Tissue Culture and Genome Editing in the Illinois Long Term Selection Experiment

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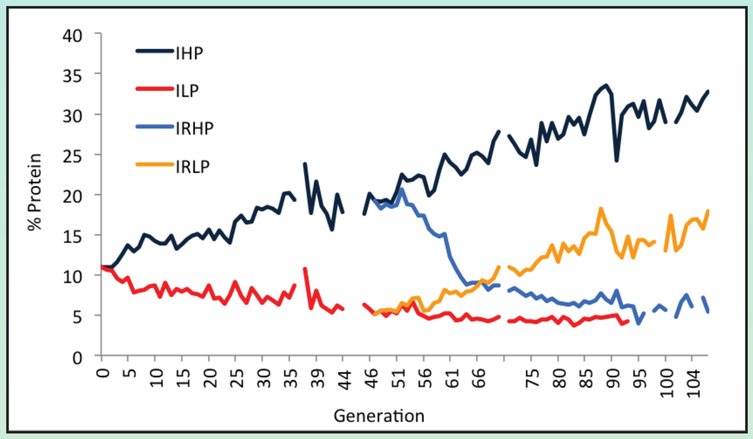
Abstract

The Illinois Long Term Selection Experiment is a unique genetic resource for identifying and characterizing genes selected for nitrogen use and protein accumulation in maize. To facilitate study of gene functions, we aim to establish a CRISPR Cas9 mediated genome-editing system in these novel genetic backgrounds. A media regime has been developed for successfully regenerating fertile plants of both Illinois High Protein (IHP) and Illinois Low Protein (ILP). Currently, putative transgenic lines expressing the Cas9 protein have been recovered using NPTII as a selectable marker. These Cas9 positive lines will be used to make targeted mutations with this germplasm. We have also initiated experiments to edit the Prolamin Box Binding Factor (PBF), which regulates zein gene expression and shows changes in both allele frequencies and mRNA expression that are consistent with PBF being a target of selection for grain protein concentration. In addition to generating knockout mutations, we are also investigating the functional significance of variation in the length of an asparagine (Asn) repeat motif found at the C-terminus of PBF. This Asn repeat shares features with triplet repeat expansions studied in Arabidopsis and trinucleotide repeat disorders in humans such as Huntington's disease. It is hypothesized that variation in the Asn rich region of PBF could act as a sensor to control α-zein accumulation in response to incoming supply of amino acids, or possibly interacting with other transcription factors such as opaque-2. To target this Asn-repeat motif, single-guide RNAs were designed to create variation in Asn repeat length in conjunction with expressed Cas9. Guide RNA constructs will be introduced via biolistics and screened by Sanger sequencing to confirm targeted edits in the genome.

A Century of Selection Has Generated Novel Maize Genotypes



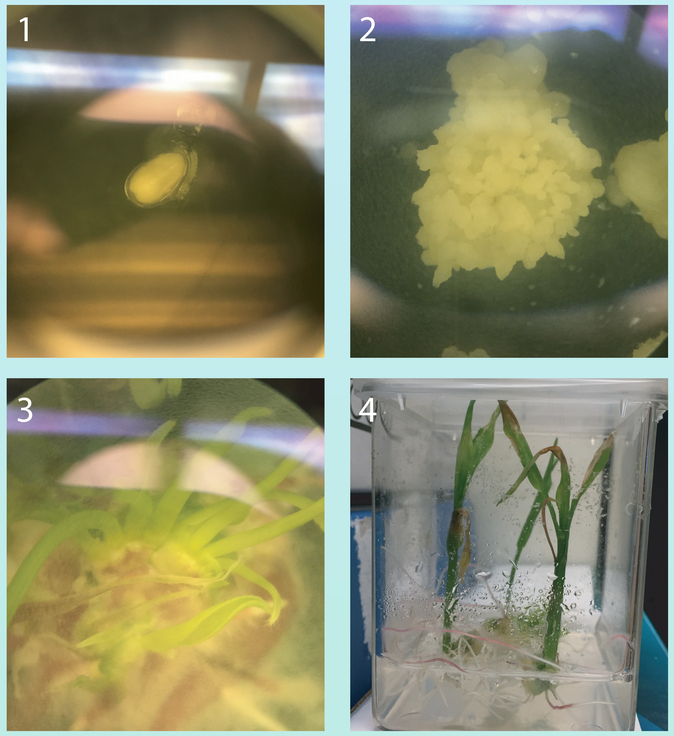
Illinois High Protein (IHP)

- ~30% kernel protein
- High N Uptake
- Poor N utilization

Illinois Low Protein (ILP)

- ~4% kernel protein
- Poor N Uptake
- High N utilization

IHP and ILP produce highly regenerable embryogenic callus



Embryos were induced and maintained (1, 2) on N6 media supplemented with 15 uM Dicamba. Callus with proper morphology (2) were transferred to regerenation media, as per ISU PTF, containing 5 mg/L 6-BAP for 4 days (3), then transferred to R media with no hormone. Once ready, plantlets were moved to magenta boxes (4) and later moved a greenhouse.

Transformation Strategies

Goal: Develop Cas9 expressing lines in IHP/ILP for genome editing

↓

Transform IHP/ILP with Ubi:Cas9 and NPTII via particle bombardment

↓

Regenerate transgenics and obtain homozygous lines

↓

Bombard Cas9+ lines with gRNAs for future targeted editing in ILTSE

Goal: Create variation in Asn-rich region of PBF with CRISPR Cas9

↓

Transform IHP/ILP with Ubi:Cas9, NPTII, gRNAs, and HDR sequences

↓

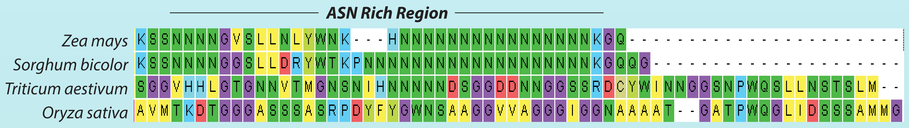
Regenerate transgenics from resistent callus

↓

Amplify C-terminus of PBF (UMC1065) and sequence to screen for edits

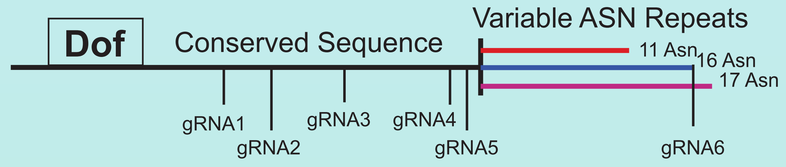
CRISPR Cas9 mediated Genome Editing of Prolamin Box Binding Factor (PBF)

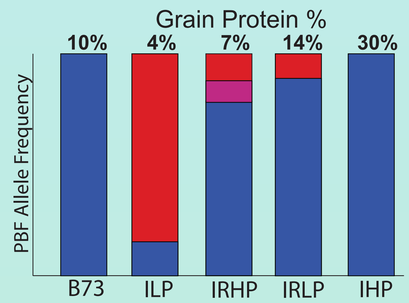
C-Terminus of PBF in Monocots

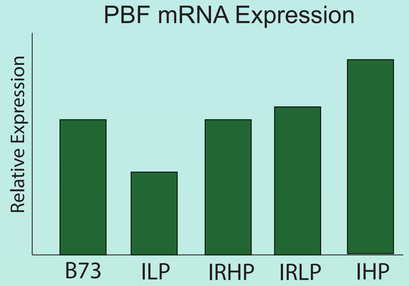


PBF protein sequences from NCBI BLAST aligned using MEGA 7 and ClustalW

PBF Gene Model







Lucas, 2014 (unpublished)

gRNA1: GTGCACATTGCACCATATGATGG
gRNA2: GAGTGGTGGCAACAATGGAATGG
gRNA3: GGTAGCAGATGAGGACGATGAGG

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Loss of function mutation

gRNA4: GGAGATGACCTTGTTCGTGTTGT
gRNA5: AACAACAACAAGGGACAATAAGG
gRNA6: TATCATTGTTGAACCTCTACTGG

↓

Variation in Asn rich region by Homology Directed Repair

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