Bioinformatic Approach to Nominating Candidate Genes for Uncloned Maize Mutants

Yaya Similien and Hank W. Bass

Department of Biological Science, Florida State University, Tallahassee, FL, USA

Introduction

Mutations occur in all living organisms no matter how big or small. Maize is a model genetic system, ideal for investigating how mutations affect organisms. Heritable traits are also called phenotypes and their genetic basis are called genotypes. A number of maize mutants have been characterized as heritable and genetically mapped to specific chromosome locations. However exactly which gene is mutated and responsible for the mutated phenotype is not always known.

Abstract

My project was to select three maize mutants and develop candidate gene lists by inspecting all the gene models in the region where the mutant maps. This involves systematic inspections of genes and their encoded gene products and known or presumed functions. I have picked three mutants for this project and report on what I found. For example, if I chose a seed-starch deficient mutant (e.g. a mutant called *sugary45*), and it was genetically linked to chromosome 3, I would look at all the known nearby genes using online maize genome websites, MaizeGDB and genomaize.org. For each gene on chromosome 3 near my mutant, I will ask "if this gene was mutated (broken or disrupted), could it result in starchless seed phenotype?" If it could, that gene gets nominated as a "**candidate**" gene, which could be tested later using molecular biology.

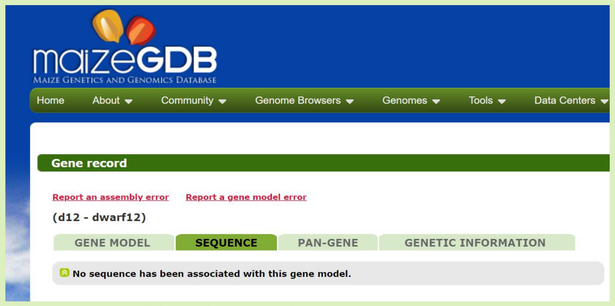

Figure 1. Candidate d12 in MaizeGDB

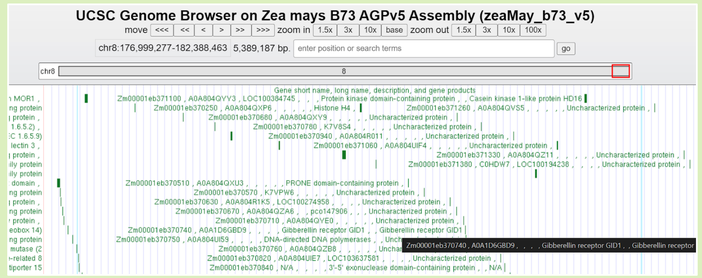

Figure 2. Candidate d12 genomic region in genome browser

Methods

For this project, the 3 uncloned mutants chosen are *albescent plant1*, *dwarf12*, and *torn leaves1*. The mutants were searched up on MaizeGDB to make sure they were still uncloned. We know they are uncloned when the sequence tab in MaizeGDB of the mutant provides no information. I searched for their linkage map on the bottom of the page in order to find their chromosomal neighborhood. I narrowed down the candidate pool to ten genes before and after the uncloned mutants. Each gene was evaluated whether it would be practical as the candidate gene for the uncloned maize mutant. With the help of literature review, the candidate genes were narrowed down to just a few.

Figure 3. The dwarf12 (d12) mutant (dashed box), from MaizeGDB

Results

During this project, one mutant chosen was cloned. That mutant was the ***albescent plant1*** (*al1*) mutation. The *al1* mutation causes partial or complete whitening of the leaves or kernel. While searching for the description of the genes in the gene neighborhood for albescent 1, *ptox1* and *ptox2* were described as the two possible genes involved in the *al1* phenotype. They are plastid terminal oxidase genes which encode *al1* (Nie et al., 2024).

The ***dwarf12*** (*d12*) mutation makes plants short and compact(Fig. 3). We looked for genes that involve plant growth regulators such as gibberellic acid and cytokinin (Fawcett et al., 2016). The genes *Gibberellin receptor/GID1* and *cytokinin oxidase 4b/Cko4b* were found in my search area and chosen as candidates. *GID1* was my most likely candidate because, "GA is involved in controlling stem elongation…GA deficiency or insensitivity to GA could easily result in different levels of dwarfism…"(Reynante Ordonio et al., 2015). Gibberellin is involved in the elongation of the stem of plants, thus, it was my most logical candidate gene.

Lastly, ***Torn leaves1*** (*Trn1*) is a mutation which causes maize leaves to be torn or have necrotic tips (dead) (Fig.4). My most likely candidate genes could be involved in causing cell death and the necrotic leaf tips. The candidate gene for this mutation is *mlo7*. *Mlo7* is a defense response capable of triggering local cell death. In addition, "...the absence of the *Mlo* wild-type allele stimulates a spontaneous cell death response in foliar tissue" (Peterhansel et al., 1997).

Figure 4. The torn leaves1 (trn1) mutant, from MaizeGDB

Figure 5. The albescent plant1 (al1) mutant from MaizeGDB

Conclusions

- The *albescent plant1* was cloned as this project was taking place. The genes are *ptox1* and *ptox2* (Nie et al., 2024).
- Candidate gene for *dwarf12* is Gibberellin receptor/GID1 because Gibberellin is involved in stem elongation of plants (Reynante Ordonio et al., 2015).
- My *Torn leaves1* candidate gene is *mlo7* because it is associated with cell death response.

References

Fawcett, J., Koopman, Z., & Miller, L. (2016, January 1). *On-farm corn and soybean plant growth regulator trials*. Iowa State University Digital Repository - Home. http://lib.dr.iastate.edu/cgi/viewcontent.cgi

Nie, Y., Wang, H., Zhang, G., Ding, H., Han, B., Liu, L., Shi, J., Du, J., Li, X., Li, X., Zhao, Y., Zhang, X., Liu, C., Weng, J., Li, X., Zhang, X., Zhao, X., Pan, G., Jackson, D., Li, Q. B., … Zhang, Z. (2024). The maize PLASTID TERMINAL OXIDASE (PTOX) locus controls the carotenoid content of kernels. *The Plant journal : for cell and molecular biology*, 10.1111/tpj.16618. Advance online publication. https://doi.org/10.1111/tpj.16618

Peterhansel, C., Freialdenhoven, A., Kurth, J., Kolsch, R., & Schulze-Lefert, P. (1997, August 1). *Interaction analyses of genes required for resistance responses to powdery mildew in barley reveal distinct pathways leading to leaf cell death.* OUP Academic. https://academic.oup.com/plcell/article/9/8/1397/5986489

Reynante Ordonio, Boudet, A. M., Fleet, C. M., George-Jaeggli, B., Hu, Y., Humphreys, J. M., Jones, D. A., Koo, B.-H., Mehlo, L., Suzuki, S., Akin, D. E., Alam, M., Farfan, I. D. B., Blomstedt, C. K., Borrell, A. K., Bout, S., Brown, P. J., Buchanan, C. D., … Lipkie, T. E. (2015, October 31). *Molecular breeding of Sorghum bicolor, a novel energy crop*. International Review of Cell and Molecular Biology. https://www.sciencedirect.com/science/article/abs/pii/S193764481500091X?via%3Dihub