GENETIC AND MORPHOLOGICAL STUDY OF APPLE FRUIT SHAPE

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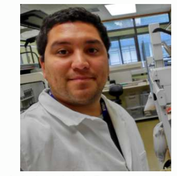

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Introduction

Apple fruit shape is an important quality trait for the producers and consumers. Fruit development from post-anthesis to harvest has been studied in numerous varieties, observing differences in fruit growth in relation with fruit shape. Only few QTLs for fruit shape have been identified so far, while candidate genes and the genetic mechanisms underlying this trait are still unknown. In addition, differential DNA methylation in gene regulatory regions can also be involved. This work aims at understanding the genetic mechanisms controlling apple fruit shape. **We study at histological level** fruit tissues at different development stages, from anthesis to harvest, in three fruit types: flat, round and cylindrical. In addition, **measures of fruit sections** of **274 genotypes** of the high-throughput genotyped apple REFPOP obtained with Tomato Analyzer software are used in a ***GWAS analysis***. This work will provide knowledge about genetic variants related with apple shape.

Materials & Methods

A) Fruit development evolution: Nine selected genotypes of the apple REFPOP (3 flat, 3 round and 3 cylindrical) were analyzed at 10 points throughout their development for height, diameter, fruit shape index, weight. Fruit Shape Index (FSI), measured as the ratio between height and diameter, was used for comparative analysis. **(Figure 1)**

B) Histological Analysis: Three of the genotypes (one representative of each shape) were selected for histological analysis at 0, 61, and 98 days after anthesis. Longitudinal fruit portions embedded in paraffin, cut in slices of 10-18 μm and dyed, were used to measure cell number, area and intercellular spaces (standardizing for 0,5 mm^2 CIA), using a LEICA-DM6 optical microscope and Image J software. **(Figure 2)**

C) GWAS analysis: Fruit sections of 274/534 genotypes from the apple REFPOP collection were secanned and their images processed with Tomato Analyzer software to obtain fruit dimensions as well shape parameters. Phenotypic and genotypic data of 303K SNP were use in a GWAS. **(Figure 3)**

Results

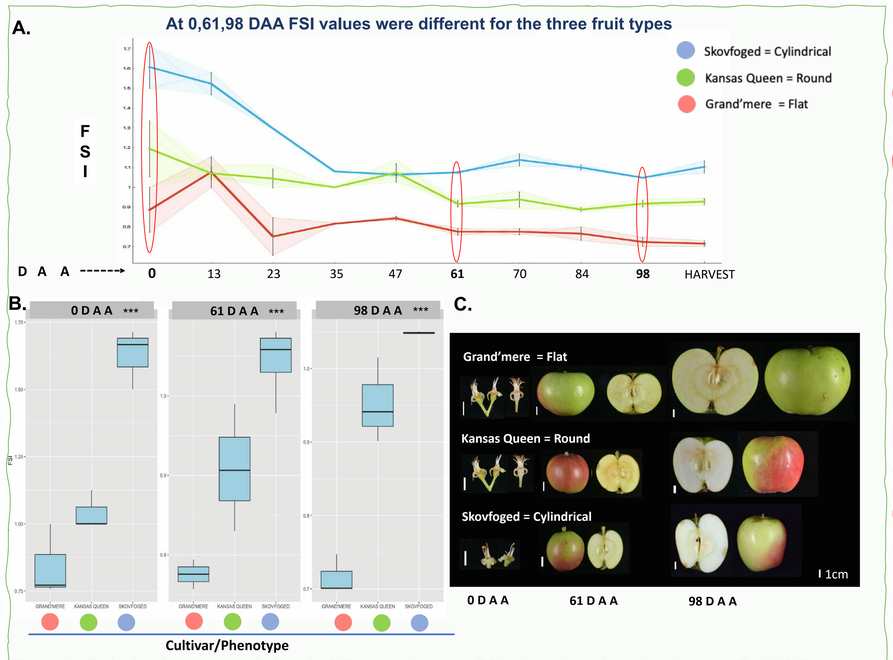


Figura 1. ***Fruit development evolution***, **A.** Differences between FSI values throughout development, **B.** ANOVA test, statistically significant *** (0,001) in the comparative analysis of shapes, **C.** Images at 0, 61 and 98 stages.

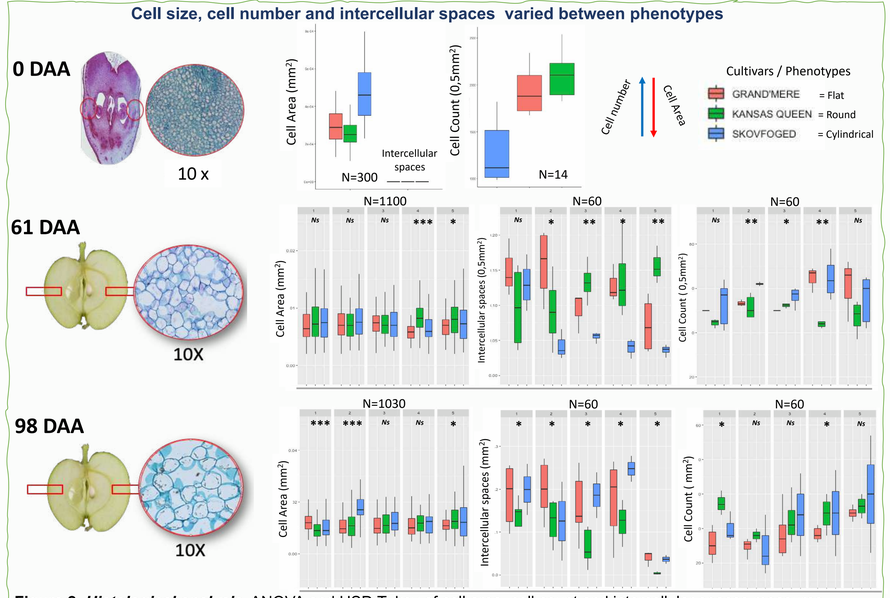
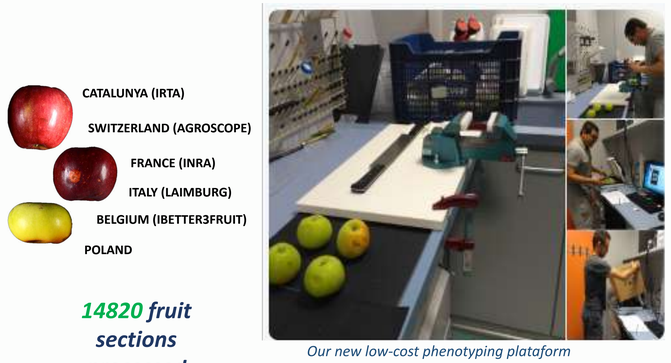
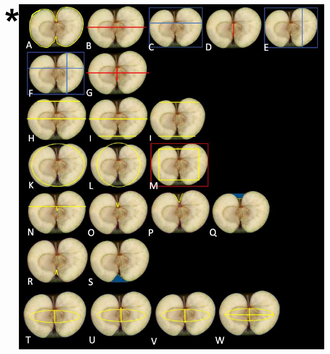


Figura 2. ***Histological analysis***. ANOVA and HSD Tukey of cell area, cell count and intercellular spaces measures analyzed in 0 , 61 and 98 DAA. (***Ns***) *not statistically significative p(0,05*, 0,01**, 0,001**).*

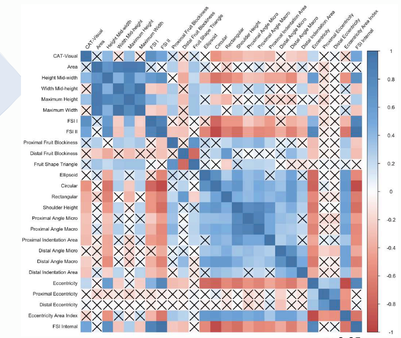
We found two QTNs for Proximal Eccentricity. These results are only for one out of the 23 apple morphological measures. At present, we have other putative QTNs for other measures.

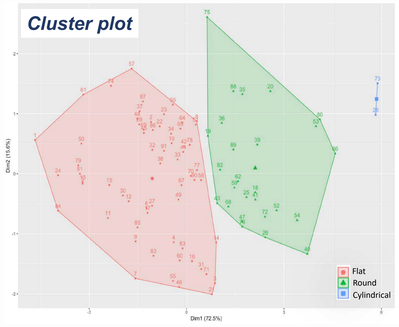
A. Apple REFPOP Collection → ***Phenotyping Plataform*** → ***Measurements***





* Measurements from Tomato Analyzer software, **23 apple morphological measures. Basic measurements: A.** Area, **B.** Widht mid height, **C.** Maximum Width, **D.** Height mid width, **E.** Maximum Height, **Fruit Shape Index: F.** Fruit shape index maximum, **G.** Fruit shape index minimum, **Blockiness: H.** Proximal fruit blockiness, **I.** Distal fruit blockiness, **J.** Fruit shape triangle, **Homogeneity: K.** Ellipsoid, **L.** Circular, **M.** Rectangular, **Proximal Fruit End Shape: N.** Shoulder height, **O.** Proximal angle micro, **P.** Proximal angle macro, **Q.** Proximal indentation area, **Distal Fruit End Shape: R.** Distal angle micro, **S.** Distal Indentation area, **Internal Eccentricity: T.** Eccentricity, **U.** Proximal eccentricity, **V.** Distal eccentricity, **W.** Fruit shape internal.





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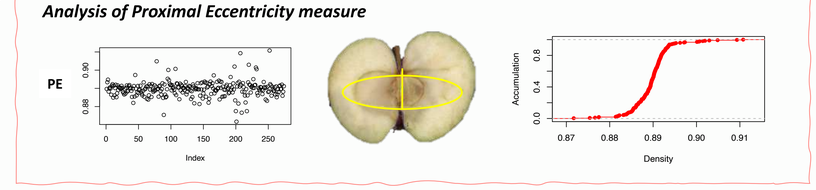
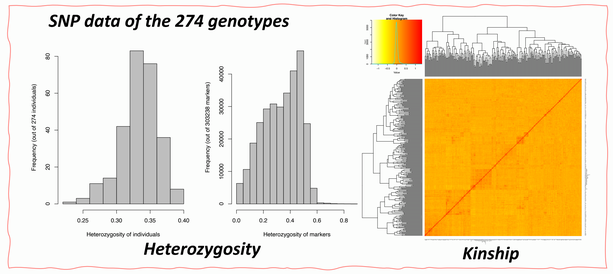
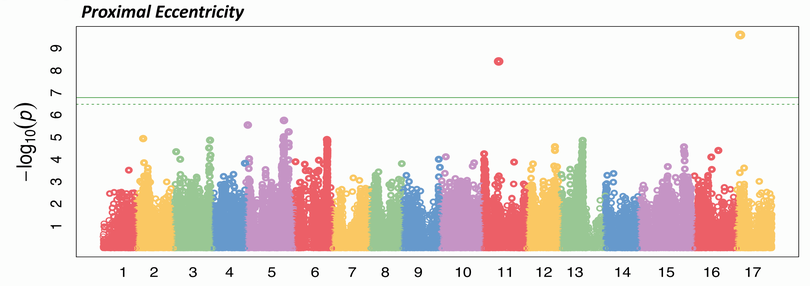




GWAS



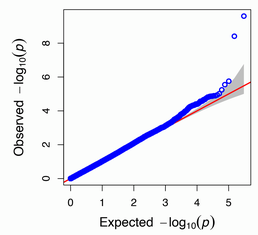


Figura 3. ***GWAS analysis***. **A.** Phenotyping plataform, Measurements, Correlation between variables p>0.05, Cluster plot grouping by shape, **B.** Analysis of the Proximal Eccentricity measure, SNP data of the 274 genotypes (Heterozygosity of individuals and markers, and Kinship), GWAS – Multi-locus Mixed-Model with Bonferroni correction p>0.05, Significant SNP data.

Acknowledgments