Unveiling conserved evolutionary branching traits among agricultural grass species

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Mutants in Agriculture

The field of plant genomics has witnessed remarkable advancements through the integration of various techniques such as quantitative trait locus mapping, genome-wide association studies, and next-generation sequencing (NGS) technologies[1]. These methodologies have proven instrumental in unveiling the genes responsible within the domestication processes and understanding evolutionary divergence of cultivated plant species.

Molecular insights reveal:

- Selection pressures acting upon these pivotal evolutionary genes in crops, along with the genes and mutations are intricately linked to these processes.
- The capacity to recreate domestication quantitative trait loci and manipulate promoter alleles, resulting in phenotypic variations has defied traditional predictive models[2].
- The optimization of yield related traits in maize shows the immense potential of genomic interventions in crop enhancement[2].
- The intricate role of conserved non-coding sequences in regulating gene expression patterns across plant species, as well as their interactions with transcription factors and other proteins, emerges as a critical avenue of exploration.

In this project, we utilize identified conserved non-coding sequences (CNSs) from Hendelman et al. (2021) to unravel the pleiotropic regulatory mechanisms governing GT1 function between the grass species *Zea mays* and *Brachypodium distachyon*. CNS regions can either interact with transcription factors (TFs) or other proteins to regulate gene expression spatial-temporally, where there is greater expression in leaves rather than in stems, quantitatively regulate the kernel row number and grain yield[3], or have conserved evolutionary function to regulate plant tillers in different grass species like *Zea mays* and *Brachypodium distachyon*. By understanding the alleles for gene expression, we can trace back where genetic divergence occurred genomically.

Modular cloning and CRISPR gene editing

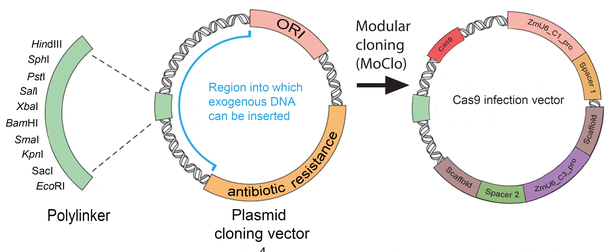


Figure 1. Plasmid cloning vector[4] basic components converted to a Cas9 infection vector. Plasmid vectors contain a antibiotic resistance selectable gene, replication origin (ORI), and a synthetic polylinker containing the recognition sequences for several different restriction enzymes. Exogenous DNA can be inserted into the bracketed region and modified to have the Cas9 protein, different promoters, and targeting spacers, which is done by modular cloning.

- Once the plasmid is modified to have Cas9 and SgRNA, it can be transformed into agrobacterium.
- Agrobactium then invades plant calli, which are similar to stem cells. These modifcations allow us to study desired alleses through CRISPR/Cas9 gene knock-out.

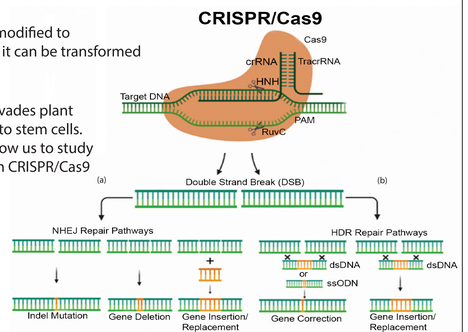
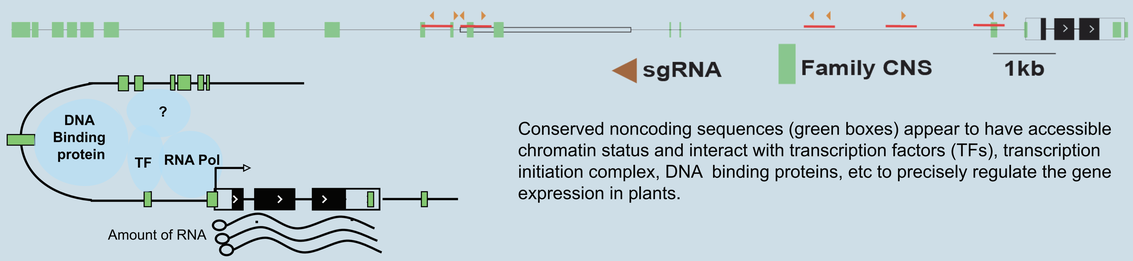


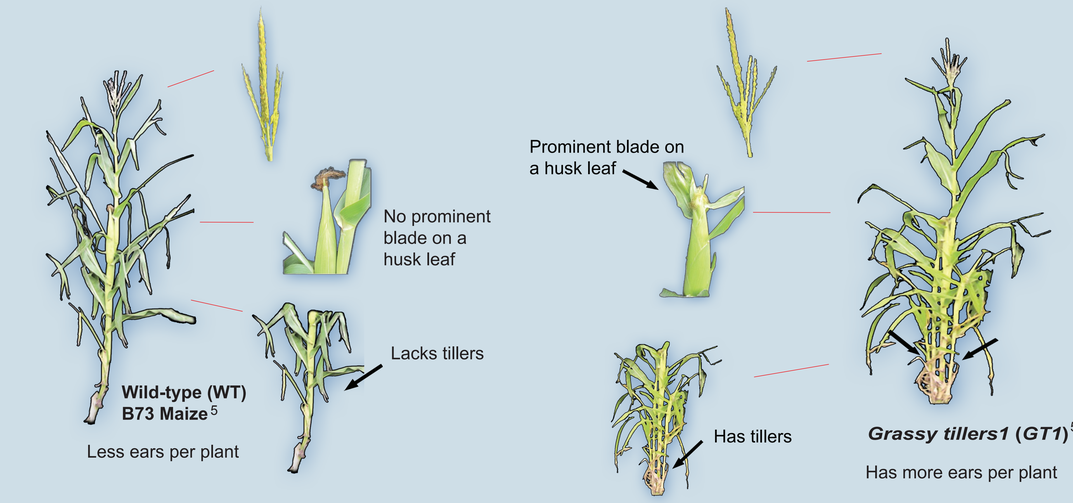
Figure 2. CRISPR/Cas9 complex with target sequence inducing double-stranded breaks (DSBs)[4]. This pathway induces either (a) non-homologous end joining (NHEJ) or (b) Homology-directed repair (HDR) in host organism. NHEJ is favored for editing as it is error-prone and edits are more likely to be introduced.

What edits do we make?



Conserved noncoding sequences (green boxes) appear to have accessible chromatin status and interact with transcription factors (TFs), transcription initiation complex, DNA binding proteins, etc to precisely regulate the gene expression in plants.

Understanding *GT1* by phenotype



Barcoding and genotyping

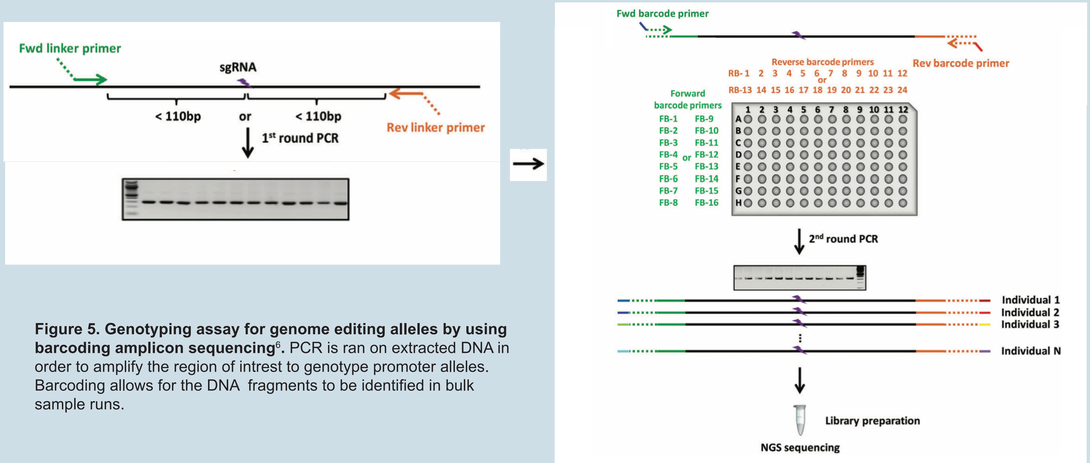
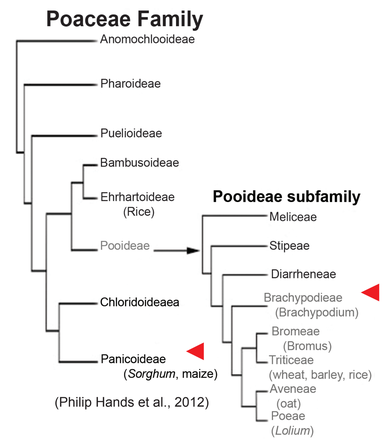


Figure 5. Genotyping assay for genome editing alleles by using barcoding amplicon sequencing[6]. PCR is ran on extracted DNA in order to amplify the region of intrest to genotype promoter alleles. Barcoding allows for the DNA fragments to be identified in bulk sample runs.

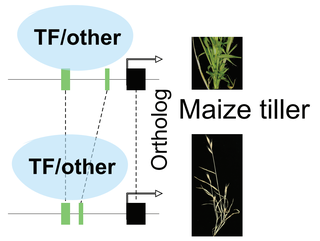
Future research in grass orthologs

WT (bd21-3)

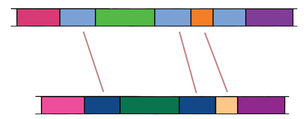
grassy tillers1 (GT1)
grassy tillers1 controls pleiotropic traits



(Philip Hands et al., 2012)



Brachypodium tiller



Orthologue genes preform the same gene function after speciation

- By understanding *GT1* in *Zea mays*, we are able to modifiy *Brachypodium distachyon* to test conserved gene function in additon to its phenotype and genotype.
- There has not been an editing effect found in the previous 6 *Brachypodium gt1* promoter sgRNA sites.
- We identified two CNS sites in *prol1* region and plan to further dissect the evolution and function of *prol1* as it is upstream of *GT1 and* underlies a major quantitative trait locus (QTL) for ear number (prolificacy) (Wills et al., 2013).
- Currently we are targeting sgRNA sites for the aligned *prol1* region and new CNS sites within *Brachypodium distachyon.*

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