Title: Identifying the Genes that Control Unisexual Flower Development in Maize			
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Non-Technical Summary

This proposal describes a next-generation sequencing (NGS)-based approach to identify genes that control unisexual flower development in Zea mays (maize). Maize develops separate male flowers in the tassel and female flowers in the ear (Klein et al., 2018). The development of unisexual flowers is important for hybrid crop production - separate tassel and ear flowers allow humans to very easily make controlled crosses (Phillips, 2010). Many cereal crops related to maize, like rice and wheat, have unisexual flowers, hampering hybrid seed production (Kellogg, 2015). In addition, the same process that leads to the development of maize flowers in the tassel - carpel suppression - also occurs in half of all ear flowers, effectively halving the number of seeds a maize ear could produce (Cheng et al., 1983). Thus, modifying the genes that control carpel suppression using CRISPR/Cas9 genome engineering could allow crop engineers to generate unisexual flowers in other grass crops, and to improve yield in maize (Gao, 2018). However, the genes that specifically control carpel suppression remain poorly understood. Here, we propose to almost double the number of genes with potential roles in carpel suppression using an NGS-based protocol for mapping genes to chromosomal locations and identifying candidate genes (Klein et al., 2018).
Until recently, the process of cloning maize genes was arduous and time-consuming (Gallavotti and Whipple, 2015). The rise of next-generation sequencing technology, as well as a fully sequenced maize genome, now makes it possible to quickly map genes to a chromosomal location and identify gene candidates using bulked segregant analysis coupled to high throughput sequencing (BSA-Seq) (Klein et al., 2018; Michelmore et al., 1991). In a BSA-Seq experiment, a mutant of interest is crossed to a wild-type individual in a contrasting genetic background. Heterozygous F1 individuals are then selfed or backcrossed, and mutants with recombinant chromosomes are identified in the resulting F2 population. These mutants can then be used to identify a region of increased homozygosity in chromosomal regions physically linked to causative lesions . DNA from a pool of these mutants is then sequenced (Fig. 1). Downstream analyses identify thousands of polymorphisms that differ between the two parental genotypes, and detect genotypes that are over-represented (i.e. linked to causative lesions) in the mutant segregant pool. Once a mapping interval has been identified, the sequencing data can be examined for potentially causative lesions. Thus, BSA-Seq allows researchers to identify small mapping intervals--and often causative lesions--relatively quickly (Klein et al., 2018).
Here, we will use BSA-Seq to map seven genes with roles in carpel suppression: two mutants that arose from an EMS mutagenesis screen in our lab, four mutants from the Maize Genetics Cooperative Stock Center (referred to hereafter as the

"Co-op"), and a naturally occurring modifier of carpel suppression from the P39 genetic background (See below for more details). Previous Hatch funding was sufficient to cover some nursery expenses and some whole genome shotgun sequencing runs. Therefore, I have limited this proposal to the field work, mutant characterization, and initial sequencing necessary to map the P39 modifier and the six focal mutant genes.

Goals / Objectives

Goal 1: Identify the naturally occurring modifier of gt1 carpel suppression in the P39 genetic background
Specific objectives:

1. Map the P39 modifier to a chromosomal region using bulked-segregant analysis coupled to high-throughput sequencing (BSA-Seq)
2. Confirm the identity of the enhancer using the Nested Association Mapping population Recombinant Inbred Lines (NAM RILs)

Goal 2: Identify candidate genes disrupted in six uncloned rzl and ts mutants

1. Quantify phenotypes and characterize development in the focal rzl and ts mutants
2. Map ts and rzl mutants to chromosomal locations using BSA-Seq

OUTPUTS

1. The location and identity of a naturally occurring modifier of carpel suppression
2. Detailed phenotypic characterization of rzl and ts mutants
3. Mapping intervals and gene candidates for six rzl and ts genes

Methods

Goal 1: Identify the naturally occurring modifier of gt1 carpel suppression in the P39 genetic background
Natural genetic variation is an important source of alleles for understanding plant development (Soyk et al., 2017; Leiboff et al., 2019). We discovered a naturally occurring modifier of gt1 in the P39 genetic background. Here, we will map the gene underlying this modifier using BSA-Seq, and use the NAM RILs (McMullen et al., 2009) to confirm the enhancer's identity.

1.1 Map the P39 modifier to a chromosomal region using BSA-Seq
To generate a mapping population for the P39 enhancer, we crossed gt1-1 in the A619 genetic background to P39, and selfed many of the resulting progeny to generate an F2 mapping population. We will use those mutants with the most extreme carpel derepression phenotypes in this population for downstream BSA-Seq. To localize the gt1 modifier to a chromosomal location, we will perform whole genome shotgun sequencing on DNA from pooled mutant leaf samples. We will sequence the mutant pool at an average depth of 30X, the standard for human genome re-sequencing (Telenti et al., 2016). After barcode removal, quality control, and trimming, we will use bowtie2 to align the reads to the maize genome and GATK4 to produce a variant call file for downstream analysis (Langmead et al., 2009; McKenna et al., 2010; Bolger et al., 2014; Jiao et al., 2017).

BSA-Seq homes in on mutant loci by identifying regions of high homozygosity in sequenced DNA pools. High homozygosity indicates a region where the mutant parental genotype is overrepresented because of linkage to the causative lesion (Gallavotti and Whipple, 2015). Thus, in the case of the P39 modifier, we expect at least two regions of high homozygosity - one region on chromosome one, where gt1 is located, and a second at the modifier locus (Whipple et al., 2011; Song et al., 2017). Critically, even if the P39 modifier is not a single locus, BSA-Seq will reveal all chromosomal regions where there is increased homozygosity (Song et al., 2017; Haase et al., 2015). Together with our phenotyping of the F2 mapping population, this analysis will confirm how many loci underlie the enhancement of gt1 in P39.

Once we have identified a map location for the P39 modifier, we will search for potentially causative DNA variants. To focus on lesions most likely to negatively affect gene function, we will use SnpEff (Cingolani et al., 2012) and SIFT (Kumar et al., 2009). The P39 modifier may be the result of a more complicated DNA variant than a SNP or a small indel, such as a genomic rearrangement, a large deletion, or another structural variant (Wingen et al., 2012; Han et al., 2012; Lunde et al., 2019; Chuck et al., 2007a). If our BSA-Seq results suggest that a complex lesion underlies the P39 enhancer, we will use additional tools and manually evaluate candidate lesions (Wu and Nacu, 2010; Tattini et al., 2015; Ye et al., 2009; Chen et al., 2009, 2016). If the modifier maps to a large chromosomal interval, and/or no single clear candidate gene emerges, we will use our sequencing data to design indel and CAPs markers for fine-mapping, using DNA extracted from stored mutant leaf samples (Gallavotti and

Whipple, 2015; Klein et al., 2018).

1.2 Confirm the identity of the enhancer using the NAM RILs
Once we have a map location and a candidate gene, will confirm the identity of the P39 modifier. To do this, we will order those P39/B73 NAM RILs that have either the P39 or the B73 haplotype, or are heterozygous, at the modifier map location (McMullen et al., 2009). In our experience, this translates to 5-20 RILs, depending on mapping interval size. We will cross gt1-1 in B73 to these RILs, self the F1 progeny, and quantify carpel derepression in gt1 mutant progeny. To verify which allele of our modifier candidate gene is present in each mutant, we will genotype for B73 vs. P39 markers at the modifier map location. If the candidate gene we identify in Goal 1.1 is the P39 modifier, we expect enhanced carpel growth in gt1 mutants that have the P39 allele of our candidate at the modifier locus, but not in gt1 mutants with the B73 modifier allele. Depending on the identity of the modifier gene, and on the nature of the P39 allele of the modifier, we may also be able to employ CRISPR/Cas9 genome editing (Gao, 2018; Yin et al., 2017), reverse genetics resources like the Mu insertion lines (Settles et al., 2007), and/or a transgene strategy to confirm the identity of the enhancer. We have direct experience with all of these methods (Bartlett et al., 2015; Abraham-Juarez et al.; Je et al., 2018; Man and Bartlett, 2019), and do not foresee any unusual difficulties in employing the most appropriate strategy.

Goal 2: Identify candidate genes disrupted in six unmapped rzl and ts mutants
To identify additional genes with roles in carpel suppression and tassel vs. ear floral development, we will use BSA-Seq to map and identify candidate genes for six maize mutants. We will focus on mutants where carpel suppression is disrupted in male flowers: two rzl mutants from a mutagenesis screen in our lab; and four ts mutants from the maize co-op (ts*1967, ts*-P1251881, ts*-br, and ts*-N2490). The ts mutants we selected from the co-op collection were those that (1) had an expressive phenotypes in our field, (2) were phenotypically distinct from known ts genes (Acosta et al., 2009; DeLong et al., 1993; Lunde et al., 2019; Chuck et al., 2007b; Nickerson and Dale, 1955; Best et al., 2016; Hartwig et al., 2011; Yan et al., 2012), and (3) exhibited partial tassel floret feminization (Fig. 4).

2.1 Characterize floral development in ts and rzl mutants
To determine which aspects of development have been disrupted in our six focal mutants, we will characterize floral development in at least two ts and two rzl mutants, and quantify adult floral phenotypes in all six mutants. We will analyze tassels from at least two ts mutants and two rzl mutants over the course of development, using scanning electron microscopy (SEM). The image data we collect will be assembled into a developmental series, which will allow us to determine when development goes awry in these mutants and, therefore, when the mutant genes might act during normal floral development (Bartlett et al., 2015).

2.2 Map ts and rzl mutants to chromosomal locations using BSA-Seq
To generate mapping populations, each mutant will be crossed to the B73 and Mo17 inbreds, and F1 hybrid progeny will be selfed to create F2 populations. B73 and Mo17 are both fully sequenced and highly polymorphic with each other (Sun et al., 2018; Schnable et al., 2009), ensuring that at least one cross will create a polymorphism-rich population. At least twenty F1 progeny will be selfed to generate large F2 mapping populations. We already have mapping populations in hand for three mutants, and will have mapping populations for the remaining three ready to screen by summer 2022. We will plant out and score one to two F2 mapping populations per year of the project (see 'Evaluation Milestones', below).

Before proceeding with BSA-Seq, we will evaluate each F2 mapping population for (1) clear expressivity and (2) distinct homozygous phenotypes that (3) clearly segregate according to Mendelian ratios (one or two loci). Mutants that meet these criteria will proceed through our BSA-Seq pipeline. We will score our mapping populations for ts or rzl phenotypes, being careful to only include the strongest phenotypes and avoid heterozygote contamination. We will isolate DNA, sequence DNA samples, map our sequencing data, and search for potentially causative mutations and candidate genes as in Goal 1. Confirming the identity of candidate genes using either second alleles or transgenic approaches is beyond the scope of this project. However, any map locations and candidate genes we identify will be important preliminary data for further grant proposals. ?

Target Audience

One target audience will be scientists working on maize genetics, crop engineering and floral development. Other target audiences include undergraduate and graduate student trainees, and local farmers in the Pioneer Valley.

Products

Products generated during the life of this project include sequencing data for multiple maize genetic mutants, scientific papers

describing our discoveries, conference presentations, and will help develop skills in maize genetics, developmental biology and bioinformatics in undergraduate and graduate students.

Expected Outcomes

There will be two important outcomes from this work:
First, we will identify at least 6 more genes with roles in carpel suppression in maize (Fig. 4), almost doubling the number of genes with roles in specifying male vs. female flower morphology in maize.
Second, Hatch funds will allow me to gather preliminary data that I will use to apply for funding from federal agencies like the USDA and the NSF. Indeed, prior Hatch funding supported the preliminary data needed for a pending NSF grant application.

Keywords

flower development ~maize ~floral sexuality ~developmental genetics

Estimated Project FTEs For The Project Duration

Role	Non-Students or Faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.8	0.8	2.4	0.0	4.0
Professional	0.0	0.0	0.0	0.0	0.0
Technical	0.0	0.0	0.0	0.0	0.0
Administrative	0.0	0.0	0.0	0.0	0.0
Other	0.0	0.0	0.0	0.0	0.0
Computed Total	0.8	0.8	2.4	0.0	4.0

Animal Health Component 0 %

Forestry Component 0 %

Is this an AREERA Section 204 Integrated Activity? No

Activities

Research	100 %
Extension	0 %
Education	0 %

Research Effort Categories

Basic	100 %
Applied	0 %
Developmental	0 %

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
201	1510	1080	100

Knowledge Area

201 - Plant Genome, Genetics, and Genetic Mechanisms

Subject Of Investigation

1510 - Corn (for sweetcorn use 1480)

Field Of Science

1080 - Genetics

Primary Critical Issue

Sustainable Agriculture and Food Systems

Accession No. 1024691 **Project No. MAS00568**

Assurance Statements

1. **Are Human Subjects Involved?** ◉ No ○ Yes

If YES to Human Subjects
Is the Project Exempt from Federal regulations?

○ Yes

If yes, select the appropriate exemption number.

○ No

If no, is the IRB review Pending?

○ Yes

○ No IRB Approval Date

Human Subject Assurance Number

2. **Are Vertebrate Animals Used?** ◉ No ○ Yes

If YES to Vertebrate Animals
Is the IACUC review Pending?

○ Yes

○ No IACUC Approval Date

Animal Welfare Assurance Number

Project Proposal:

Filename	Size	Type
Bartlett_Hatch_2020.pdf	4956884	application/pdf

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