

The role of phospholipids in maize adaptation to highlands of Mexico

Phospholipids constitute around 1/3 of the available phosphorus in the plant and are the major component of membranes, specially in chloroplasts¹. Besides their structural role, some of the most abundant phospholipids such as phosphatidylcholine have interesting signaling functions and are involved in multiple regulatory processes². Their relative concentrations changes in response to several environmental factors. Plants challenged with low temperatures tend to increase the proportion of phospholipids in the composition of membranes with respect to other polar lipids such as galactolipids and sulfolipids³ probably as a mechanism to modulate membrane fluidity. On the other hand, plants under phosphorus deficiency recycle phospholipids -to free up phosphate- and substitute them with galactolipids and sulfolipids¹. Maize adaptation to the highlands in México involved both adaptation to colder temperatures as a consequence of high altitude and low phosphorus since the area colonized by maize coincides with the Mexican volcanic belt characterized by Andosolic soils that have low levels of bio-available phosphate. We hypothesize then, that fine tuning of phospholipid metabolism was important in the process of maize adaptation to the Mexican highlands.

There are several lines of evidence that support this hypothesis. Takuno and collaborators found a group of around 90 common maize genes showing marks of adaptation to highlands in both South America and Mesoamerica landraces⁴. A search of GO terms for this list of genes showed that a third of the terms found were related to the synthesis of lipids / phospholipids and phosphorus acquisition. Furthermore, one of these genes is described as a CONSTANS interacting protein, a transcription factor that is upstream of FLOWERING LOCUS T (FT), which control flowering time integrating information about the photoperiod. In Arabidopsis, FT binds a specific species of phosphatidylcholine⁵. When the ratio between phosphatidylcholine and phosphoethanolamine is genetically increased flowering times are reduced, probably because the FT complex with phosphatidylcholine enhances the union of FT with the promoters of its target genes accelerating flowering. In maize, lipid

composition is also a good predictor of flowering time⁶. Furthermore, both phosphatidylcholine and other intermediate metabolites of phosphatidylcholine synthesis are essential for proper embryo development⁷ and root development⁸. Phosphatidylcholine

Plans for the future in collaboration with Ruairidh Sawers, other colleagues here in Langebio and Oliver Fiehn at the Genome Center in UC Davis include:

- A first characterization of phospholipid profiles in PT and B73 plants grown in highland and lowland conditions (Metepéc and Nayarit) and plants grown in growth chambers simulating highland and lowland conditions (mainly temperature) in combination with low and control P conditions. Possibility to also use F1 plants, specially if samples are taken in parallel for allele specific expression I am thinking here on some sort of targeted capture -such as CaptureSeq⁹- of genes involved in phospholipid pathways. This first experiment should give us an idea of the genetic and environmental variation in phospholipids that we might expect.
- We could then move to QTL, association mapping analysis using the mapping populations that Ruairidh has been developed or other material that will be produced by the hilo project using the phospholipid profiles as biochemical phenotype. The idea here is that this biochemical phenotypes are much closer to the underlying causative genes. One can use concentrations of metabolites as input but also ratios between two metabolites that have been shown to provide more information than the concentration of the individual metabolites¹⁰.
- Use evo/pop genomic tools to look for marks of adaptation to highlands specifically for the group of genes involved in phospholipid metabolism. As we talked before I would be interested here to see with folks like Graham if selection -to highlands- could happen at the pathway rather than the single gene level as suggested by B. Gaut¹¹.
- We are also using reverse genetics using UniformMu and Ac/Ds tagging of the genes of interest. Ruairidh's lab has already done work in the XIPOTL genes and this winter we have planted around 40 UniformMu stocks in Nayarit to clean and advance them.

- Finally we are using heterologous expression in Arabidopsis using the CDS of the genes of interest fused with the Arabidopsis promoter of that gene and expressing it in Arabidopsis mutants for that gene. This allows us to test on a “neutral” background the effects of different maize alleles.

In summary, I see good opportunities to use data, material and the experience of the hilo project members in population and evolutionary genetics and genomics in combination with a very targeted functional validation approach of an specific metabolic pathway that seems to be important in the adaptation of maize to the highlands.

References

1. Veneklaas, E. J. *et al.* Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytologist* **195**, 306–320 (2012).
2. Xue, H.-W., Chen, X. & Mei, Y. Function and regulation of phospholipid signalling in plants. *Biochemical Journal* **421**, 145–156 (2009).
3. Degenkolbe, T. *et al.* Differential remodeling of the lipidome during cold acclimation in natural accessions of Arabidopsis thaliana. *The Plant Journal* **72**, 972–982 (2012).
4. Takuno, S. *et al.* Independent Molecular Basis of Convergent Highland Adaptation in Maize. *Genetics* genetics.115.178327 (2015).
5. Nakamura, Y. *et al.* Arabidopsis florigen FT binds to diurnally oscillating phospholipids that accelerate flowering. *Nature Communications* **5**, 3553 (2014).
6. Riedelsheimer, C., Brotman, Y., Méret, M., Melchinger, A. E. & Willmitzer, L. The maize leaf lipidome shows multilevel genetic control and high predictive value for agronomic traits. *Scientific reports* **3**, 2479 (2013).
7. Lin, Y.-C., Liu, Y.-c. & Nakamura, Y. The Choline/Ethanolamine Kinase Family in Arabidopsis: Essential Role of CEK4 in Phospholipid Biosynthesis and Embryo Development.

The Plant Cell ... **27**, 1497–1511 (2015).

8. Cruz-Ramirez, A. *et al.* The xipotl mutant of Arabidopsis reveals a critical role for phospholipid metabolism in root system development and epidermal cell integrity. *The Plant Cell* **16**, 2020–2034 (2004).

9. Mercer, T. R. *et al.* Targeted sequencing for gene discovery and quantification using RNA CaptureSeq. *Nature Protocols* **9**, 989–1009 (2014).

10. Petersen, A.-K. *et al.* On the hypothesis-free testing of metabolite ratios in genome-wide and metabolome-wide association studies. *BMC Bioinformatics* **13**, 120 (2012).

11. Gaut, B. S. Evolution Is an Experiment: Assessing Parallelism in Crop Domestication and Experimental Evolution: (Nei Lecture, SBE 2014, Puerto Rico). *Molecular Biology and Evolution* **32**, 1661–1671 (2015).