The Genetics of Phospholipid Metabolism In Maize Highland Adaptation

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Background and Introduction

Maize, a C4 tropical grass was domesticated ∼10,000 years ago from lowland teosinte in the warm, humid, low-elevation Mexican southwest [1]. Maize, , spread throughout the entire world including the highlands of Mexico and South America where is still grown at altitudes that can reach 4000 masl. In the highlands, maize was exposed to a whole range of environmental factors that differ from the site of domestication, including, among others, lower temperatures, areas with low phosphorus availability and different biological pressures. We hypothesize that phospholipid metabolism remodeling was important in the process of maize adaptation to highlands. This hypothesis is based on the role of glycerolipids in plant adaptation to low temperatures, low phosphorus, flowering time determination and plant biotic defense.

Common garden experiment.

We grew two mapping populations in two common gardens located at high (Metepec 2600 masl) and low elevations (Valle de Banderas 50 masl). Tissue from this plants was collected and glycerolipids were analyzed using HPLC-QTOFMS. We are able to identify 120 glycerolipid species.

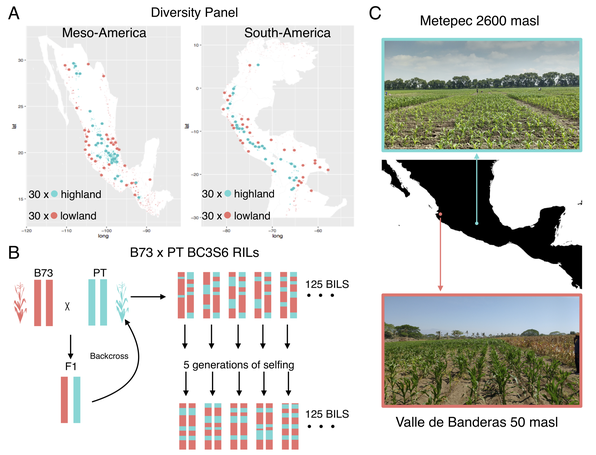

Figure 1: A) Geographic origin of the diversity panel and B) crossing scheme of the B73 x Palomero Toluqueño BIL mapping population. C) Geographic location of the two common garden fields were the two mapping populations were grown

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A major QTL at chr 3 controls PC and LPC levels

We used a B73 x Palomero Toluqueño RIL mapping population to identify QTLs controlling glycerolipid content in plants growing in highland and lowland conditions. Using the sum of lyso-phosphatidylcholines species (LPCs) we found a major QTL peak located at 8.5 Mb of chromosome peak -qLPCs3- (Fig 2A). We also found a major QTL peak -qLPCs3- when we use the sum of phosphatidylcholine species (PCs) (Fig 2B). At the peak of the QTL we identified GRMZM2G353444, a gene with predicted Phospholipase A1 activity. PLA1 enzymes cut fatty acids in the sn-1 position of phospholipds and produce a fatty acid and a lyso-phospholipid. We named the gene *ZmPLA1.2*. Effect size plots in the QTL peaks show opposite behaviours. In the case of LPCs (Fig 2C), RILs that are homozygous B73 at the QTL peak, have higher concentrations of LPCs that RILs that are homozygous PT. In the case of PCs (Fig 2D), homozygous B73 RILs at the QTL peak have lower concentrations of PCs than homozygous PT RILs. Both, qLPCs3 and qPCs3, are environment independent and are found when plants are grown in highland and lowland conditions. Fig 2E shows qLPCs3. The ratio between the two species is highly correlated across the RILs and parents and high ratios are mainly due to low levels of LPCs (Fig 2F)

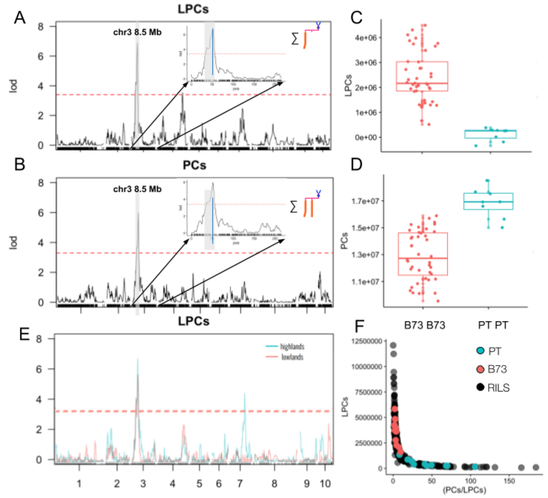

Figure 2: QTL analysis of the sum of lyso-phosphatidylcholine A) and phosphatidylcholine B) species. Insets show a zoom in chromosome 3 with the major QTL peak for both types of species. Lyso-phosphatidylcholine C) and phosphatidylcholine D) effect sizes of RILs homozygous B73 or PT at marker in the QTL peak. E) LPCs QTL analyis of RILs grown in highland and lowland conditions. F) Correlation between LPCs and PCs/LPCs ratio in RILs and parental lines

A second QTL for LPC 18:1 interacts with qLPCs3

Using LPC 18:1 as a phenotype we identified a second QTL at 195.5 Mb in chr 5. A candidate gene, GRMZM2G481755 with predicted Lyso-phosphatidylcholine acyl transferase activity, (the reverse reaction of *ZmPLA1.2*) was identified at the peak of the QTL. We named the gene *ZmLPCAT1*. qLPC18:1(5) is controlled epistatically by qLPC18:1(3). If plants are homozygous B73 at the chr3 locus, LPC18:1 levels are low regardless of the genotype in the chr5 locus.

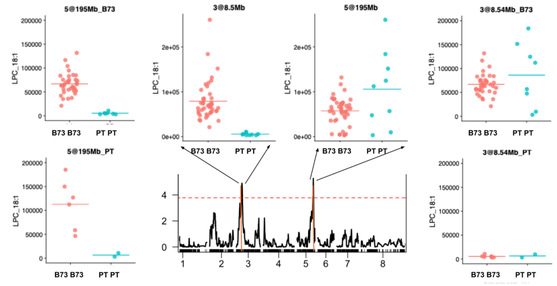

Figure 3: QTL analysis of LPC 18:1. Central figure: two QTLs where found in Chr3 (same as qLPCs). Lateral figures show epistatic interactions between both QTLs. Location of candidate genes in marked with a vertical line

Highland landraces have high PC/LPC ratios

We analyzed glycerolipid data from the landraces of the diversity panel and we observed that high PC/LPC ratios are more prevalent in highland landraces (Fig 4A) and particularly in Mexican landraces Fig 4B-C). This data shows that the high PC/LPC ratio phenotype is not exclusive of PT and is common in Mexican highland landraces to a lesser extent in South American landraces.

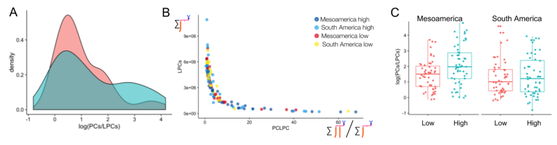

Figure 4: A)Density distribution of PC/LPC ratio of all highland and lowland landraces grown in both experimental fields. B) Correlation of LPCs and PC/LPC ratios in the diversity panel C) Boxplots of log2 PC/LPC ratios. The whole set of landraces was grown side by side the RIL mapping population in the summer of 2016 in our highland site and winter of 2016 of the same year in our lowland site. Samples were fast frozen in the field and analyzed using Ultra Performance Liquid Chromatography coupled with a Quadropole Time of Flight Mass Spectrometer.

Genes determining PC/LPC are under selection

Using genome sequences of 33 landraces from highlands and lowlands across the Americas [2] (Fig 5A) we found that a collection of 215 genes involved in glycerolipid pathways show higher Population Branch Excess values in all 4 highland populations than the average distribution of PBE values (Fig 5B). Genes showing significant PBE values in at least 3 of the 4 highland populations code for enzymes controlling PC/LPC ratio including *ZmPLA1.1* and *ZmLPCAT1* (Fig 5C). In temperate inbreds outlier PBE genes coding for enzymes that convert PCs into LPCs are up-regulated in cold conditions while outlier genes coding for enzymes converting LPCs into PCs are down regulated [3] Fig 5D.

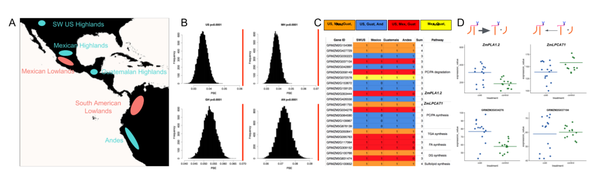

Figure 5: A) 30 WGS of 6 groups were used to conduct PBE analysis. B) Average glycerolipid PBE values (red line) vs average PBE distribution. C) Glycerolipid genes with selection signals in at least 3 highland populations. D) Expression values of genes from (C) coding for enzymes controlling PC/LPC ratios. Data from [3] was obtained from temperate inbreds grown in control and cold conditions

ZmPLA1.1 and *ZmLPCAT1* are outliers in *pcadapt* analy

We then used GBS data from 4000 landrace accessions [4] to run a *pcadapt* [5] analysis that can identify SNPs under selection. PC1 mainly explains elevation adaptation and therefore SNPs with high scores in PC1 should show selection with altitude. We observed both *ZmPLA1.1* and *ZmLPCAT1* are clear outliers in the *pcadapt* PC1 score (Fig6 1A-D). PT alleles of the SNPs falling into the CDS of ZmPLA1.1 are more frequent at high altitude than the B73 allele (Fig 6E-F).

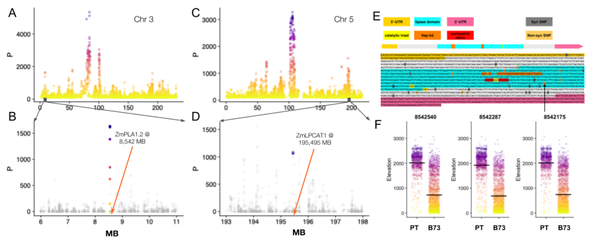

Figure 6: *pcadapt* PC1 scores of *ZmPLA1.1* (A-B) and *ZmLPCAT1* (C-D). SNPs of *ZmPLA1.1* identified using Sanger sequencing of B73xPT RILs (E). Elevation frequency of some of the SNPs shown in B and E using the GBS data of the 4000 geo-referenced landraces