

## INDEX

<b>ABSTRACT</b>	ii
<b>RESUMEN</b>	iv
<b>RESUM</b>	vi
<b>INTRODUCTION</b>	1
The ABA signaling pathway.	2
PYR/PYL/RCAR ABA receptors.	3
The ABA Core Signaling Pathway.	5
Clade A protein phosphatases type 2C.	7
SNF1-related protein kinases 2.	9
Transcription factors involved in ABA response.	11
Regulation of ABA core components by post-transcriptional mechanisms and interacting proteins.	14
The ubiquitin system.	20
E3 ubiquitin ligases.	28
Monomeric E3 ligases.	28
RING DOMAIN LIGASE (RGLG): RING-type E3 ubiquitin ligase family.	33
Multimeric E3 ligases.	38
BTB/POZ-MATH (BPM): substrate adaptors of CRL3BTB ubiquitin ligase.	50
<b>OBJECTIVES</b>	55
<b>RESULTS I: BPMs</b>	59
BPM3 and BPM5 interact with clade A PP2Cs.	60
BPM3 and BPM5 promote degradation of clade A PP2Cs in vivo.	73
BPM3 and BPM5 gain-of-function leads to enhanced sensitivity to ABA.	83
bpm3 bpm5 loss-of-function shows reduced sensitivity to ABA.	88
BPM3 and BPM5 are required for in vivo ubiquitination of PP2CA and HAB1.	96
<b>RESULTS II: RGLG1</b>	101
RGLG1 localizes at the plasma membrane under non-stress conditions.	102
RGLG1 is myristoylated under non-stress conditions.	106
RGLG1 shuttles to the nucleus after ABA and salt stress treatment.	109
RGLG1 interacts with PP2CA and ABA receptors in the nucleus.	117
<b>DISCUSSION</b>	125
<b>CONCLUSIONS</b>	137
<b>MATERIALS AND METHODS</b>	141
<b>BIOLOGICAL MATERIALS</b>	142
Bacterial Strains	142
Yeast Strain	142
Plant Material	142
Growing Conditions and Transformation	144
Bacterial Culture	144
Bacterial Transformation	144
Yeast Culture	145
Yeast Co-Transformation	146
Arabidopsis thaliana	147
In Vitro Tissue Culture	147
Plant Treatments	147
Greenhouse Culture	148

Generation of Mutants	148
Physiological Assays	149
Seed Germination and Seedling Establishment Assays	149
Root Growth Assays	149
Water Loss and Drought Stress Experiments	150
Infrared Thermography	150
Gas Exchange Experiments	151
Nucleic Acids Extraction and Analysis Methods	152
DNA Extraction	152
Gene Expression Analysis by PCR Reaction	153
RNA extraction	155
RT-qPCR	155
Generation of new constructs	156
GATEWAY™ Cloning	156
Constructions for Yeast-Two Hybrid	157
Constructions for Plant Transformation	158
Constructions for Bi-molecular Fluorescence Complementation (BiFC) and Multicolour BiFC	158
Constructions for Split-Luciferase	159
Constructions used for Protoplast Transformation	160
Constructions for Protein Purification in <i>E. coli</i>	161
Protein Technology	161
His-tag Proteins Culture and Purification	161
GST-tag Proteins Culture and Purification	162
Coomassie Staining	163
Transient Protein Expression in <i>N. benthamiana</i>	163
Protein Expression in Protoplasts	164
Protein Extraction from Plant Material	164
SDS-PAGE Electrophoresis	165
Western Blot Analyses	165
Antibodies	166
Pull-down Assays	167
Coimmunoprecipitation (coIP)	168
Split-luciferase (LUC) Complementation Assay	168
In vivo Protein Degradation Assays	169
Protein Stability Kinetics	170
In vitro Ubiquitination Assay	170
In vivo Ubiquitination Assay of PP2CA-GFP in <i>N. benthamiana</i>	171
Affinity Purification of Ubiquitinated Proteins Using p62-agarose	172
In vivo Myristoylation Assay	172
Yeast Two-Hybrid (Y2H) Assays	146
Confocal Laser Scanning Microscopy	173
Nuclei Staining and Counting	173
Mass Spectrometry Analysis	174
Statistics	175
<b>REFERENCES</b>	177
<b>ABBREVIATIONS</b>	211
<b>APPENDIX</b>	221