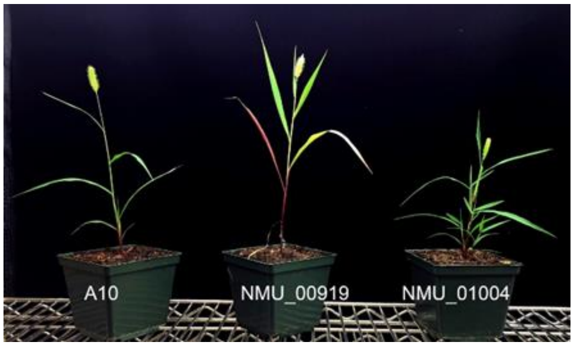

Figure 1 *- The phenotypes of the wild species (A10) and the two mutants (NMU_00919 and NMU_01004).*

leaf angle to identify the tips of the leaves. Measurements of leaf length were then computed.

Once the plants reached maturity, the comparison of digitally recorded growth length to manually measured leaf length were graphed to identify the similarities and margin of error between the two methods.

Results

Hand measurements

We measured leaf lengths for each leaf position at four different time-points. Greater differences between accessions were seen for older leaves (e.g. leaves 1 and 2) than for the youngest leaves (e.g. leaves 5 and 6) (Figure 3). Leaf length was also analyzed using a repeated measures ANOVA design in SPSS and significant differences were found between accessions.

Digital analysis

We analyzed leaf length, leaf angle and tiller number daily from midway through growth until flowering, using digital images and the Acute landmark identification package (Hodge, unpublished).

Comparison of hand and digital measurements of leaf length

It was not possible to directly contrast hand and digital measurements as the plants were grown in different light conditions. However, the amount of variation in leaf elongation explained by growth time for each leaf position in each of the data sets was roughly similar ($R^2 = 0.4 - 0.65$), suggesting that digital analysis is equivalent to hand measurements in terms of precision.

At flowering, A10 and NMU_00919 are a similar height and have few or no tillers respectively, and noticeably differ in the angle of their leaves. By contrast, A10 and NMU_01004 have similar leaf angles but differ in the number of tillers.

Discussion

Hand measurements and digital analysis were equivalent in their precision in calculating leaf length, although differences in growing conditions made it impossible to directly compare accuracy of the measurements. However, digital analysis also allowed us to easily gain additional architectural features such as leaf angle and tiller number over

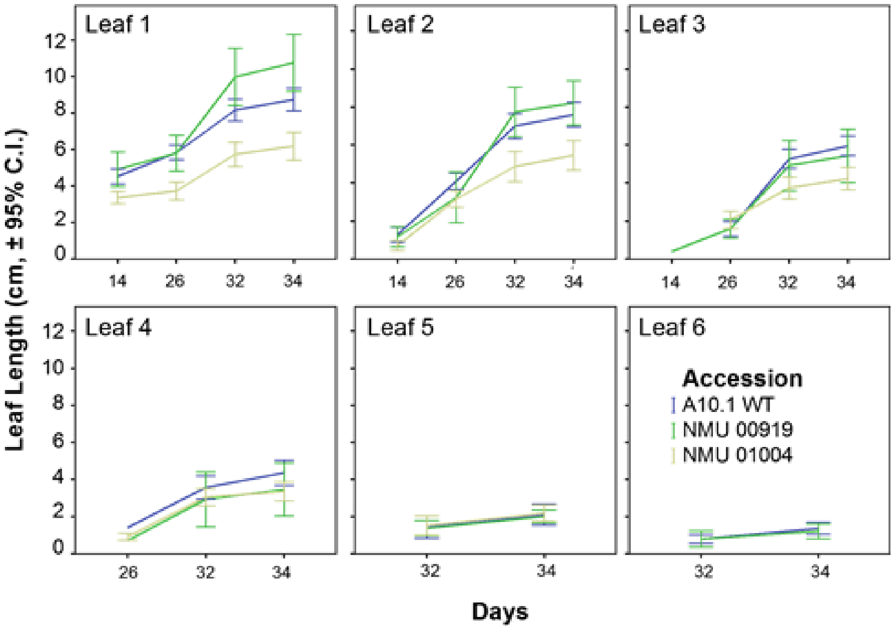

Figure 2 *- The length of each leaf at the four measuring points.*

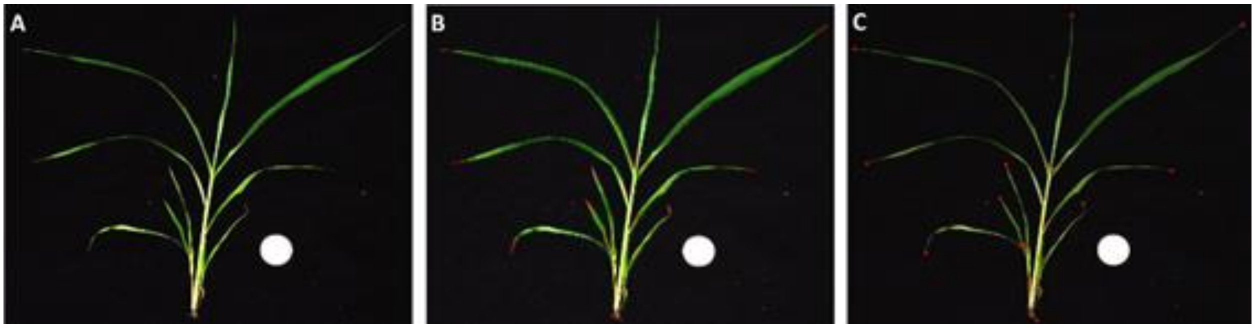

Figure 3 *- Example of landmark identification method. Red points represent Raspberry Pi detection of leafs end point. (A) Raw image file used for landmark identification. (B) Acute region detection along contour of plant to flag positions of interest. (C) Reduction to a single informative pixel that can be used for analysis.*

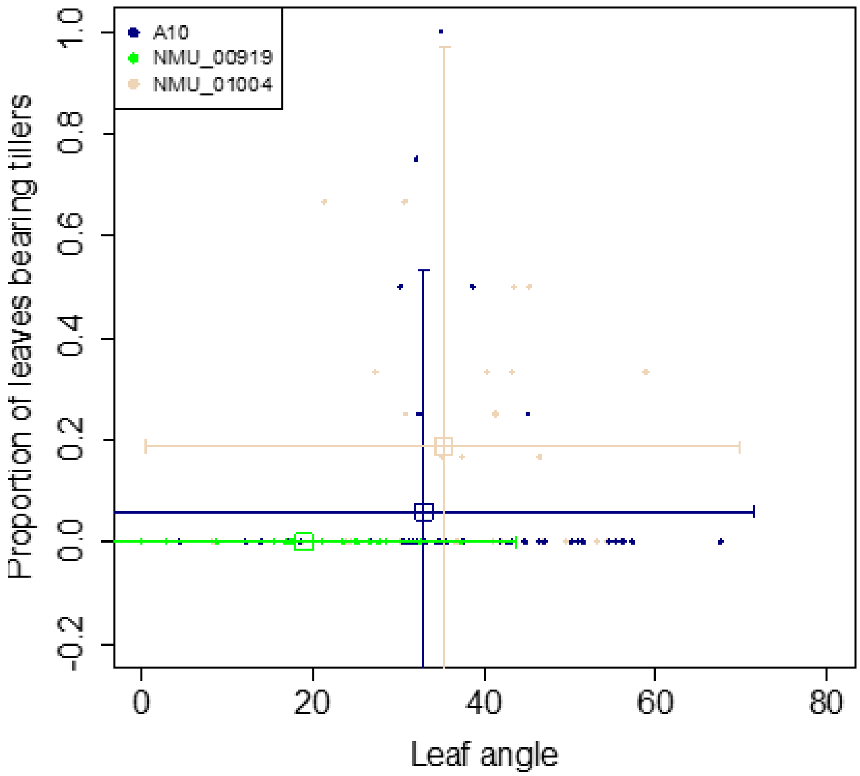

Figure 4 *- Comparison between blade angle of each leaf series and the proportion of branches associated with it (points). Mean + 95% C.I. of all leaf series across development (squares).*

developmental time. More work is required to calibrate hand and digital analyses, and this forms part of on-going research in the lab.

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