Short Communication Open Access

Quantifying *Zea mays* L tassel development and correlation with anther developmental stages as a guide for experimental studies

Rachel L Egger[1]*, Virginia Walbot[1]

[1]Stanford University Department of Biology, Stanford, CA, USA, 94305
*Corresponding author: E-mail: egger@stanford.edu

Abstract

In the study of *Zea mays* L early anther development, anther length is tightly correlated with developmental stage over the initial eight days of ontogeny. The developing tassel is enveloped inside the whorl of developing leaves, and the pace of its growth is environmentally sensitive. Determining the size of a young tassel without sacrificing the plant remains difficult. This obstacle can present problems when trying to study pre-meiotic and meiotic stages of anther development in planta or when conducting sequential sampling from the same tassel. In this study a range of exterior characteristics (leaf number, stem circumference, and tassel height above the soil line) were measured and correlated with tassel length. Anther lengths at seven tassel locations were also measured to correlate tassel length with anther development at these locations. Results indicate that tassel length can occupy a wide-range for a given exterior measurement, but also that anther development at specific tassel locations is highly reproducible for a tassel of known length. This information provides a useful guide for the study of anther development in the context of the maize tassel.

Keywords: tassel, anther, development, maize, pre-meiotic

Introduction

In plants, the germ line is established in the gametophytes, and even the germinal, pre-meiotic cells are not established until late in flower development (Ma et al, 2005; Feng and Dickinson, 2007). The anther is a specialized organ responsible for the specification, nourishment, and maturation of the male germinal cells, which originate from somatic precursors (Feng et al, 2007; Kelliher and Walbot, 2011). The developmental timeline for the patterning of the anther cell types is well-documented in maize (Kelliher and Walbot, 2011; Zhang et al, 2014). Anthers consist of a four-lobed structure, with layers of cells consisting of five different cell types at the onset of meiosis.

Pre-meiotic patterning of these anther cell types requires a series of perfectly timed periclinal divisions and cell fate decisions influenced by positional information (Kelliher et al, 2014). The nature of this positional information remains an active area of study, with known roles for leucine-rich-repeat receptor-like-kinases (LRR-RLK) – ligand pairs (Jia et al, 2008; Wang et al, 2012; Zhao et al, 2008), peptide factors (Jia et al, 2008; Hong et al, 2012; Wang et al, 2012), and hypoxia gradients (Kelliher and Walbot, 2012), as well as suggestions for hormone involvement (Zhang et al, 2014) and possible roles for small RNA molecules (Zhai et al, 2015). Studying these and other possible types of positional information often requires perturbation of anther development at specific stages, followed by tissue collection hours or days later. In maize, such experiments are simplified by the large number of anthers present at a range of stages on any single tassel (Bedinger and Fowler, 2009). But, not all anther stages are present on every tassel. Moreover, locating the tassel inside the developing whorl, without disrupting the whorl's integrity, can be difficult. Thus, for studies requiring the perturbation of the air space surrounding the tassel, either by adding gaseous components (Kelliher and Walbot, 2012), injecting hormones or other solutions (Walbot and Skibbe, 2010; Kelliher and Walbot, 2012), or even the introduction of pathogens (Skibbe et al, 2010), a method of reliably determining tassel size based on exterior morphological characteristics would be invaluable.

Existing staging systems rely primarily on leaf emergence and growth (Tranel et al, 2008). But, given that the length of time between emergence of successive leaves (the plastochron) varies in plants grown under ideal conditions, the resolution is not ideal for reliably acquiring anthers at specific stages. Furthermore, the intervals between leaf emergence can expand to many days when there is low water availability or other adverse environmental conditions.

We sought to improve reliability by assessing additional external plant characteristics to determine if they correlate with tassel length. Furthermore, collection of relevant anther stages for biochemical and microscopic analyses after treatment will also be aided by a reliable correlation of anther sizes and tassel length. These are the tools we set out to establish in this study.

 RECEIVED 11/13/2015

Materials and Methods

Ninety-five wild type *Zea mays* L plants (inbred line W23 *bz2*) were potted directly into soil and grown in five cohorts in the greenhouse during the winter of 2012-13. Greenhouse conditions were as specified in Kelliher and Walbot (2011). Beginning 30 days after planting, plants were sacrificed in cohorts of five every two days, and the following external and tassel characteristics were recorded for each plant: stem circumference (cm), number of emerged leaves, tassel height above the soil – the distance from the soil line to the basal tassel node (cm), tassel length (cm), number of tassel branches, and lengths of longest and shortest branches (cm). Seven readily reproducible positions were specified on the tassel, and from each plant lower and upper floret anthers were sampled at each of these locations to determine what correlation might exist between tassel length and anther length at a particular location.

Results

Seven locations were chosen to sample anther sizes on the tassel, in addition to measuring the external characteristics for each plant sampled. These locations were (1) the center of the central spike, (2) halfway between the center and the tip of the central spike, (3) the tip of the central spike, (4) the base of the central spike, (5) the tip of the longest side branch, (6) the center of the longest side branch, and (7) the center of the shortest side branch (Figure 1). Based on experience dissecting anthers, these locations correspond in numerical order to locations with the largest (Location 1) and smallest (Location 7) anthers. At each of these locations, both upper and lower anther florets were sampled and measured.

A comparison of external characteristics to tassel length detected no relationship between tassel length and stem circumference or leaf number (Figure 2A). There was a correlation between tassel length and tassel height, however, with large standard errors in tassel height, this correlation is not precise enough for experimental use (Figure 2A). The correlations between tassel length and tassel architecture were similarly either independent (branch number) or not experimentally useful (branch lengths) (Figure 2B).

With the exception of the smallest side branch (Location 7), the correlation between anther length at a particular location in an upper or lower floret and tassel length was very high (average R^2=0.81) (Figure 3). Omitting location 7, 11/12 locations sampled had correlation coefficients greater than 0.71, and 5/12 greater than 0.91. This indicates a strong linear relationship between tassel length and anther size at a given tassel location with strongest correlations observed near the center of the central spike and long side branches (Locations 1, 2, and 6), and a slightly

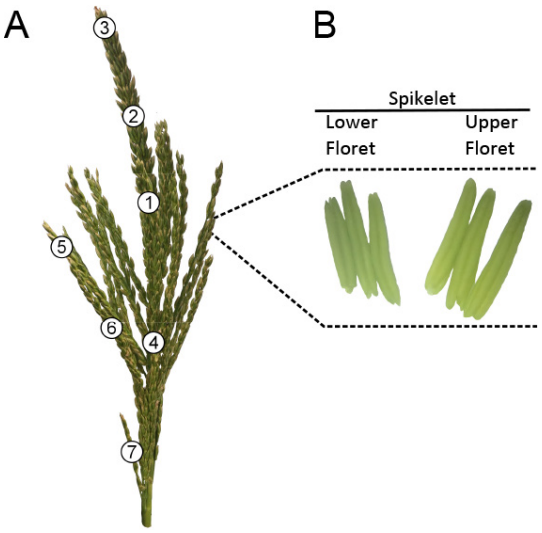


Figure 1 - Anther and tassel morphology. A: The locations sampled on each tassel numbered 1-7; B: A single spikelet with the glumes, lemma, and palea removed and upper and lower florets of anthers separated.

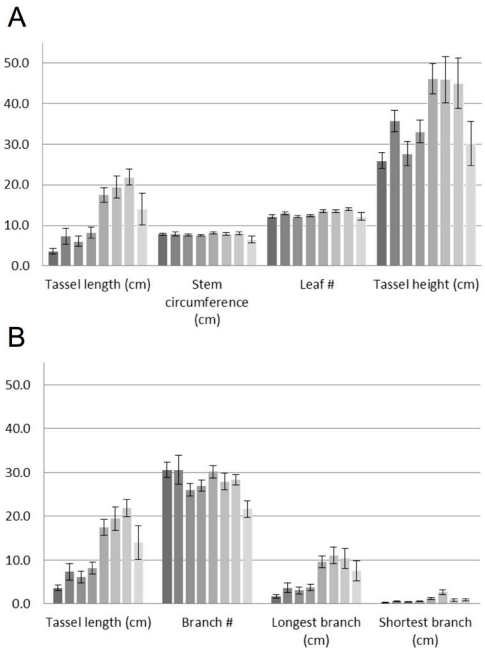


Figure 2 - Comparison of external characteristics and tassel architecture with tassel length. A: Comparison of external plant characteristics (stem circumference, emerged leaf number and tassel height above the soil line) compared with tassel length; B: Comparison of tassel architechture characteristics (branch number, length of longest branch, and length of shortest branch) compared with tassel length. For both plots, in each character group the bars from left to right (darkest to lightest) are averages of plants sampled at 30, 32, 34, 36, 38, 40, 42, and 44 days after planting. Error bars indicate standard error of the mean. The number of individual plants sampled in each group was: 30 (n=5), 32 (n=5), 34 (n=19), 36 (n=19), 38 (n=18), 40 (n=16), 42 (n=9) and 44 (n=4).

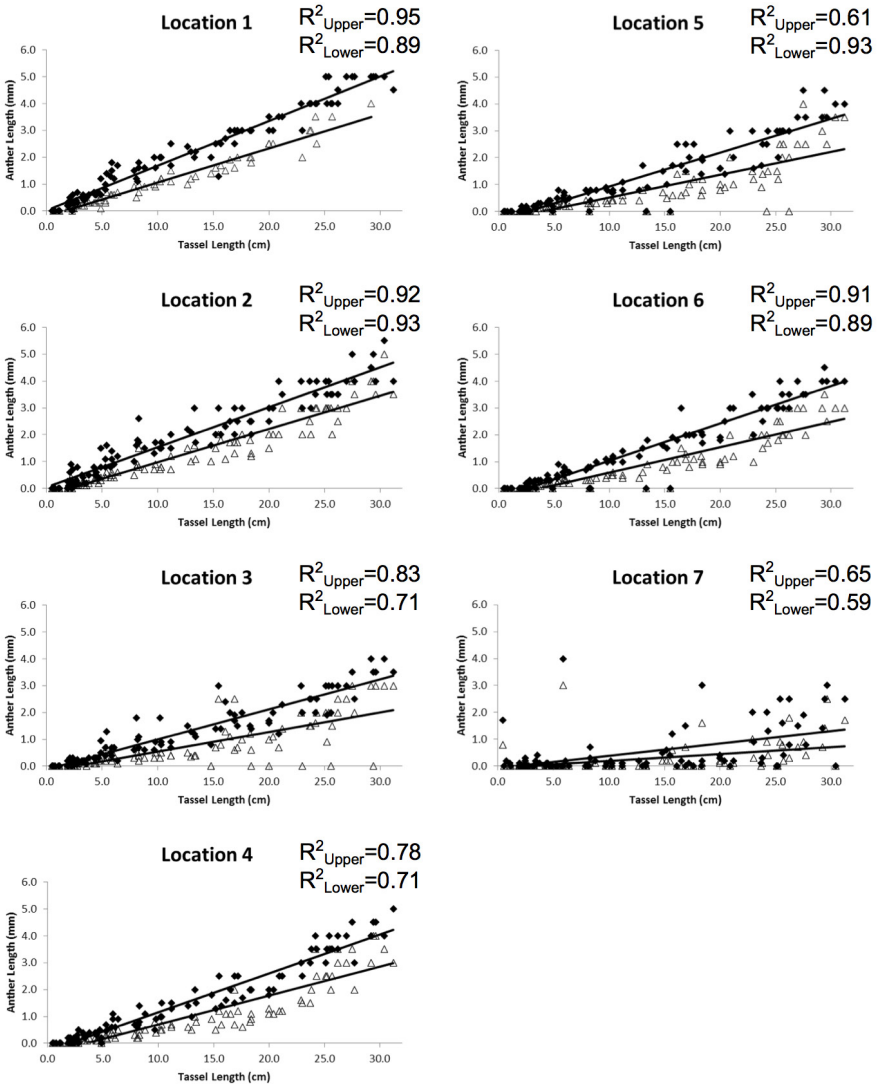


Figure 3 - Correlations of upper and lower floret lengths at seven tassel locations. Anther lengths in both upper and lower florets were measured from the seven sampled tassel locations. On each plot upper floret anther lengths are indicated with solid diamond markers, and lower florets with open triangles. For each dataset a linear regression calculation was used to generate a line-of-best-fit. The correlation coefficients of these calculations are indicated in the upper corner of each plot.

weaker relationship near tips and bases of the spike and side branches (Locations 3, 4, and 5) (Figure 3). These data were used to generate a reference table of anther lengths at each of the seven locations as a function of tassel length (Table 1).

The anther developmental literature has long asserted that the upper floret is larger and develops roughly a day ahead of the lower floret. In maize, this would translate to upper floret anthers being twice the size of the lower floret within a single spikelet at any point on the tassel. To the best of our knowledge, this concept has not been tested across the whole of pre-meiotic anther development. A ratio was calculated for each sample collected for all seven locations sampled (Figure 4). While there is some deviation around the mean, the average for all locations and all tassel lengths was a ratio of 1.98 (standard deviation = 0.63, and standard error of the mean = 0.03), confirming the assertion that upper floret anthers within a spikelet are twice the size of the lower floret.

Discussion

None of the external features measured (stem circumference, leaf number or tassel height above the soil line) was found to be a sufficiently good predictor of tassel length inside the maize leaf whorl for experimental use (Figure 2A). Therefore, none of these parameters could be used to improve the staging based on leaf emergence. Aspects of tassel architecture (number of branches, lengths of longest and shortest branch) were also sampled. The number of

Table 1 - Anther lengths according to tassel size.

Tassel Length (cm)	Upper (mm)	Lower (mm)	Upper (mm)	Lower (mm)	Upper (mm)	Lower (mm)	Upper (mm)	Lower (mm)	Upper (mm)	Lower (mm)	Upper (mm)	Lower (mm)	Upper (mm)	Lower (mm)
0.5	0.1	0.3	0.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1.0	0.2	0.3	0.2	NA	NA	NA	NA	NA	NA	NA	0.5	NA	NA	NA
1.5	0.3	0.4	0.3	NA	NA	NA	NA	NA	NA	NA	0.5	NA	NA	NA
2.0	0.4	0.5	0.4	NA	0.1	NA	NA	NA	NA	NA	0.6	NA	NA	NA
2.5	0.4	0.5	0.4	NA	0.2	NA	0.1	NA	NA	NA	0.7	NA	NA	NA
3.0	0.5	0.6	0.5	0.1	0.2	NA	0.2	NA	NA	NA	0.8	NA	0.1	NA
3.5	0.6	0.6	0.6	0.2	0.3	0.1	0.2	NA	0.1	0.0	0.8	NA	0.1	NA
4.0	0.7	0.7	0.7	0.2	0.3	0.1	0.3	NA	0.2	0.0	0.9	NA	0.1	NA
5.0	0.9	0.8	0.8	0.4	0.4	0.2	0.4	0.2	0.3	0.1	1.0	0.1	0.1	0.1
6.0	1.0	1.0	0.9	0.5	0.5	0.2	0.6	0.3	0.4	0.2	1.2	0.2	0.2	0.1
7.0	1.2	1.1	1.1	0.6	0.7	0.3	0.7	0.4	0.6	0.3	1.3	0.3	0.2	0.1
8.0	1.4	1.2	1.2	0.7	0.8	0.4	0.9	0.5	0.7	0.3	1.4	0.4	0.3	0.1
9.0	1.5	1.3	1.4	0.9	0.9	0.5	1.0	0.6	0.8	0.4	1.6	0.5	0.3	0.2
10.0	1.7	1.5	1.5	1.0	1.0	0.5	1.2	0.7	0.9	0.5	1.7	0.6	0.4	0.2
11.0	1.9	1.6	1.7	1.1	1.1	0.6	1.3	0.8	1.1	0.6	1.9	0.7	0.4	0.2
12.0	2.0	1.7	1.8	1.2	1.2	0.7	1.5	0.9	1.2	0.7	2.0	0.8	0.5	0.2
13.0	2.2	1.8	2.0	1.3	1.3	0.8	1.6	1.0	1.3	0.8	2.1	0.9	0.5	0.3
14.0	2.4	2.0	2.1	1.5	1.4	0.8	1.7	1.1	1.4	0.9	2.3	1.0	0.6	0.3
15.0	2.5	2.1	2.3	1.6	1.6	0.9	1.9	1.2	1.6	0.9	2.4	1.1	0.6	0.3
16.0	2.7	2.2	2.4	1.7	1.7	1.0	2.0	1.3	1.7	1.0	2.5	1.2	0.7	0.3
17.0	2.8	2.3	2.6	1.8	1.8	1.1	2.2	1.4	1.8	1.1	2.7	1.3	0.7	0.4
18.0	3.0	2.5	2.7	2.0	1.9	1.1	2.3	1.6	1.9	1.2	2.8	1.3	0.7	0.4
19.0	3.2	2.6	2.9	2.1	2.0	1.2	2.5	1.7	2.1	1.3	3.0	1.4	0.8	0.4
20.0	3.3	2.7	3.0	2.2	2.1	1.3	2.6	1.8	2.2	1.4	3.1	1.5	0.8	0.4
R^2	0.96	0.95	0.92	0.91	0.88	0.61	0.94	0.83	0.88	0.70	0.92	0.86	0.26	0.20

No Anthers
0.1-0.5 mm
0.5-1.0 mm
1.1-2.5 mm
2.5+ mm

This table uses the linear regressions extrapolated in Figure 3 to generate estimated anther sizes for upper and lower florets at each of the seven tassel locations as a function of tassel length. Gray boxes indicate locations for which anthers do not yet exist, blue for anthers ranging from 0.1-0.5 mm (during initial germinal and somatic cell specification events), green for anthers 0.5-1.0 (proliferation and patterning of the somatic cell layers), and 1.1-2.5 mm (maturation of archesporial cells to Pollen Mother Cells and progression through meiosis).

branches appears to be independent of tassel length; however, the lengths of both the longest and shortest branch were directly related to the length of the tassel sampled (Figure 2B). Future experiments requiring treatments of specific anther stages will require large cohorts of plants to compensate for environmental variations that influence plant architectural development.

By contrast, the anther lengths of the upper and lower florets at a particular tassel location for a tassel of a known length were highly reproducible with an average correlation coefficient of 0.81 (Figure 3). The locations on the central spike of the tassel and the center of the longest side branches had higher correlation coefficients and represent the best locations for reproducible anther sampling. Furthermore, in all locations sampled, upper florets were roughly twice the length of the paired lower florets, allowing for sample collection without measuring the length of both upper and lower florets. These data are summarized in Table 1, which can be used as a reference when dissecting anthers for stage-specific analyses. While difficulty in estimating the tassel length based on external characteristics remains, the high level of reproducibility in locating anthers of a particular size is extremely useful for collecting anther samples in which precise developmental staging is critical.

Acknowledgements

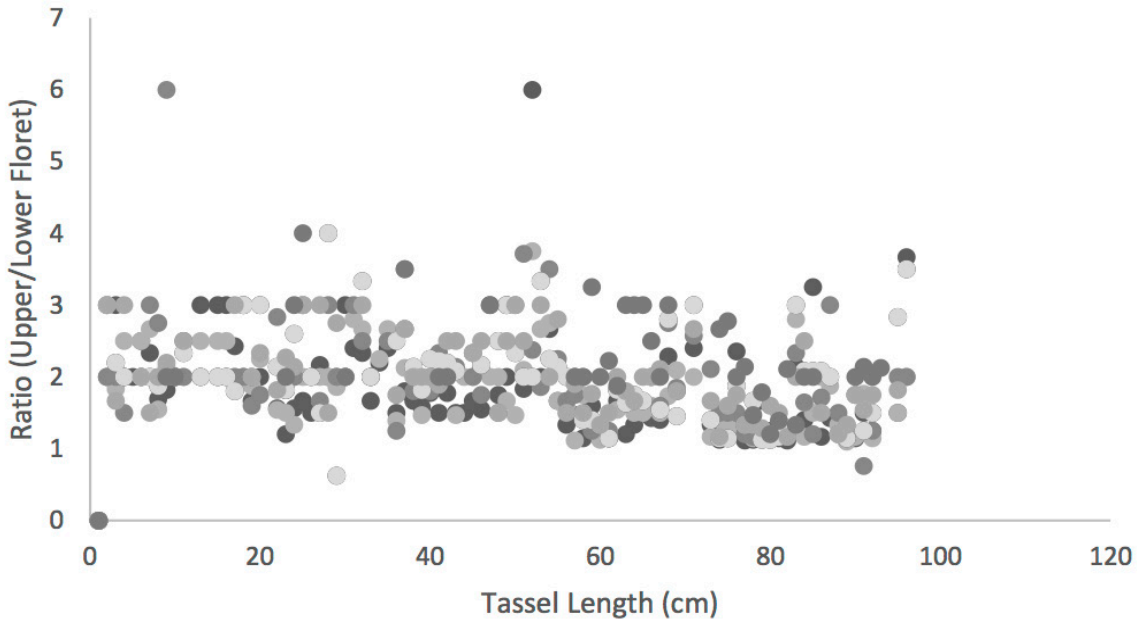
Rachel Egger was supported in part by an NIH Cell and Molecular Biology Training Grant (5T32GM007276-36), by the 2013 American Society of Plant Biologists-Pioneer Hi-Bred Graduate Student Fellowship, and by an Agriculture and Food Research Initiative Competitive Grant from the USDA National Institute of Food and Agriculture (2013-67011-21096). Maize anther research was supported by National Science Foundation grants PGRP07-01880 and PGRP13-39229.

References

Bedinger P, Fowler J, 2009. The Maize Male Gametophyte, pp. 57-77. In Handbook of Maize: Its Biology. JL Bennetzen and SC Hake eds. Springer New York, New York

Feng X, Dickinson HG, 2007. Packaging the male germline in plants. Trends Genet 23: 503–10

Hong L et al, 2012. MIL2 (*microsporeless2*) regulates early cell differentiation in the rice anther. New



Phytol 196: 402–13
Jia G, Liu X, Owen Ha, Zhao D, 2008. Signaling of cell fate determination by the TPD1 small protein and EMS1 receptor kinase. Proc Natl Acad Sci USA 105: 2220–2225
Kelliher T, Egger RL Zhang H, Walbot, V, 2014. Unresolved issues in pre-meiotic anther development. Front Plant Sci 5: Article 347
Kelliher T, Walbot, V, 2011. Emergence and patterning of the five cell types of the *Zea mays* anther locule. Dev Biol 350: 32–49
Kelliher T, Walbot, V, 2012. Hypoxia triggers meiotic fate acquisition in maize. Science 337: 345–48
Ma H, 2005. Molecular genetic analyses of microsporogenesis and microgametogenesis in flowering plants. Annu Rev Plant Biol 56: 393–434
Skibbe DS, Doehlemann G, Fernandes J, Walbot, V, 2010. Maize tumors caused by Ustilago maydis require organ-specific genes in host and pathogen. Science 328: 89–92
Tranel D, Perdomo A, Knapp A, 2008. Tassel development events leading to pollen production: A timeline. Maydica 53: 207–16
Walbot, V and Skibbe, DS, 2010. Maize host requirements for Ustilago maydis tumor induction. Sex Plant Reprod 23: 1–13
Wang C-JR et al, 2012. Maize multiple archesporial cells 1 (*mac1*), an ortholog of rice TDL1A, modulates cell proliferation and identity in early anther development. Development 139: 2594–2603
Zhai J, Zhang H, Arikit S, Huang K, Nan G-L, Walbot V, Meyers, BC, 2015. Spatiotemporally dynamic, cell-type–dependent premeiotic and meiotic phasiRNAs in maize anthers. Proc Natl Acad Sci USA 112: 3146–51
Zhang H, Egger RL, Kelliher T, Morrow D, Fernandes J, Nan G-L, Walbot, V, 2014. Transcriptomes and proteomes define gene expression progression in pre-meiotic maize anthers. G3 4: 993–1010
Zhao X et al, 2008. OsTDL1A binds to the LRR domain of rice receptor kinase MSP1, and is required to limit sporocyte numbers. Plant J 54: 375–87