

# Zein-RFP reporter lines as tools to study 22 kD $\alpha$ -zein gene regulation



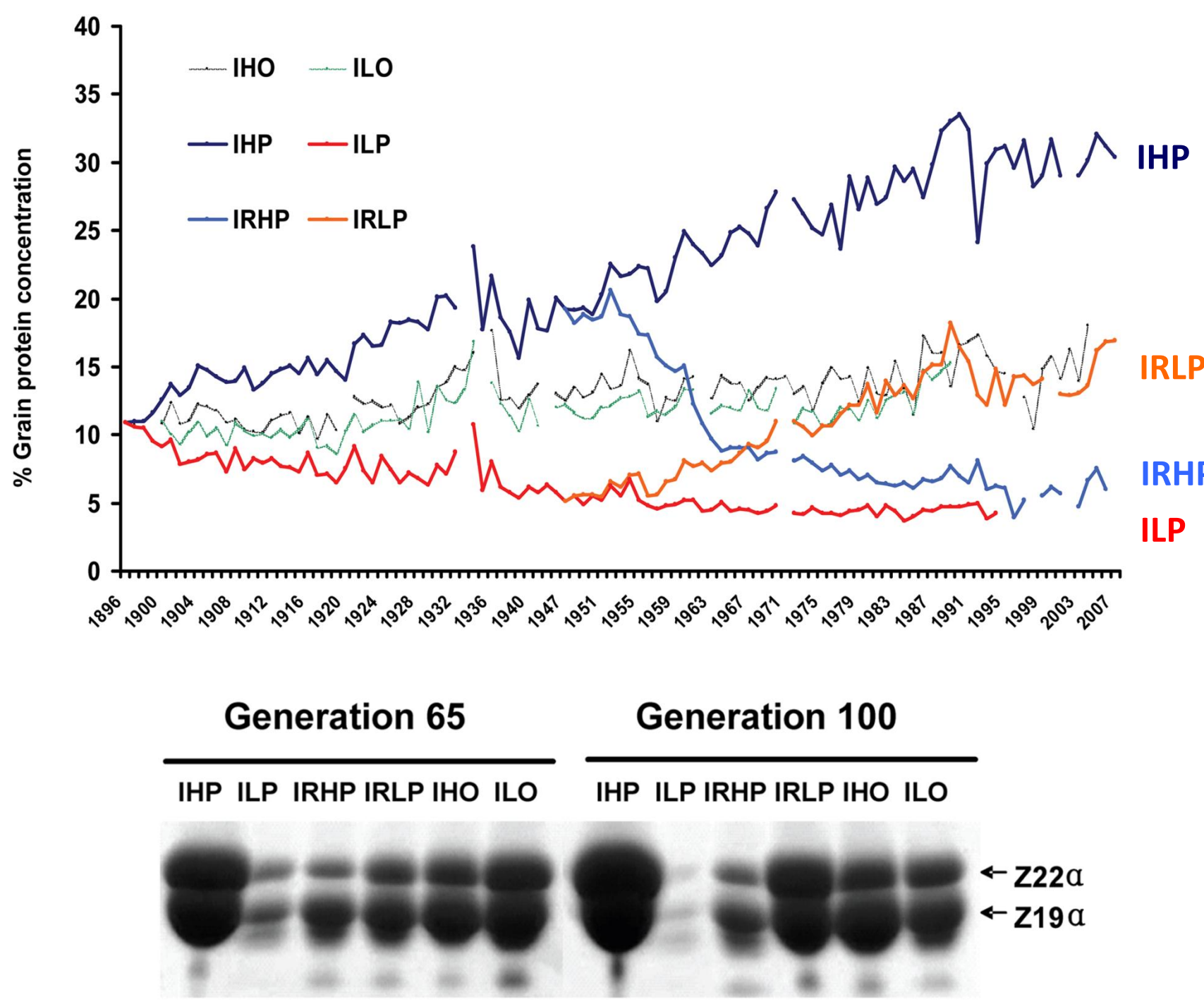
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## Abstract

Long-term divergent selection for grain protein concentration has produced populations with the known phenotypic extremes for this trait, and also illustrates the nature of responses to phenotypic selection. Mapping studies suggest that the response to selection in this experiment is dependent upon many small-effect genes. An alternative theory explored here is that the response depends on quantitative expression variation of a few major regulators. Selection for grain protein concentration most dramatically affects the  $\alpha$ -zeins. Of the 22 kD  $\alpha$ -zeins, the few known regulators are *OPAQUE2* (*O2*), the *Prolamin-box Binding Factor* (*PBF*), and factors influencing the folding of zeins into endosperm protein bodies. However, QTL studies do not show these factors as having strong genetic effects on variation in seed protein concentration, suggesting the possibility of other key regulators of zein expression. RNA analysis and measuring protein abundance are two effective approaches for studying the regulation of zein expression, but they are also expensive, destructive, and laborious. An alternative inexpensive and nondestructive approach to investigate the regulation of zein expression is the use of *FLOURY2* -RFP reporter lines, courtesy of Dave Jackson's lab at Cold Spring Harbor, which have been introgressed into inbred lines derived from the Illinois Protein Strains (IPS). We found that RFP expression not only correlates with grain protein concentration, but also follows the accumulation patterns of endogenous zein genes throughout development. At all developmental stages, RFP expression was strongest in Illinois High Protein (IHP), the lowest in Illinois Low Protein (ILP) and intermediate in Illinois Reverse High Protein (IRHP) and Illinois Reverse Low Protein (IRLP). RFP expression was also visibly detected the earliest in IHP. Additionally, by crossing *F12* zein-RFP to an  $\alpha 2$  mutant introgressed into IHP, we show that its expression is strongly activated by *O2*, further supporting the conclusion that the *F12* -RFP transgene reporter is regulated in the same manner as endogenous zein genes. Similar tests are being done with a transgenic line that over expresses *PBF*. Future experiments will use the *F12* -RFP transgene as a tool for identifying regulators in ongoing genetic mapping studies.

## Illinois long-term selection for grain protein in maize

More than 100 generations of selection in the open-pollinated variety Burr's White have generated four populations that span the known extremes for grain protein concentration.

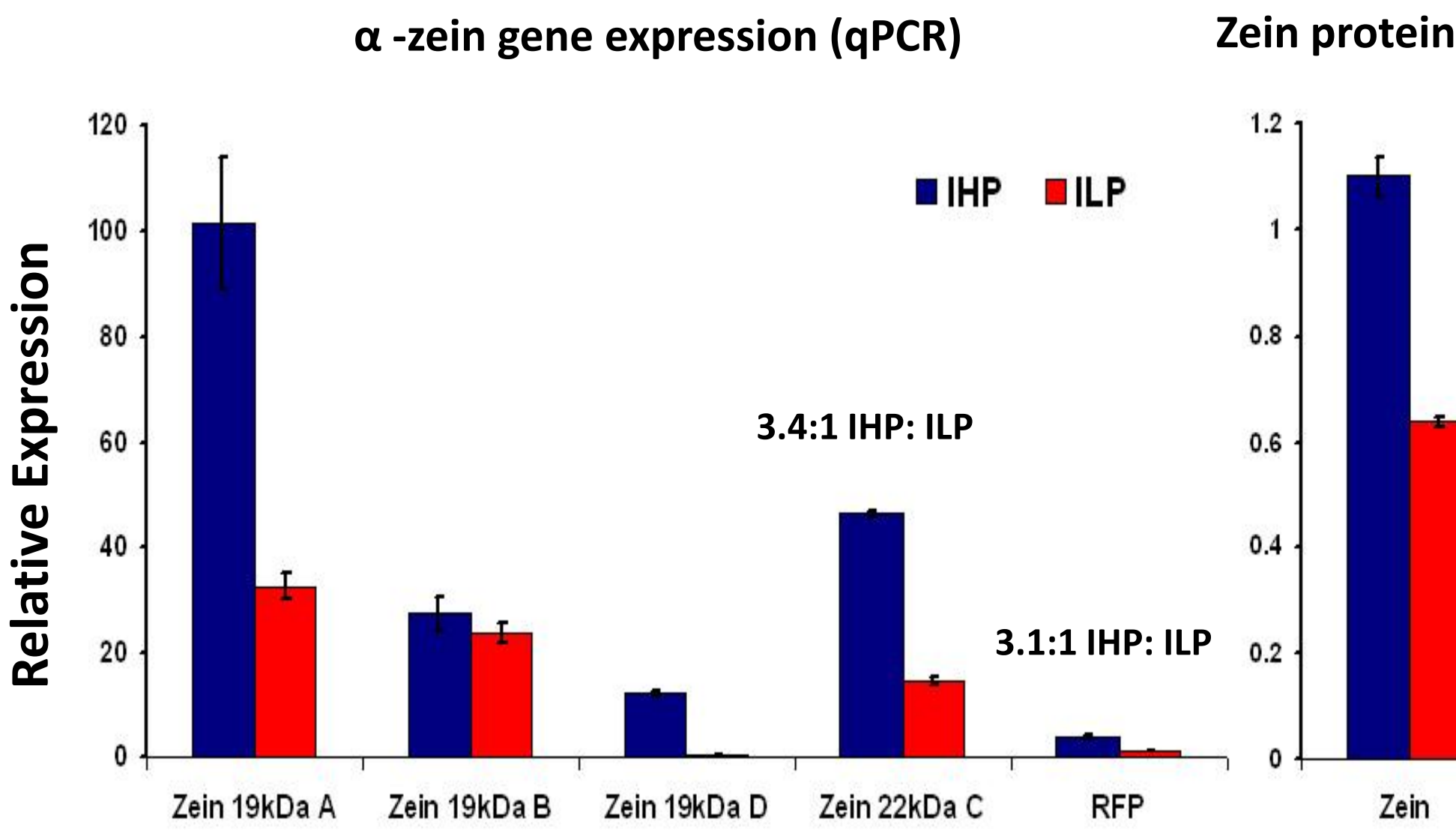


### Divergent Recurrent Selection:

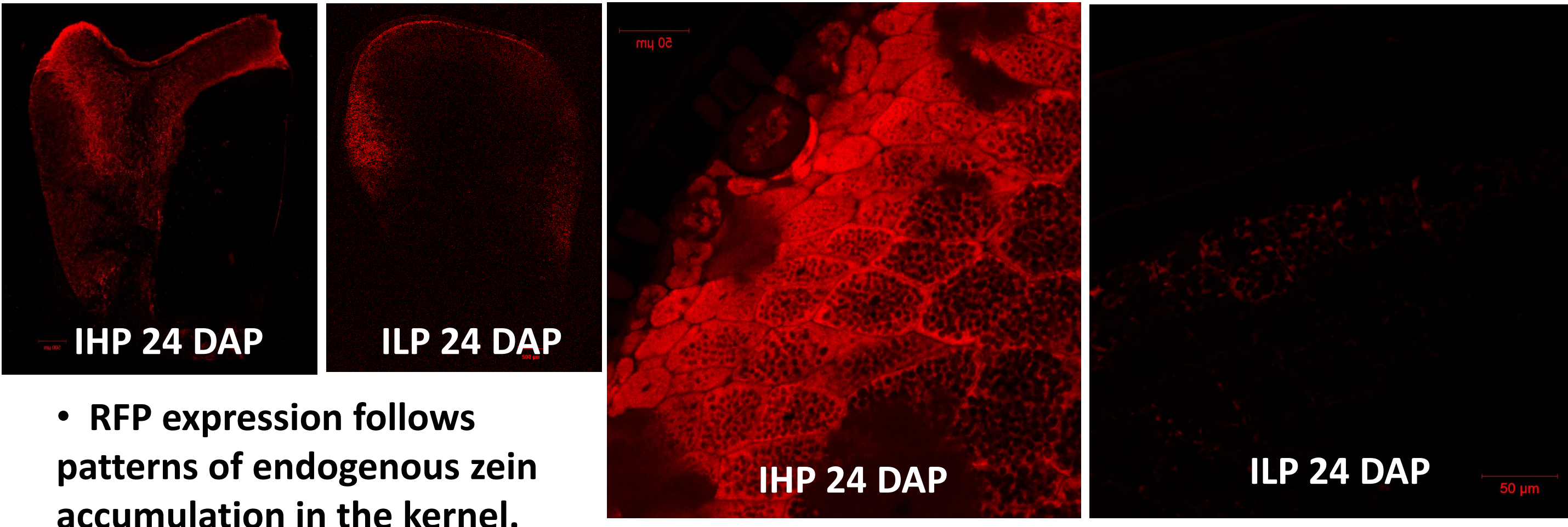
- Each population was pollinated in isolation using schemes to minimize inbreeding.
- 60-120 ears were analyzed from each population per cycle.
- 20% selection index on an ear basis

The Illinois selection strains exhibit large differences in the accumulation of  $\alpha$ -zein (19 kD and 22 kD) and total N at two generations of selection (~65 and ~105).

## Floury2:RFP expression in IHP and ILP 24 DAP kernels



- Zein gene expression greater in IHP than ILP
- Zein protein concentration greater in IHP than ILP
- RFP expression greater in IHP than ILP



- RFP expression follows patterns of endogenous zein accumulation in the kernel.

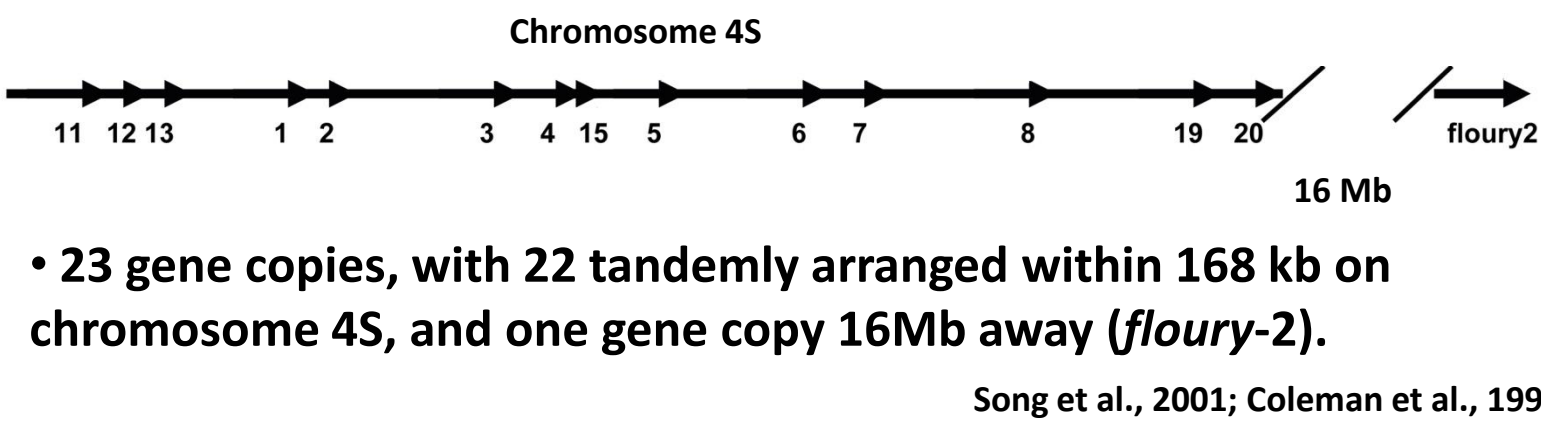
- RFP expression greater in IHP than ILP

## Does the maternal parent control zein regulation?

### 2 Possibilities

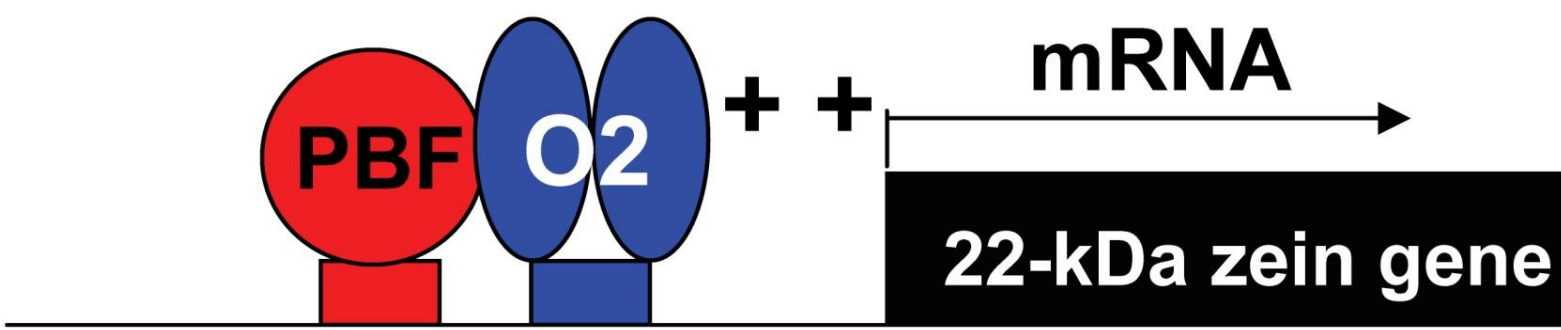
- Epigenetic control by maternal imprinting
- Nutrient supply of the plant

## Does the clustering of the 22 kD $\alpha$ -zeins affect their regulation?

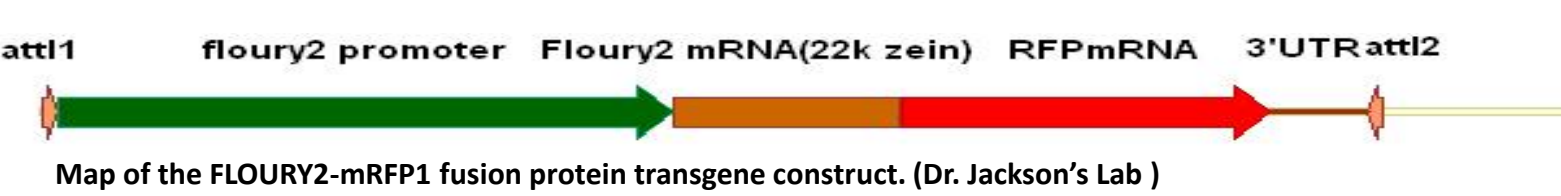


- *Opaque2* (*O2*) is a transcriptional activator that binds a highly conserved sequence element present in the promoters of all 22 kD ( $\alpha 1C$ )  $\alpha$ -zeins, but not other zein genes (Schmidt et al., 1992).
- The *Prolamin-box Binding Factor* (*PBF*), a Dof zinc-finger DNA binding protein, interacts *in vitro* with the *O2* protein in the  $\alpha$ -zein promoter region, suggesting that protein: protein interaction may play a role in regulating  $\alpha$ -zein expression (Vicente-Carbajosa et al., 1997).

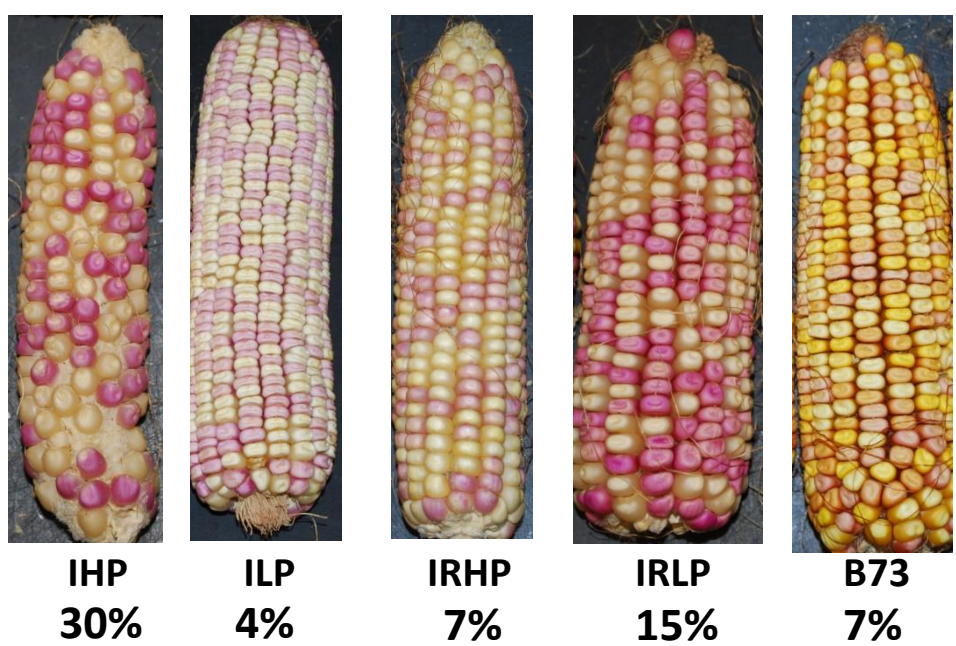
## Known regulators of 22kD $\alpha$ -zeins: *O2* and *PBF*



## The *floury2*: RFP transgene is a tool for investigating 22kD $\alpha$ -zein gene regulation

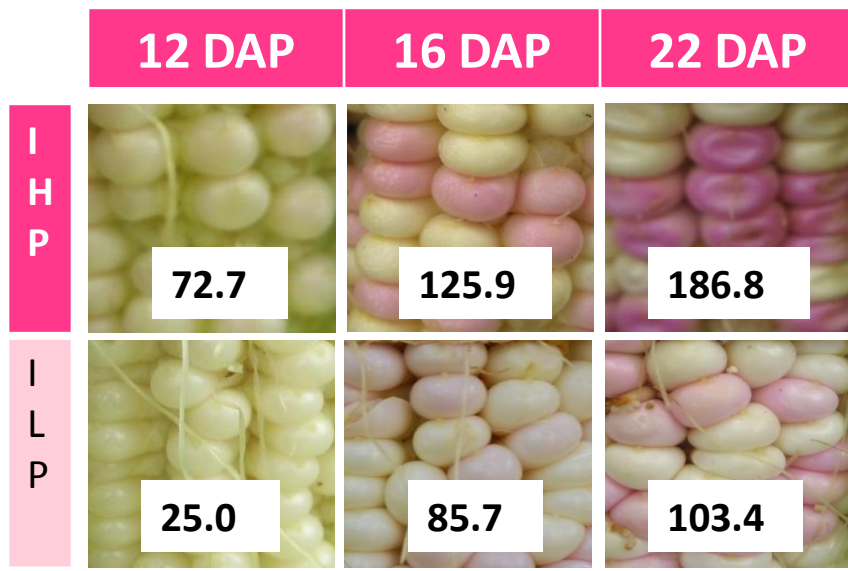


## *floury2*:RFP backcrossed into the inbred- derived Illinois Protein Strains



- Percentage indicates grain protein concentration
- RFP expression correlates with grain protein concentration

### RFP Quantification



- Quantification of relative expression from *floury2*:RFP transgene in developing kernels.
- The number within each panel indicates the mean intensity in the magenta channel for the RFP-expressing kernels in Adobe Photoshop images (CMYK scale 1- 255).

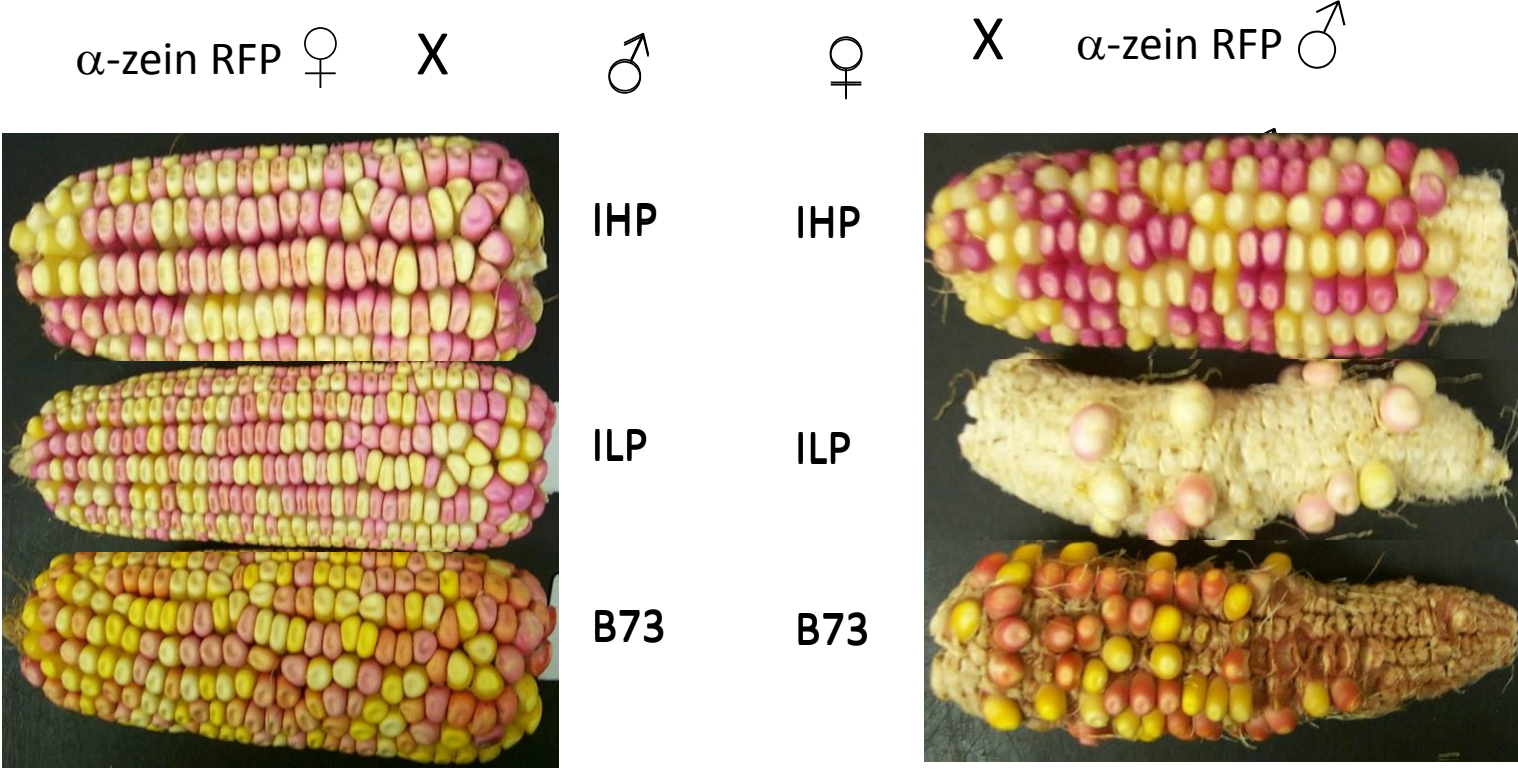
## RFP expression follows zein expression throughout development



- IHP expresses *floury2*: RFP earlier.
- IHP *floury2*: RFP expression remains higher throughout development.
- *Floury2*: RFP expression tracks developmental trends of endogenous zein accumulation.

## The zeins respond to the nutritional status of the plant

- Reciprocal crosses show differences of *floury2*: RFP expression when transmitted through the male or female.



No change in RFP expression when Protein Strains used as fathers

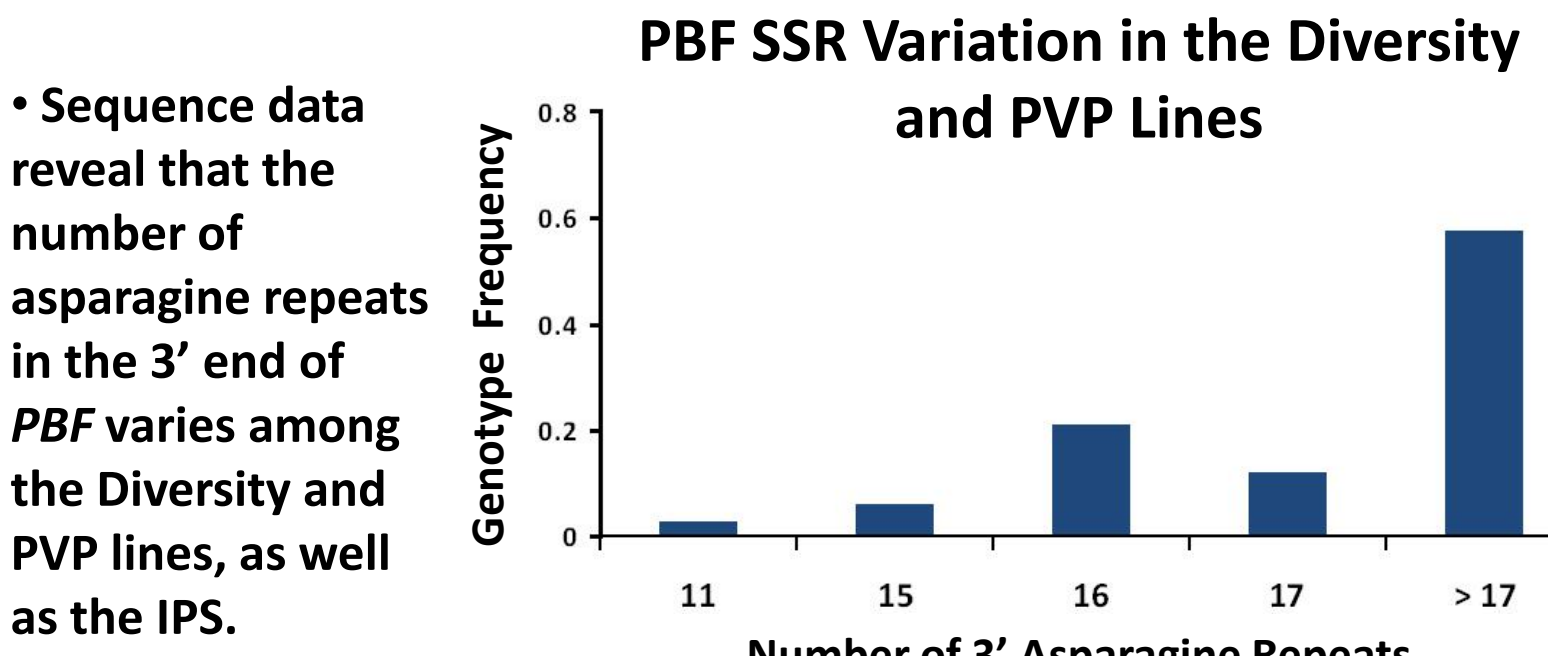
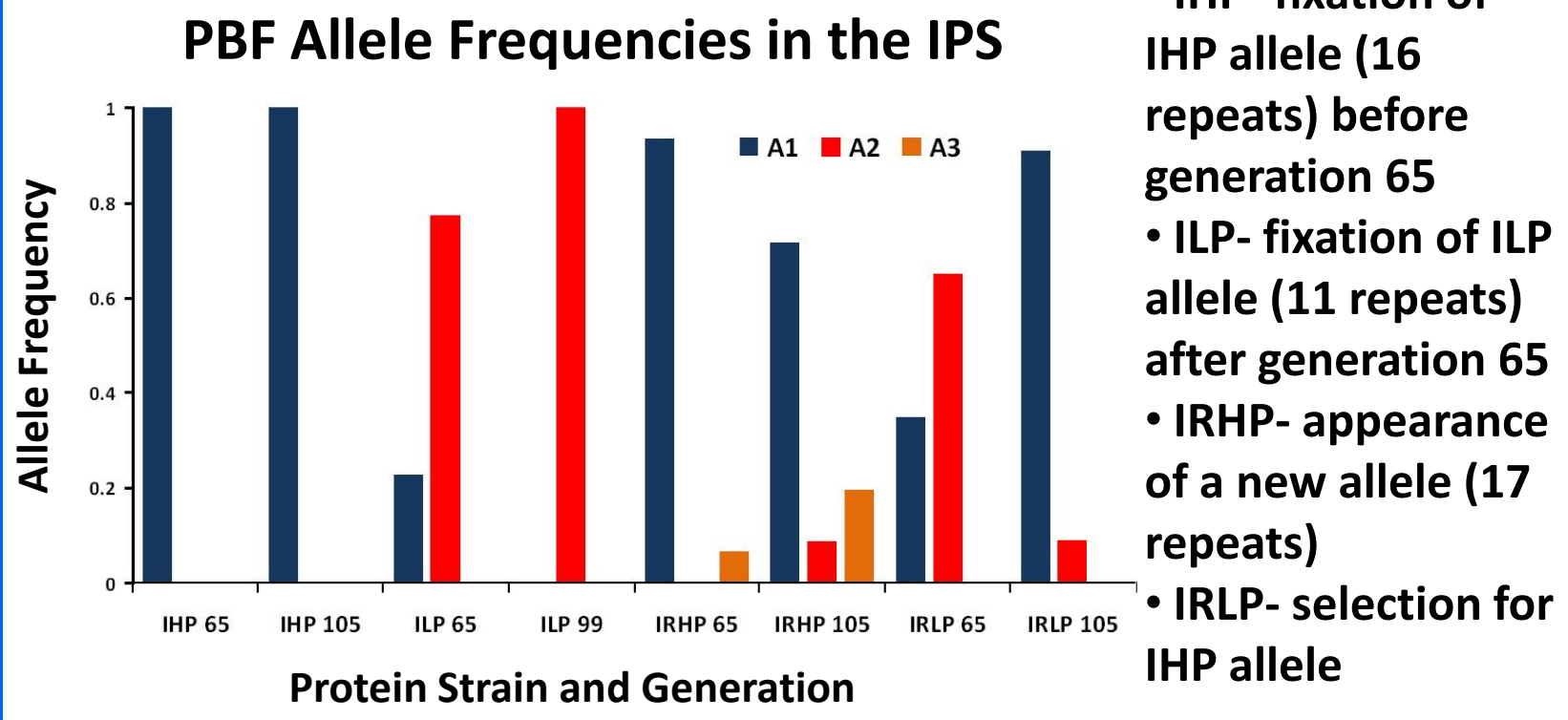
Changes in RFP expression depend on maternal genotype, which differ in N status

## Location within the 22 kD tandem cluster is not required for proper regulation of zein expression



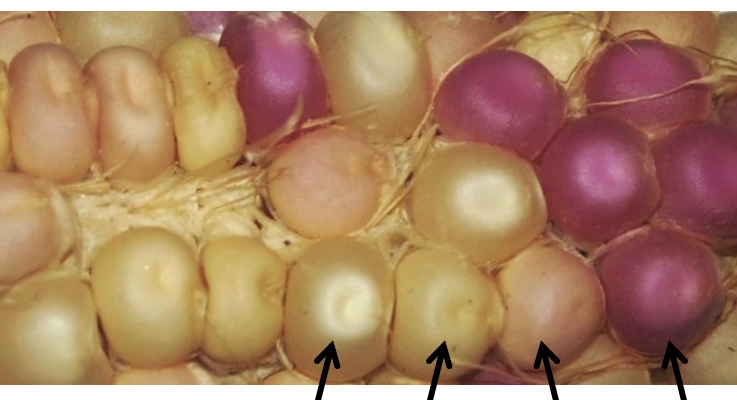
- No difference in RFP expression among the three transgenic events in the *floury2*:RFP Illinois Protein Strains
- Regulation of the *floury2*:RFP transgene does not depend on location in the genome in the Illinois Protein Strains.

## PBF regulation



- Sequence data reveal that the number of asparagine repeats in the 3' end of *PBF* varies among the Diversity and PVP lines, as well as the IPS.

## O2 regulation



- $\alpha 2$  mutation introgressed into the *floury2*:RFP Illinois Protein Strains
- RFP illustrates reduction in zein due to the  $\alpha 2$  mutation
- $\alpha 2$  mutants still express RFP, evidence for other regulators

## Future Work

- Histochemical staining of the *floury2*:RFP IPS to determine if zein-RFP protein accumulates properly in protein bodies.
- Introgress of PBF over expression construct into the *floury2*:RFP IPS to determine the regulatory role of PBF.
- Further quantification of RFP.
- Use RFP to map QTL for grain protein.

## Acknowledgements

We gratefully acknowledge the efforts and perseverance of Cyril G. Hopkins, Louie Smith, Clyde Woodworth, Earl Leng, Denton E. Alexander and Robert J. Lambert, the researchers who have served as principal investigators of the Illinois Long-Term Selection Experiment.