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* originataed[1]. From that region maize spread throughout Mexico, the rest of the Americas and nowadays it grows on every continent except Antarctica[2]. 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 conditions,[3]. 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 phosphorus[4].

Plants growing in phosphorus limiting soils develop a series of metabolic strategies including recyling 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 chloroplasts[5]. Besides their structural role, some of the most abundant phospholipids such as phosphatidylcholine have interesting signaling functions and are involved in multiple regulatory processes[6]. 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 sulfolipids[7] 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 sulfolipids[5]. 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 expect[8].

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 America[9]. 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 time[10]. 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 development[11]. In maize, lipid profiles can also be used as good predictors of flowering time[12].

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 throughout 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 (Metepec, 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.

The current project brings together a young research group in México (Rubén Rellán-Álvarez) interested in understanding the biochemical basis of maize local adaptation with a well stablished group (Oliver Fiehn) in the Genome Center at UC Davis. Rellán-Álvarez has a proven record on the study of how abiotic stresses shape different plant metabolic pathways and is starting an ambitious research program to understand at the biochemical level how maize has adapted to highland elevations in México. Oliver Fiehn is a world renowed expert in the development and application of metabolomics techniques. His lab has developed analytical methods and data pipelines to identify a great variety of lipids[13]. This project will enable precise, high troughput biochemical analysis in the UC Davis Genome Center of plants grown in different field locations in México. Rubén Rellán-Álvarez and Oliver Fiehn have experience in carrying out this type of collaborative research. During his PhD Rubén Rellán-Álvarez visited the lab of Oliver Fiehn several times to perform similar types of work and perefectioned the logistics of preparing, sending and analyzing the samples in collaboration. Now that Rellán-Álvarez has started his own lab in México this project would provide a fantastic opportunity to establish new collaborations trough common visits and stays of Mexican students that are starting their Masters program in the laboratory of Rellán-Alvarez. In particular, the work of two students, Karla Juarez and Estefany Sánchez

are expected to benefit from this collaboration.

Furthermore, is expected that the techniques developed in the Fiehn lab will be transferred to Langebio´s metabolomics lab.

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 adapatation.

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, design and potential problems

In this aim we will get a first characterization of the effect of phosphorus deficiency and cold 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. So each genotype will be grown under two different phosphorus and temperature conditions. Before this experiment we will optimize lipid extraction procedures to adapt it to maize leaf tissues but once this is optimized we don´t expect too much complications as the rest of the procedures are already

performed regularly in both labs. This experiment will set the basis for future experiments using B73xPT F1s to study Allele Specific Expression (ASE) using targeted RNA-Seq in collaboration with the laboratory of Daniel Runcie at the department of Plant Sciences UC Davis. Samples will also be collected to study methylation patterns of phospholipid pathway genes in collaboration with the laboratory of Luis Herrera-Estrella that have recently found interesting methylation pattens of these genes in Arabidopsis plants under P deficiency[???].

Methodology.

Plants will be grown for a period of 2-3 weeks. Samples will be collected at V3 stage from the youngest full developed leaf. Maize samples will be extracted according to the method of Matyash et al.[14]. 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 base[13]. Samples will also be collected from the opposite side of the leaf midrib for RT-qPCR analysis of the genes involved in phosphatidylcholine biosynthesis pathway See Figure 1.

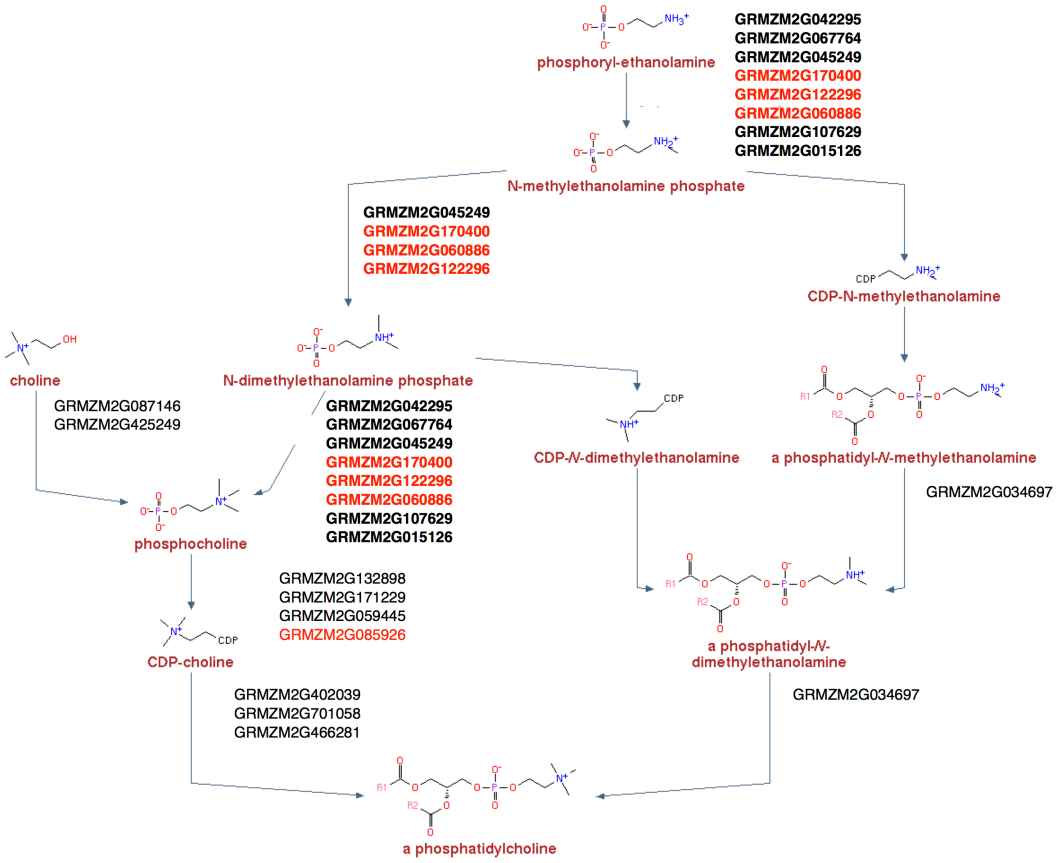

Figure 1 Phosphatidylcholine biosynthesis super pathway indicating the different pathways by which PthDChol can be synthesized in maize. Predicted genes are indicated. (In bold those that can perform different steps of the pathway, in red those for which we are already homozygous single insertional mutants).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

Aim 2. Characterize the natural variation and genetic architecture of phospholipid metabolism and identify regions of the genome (QTLs) controlling the re-organization of polar lipids in response to highland conditions.

Rationale, design and potential problems.

In this aim we will study the natural variation of lipid profiles from a collection of 30

highland and 30 lowland adapted Mexican landraces. This landraces (15 plants each) will be planted in March 2017 in a common garden experiment in both lowland (Nayarit field site) and highland conditions (Metepec field site). Along side these landraces we will grow a collection of BC1S5 100 Recombinant Inbred Lines of B73xPT. Samples will be collected at V3-V5 (we expect different rates of development) from 3-5 plants per landrace and RILS in the field, stored in dry ice and transported to the laboratory in Langebio for extraction. Extracted samples will then be shipped to the UC Davis Genome Center for analysis by the laboratory of Oliver Fiehn.Lipid analysis will be performed as described in the previous aim. Phospholipid profiling data from the landraces will give us a very valuable information about genetic highland/lowland genetic variability in the synthesis of these compounds and on the environmental effect of highland and lowland growth conditions. Given the great genetic diversity of our panel and the fact that previous data point to an important role of phospholipid metabolism since highland conditions imposed a high selective pressure in these pathways we expect to find great biochemical diversity and even new compounds as recently shown in the model plant Arabidopsis[8]. For the RILS we will use a mapping population, developed by Ruairidh Sawers laboratory at Langebio. This population is composed by 100 RILS (Recombinant Inbred Lines) BC1S5 (Backcross with B73 and then selfed during 5 generations), from a cross of B73 (reference line) with Palomero Toloqueño. Lipid profiles will be analyzed and used for a QTL analysis with R/QTL program[15] 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. Once QTLs are identified we will these QTLs to run association mapping on the set of 60 landraces (for which there is already genotypic (GBS) data from Cimmyt Seeds of Discovery project).

The problems that we expect here are related with the nuances of field sampling experiments. However we have already grown and collected samples from both field sites in collaboration with the laboratory of Ruairidh Sawers at Langebio.

Aim 3. Identify th effect of mutations of genes involved in the Phosphatydil-Choline biosynthesis pathway.

Rationale, design and potential problems.

In collaboration with the laboratory of Ruairidh Sawers, the lab of Rubén Rellán-Álvarez is developing different lines carrying insertional mutations in key genes of the Phosphatydil-Choline biosynthesis. We have mutants of different alleles homologues to the Arabidopsis gene xipotl[16], that encodes an enzyme with phospho-ethanolamine N-methyltransferase activity. In Arabidopsis, three different enzymes are needed to complete three consecutive N-methylation steps that are carried out on phospho-bases, phosphoethanolamine, phospho-N-methylethanolamine, and phospho-N-dimethylethanolamine produce PtdCho, but in maize, all three steps can be carried out by at least 4 different xipotl alleles: GRMZM2G045249, GRMZM2G170400, GRMZM2G122296 and GRMZM2G060886. We have UniformMu or Ds insertional mutant stocks for the last three of them and we are in the process of finding homozygous lines for all of them. We also have a UniformMu insertional mutants of GRMZM2G085926 that encodes an enzyme in the Kennedy pathway that synthesizes PtdChol *de novo* from choline. Using the same experimental design as in Aim 1 all these mutants will be grown in high/low temperature and phosphorus conditions. Samples will be taken for phospholipid analysis to study the effect of the mutations in phospholipid profiles. Samples will also be collected for RT-qPCR of the genes involved in the PtdChol super pathway. These data will allow us to study if: - we can modify PtdChol concentrations in maize plants. - compensantion mechanisms between the different branches of the PtdChol pathway exist and what os the importance of each of these pathways. Morphological traits of seedling will also be scored to identify effects in leaf and root development.

In the future this information will be used to design field studies where mutants of these genes will be evaluated in highland and low lowland conditions and directly test the effect of the genes real field conditions. We have already started making crosses between these insertional mutants and selected RILS of the PTxB73 mapping population with extreme flowering times to test if changes in PthChol concentrations can indeed modify flowering

times in field conditions[10].

Crosses and genptyping of all the mutants is well underway. Field experiments won´t be possible within the timeframe of the UC-Mexus project but data generated in the growth chambers will guide theses experiments in the future.

Summary and synthesis

We consider also the possibility of rapid functional change between mexicana and highland maize as a result of the pressures of cultivation, in the absence of recombination. This later hypothesis will be investigated contingent of the availability of mexicana sequence data.

Work Group Contributions

RRA group, will carry out growth chamber and field experiments, perform lipid and DNA isolation and make maize crosses and genotyping to generate homozygous mutants of PtdCho sysnthesis OF group will perform HPLC-MS analysis of samples to generate lipid profile data and train visiting students from RRA lab in data analysis. RRA and OF will work jointly on data analysis of data. RRA group will work closely with the metabolic services at Langebio to implement lipid profile analysis in Langebio.

Timeline.

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