

The role of phospholipids in maize adaptation to Mexican highlands

Background

The ancestor of maize, teosinte, is native to southeast Mexico, specifically from the Balsas River Valley at a low altitude, located in Guerrero state. This species is known as teosinte *parviglumis* (*Zea mays ssp. parviglumis*). Another subspecies of teosinte named *mexicana* (*Zea mays ssp. mexicana*) that colonized the highlands of Mexico over 1800 meters. Maize (*Zea mays ssp. mays*) was domesticated from *parviglumis* about 8,000 years ago in the low valleys where *parviglumis* originated¹. From that region maize spread throughout Mexico, the rest of the Americas and nowadays it grows on every continent except Antarctica². Therefore, maize represents a great model to study local adaptation processes. Local adaptation can be defined as the mechanisms of how plants modify their metabolism to develop in the biotic and abiotic conditions of the ecological niche they occupy. After its domestication in the Balsas River, maize colonized Mexican highlands thanks in part to introgression events with teosinte *mexicana*, already adapted to these highland conditions³. In this highland conditions maize was exposed to higher levels of radiation, lower air pressure, temperature and precipitation; furthermore, in some areas close to the Mexican volcanoes chain, maize had to adapt to Andosol soils, characterized by low bio-availability of phosphorus⁴.

Plants growing in phosphorus limiting soils develop a series of metabolic strategies including recycling of P containing compounds such as RNA and phospholipids. Phospholipids constitute around 1/3 of the available phosphorus in the plant and are the major component of membranes, specially in chloroplasts⁵. Besides their structural role, some of the most abundant phospholipids such as phosphatidylcholine have interesting signaling functions and are involved in multiple regulatory processes⁶. 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 sulfolipids⁷ probably as a mechanism to mod-

ulate membrane fluidity. On the other hand, plants under phosphorus deficiency recycle phospholipids -to free up phosphate- and substitute them with galactolipids and sulfolipids⁵. Although the metabolic pathways involved in these processes are more or less known, recent results suggest that the diversity of polar lipids involved may be greater than we expect⁸.

There are several studies that point to an important role of phospholipids in maize adaptation to highlands. Takuno and colleagues studied genes showing selective breeding marks in maize populations from highlands in Mesoamerica and South America⁹. Genes that showed marks of adaptation to highlands in the two groups only agreed a group of about 90 genes. When performed a search on Gene Ontology over a third of the terms found were related to the synthesis of lipids / phospholipids. Furthermore, one of these genes is described as a CONSTANS interacting protein, a transcription factor that is upstream of FLOWERING LOCUS T (FT) transcription factor, which controls flowering time according to photoperiod. In *Arabidopsis thaliana*, FT binds to phosphatidylcholine (PtdCho) *in vitro*, having a direct correlation between PtdCho levels and flowering time¹⁰. When the proportion of PtdCho is increased, relative to the phosphoethanolamine (PEA), flowering time is reduced, probably because the FT complex with PtdCho favors the union of FT to the promoter of FT target genes, accelerating flowering. Additionally, both PtdCho and other intermediate metabolites of PtdCho synthesis are essential for proper embryo development¹¹. In maize, lipid profiles can also be used as good predictors of flowering time¹².

In this project we are proposing to systematically study the differences in the metabolic phospholipids pathways of maize plants grown in highland and lowland conditions. We will use high throughput liquid chromatography coupled to mass spectrometry and lipid mass spectra databases to identify a wide range of phospholipids in different plant maize materials including:

- A collection of 60 highland and lowland maize landrace diversity panel grown in common gardens experiments at highland (Metepéc, 2500 m) and lowland (Nayarit, 25 m) - A mapping population composed of 100 Palomero Toluqueño (PT, a Mexican highland landrace) x B73 (sequenced US inbred line) Recombinant Inbred Lines mapping populations grown

in the same locations. - PT and B73 parents and mutants of genes involved in PtdCho biosynthesis grown in growth chamber with a factorial experiment combining low and high temperature and low and high phosphorus concentrations simulating highland and lowland conditions. In this plant material we will also collect tissue to study the expression of genes involved in the PtdCho pathway.

Hypothesis

We hypothesize that phospholipid metabolism reorganization played a key role in the adaptation of maize to the highlands of Mexico.

Relevance to the UC-Mexus program goals.

This proposal is considered as a border between combining genetic approaches using quantitative techniques, molecular biology, population genetics, evolutionary genetics and polar lipid analysis with liquid chromatography techniques coupled to mass spectrometry and use of libraries of mass spectra of lipids. Also, it is necessary to develop controlled experiments, in fields, that match the natural environments in which these adaptation processes originally arose. It should highlight the importance of combining these approaches here in Mexico, which is an advantage over other groups working on similar projects.

As results of this project, understanding the role of phospholipids in response to phosphorus deficiency is key for developing new strategies that may be used to design basic improvement projects of maize races or to specifically incorporate desirable traits in maize hybrids. At present there are few projects studying local adaptation phenomena, this due to researches have focused on specific processes using local adaptation just as a complementary approach to understand other processes. The current basic science project we are working on, is studying some of these issues using as the model of study *Arabidopsis*, nevertheless, maize is the main C4 crop worldwide, reason enough to directly study all these processes in it.

Table 1: My caption

		Environment			
		Phosphorus		Temperature	
Genotype	B73	high	low	high	low
	PT	high	low	high	low

General Aim

The general aim of this proposal is to systematically characterize the changes in phospholipid profiles and expression of genes involved in phospholipid synthesis to shed light on the possible role of phospholipids in Maize highland adaptation.

Specific Aims and relevant methodology

Aim 1. Characterize the profile of phospholipids and other polar lipids, as well as the expression patterns of genes involved in the synthesis of those lipids in B73 and Palomero Toluqueño,

Rationale and design.

In this aim we will get a first characterization of the effect of phosphorus deficiency and cold on the phospholipid profiles and the expression of genes involved in PtdCho synthesis in two well characterized genetic backgrounds: B73 and Palomero Toluqueño (a highland adapted landrace). Plants will be grown in a growth chamber simulating weather conditions of highlands and low valleys, mainly by regulating the chamber's temperature and photoperiod. The growth chamber's conditions for highlands will be 20°C during the daylight and 10°C during the night time. While for low valleys daylight temperature will be 30°C and 20°C during the night. For both experiments the photoperiod will be 14 hours of light and 10 h of darkness. At each of the temperature conditions half of the plants will be grown under low phosphorus and half under control phosphorus concentrations.

Plants will be grown for a period of 2-3 weeks. Samples will be collected at V3 stage from youngest full developed leaf. Maize samples will be extracted according to the method of

Matyash et al.¹³. Phospholipid analysis will be performed at the West Coast Metabolomics Center in UC Davis. Separation and identification of lipids will be accomplished using a method that has been optimized, in the laboratory of Oliver Fiehn, with UPLC-QTOFMS electrospray on modes negative and positive. The method allows the identification of about 500 types of lipids including phospholipids, galactolipids, and sulfolipids. Further identification was complemented using LipidBlast data base¹⁴.

B73xPT F1s and mutants of genes involved in in the phosphatidylcholine biosynthesis pathway.

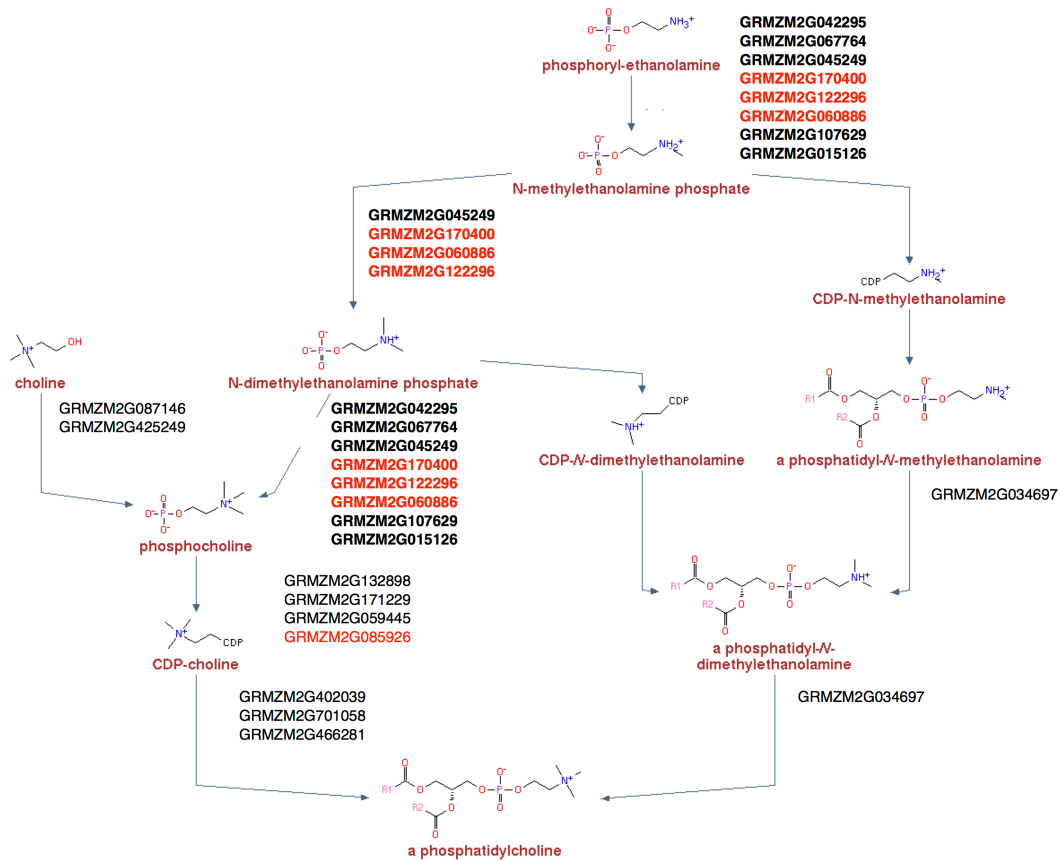


Figure 1 Phosphatidylcholine biosynthesis super pathway indicating the different pathways by which PthDChol can be synthesized in maize. Predicted genes are indicated. (in bold those that can perform different steps of the pathway, in red those for which we are already homozygous single insertional mutants)

2. Characterize the genetic architecture of phospholipid metabolism, in order to identify regions of the genome (QTLs) and/ or genes that may be ruling re-organization of polar lipids in response to conditions of highlands.
3. Study the differentiation signals of genes involved in the synthesis of phospholipids, glycerolipids and sulfolipids between maize races of highlands and lowlands.
4. Analyze the effects of abnormal levels of phosphatidylcholine (PtdCho) on the flowering time, in conditions of highlands and lowlands.
5. Study what is the effect of natural variation in genes involved in the synthesis of phosphatidylcholine (PtdCho), on the expression of lipids and other phenotypes such as flowering time.

Relevant Methodology

2. For this objective, a mapping population, developed by Ruairidh Sawers' Maize Genetics and Genomics laboratory, will be analyzed. This population is composed by 100 RILS (Recombinant Inbred Lines) BC2S4 (Backcross twice with B73 and then selfed during four generations), from a cross of B73 (reference line) with Toluqueño Dovecote (maize breed adapted to highlands). Based on the results obtained in the above objective, samples of plants grown in an experimental field of Valle de Banderas (50 meters above sea level, low valley) and other field in Metepec (2600 m.a.s.l, high valley) will be taken at the same stage of development. Once taken the maize samples, lipid profiles will be analyzed and used for a QTL analysis with R/QTL program [[13]] (<https://paperpile.com/c/X0kwCD/Csme>). The concentrations of each of the lipid types, and the ratios between different environments (Metepec vs Valle de Banderas) are going to be used as the phenotype. A future goal is to do the same analysis using a NAM mapping population (Nested Association Mapping), also created by R. Sawers Laboratory, where 15 races of highlands maize have crossed with CML312 hybrid which will allow to capture a wider range of alleles from a larger number of parents. It is expected to identify genes that fall within identified QTL regions, then, mutants for

the candidate genes will be developed by using insertional mutant lines UniformMu or Ac/Ds. Alternatively, mutants using the CRISPR-CAS9 technology will be developed in collaboration with the laboratory of Luis Herrera-Estrella at LANGEBIO.

3. For this purpose the genomes to be used (with a depth of sequencing 30X) are from 30 races of maize grown in highlands and lowlands, not published yet but available at Jeffrey Ross-Ibarra's laboratory at UC Davis. With these data, F_{st} and P_i values will be calculated to assess the diversity in the sequences of the genes (and their corresponding regulatory elements) involved in the synthesis of phospholipids, also, data will be analyzed to check if there are marks of selection (low diversity) along the races adapted to highlands, which would indicate that these genes have been evolutionarily selected as beneficial for highlands conditions.
4. In order to check the possible effects, under field conditions, of changing levels of phosphatidylcholine (PtdCho)/phosphoethanolamine (PE) in flowering times [8], crosses will be made between UniformMu insertional mutants in XIPOTL genes and RILS of the population B73xPT, only the ones which present extreme flowering times, both in highlands and lowlands conditions. After these crosses are made, they will again grow under the same conditions to evaluate the effects of the mutation in flowering times.
5. For this point, the data obtained at objective 3 will be also used. On one hand, the genes which have been found to present differentiation will be selected and re-sequenced, along the 30 maize races group. Having these data, genetic expression studies will be possible using real-time PCR, in order to verify if differentiation levels correspond with expression levels. The coding regions of genes from highlands and lowlands with higher levels of differentiation will be cloned and expressed in mutants of *Arabidopsis* orthologous genes, this under the control of the endogenous promoter of *Arabidopsis*. Done this, genetic natural variation of lipids and other phenotypes, as flowering time and root development, can be evaluated using a neutral background (*Arabidopsis* mutant) In order to achieve this objective we will maintain collaboration with Dr. Fulgencio Alatorre Laboratory at the Graduate College of Campeche

in Mexico, and also with Dr. Alfredo Cruz Ramírez Laboratory at LANGE BIO in Mexico.

Expected Results

This project is expected to clarify to what extent the phospholipids metabolism is involved in the adaptation of maize to highlands in Mexico. The results of the various goals complement each other, but represent independent units that will lead to individual deliverables.

1. A map of the phospholipids content and expression will be obtained, this along different maize developmental stages.
 2. Genetic architecture of phospholipids synthesis will be obtained through QTLs identification. Within two years, time that is being proposed for this project, may be difficult to map finely potential candidates and test their function, but this can be achieved on possible extensions of the project.
 3. Data on the differentiation of the genes involved in synthesis of phospholipids will be obtained, which may explain adaptation to highlands.
 4. We expect to design mutants with altered phosphatidylcholine levels, using PtdCho mutants, field data can be obtained to explore the effects of the levels of phosphatidylcholine in flowering time and the consequences in terms of adaptation to highlands.
 5. Data generated will help to verify if natural variation in the sequences of the genes involved in phospholipids synthesis have effects on the expression levels, the phospholipids content and other phenotypes, such as flowering time and root development.
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