

INDEX

INTRODUCTION

1.	The use of <i>Arabidopsis thaliana</i> as a model to study complex biological processes.....	1
2.	Genetic control of flowering	3
3.	The photoperiod pathway.....	5
3.1.	Hormonal control of flowering time	9
3.2.	The age pathway	10
3.3.	The sugar/carbohydrate pathway	11
4.	Omics tools to investigate plant development and signaling	12
4.1.	Metabolomic studies	12
4.2.	Chemical genetics	15
5.	Pipecolic acid as a signaling molecule involved in the control of plant development	19

MATERIAL AND METHODS

1.	Plant material and growth conditions.....	22
1.1.	Arabidopsis growth in the greenhouse or growth chambers.....	25
1.2.	Arabidopsis crosses.....	25
1.3.	<i>In vitro</i> growth conditions.....	26
2.	Bacterial cultures.....	26
2.1.	Long-term preservation of microorganisms.....	27
3.	Molecular Biology Methods	27
3.1.	Bacterial DNA extraction	27
3.2.	Arabidopsis total RNA and genomic DNA extraction	28
3.3.	cDNA synthesis	28
3.4.	Polymerase chain reaction (PCR) and amplicon amplification	28
3.5.	Reverse Transcription-Quantitative Real-Time PCR (RT-qPCR) conditions .	29
3.6.	Analysis of DNA by digestion with restriction enzymes	30
4.	Generation of genetically modified organisms	30
4.1.	Bacterial transformation.....	30
4.2.	Arabidopsis transformation.....	30
5.	Histological sections and RNA <i>in situ</i> hybridization.....	30
6.	Phenotypic analysis of plants.....	32

6.1.	Flowering time evaluation	32
6.2.	Evaluation of the number of fruits and seeds.....	32
6.3.	Estimation of Arabidopsis rosette area	32
6.4.	Estimation of cell size and cell number in Arabidopsis leaves.....	32
7.	Plant treatments	33
7.1.	Dexamethasone treatment.....	33
7.2.	Abscisic acid (ABA) treatments.	33
7.3.	Pipecolic acid treatment.....	33
8.	Chemical genetic screenings	34
8.1.	Conditions and experimental design for the primary chemical genetic screening.	34
8.2.	Conditions and experimental design for the secondary chemical genetic screening.	34
9.	Reporter gene analysis techniques	35
9.1.	β -glucuronidase (GUS) activity assay	35
9.2.	Luciferase (LUC) activity assay	35
10.	Bioinformatic analysis.....	36
10.1.	Sequence analysis.....	36
10.2.	Statistical analysis (Student's t-test/ANOVA).	36
11.	Treatment and sampling for metabolomics, lipidomics, transcriptomics and hormone profiling.....	37
11.1.	Extraction of metabolites and preparation of samples	37
11.2.	Targeted metabolomics by GC-MS	38
11.3.	Targeted and untargeted metabolomics and lipidomics by LC-MS.....	38
11.4.	Hormone quantification	39
11.5.	Transcriptome analysis.....	40
12.	Pathway enrichment analysis	42
12.1.	Pathway enrichment analysis for targeted metabolomics and lipid classification.	42
12.2.	Processing files and pathway enrichment analysis by MetaboAnalyst (untargeted metabolomic data).	42
12.3.	Pathway enrichment analysis by Plant Metabolomic Network (targeted metabolomic, lipidomic and transcriptomic data).	43
13.	Extraction and analysis of sugar content by GC-MS.	43

RESULTS

Chapter 1: The search for new candidates to regulate flowering time by chemical genetics.

1.1. A small library of bioactive molecules to screen for regulators of floral transition.	46
1.1.1. Characterization of the expression of floral marker genes under <i>in vitro</i> culture conditions by RT-qPCR.....	47
1.1.2. Screening for induction of β-glucuronidase expressed under the <i>FT</i> promotor.....	48
1.2. Developing tools for a secondary screening with a luciferase reporter system...	52
1.2.1. Re-testing the molecules selected among the positive hits in the first and second screenings.....	55
1.3. An additional screening based on the <i>LUC</i> reporter gene to analyze the effect of the molecules on <i>FT</i> expression.	57
1.4. Effect of CF5 and CF11 on flowering.....	59

Chapter 2: Effect of Pipecolic acid on plant development.

2.1. Studying the effect of Pipecolic acid on flowering time.	62
2.1.1. Analysis of growth of <i>Arabidopsis</i> under different Pip concentrations <i>in vitro</i> . ..	62
2.1.2. Flowering time in knock-out mutants of genes involved in pipecolic acid biosynthesis.....	63
2.2. Characterization of the effect of <i>ald1-1</i> and <i>sard4-5</i> mutations on the area of the rosette.	65
2.3. Study of the effect of Pip on rosette growth.....	67
2.4. Cellular basis of the larger rosette phenotype of pipecolic biosynthesis mutants.	
70	
2.5. Characterization of additional mutants affecting genes involved in the biosynthesis of Pip.....	71
2.6. Analysis of the effect of Pip on the development in <i>Marchantia polymorpha</i> ..	73

Chapter 3: A multi-omics approach to decipher the metabolic changes during the floral transition in *Arabidopsis*.

3.1. Generation of dexamethasone-inducible transgenic lines expressing CO or FT proteins under the control of phloem specific promotors (pSUC2, pCO or pFT).....	76
3.1.1. Selection of the best inducible system to trigger floral induction.....	78

3.1.2. Characterization of the expression pattern driven by the <i>CO</i> promoter used in the inducible p <i>CO</i> :: <i>CO-GR</i> #9.....	79
3.2. Assessment of experimental conditions to induce floral transition in Col-0 and <i>co-10</i> backgrounds.....	81
3.2.1. Setting up the developmental stage and timing to induce flowering in the CO-GR inducible system.....	81
3.2.2. Molecular characterization of floral induction in the p <i>CO</i> :: <i>CO-GR</i> line #9 at different time points after Dexamethasone and Mock treatments.	83
3.3. Experimental design for the integrated analysis of changes associated with floral transition: differential metabolomics, lipidomics, hormone quantification and transcriptomics analysis in apices and leaves of induced and non-induced p <i>CO</i> :: <i>CO-GR</i> plants.....	85
3.3.1. Identification of the optimal timepoints to perform the multi-omic analysis in this experimental system.....	86
3.3.2. Identification of changes in metabolite abundance associated with floral transition.	88
3.3.2.1. Identification of pathways altered during floral transition based on metabolites detected using a targeted method.	92
3.3.3. Untargeted metabolomic analysis by LC-MS (negative).....	94
3.3.3.1. Identification of pathways altered during floral transition based on metabolites detected using an untargeted method.	97
3.3.4. Identification of changes in lipids associated with floral transition.....	100
3.3.4.1. Identification of lipid pathways altered during floral transition.....	102
3.3.5. Characterization of hormone profiles (IAA, jasmonic acid, salicylic acid and abscisic acid) by LC-MS.....	103
3.3.6. Changes in the transcriptome associated with the floral transition by RNA-seq analysis.	105
3.4. Identification of perturbed pathways by integration of transcriptomic data with metabolomic and lipidomic data.....	107
3.5. Selection and phenotyping of loss-of-function mutants of the main identified pathways.....	109
3.5.1. ABA degradation.	115
3.5.2. RFOs Biosynthesis and degradation of raffinose oligosaccharides.	118
3.6. Characterization of loss-of-function and early flowering phenotype of the rs5-2 mutant.....	122
3.6.1. Validation of expression changes in <i>GOLS</i> and <i>RS</i> genes during floral transition.	122
3.6.2. Assessment of the effect of exogenous addition of raffinose biosynthesis-related metabolites on flowering time in Col-0 plants.....	123
3.6.3. Molecular characterization of rs5-2.	124

3.6.4.	Characterization of the <i>RS5</i> expression pattern during floral transition. ...	127
3.6.5.	Molecular characterization of floral marker genes in <i>rs5-2</i> mutant by RT qPCR.....	129
3.6.6.	Endogenous sugars quantification in <i>rs5-2</i> mutant by GC-MS.	131
3.6.7.	Study of the circadian clock in <i>rs5-2</i> mutant.	132

DISCUSSION

The identification of small molecules (CF5 and CF11) as regulators of flowering time signals by a chemical genetic screening.	137
Three potential novel functions described for pipecolic acid in <i>Arabidopsis</i> beyond SAR: regulation of flowering time, rosette area and cell cycle.....	139
The floral transition metabolome showed significant changes in the abundance of metabolites in the apex but not in the leaf.	141

CONCLUSIONS

First:.....	151
Second:	151
Third:	151
Fourth:	151
Fifth:	151

BIBLIOGRAPHY.....153

GLOSSARY OF ABBREVIATURES.....200

OTHERS INDICES

1. FIGURES INDEX.....	208
2. TABLES INDEX.....	212