

Characterization of a mitochondrial PPR protein in *Arabidopsis thaliana*

Dissertation

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Hossein Emami

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First examiner: Prof. Dr. Frank Kempken

Second examiner: Prof. Dr. Dietrich Ober

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To My Mother, To My Father

To my love

Table of contents

1. Introduction	1
1.1. Mitochondria in plants	1
1.1.1. The endosymbiotic origin of mitochondria	1
1.1.2. The plant mitochondrial genome	1
1.1.3. Mitochondrial gene expression in plants.....	2
1.2. Pentatricopeptide repeat (PPR) proteins	5
1.3. Flowering in land plants	9
1.3.1. Pathways controlling flowering time in <i>Arabidopsis thaliana</i>	10
1.3.2. Regulation of flowering through <i>FLC</i>	13
1.4. Influence of mitochondria on flowering	15
1.5. Mitochondrial contribution to the stress response	16
1.6. Aim of the study	19
2. Material and methods	21
2.1. General molecular biology methods	21
2.1.1. General solutions and buffers.....	21
2.1.2. Media	21
2.1.3. Devices, chemicals and Enzymes	22
2.1.4. Bacterial strains and cultivation	22
2.1.5. Isolation of plasmid-DNA from liquid culture of <i>E. coli</i> cells.....	23
2.1.6. DNA elution from an agarose gel	23
2.1.7. DNA hydrolysis by endonuclease restriction enzymes.....	23
2.1.8. DNA ligation	24
2.1.9. DNA gel-electrophoresis	24
2.1.10. Transformation of <i>E. coli</i>	24
2.1.11. Transformation of <i>A. tumefaciens</i>	24
2.1.12. Polymerase chain reaction (PCR)	25
2.1.13. Reverse transcriptase-polymerase chain reaction (RT-PCR)	26
2.1.14. Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR).....	26
2.1.15. RNA editing analysis using RT-PCR	27
2.1.16. Sequencing of DNA fragments	28
2.1.17. Quantification of the nucleic acids	28
2.1.18. Control of RNA quality	28
2.2. Protein analysis	28
2.2.1. Determination of protein concentrations	28
2.2.2. GFP pull-down assay	29

2.2.3.	SDS polyacrylamide gel electrophoresis (SDS-PAGE).....	30
2.2.4.	Staining of proteins in the SDS gel by Coomassie Brilliant-Blue.....	30
2.2.5.	Protein identification by LC-MS	31
2.3.	Experiments with <i>Arabidopsis</i>	31
2.3.1.	Culturing of <i>Arabidopsis thaliana</i>	31
2.3.2.	Flowering time measurements	31
2.3.3.	Stable transformation of <i>A. thaliana</i>	32
2.3.4.	<i>Arabidopsis</i> root culture (ARC).....	32
2.3.5.	Isolation of RNA from <i>A. thaliana</i>	33
2.3.6.	Detecting superoxide radicals in leaves	33
2.3.7.	Measurement of respiration.....	34
2.3.8.	ATP level measurement	34
2.3.9.	Isolation of Mitochondria from ARC.....	34
2.3.10.	Isolation of protein from mitochondria.....	35
2.4.	Studying the transcriptome with RNA sequencing	36
2.4.1.	Analysis of RNA-Sequencing data.....	36
2.4.2.	RNA editing analysis from RNA-seq data	37
2.5.	Data banks and software	37
3.	Results	38
3.1.	<i>poco1</i> displays an early-flowering phenotype under long- and short-day photoperiods	39
3.2.	Complementation of <i>poco1</i> restored the normal flowering time	40
3.3.	Generating a POCO1:GFP transgenic line	43
3.4.	Identification of putative <i>in vivo</i> interaction partners of POCO1	45
3.5.	POCO1 affects the editing efficiency of <i>rps3</i>	46
3.6.	POCO1 affects several mitochondrial editing sites	47
3.7.	Analysis of the expression of flowering marker genes	49
3.8.	Analyzing the expression level of the <i>FLC</i> promoting genes	50
3.9.	<i>poco1</i> plants show altered sensitivity to ABA	52
3.10.	<i>poco1</i> plants show a higher sensitivity to drought stress	54
3.11.	<i>poco1</i> plants show an altered mitochondrial respiratory activity	54
3.12.	Studying the gene expression by RNA sequencing	56
3.12.1.	Analysis of differentially expressed genes.....	57
3.12.2.	Identification of genes associated with flowering	61
3.12.3.	<i>poco1</i> inactivates ABA signaling and response	63
3.12.4.	Identification of genes associated with drought and oxidative stresses	65
3.12.5.	Altered expression of genes associated with cellular regulation in <i>poco1</i>	66
3.12.6.	<i>poco1</i> affects the expression of genes associated with cellular redox state....	68

3.12.7.	<i>poco1</i> altered expression of genes associated with Stomatal function	69
3.12.8.	Altered expression of marker genes associated with the mitochondrial perturbation	70
3.12.9.	Analysis of the mitochondrial gene expression by RNA-seq	71
4.	Discussion	72
4.1.	Flowering time is promoted by <i>POCO1</i> mutation	72
4.2.	<i>FLC</i> and its positive regulator, <i>ABI5</i>, are down-regulated in <i>poco1</i>	73
4.3.	<i>poco1</i> plants show altered sensitivity to ABA and drought stress	75
4.4.	<i>POCO1</i> is required for the mitochondrial RNA editing	76
4.5.	Mitochondrial respiratory activity is disturbed in <i>poco1</i> plants	79
4.6.	Putative interaction partners of <i>POCO1</i>	81
4.7.	Differential gene expression analysis by RNA-seq	82
4.7.1.	Effect on genes involved in flowering	84
4.7.2.	ABA signaling and response is impaired in <i>poco1</i>	85
4.7.3.	Effects on drought and oxidative stresses-associated genes	89
4.7.4.	Cellular regulation genes are affected by <i>poco1</i>	92
4.7.5.	Altered expression of genes associated with the mitochondrial perturbation ..	95
4.7.6.	Cellular redox genes are differentially regulated in <i>poco1</i>	98
5.	Summary	101
6.	Zusammenfassung	103
7.	Abbreviations	105
8.	Bibliography	108
9.	Appendix	136
10.	Acknowledgements	165

1. Introduction

1.1. Mitochondria in plants

1.1.1. The endosymbiotic origin of mitochondria

The last eukaryotic common ancestor (LECA) incorporated a 'universal' facultative anaerobic mitochondrion of alphaproteobacterial origin. Other types of mitochondria-related organelles such as aerobic, facultatively anaerobic, anaerobic, hydrogenosomes and mitosomes evolved later on independently from the LECA and exist in different taxa of all eukaryotic supergroups. Based on the endosymbiotic theory, the physiological and biochemical similarities of mitochondria to free-living prokaryotes point to their single prokaryotic origin (Zimorski et al. 2014).

The evolution from an endosymbiotic bacterium to a permanent organelle involved a variety of huge evolutionary alterations, including the origins of new genes and a protein import system, insertion of membrane transporters, integration of metabolism and reproduction, genome reduction, endosymbiotic gene transfer, lateral gene transfer and the retargeting of proteins (Roger et al. 2017). Although the majority of the genetic information of the organelle has been transferred to the nuclear genome, the core set of genes for the oxidative phosphorylation has remained in mitochondria (Liberatore et al. 2016).

1.1.2. The plant mitochondrial genome

Due to constant DNA recombination and subsequent rearrangement, plant mitochondrial genome exists in a complex physical form, which leads to the variation in the gene order (Hazle and Bonen 2007). During the symbiont evolution, a high portion of the genetic information of mitochondria was transferred to the nucleus. The mitochondrial genome in angiosperms underwent gene loss processes and a simultaneous intron acquisition by horizontal gene transfer (Palmer et al. 2000). Whereas most animals possess a mitochondrial genome size of about 15-16 kb, higher plant mitochondria have a rather larger genome size, for instance, in *Arabidopsis*, it is about 366 kb (Figure 1). The increase in size is mostly due to the existence of repetitive regions, AT-rich non-coding regions, large introns and non-coding sequences (Gualberto et al. 2014). This leads to the presence of a different number of molecular products in mitochondria (Unsold et al. 1997). The plant mitochondrial genome of the present-day contains 57 genes, representing only a small number of their ancestral genes encoding for components of complexes I to V, cytochrome c biogenesis, ribosomal RNAs, ribosomal

proteins, transfer RNAs and intronic ORFs (Unsold et al. 1997). Expression and regulation of these genes in mitochondria highly rely on various post-transcriptional processes and require both mitochondrial and nucleus-encoded proteins. Therefore, the gene expression in both genomes need to be properly coordinated (Hammani and Giegé 2014). Nucleus-encoded proteins can post-translationally be imported to the mitochondria via a transitpeptide sequence and are present in the majority of the mitochondrial proteomes.

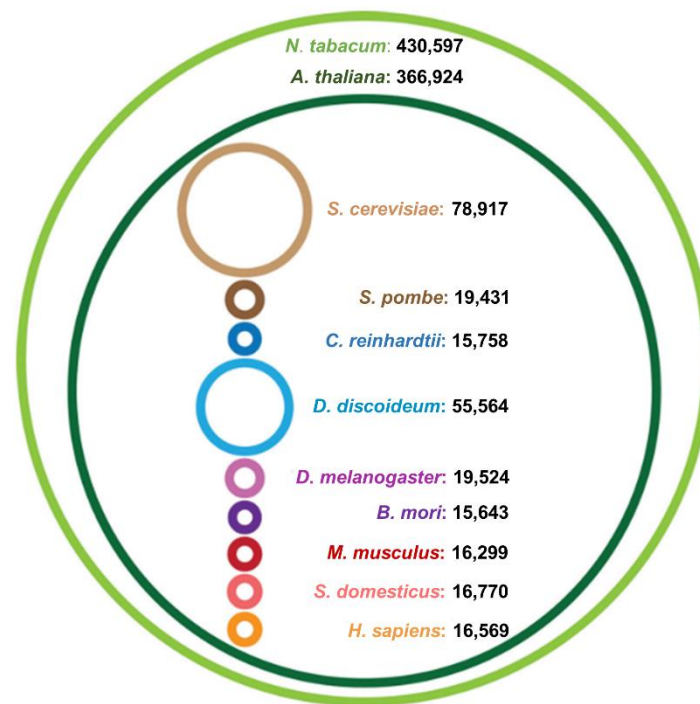


Figure 1: Scale representation of mitochondrial genome size in different organisms. The mitochondrial genome in the plant lineage is remarkably bigger compared to other organisms from different phyla. Figure from Morley and Nielsen 2017.

1.1.3. Mitochondrial gene expression in plants

Gene expression in plant mitochondria involves several transcriptional and post-transcriptional processes including RNA transcription, RNA processing and translation. In plant mitochondria, post-transcriptional RNA metabolism plays a vital role in gene product abundance and functionality. Therefore, loss of these processes often leads to an extreme effect on plant growth, development and fertility (Binder and Brennicke 2003; Hammani and Giegé 2014).

Plant mitochondrial genes are transcribed by nucleus-encoded phage-type RNA polymerases (NEPs). Three types of NEPs exist in flowering plants, RpoTm, RpoTmp and RpoTp. RpoTm is localized to mitochondria, RpoTmp is localized to mitochondria and chloroplasts and RpoTp is localized to chloroplasts. The most essential NEP in the mitochondrial transcription is RpoTm (Hammani and Giegé 2014). Although RpoTmp is dual targeted to mitochondria and chloroplasts, its function is more critical in mitochondria as only *rpotmp* mutant plants

expressing *RpoTmp* with mitochondrial targeting could restore the wild-type phenotype and mitochondrial transcript level. However, the activity of RPOTmp in *rpotp* mutants was not sufficient to restore the phenotype of these mutants (Courtois et al. 2007; Millar et al. 2008; Tarasenko et al. 2016).

In plant mitochondria, several post-transcriptional processes are required to obtain functional mRNAs, rRNAs and tRNAs. These maturation processes in plant mitochondria consist of splicing of introns, 5' and 3' end maturation as well as cytidines (C) to uridines (U) RNA editing.

Two classes of introns, group I and group II introns exist in organelles. Introns in mitochondrial genes of land plants are mostly represented by group II introns. The splicing mechanism of the group II introns is mediated via two transstratification reactions and the release of the intron as a lariat (Michel and Ferat 1995). The *Arabidopsis* mitochondrial genes are interrupted by 23 introns. Some genes such as *NAD7*, are interrupted by more than one intron (Giege and Brennicke 2001). The splicing factors in the plant mitochondria include MatR (maturase), putative DEAD-box proteins, WHAT'S THIS FACTOR? 9 (WTF9), RCC1/UVR8/GEF-LIKE 3 (RUG3), a member of the regulator of chromosome condensation 1 (RCC1) protein family, MITOCHONDRIAL CAF-LIKE SPLICING FACTOR 1 (mCSF1), plant organelle RNA recognition (PORR) domain proteins, pentatricopeptide repeat (PPR) proteins and the chloroplast RNA splicing and ribosome maturation (CRM) domain proteins. The last three factors have RNA-binding activity (Barkan et al. 2007; Hammani and Giegé 2014).

After termination of transcription in the plant mitochondria, many transcripts accumulate, of which the majority are rapidly degraded (Holec et al. 2006). Therefore, the desirable transcripts have to be saved by 5' and 3' end maturation, which can be achieved by the exonucleolytic or endonucleolytic activity. 5' ends in plant mitochondria can be generated by de novo transcription or by post-transcriptional processing. Due to the absence of experimental data regarding exonucleolytic activity, it is assumed that the 5' end processing in plant mitochondria is generated by a kind of endonucleolytic activity. 3' ends often possess inverted sequences. In bacteria, these elements act as independent terminators. Two main exoribonucleases are the major factors in 3' end maturation in plant mitochondria: POLYNUCLEOTIDE PHOSPHORYLASE (PNPase) and RIBONUCLEASE II (RNase II). The unravelled details suggest that the PNPase would first eliminate the extensions of precursor transcripts and then RNase II would subsequently trim short nucleotide extensions to produce mature 3' ends. RNA secondary structures (RNA stem-loop) and pentatricopeptide repeat (PPR) proteins are other factors involved in defining the transcript end (Cohen 1995; Giegé et al. 2000; Hammani and Giegé 2014). Furthermore, for functional translation 5' and 3' ends of tRNAs need to be matured. This requires two endonucleases, which are also involved in mRNA processing:

RNase Z and RNase P (Barbezier et al. 2009; Gutmann et al. 2012). The mature 5' end of 18S and 5S rRNAs in plant mitochondria are generated by endonucleolytic cleavage (Binder et al. 2011).

Organelles of land plants undergo a number of nucleotide changes that differentiate the RNA sequence from the genome. Mostly mRNAs and tRNAs are subjected to RNA editing, rRNAs are rarely affected. The majority of this phenomenon is seen for coding sequences of mRNAs rather than UTRs or introns. In angiosperms, RNA editing changes hundreds of specific Cs to Us, from which 400-600 editing events occur in mitochondria and 30-40 editing events occur in the plastid. In *Arabidopsis*, 34 in plastids and more than 500 in mitochondria C-to-U conversions were observed (Giegé and Brennicke 1999; Chateigner-Boutin and Small 2007). A different type of RNA editing, the U to C conversion does not exist in flowering plants, but in ferns, hornworts and lycopods. (Takenaka et al. 2013b; Ichinose and Sugita 2017). RNA editing is considered to be a correction mechanism of mutations to restore conserved codons or create start or stop codons. Despite the discovery of RNA editing and editing sites, its complete mechanism has not yet been understood. The plant-specific editing factors (the editosome) is a hypothetical protein complex that associates with mitochondrial or plastid RNA and consists of: RNA editing factor interacting proteins (RIPs) or multiple organellar RNA editing factors (MORFs), which are essential for the efficient editing process (Bentolila et al. 2012), organelle RNA recognition motif (ORRM) proteins that interact with RIP/MORF to form homo- or heterodimers (Shi et al. 2016), organelle zinc-finger (OZ) proteins, that play essential role in RNA editing as Zn²⁺ ions are highly required in the active centre of cytidine deaminase (Vasudevan et al. 2013), PROTOPORPHYRINOGEN OXIDASE 1 (PPO1), which is essential for the RNA editing efficiency in the plastids (Zhang et al. 2014) and PPR proteins, that specially recognize *cis*-elements about 30 nucleotides upstream of the editing sites in a combinatorial pattern (Barkan et al. 2012; Takenaka et al. 2013a; Sun et al. 2016). However, the specific enzyme catalyzing the editing reaction by presumably a deamination reaction has not been identified yet. PPR proteins of the PLS-type are involved in the recognition of *cis*-elements as *trans*-factors (Lurin et al. 2004) and are seen as a candidate to perform the deamination reaction due to the conserved cytidine deaminases motif in their C-terminus (DYW-domain) (Salone et al. 2007; Boussardon et al. 2012). Furthermore, the E-domain of PPR proteins has been shown to be essential for the RNA editing, since it is required for protein-protein interaction or RNA binding activity (Okuda et al. 2007; Wagoner et al. 2015). However, based on another model proposed, PPR proteins recognize and bind in vicinity of RNA editing sites and recruit other factors essential for enzymatic activity by protein-protein interaction (Kotera et al. 2005; Okuda et al. 2007). C-to-U editing has been revealed to vary in different plant tissues (Howad and Kempken 1997; Peeters and Hanson 2002), different environmental conditions (Karcher and Bock 2002) and between ecotypes within the same

species (Bentolila et al. 2005; Zehrmann et al. 2008). Therefore, C-to-U RNA editing in plant mitochondria seems to have several different functions in modulation of gene expression and generating protein diversity, which subsequently affects plant development, cell signaling and stress tolerance regulation (Tang and Luo 2018). An interesting feature of RNA editing is that not all the transcripts for a particular gene are edited at the same rate. Various developmental stages, plant tissue type and growth conditions influence the extent of editing (Grosskopf and Mulligan 1996).

In plant mitochondria, a steady-state level of functional transcripts exists. The rate of degradation varies upon different factors, among which the polyadenylation at the transcript's 3' end or in their vicinity is the general parameter. Other parameters like presence or absence of specific secondary structures, processing steps and RNA-binding factors might influence the stability of the transcripts in plant mitochondria (Giegé et al. 2000). In the plant mitochondria, PNPase is the major component of the degradation machinery and its down-regulation leads to the inhibition of polyadenylation-related RNA degradation (Binder et al. 2011).

On the base of genetic observations, it is believed that translation in plant mitochondria is performed by a completely different mechanism compared to that in bacteria. Firstly, the mitochondrial ribosomes possess different subunit (26S and 18S) (Bonen and Calixte 2006). Also, the conserved sequences like the bacterial-type *Shine-Dalgarno* sequence, which facilitate ribosomes to bind the mRNA, are absent in most mitochondrial genes (Hazle and Bonen 2007). Furthermore, mitochondrial transcripts missing the stop codon are correctly translated (Raczynska et al. 2006). These unique features highlight the participation of *trans*-factors to the translation process in mitochondria. For instance, maize MPPR6 facilitates the translation of mitochondrial *ribosomal protein s3 (rps3)* by binding to the 5' untranslated region (Manavski et al. 2012). The dual-localized PPR protein *PNM1* may be involved in the translation, as it is associated with the mitochondrial polysomes (Hammani et al. 2011).

1.2. Pentatricopeptide repeat (PPR) proteins

Early identification of PPR proteins was pioneered by Small and Peters 2000 and Aubourg et al. 2000 simultaneously. PPR proteins harbour repeated 35 amino acid sequences, which are arranged in tandem motifs with an average of 12 repeats per protein in plants (Small and Peters 2000; Lurin et al. 2004). PPR proteins belong to the alpha-solenoid superfamily, which comprises degenerate repeats of 30-40 amino acids, each of which forms two or three alpha-helices. The other members of the alpha-solenoid superfamily are transcription-activator-like effectors (TALEs) and pumilio and FBF homology (PUF) proteins, which bind to DNA and RNA

sequences respectively (Lu et al. 2009; Moscou and Bogdanove 2009).

The majority of the ancient PPR genes in the context of evolution harbour several introns while the PPR genes diverged later in the plant lineage are intron-less. The evidence has been reported from the comparison of the genes encoding PPR proteins in flowering plants; *Arabidopsis* and *Oryza*, in which a large proportion of genes encoding PPR proteins are intron-less. On the other hand, the moss *physcomitrella patens* that has diverged earlier in the plant lineage contains many intron-rich PPR genes (Lurin et al. 2004; O'Toole et al. 2008). The mechanism proposed for the expansion of the PPR genes is retrotransposition, in which the PPR genes were reverse transcribed and integrated into the genome (O'Toole et al. 2008). Another scenario, which has been discussed regarding the expansion of PPR proteins is genome duplication, which led to the multiplication of the conserved loci as paralogous copies (Geddy and Brown 2007). The PPR protein family is specific to eukaryotes and particularly widespread in land plants (Andrés et al. 2007). There are rare examples of the presence of PPR proteins in prokaryotes, but their function remains to be elucidated (Manna 2015).

Many of the PPR proteins are targeted to mitochondria and chloroplasts through signal sequences that reside in the N-terminus part of the protein, so-called transit peptide sequences. PPR proteins in land plants are classified into two subfamilies, the P and PLS subfamilies on the base of their domain structure (Figure 2). P-type composed of the classical 35 amino acid motif and the majority of them lack additional domains. Only a few numbers of P-type PPR proteins contain an additional domain; a small MutS-related (SMR) domain. Generally, this domain confers DNA and RNA endonuclease activity but their specific function in PPR proteins has not yet been identified (Fukui and Kuramitsu 2011; Liu et al. 2013). On the other hand, PLS-type PPR proteins have three different motifs varying in length, which are unique to this class; P (35 amino acids), L (long, 35-36 amino acids) and S (short, ~31 amino acids). As they are often arranged in P-L-S order, they got the PLS name (Lurin et al. 2004). The PLS-type PPR proteins are plant-specific and contain additional C-terminal domains; E/E⁺ and/or DYW domains. An E motif accompanies the final PPR motif, an E⁺ motif always accompanies an E motif and a DYW motif always accompanies an E⁺ motif (Andrés et al. 2007; Manna 2015). Although E/E⁺ domains are characterized by their degeneracy, the DYW domain possesses a highly conserved tripeptide region with the amino acids aspartate-tyrosine-tryptophan (Aubourg et al. 2000; Lurin et al. 2004). The DYW domain has long been proposed to have a catalytical activity as it has the same catalytic residues as previously identified cytidine deaminase enzymes, necessary for RNA editing (Salone et al. 2007). In a recent study it was shown that a PPR protein from *Physcomitrella patens* with a DYW domain is sufficient to perform RNA editing *in vitro* and it was concluded that the DYW-deaminase domain is the sole catalytic component of the editosome (Hayes and Santibanez 2020).

PPR proteins are required for diverse post-transcriptional processes in DNA-containing organelles to modulate gene expression (Schmitz-Linneweber and Small 2008). Members of the P-type have been implicated in RNA stabilization, processing, splicing, and translation, while PLS-type PPR proteins typically are involved in RNA editing (Andrés et al. 2007; Barkan et al. 2012). However, P-type PPR proteins involved in RNA editing events have been previously reported (Doniwa et al. 2010; Leu et al. 2016; Guillaumot et al. 2017).

PPR tracts bind to a specific sequence of RNA bases on a one repeat one nucleotide modular mode, in which two amino acids in each repeat are involved (Figure 2). These two amino acids are at the position of 6 and 1' of the next repeat (position 1') on the base of the nomenclature introduced by Lurin et al. 2004.

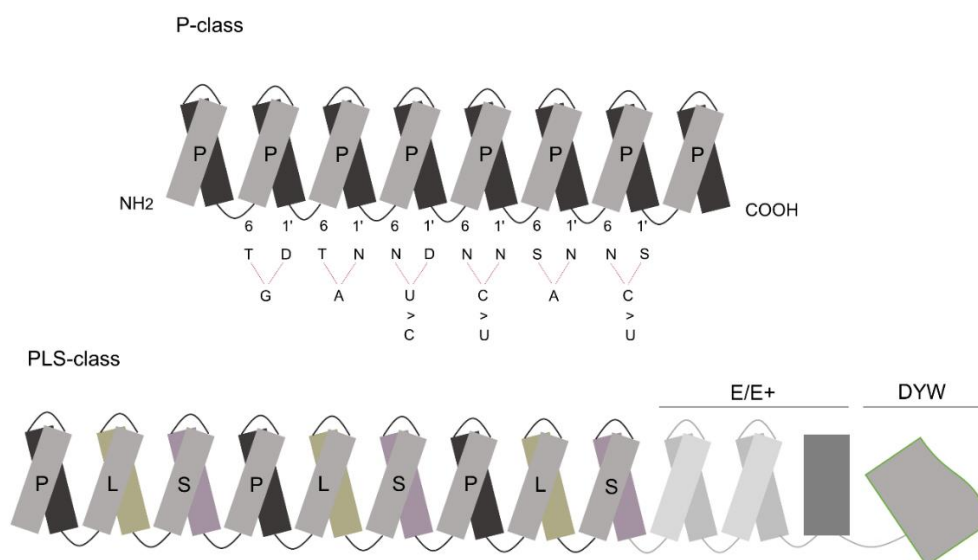


Figure 2: Schematic motif structure of plant PPR proteins and RNA recognition mechanisms. The model shows two different subfamilies of PPR proteins and different composition of motifs. From each subfamily, the typical structure has been shown. P-type (from 'Pure') PPR proteins composed of only 35 amino acid PPR motif, whereas PLS-type of PPR proteins besides P motifs harbour longer (L) and shorter (S) motifs and C-terminal domain extensions; E/E⁺ and/or DYW. The number and the order of motifs can vary in individual proteins. PPR tracts interact with a target RNA in a one PPR repeat-one nucleotide manner. Residues at the position of 6 and 1' specify the binding site on the RNA tracts. Different combination of amino acids, as well as the predicted nucleotides, are shown. The model was modified from Barkan and Small 2014.

Most of PPR proteins are localized to mitochondria and chloroplasts. Therefore, the characterization of these proteins may reveal much information about organelles and the interaction between organelles and the nucleus. Loss of function of PPR proteins have diverse effects on the physiology of plants and cause a variety of molecular dysfunctions and severe mutant phenotypes (Andrés et al. 2007; Barkan and Small 2014). Some of the PPR protein mutants with respective phenotypes are listed below (Table 1).

Table 1: Plant PPR protein mutants with their phenotypes and references.

PPR mutants	phenotype	Reference
<i>aba overly-sensitive 5</i> (<i>abo5</i>)	altered ABA-signalling/stress tolerance	(Liu et al. 2010)
<i>buthionine sulfomixine-insensitive roots 6</i> (<i>bir6</i>)	retarded growth	(Koprivova et al. 2010)
<i>chloroplast biogenesis 19</i> (<i>clb19-1</i>)	impaired leaf pigmentation	(Chateigner-Boutin et al. 2008)
<i>chlororespiratory reduction 2</i> (<i>crr2</i>)	photosynthetic defect	(Hashimoto et al. 2003)
<i>defectively organized tributaries</i> (<i>dot</i>)	aberrant leaf development	(Petricka et al. 2008)
<i>early chloroplast biogenesis</i> (<i>ecb2</i>)	impaired leaf pigmentation	(Yu et al. 2009)
<i>embryo-defective 175</i> (<i>emb175</i>)	defective embryo	(Cushing et al. 2005)
<i>growing slowly1</i> (<i>grs1</i>)	delayed growth, altered ABA-signalling	(Xie et al. 2016)
<i>mitochondrial stability factor 1</i> (<i>mtsf1</i>)	retarded growth	(Haïli et al. 2013)
<i>mutation of RBCL1</i> (<i>mr1</i>)	photosynthetic defect	(Johnson et al. 2010)
<i>organelle transcript processing 43</i> (<i>otp43</i>)	reduced germination rate, retarded growth	(de Longevialle et al. 2007), (Colas des Francs-Small et al. 2014)
<i>ppr4</i>	defective embryo	(Schmitz-Linneweber et al. 2005)
<i>ppr40-1</i>	altered ABA-signalling/stress tolerance	(Zsigmond et al. 2008)
<i>ppr protein for germination on nacl</i> (<i>pgn</i>)	altered ABA-signalling/stress tolerance	(Laluk et al. 2011)
<i>proton gradient regulation 3</i> (<i>pgr3</i>)	photosynthetic defect	(Yamazaki et al. 2004)
<i>slow growth 1</i> (<i>slg1</i>)	delayed growth, altered ABA-signalling/stress tolerance	(Yuan and Liu 2012)
<i>slow growth 2</i> (<i>slo2</i>)	delayed growth, altered ABA-signalling/stress tolerance	(Zhu et al. 2012)

<i>suppressor of the ABA receptor overexpressor (soar1-2/3)</i>	altered ABA-signalling/stress tolerance	(Mei et al. 2014)
<i>tang2</i>	retarded growth	(Colas des Francs-Small et al. 2014)

1.3. Flowering in land plants

Land plants have established an intricate manner of reproduction from specialized organs (flowers and its components) to various controlling pathways (exogenous and endogenous). Flowering, or more broadly transition from vegetative to reproductive phase, is a crucial step in the plant life cycle, which is highly regulated and ensures seed production and consequently leads to the survival of the plant species. This transition undergoes genetic and epigenetic modulation as well as the replacement of biochemical supplements throughout the plant (Andrés and Coupland 2012). Formation of flowers is the key prerequisite for sexual reproduction and the accurate time of this event is of great adaptive value. In view of that, the synchronization of flowering time is crucial for non-self-fertile species. Also, it is critically important regarding self-fertile plants due to environmental and endogenous factors.

Plants gain reproductive competence gradually by a combined exogenous and endogenous signals (Martín-Trillo and Martínez-Zapater 2002). After shoot maturation plants are competent for the transition to reproductive phase. Throughout plant development, the inflorescence meristem gives rise to floral meristem. Afterwards, through the function of floral organ homeotic genes, the floral organ identity of four concentric whorls are determined (Weigel and Meyerowitz 1994). Plants ensure maximum chances to survive by reprogramming the development according to various adversities. Many plant species are induced to flower by different stresses such as drought, high and low light-intensities, poor-nutrition, low temperature, to name a few only (Wada and Takeno 2010; Riboni et al. 2014). The underlying complexity of flowering is established by an intricate network of signaling pathways. It is well known that different flowering pathways are converged to the same genes, which cause the pleiotropic effect of genes. This further leads to the difficulty of specification of these genes to only one pathway (Mouradov et al. 2002; Bernier and Périlleux 2005).

Genetic and molecular studies have led to the elucidation of floral transition regulation in *Arabidopsis*. Several mutants with different responses to environmental conditions were isolated (Koorneef et al. 1998). Further studies using late-flowering mutants and interaction studies resulted in the identification of four different major initial flowering pathways including photoperiod, vernalization, autonomous and gibberellic acid (Boss et al. 2004). Afterwards, the

age pathway was identified by sequentially operating miRNAs typically miR156 and miR172 (Wu and Poethig 2006; Wu et al. 2009). In addition to the five major pathways mentioned above, which are considered as primary pathways, secondary pathways have been identified: the ambient temperature and the light quality, indirectly affect flowering time (Blázquez et al. 2003; Thingnaes et al. 2003; Bernier and Périlleux 2005).

1.3.1. Pathways controlling flowering time in *Arabidopsis thaliana*

Most of the recent data regarding the flowering time and genetic pathways behind it, come from annual plants, in particular, the model plant, *A. thaliana*. Flowering time in *Arabidopsis* is affected by various genes and environmental factors (Figure 3), which make it a classical organism to study flowering time pathways. These data have been extended to the perennials like crop plants (Tan and Swain 2006).

Photoperiod is one of the most important factors for the regulation of flowering time and allows the plant to prepare for the changes by differentiating long- and short-day conditions, as a sign of seasons. *Arabidopsis* is a facultative long-day plant. Therefore, long-day photoperiods stimulate flowering. However, *Arabidopsis* is able to eventually flower under short-day conditions. Genetic approaches have identified the genes, which respond to the day length (Koornneef et al. 1991). Regulation of flowering time by photoperiod is mediated by a network of complex interactions between “external environmental stimuli,” such as light and temperature and internal timekeeping systems termed “circadian clocks” (Mouradov et al. 2002). The light is perceived by phytochromes (PHY) A through E and cryptochromes (CRY) 1 and 2. The day and night duration is measured by the circadian clock (oscillator). These factors have an influence on the floral promoter CONSTANS (CO), which is the key regulator in photoperiodic regulation of flowering (Putterill et al. 1995; Coupland 1997; Turck et al. 2008; Song et al. 2013). The circadian clock-regulated transcriptional regulation of the CO and the light-dependent posttranslational regulation of CO protein are the most crucial mechanisms for day-length measurement in photoperiodic flowering in *A. thaliana* (de Montaigu et al. 2010). Furthermore, circadian clock-regulated GIGANTEA (GI), LATE ELONGATED HYPOCOTYL (LHY) and EARLY FLOWERING 3 (ELF3) proteins are involved in the CO expression (Mouradov et al. 2002; Song et al. 2013). CO mediates between the circadian clock and one of the major promoters of flowering *FLOWERING LOCUS T* (*FT*) in photoperiodic regulation of flowering time. Activation of the photoperiodic flowering pathway by regulating CO expression results in the transcriptional activation of *FT* (Andrés and Coupland 2012; Pin and Nilsson 2012). Subsequently, FT interacts with *FLOWERING LOCUS D* (*FD*) in the shoot apical meristem, which leads to the up-regulation of *APETALA 1* (*AP1*), one of the floral

meristem identity genes, leading to the conversion of the shoot apical meristem and lateral meristem to the flower meristem (Weigel and Nilson 1995).

Many plant species have to sense the winter cold to ensure flowering at the right time and to prevent premature flowering. This process is known as vernalization (Kim et al. 2009). Vernalization is a permissive process, which prepares the plant to flower. After vernalization, plants are induced to flower through the repression of *FLOWERING LOCUS C (FLC)* (Amasino and Michaels 2010). FLC is a MADS-domain transcription factor that functions as the repressor of flowering (Michaels and Amasino 1999). FLC has an inhibitory effect on flowering by the repressing of key elements of the floral integrators *FT*, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1)* and *FD*. A distinct separation between cold treatments and flowering processes, suggests that the function of vernalization happens through epigenetic regulations (Simpson and Dean 2009). During and after cold exposure, two repressive histone modifications, trimethylation of histone H3 at Lys-9 (H3K9) and Lys-27 (H3K27) are increased in *FLC* chromatin, which negatively affects its expression (Bastow et al. 2004). Furthermore, it has been shown that during the cold exposure, there is an increase in the level of a long non-coding antisense mRNA complementary to *FLC*, which is known as COOLAIR as well as an increase in the sense direction expressed mRNA, COLDAIR (Swiezewski et al. 2009; Ietswaart et al. 2012). *FLC* mRNA levels remain low after plants are exposed to warm temperatures. Vernalization in an *flc* null background induced flowering, which indicates that FLC is not the only target of the vernalization pathway (Michaels and Amasino 2001; Reeves and Coupland 2001).

The autonomous pathway was initially identified to enhance flowering independently of environmental factors and thus was named autonomous (He et al. 2003; Bernier and Périlleux 2005). Genes of the autonomous pathway including *FCA*, *FY*, *FPA*, *FVE*, *FLD*, *LD* and *FLK* (Michaels and Amasino 1999; Lim et al. 2004) were identified through their mutants, which flower later in all photoperiods (Lee et al. 1994; Koorneef et al. 1998). Therefore, the hastening effect of these genes on flowering is mainly due to their function in down-regulating *FLC* mRNA. However, by the analysis of mutants of autonomous pathway genes, it is shown that the mechanism of *FLC* down-regulation by autonomous pathway genes is not a simple linear pathway but rather contains several signaling cascades (He et al. 2003; Lim et al. 2004).

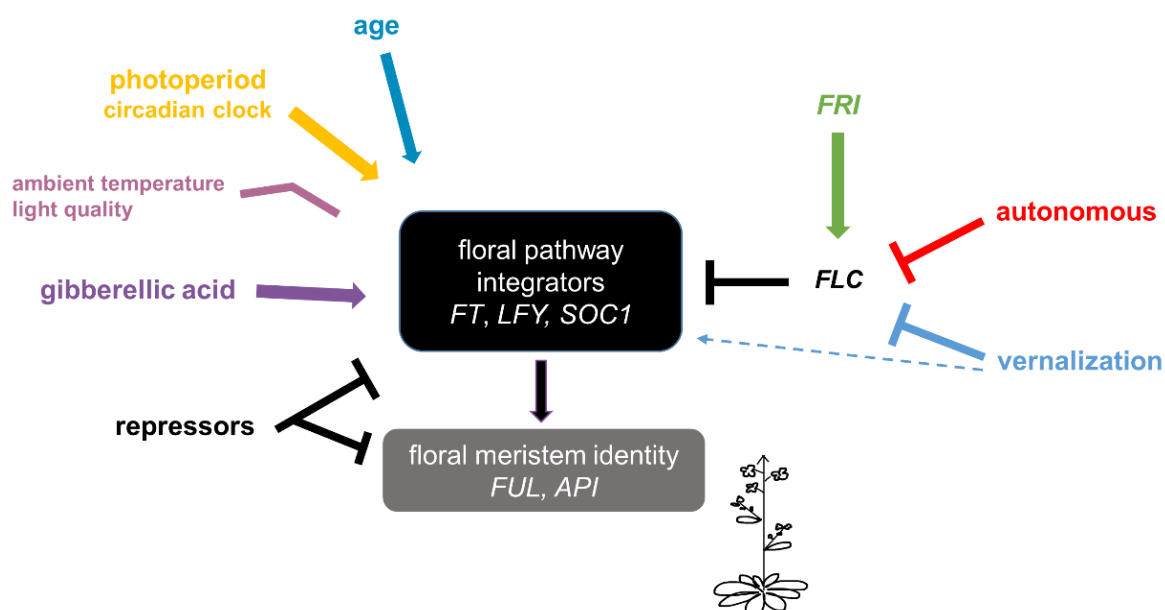


Figure 3: Pathways controlling flowering time in *A. thaliana*. A simplified chart showing the major pathways controlling flowering in *A. thaliana*. Flowering time is regulated by molecular mechanisms in multiple pathways. Photoperiod and circadian clock regulate *CO* expression, which activates both *FT* and *SOC1*. The gibberellic acid pathway transcriptionally activates *FT*. The autonomous pathway acts to down-regulate *FLC* expression, the potent repressor of flowering. Also, prolonged exposure to low temperatures (vernalization pathway) accelerates flowering contributes to the transcriptional silencing of *FLC*. The age pathway differentially regulates the expression of miRNA156 and miRNA172 regarding the plant age and induces the expression of flowering integrators. *FLC* integrates signals from various pathways and represses the expression of *FT* and *SOC1*. Floral integrators also activate the function of floral meristem identity genes like *AP1* and *LFY*, which leads to the transition from vegetative to the reproductive phase. The components of different input pathways have been omitted to simplify the diagram. Unbroken lines with arrows indicate up-regulation of gene expression, whereas lines ending in bars indicate repression. Broken line indicates the indirect effect. The dashed arrow indicates the established relationship, which needs to be further explored. The pathway is adapted from Simpson and Dean 2009.

The phytohormone gibberellic acid is a member of the tetracyclic diterpenoid family, which is involved in different processes of growth and development, such as seed germination, cell elongation and flowering (Finkelstein and Zeevaart 1994). Previous studies showed that by the exogenous application of the gibberellic acid to *Arabidopsis* plants, the flowering time is severely accelerated (Langridge 1957). In addition, any mutation that disrupts the gibberellic acid signaling or biosynthesis causes an alteration of flowering time (Jacobsen and Olszewski 1993). The gibberellic acid signals are perceived by a soluble nuclear receptor protein GIBBERELLIN INSENSITIVE DWARF1 (*GID1*). Gibberellic acid signaling promotes growth and flowering by initiating the degradation of the DELLA protein. Studies showed that bioactive gibberellic acids induce binding of *GID1* to the DELLA protein, which initiates DELLA degradation and eventually promotes flowering (Mutasa-Göttgens and Hedden 2009). DELLA negatively regulates flowering through an interaction with the miR156-targeted SQUAMOSA PROMOTER BINDING-LIKE (*SPL*) transcription factor. The interaction between DELLA and *SPL* leads to the transcription inactivation of MADS-box genes and miR172 and results in the inhibition of flowering (Yu et al. 2012). Also, gibberellic acid was reported to activate genes of

the floral integrators such as *SOC1* in the inflorescence, *FT* in the leaves and *LEAFY (LFY)* in the floral meristem (Yu et al. 2012).

The newly identified age pathway ensures flowering of the plant under non-inductive conditions. The age pathway is regulated by miRNAs in plants. Two major players of the age pathway in *Arabidopsis* are miR156 and miR172. These two miRNAs repress their downstream targets and function antagonistically but linked to each other in the regulation of flowering time. The significance of miR156 in control of flowering is related to the observation that the over-expression of miR156 leads to a reduced apical dominance and delay in flowering (Schwab et al. 2005; Schwarz et al. 2008). Contrary to miR156, the over-expression of miR172 leads to early flowering under both long- and short-day conditions (Jung et al. 2007). Interestingly, the expression of miR156 is maximized during the juvenile phase and declines before the transition to flowering. In contrast, the expression of miR172 is significantly at the highest level during the transition to flowering. The higher amount of miR156 results in the reduced expression of miR172, suggesting a feedback loop in flowering time control. SPLs, which are known as “ageing genes”, are targets of miR156. miR156-targeted SPLs permit the activation of miR172, whose function is downstream of the miR156/SPL (Hyun et al. 2017). The miR172 targets an APETALA2 (AP2)-like transcription factor and closely related proteins (Park et al. 2002; Wang 2014). In the adult phase of the plant, miR156-targeted SPLs induce flowering through the promotion of flowering integrator genes (Wang 2014; Hyun et al. 2017). Overall, these data indicate that miR156 and miR172 serve as age indicators for control of flowering time.

The signals from different pathways are integrated to a set of genes including the flowering repressor *FLC* and commonly known floral integrators *LFY*, *FT* and *SOC1*, which in turn determine switching to the flowering phase or to remain in the vegetative phase (Lee et al. 2006a; Andrés and Coupland 2012; Khan et al. 2014).

1.3.2. Regulation of flowering through *FLC*

FLC is a MADS-box transcription factor (Michaels and Amasino 1999), which functions as a repressor of flowering through inhibiting the expression of genes needed for the transition of the apical meristem to the floral meristem (Michaels and Amasino 1999; Hepworth 2002). *FLC* is mostly considered as a strong repressor of flowering as it binds to two main promoters of flowering genes, *FT* and *SOC1* (Lopez-Vernaza et al. 2012). *FLC* is expressed in all parts of the plant and through all developmental stages, which hints towards a variety of its direct or indirect functions additional to flowering initiation (Deng et al. 2011). Regulation of *FLC*

involves a variety of factors including genetic and epigenetic pathways (Rouse et al. 2002; Finnegan et al. 2005).

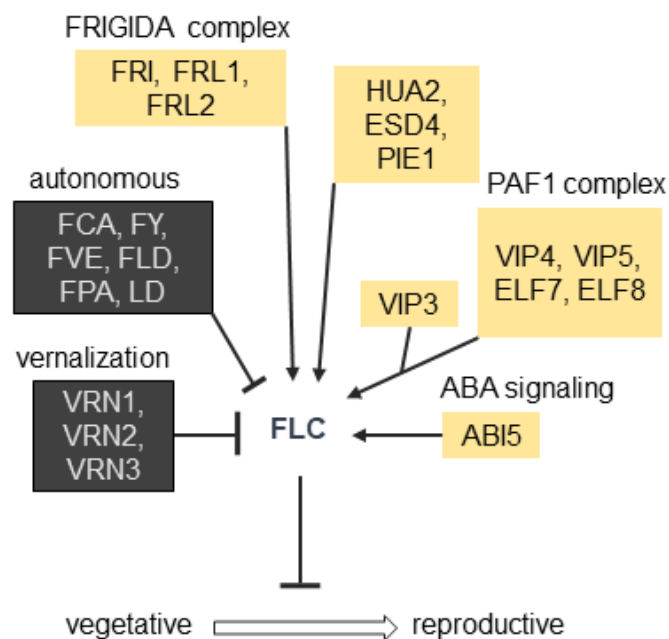


Figure 4: *FLC* regulatory pathway. *FLC* expression is down-regulated by the genes of vernalization and the autonomous pathway. On the other hand, *FLC* expression is up-regulated by the *FRI* gene family, including *FRI* and two related genes, *FRL1* and *FRL2*, which all function dependent on the genes affecting chromatin structure of *FLC* locus, PAF1 complex genes including *VIP4*, *VIP5*, *ELF7* and *ELF8*, *VIP3*, and other genes, including *PIE1*, *ESD4* and *HUA2*, which are required in autonomous pathway mutants and *FRI* active plants (He and Amasino 2005; Finnegan et al. 2005). Also, *ABI5*, part of an ABA-signaling transduction pathway, promotes *FLC* expression.

In Figure 4, the regulatory pathways affecting *FLC* expression are depicted. Vernalization and autonomous pathways converge on the activity of *FLC* and down-regulate it epigenetically (Bernier and Périlleux 2005; Quesada et al. 2005; Kazan and Lyons 2016). After the prolonged period of cold temperature, expression of *FLC* is down-regulated and plants are capable to enter the reproductive phase. Repression of *FLC* is correlated with changes in the chromatin level, specifically, a histone modification that represses the transcriptional activity of the *FLC* gene (Amasino 2004; Bastow et al. 2004). Additionally, the interaction between disparate autonomous pathway genes is required for down-regulation of *FLC* expression (Koorneef et al. 1998; Simpson et al. 2003). Up-regulation of *FLC* is mediated by the *FRIGIDA* (*FRI*) gene family, including *FRIGIDA* (*FRI*) and two related genes, *FRIGIDA LIKE 1* (*FRL1*) and *FRL2*, which function dependent on genes affecting *FLC* chromatin structure, such as PAF1 complex genes including *VERNALIZATION INDEPENDENT 4* (*VIP4*), *VIP5*, *EARLY FLOWERING 7* (*ELF7*) and *ELF8*, *VIP3*, and other genes, including *PHOTOPERIOD INDEPENDENT EARLY FLOWERING 1* (*PIE1*), *EARLY IN SHORT DAYS 4* (*ESD4*) and *HUA2*, which are required in autonomous pathway mutants (He and Amasino 2005; Finnegan et al. 2005) (Figure 4).

FLC transcription is also influenced by a novel pathway. Studies demonstrated that *FLC* is

directly activated by transcription factors ABI5 and ABI4, which are two main components of the abscisic acid (ABA) signal transduction (Wang et al. 2013a; Shu et al. 2016). It was shown that *FLC* expression is promoted by direct binding of ABI5 to the promoter region of *FLC*, which is a critical step for *FLC* expression leading to a delay in flowering time (Wang et al. 2013a). The *FLC* promoter region is also targeted by ABI4, but the binding site is different compared to ABI5. As ABI4 can also bind to the promoter region of *ABI5* and activate its transcription (Bossi et al. 2009), the effect of ABI4 on *FLC* expression can be through the transcription activation of *ABI5* (Shu et al. 2016). It was reported that the most ABA-responsive element (ABRE)-binding factors (ABFs) such as ABF1, ABF3 and ABF4 are able to promote *FLC* expression (Wang et al. 2013a).

FLC binds to the first intron and the promoter region of *FT* and *SOC1* respectively. *FLC* was also reported to have an influence on the floral architecture through the binding to the floral homeotic genes (Liu et al. 2009).

1.4. Influence of mitochondria on flowering

Successful transition from the vegetative phase to the reproductive phase (formation of male and female gametes, floral organs, seeds and fruit development) requires a source of energy, which illustrate the importance of mitochondrial function on flower development. In the case of mitochondrial defects, reproductive tissues are the most affected tissues of plants (Liberatore et al. 2016). Furthermore, dysfunction of the mitochondrial activity prevents the formation of normal viable flowers, which suggest a close link between mitochondrial function and floral development. All so far identified genes involved in classical flowering pathways are encoded in the nucleus. However, the phenomenon cytoplasmic male sterility (CMS) is an indication of the contribution of additional genes to the nuclear ones. CMS is determined by the maternally inherited mitochondrial genome (Budar et al. 2003) and refers to plants, which have male sterile flower organs but usually have normal female organs. In other words, in the CMS system floral organogenesis is affected by nuclear-mitochondrial interaction. In addition to the unviable pollen, aberrant floral organ development, which is very resemblant to *Arabidopsis* floral homeotic gene mutants, was reported for CMS plants (Linke et al. 2003; Leino et al. 2003; Linke and Börner 2005). It is demonstrated that due to the alteration in the expression of floral homeotic genes in many CMS systems, stamens are homeotically converted to either petaloid or carpeloid (Chase 2007). It has been reported that the defect in the floral morphology of CMS lines can be either due to the retrograde signaling, which targets nuclear floral homeotic regulators (Murai et al. 2002; Zubko 2004) or due to the affected ATP (adenosine triphosphate)-dependent proteolysis of the floral regulators (Teixeira et al. 2005). CMS is

repressed by the restorer of fertility (*Rf*) genes, which are encoded in the nucleus and reduce the accumulation of CMS proteins (Chase 2007). Most of the characterized *Rf* genes belong to the PPR protein family (Bentolila et al. 2002; Brown et al. 2003; Komori et al. 2004; Woodson and Chory 2008). Although there is not a conserved mechanism for the reduction of the CMS effects, PPR proteins act post-transcriptionally on CMS-related transcripts to increase endonucleolytic cleavage (Kazama and Toriyama 2003), affect editing, destabilization (Akagi et al. 2004) and the reduction of translation (Zhonghua et al. 2006).

The number of mitochondria and transcripts of mitochondrial proteins is increased during flower development (Huang et al. 1994; Mackenzie and McIntosh 1999). According to these observations, reproductive development has been shown to be particularly sensitive to changes in mitochondria. Mutations affecting mitochondria were demonstrated to cause the abortion of ovules (Landschütze et al. 1995; Skinner et al. 2001) and disruption in tapetum and pollen development, which in turn causes a CMS phenotype (reviewed in Mackenzie and McIntosh 1999). There is evidence from several plant species studies that the development of floral organs is regulated by mitochondria. For instance, an *Arabidopsis* gene, *HUELLENLOS*, which encodes a mitochondrial ribosomal protein is essential for the growth, development and patterning of the ovule (Skinner et al. 2001).

There are abundant data, which confirm that the insufficient mitochondrial function leads to the formation of aberrant flowers. It was revealed that the editing of the mitochondrial *ATP6* mRNA was reduced in the male organs of a CMS type of *Sorghum* (Howad and Kempken 1997). Flower development has been affected by the alteration of mitochondrial RNA editing (Chakraborty et al. 2015). Over-expression of antisense mitochondrial *ATP9* mRNA rescued male-sterile tobacco plants, which contained unedited *ATP9*. In addition, it was previously demonstrated that the incompletely edited mitochondrial RNAs are too unstable to accumulate and affect flower development (Zabaleta et al. 1996). Plant transformation with unedited *ATP9* mRNA leads to abnormal anthers and pollen abortion (Hernould et al. 1998). Silencing of two mitochondrial editing factors, Organelle RNA recognition motif-containing (ORRM2) and (ORRM3) proteins, affects RNA editing efficiency at approximately 6% of mitochondrial C targets, which was shown to be essential for normal flower development (Shi et al. 2016). Furthermore, it is shown that the *Arabidopsis* PPR protein REQUIRED FOR EFFICIENCY OF MITOCHONDRIAL EDITING 2 (REME2) specifically edits two mitochondrial ribosomal protein genes (*rps3-1534* and *rps4-175*) during flowering in plants (Bentolila et al. 2013).

1.5. Mitochondrial contribution to the stress response

Stress conditions including biotic and abiotic stresses result in severe productivity limitation

and extreme damages to plants. Mitochondria play an important role in the cell adaptation to stress conditions by acting as sensors of environmental perturbation, which can be followed by translating stress perception into energy-deficiency signals and regulating programmed cell death (Jones 2000; Rhoads et al. 2006; Pu et al. 2015).

Formation of mitochondrial reactive oxygen species (ROS), already occurs under normal respiration. However, it can increase under a range of abnormal conditions, including exposure to biotic and abiotic stresses and mitochondrial dysfunction. Stress conditions affect the donation and acceptance rate of electrons by the mitochondrial electron transport chain (mETC) complexes, which further leads to the excessive reduction of specific mETC sites and subsequently causes electron leakage to O₂ and the over-production of ROS (Jacoby et al. 2011). ROS include diverse types of molecules such as superoxide, hydrogen peroxide and hydroxyl radicals (Møller and Sweetlove 2010; Suzuki et al. 2012). The primary sites of ROS production in the mETC are complex I (NADH dehydrogenase) and III (cytochrome c reductase) (Zsigmond et al. 2008). However, another study indicated that ROS also can be produced by complex II (succinate dehydrogenase) (Gleason et al. 2011). ROS generated by the complex I, II and III are required for appropriate responses to stress conditions (Pastore et al. 2007; Møller et al. 2007; Gleason et al. 2011; Liberatore et al. 2016). The high amount of ROS leads to damages to the cellular compartments, macromolecules, nucleic acids, proteins and lipids. In plants, alternative oxidases (AOXs) (Pastore et al. 2001), alternative NAD(P)H dehydrogenases (NADs) (Møller 2001), ATP-sensitive potassium channel (Pastore et al. 1999) and uncoupling proteins (UCPs) (Pastore et al. 2000) play a critical role during stress to lessen the amount of ROS. During excess energy demands because of stress conditions, AOXs and NADs decrease ROS levels by preventing the over-reduction of the ubiquinone pool and allow for electron transport and oxygen reduction. ATP-sensitive potassium channel and UCP deplete the excess of energy through mETC and dissipate the transmembrane potential made by respiration respectively (Pastore et al. 2007; Vanlerberghe 2013; Schertl and Braun 2014). Besides these known mechanisms, plants have enzymes such as Mn SUPEROXIDE DISMUTASE (MnSOD), ASCORBATE PEROXIDASE (APX) (Chew et al. 2003), GLUTATHIONE PEROXIDASE (GPX) (Navrot et al. 2006) and GLUTATHIONE S-TRANSFERASE (GST) (Marrs 1996) to alleviate the excess of ROS.

Lacking a fully functional mETC leads to a new cellular homeostasis demand, which requires an alteration in the nuclear gene expression. Mutants having altered cellular homeostasis showed an altered expression of AOX, heat shock proteins (*HSPs*) and antioxidant enzymes genes (Karpova et al. 2002; Dutilleul et al. 2003; Kuzmin et al. 2004). ROS generated in mitochondria by encountering various stress conditions act as signals and/or lead to the formation of secondary signals (signaling components involved in oxidative stress sensing

and/or redox signaling), which further causes a modified gene expression in the nucleus (Rhoads et al. 2006; Zsigmond et al. 2008; Kmiecik et al. 2016). Exceeding the normal level, ROS leads to oxidative stress and changes in the redox state (Foyer and Noctor 2003; Ng et al. 2014). Subsequently, alteration of the mitochondrial redox status was reported to change the expression of antioxidant enzymes such as enhanced expression of AOX, which further causes plant tolerance to biotic and abiotic stresses. Conversely, the lower expression of *AOX1a* results in the alteration of defence and stress-responsive genes (Giraud et al. 2008).

It is widely accepted that mitochondria are important targets of various stresses. Consistently, many nuclear genes, which encode mitochondrial proteins (NGEMPs) have been identified to function in different stress conditions. The classical instances are *AOX* and *HSPs* (Clifton et al. 2005; Taylor et al. 2009). Altered expression of NGEMPs affects mitochondrial functions and thus emphasizes their involvement in retrograde signaling (Van Aken et al. 2009b). Mitochondrial retrograde signals regulate not only the expression of mitochondrial proteins but also reprogram nuclear gene expression of various genes belonging to different cellular functions (Schwarzländer and Finkemeier 2013). The signal cascades activated to modulate the specific transcriptional regulation are unique to the type of stress (Liberatore et al. 2016). It increasingly appears that stress-tolerant species showed enhanced gene expression of mitochondrial antioxidant proteins and a higher accumulation of these proteins compared to non-tolerant species (Liberatore et al. 2016).

Further evidence of the mitochondrial contribution to cope with stress conditions arises from the involvement of several mitochondrial-targeted PPR proteins in the plant stress response. There are several functional studies on mitochondrial-targeted PPR proteins, which have been implicated to the stress response such as PPR40 (Zsigmond et al. 2008), ABO5 (Liu et al. 2010), PGN (Laluk et al. 2011), HOT ABA-DEFICIENCY SUPPRESSOR2/LOVASTATIN INSENSITIVE1/ MITOCHONDRIAL EDITING FACTOR11 (HAS2/LOI1/MEF11) (Sechet et al. 2015), SLG1 (Yuan and Liu 2012) and PPR96 (Liu et al. 2016), which further implies the importance of a proper mitochondrial metabolism in the plant stress response.

Plant hormones are highly involved in the regulation of stresses (Vanstraelen and Benková 2012) and, hence, it would be expected that mitochondrial function interacts with the hormonal pathways in response to stresses. Plant hormones also have remarkable roles in many cellular processes. Therefore, the involvement of plant hormones in mitochondria to nucleus communication and vice versa can be expected. In addition, ROS generated in mitochondria in response to stress was reported to interact with hormonal pathways (Xia et al. 2015). The phytohormone ABA is widely accepted to have a major role in plant adaptation to stress conditions, which was demonstrated to be one of the major regulators of mitochondrial function

(Berkowitz et al. 2016). A direct influence of ABA application on mitochondrial function was observed in *Zea mays* (Prasad et al. 1994). An altered gene expression for many genes encoding mitochondrial proteins was shown after ABA treatment in *Arabidopsis* (Berkowitz et al. 2016). Also, many genes encoding mitochondrial proteins such as *ABO5* (Liu et al. 2010), *PGN* (Laluk et al. 2011), *ABA OVERLY SENSITIVE 6 (ABO6)* (He et al. 2012), *SLG1* (Yuan and Liu 2012), *ABA HYPERSENSITIVE GERMINATION 11 (AHG11)* (Murayama et al. 2012), *SLOW GROWTH 2 (SLO2)* (Zhu et al. 2014) and *HAS2/LOI1/MEF11* (Sechet et al. 2015) were demonstrated to have altered responses after ABA treatment. All of these proteins were revealed to be involved in mitochondrial processing and contributed to altered retrograde signaling and ROS production. According to all above-mentioned reasons, the contribution of mitochondria in stress responses is strongly evident.

1.6. Aim of the study

Most of our knowledge of characterized PPR proteins comes from studies on individual mutants (Kocábek et al. 2006). Since the identification of PPR proteins, only a few of them have been functionally characterized. Most of the plant PPR proteins are trafficked to mitochondria and chloroplasts. Therefore, characterization of PPR genes unravels many aspects of interactions between organelles and the nucleus. A T-DNA insertion mutation in *AT1G15480*, a PPR protein-encoding gene, led to an early-flowering phenotype in *A. thaliana* (Bruhs 2012), which is a rare phenotype for a PPR protein mutant. In this thesis, the functional characterization of *AT1G15480* was studied.

The transition from the vegetative to the reproductive phase is a crucial step in the life of a plant and one of the major research interests in plant breeding for crops and trees breeding researches. Nearly nothing is known about the impacts of mitochondrial proteins, affecting flowering. By characterization of *AT1G15480* and unravelling the underlying regulatory mechanisms, our knowledge about the influence of a mitochondrial PPR protein on the flowering will be enhanced. It is, therefore, necessary in the first place, to assure that the early-flowering phenotype is caused by the T-DNA insertion by the mean of a genetic complementation analysis. Since many of PPR proteins function as a constituent of a complex (Andrés-Colás et al. 2017), the putative *in vivo* interaction partners will be investigated. It is known that PPR proteins are involved in diverse post-transcriptional processes in chloroplasts and mitochondria, thereby influencing the growth, development and physiological characteristics of the plants. Thus, one of the aspects of RNA processing, RNA editing status will be checked. By then, to study the molecular mechanism, by which *AT1G15480* influences on flowering time the expression level of flowering marker genes in wild-type and the PPR

mutant plants will be analyzed. Furthermore, to analyze the effect of *AT1G15480* T-DNA insertional mutation on mitochondrial functions, physiological aspects of mitochondria in wild-type and mutant plants will be investigated. Additionally, RNA-seq data will be analyzed to investigate differences in the gene expression profiles between wild-type and PPR mutant plants.

2. Material and methods

2.1. General molecular biology methods

2.1.1. General solutions and buffers

The following solution and buffers were used in this thesis:

10 x TBE buffer: 1 M Boric acid, 1 M Tris-HCl, 20 mM EDTA (pH 8.3)

DNA-loading buffer: 10 mM Tris-HCl, 0.03% Bromophenol blue, 0.03% Xylene cyanol, 0.15% Orange G, 60% Glycerol, 60 mM EDTA (pH 7.6)

SOC-medium: 2% (w/v) Tryptone, 0.5% (w/v) Yeast extract, 20 mM Glucose, 10 mM NaCl, 10 mM MgCl₂, 2,5 mM KCl, 10 mM MgSO₄ (pH 7.0), Autoclave at 112 °C

2.1.2. Media

The following media were used in this thesis:

Luria-Bertani (LB) medium: 1% (w/v) Tryptone, 0.5% (w/v) Yeast extract, 0.5% (w/v) NaCl, (pH 7.2)

To make LB plates 1.5% (w/v) of Agar-Agar was added to the medium. The medium was autoclaved at 121 °C prior to use.

Murashige-Skoog (MS) medium: 0.22% (w/v) MS basal medium (Sigma-Aldrich), 1% (w/v) sucrose, 0.05% (w/v) MES, (pH 5.7)

To make MS plates 1% (w/v) of Agar-Agar was added to the medium. The medium was autoclaved at 112 °C prior to use.

2x Yeast extract-Tryptone (2x YT) medium: 1.6% (w/v) Tryptone, 1.0% (w/v) Yeast extract 0.5% (w/v) NaCl, (pH 7.5)

To make 2x YT plates 1.5% (w/v) of Agar-Agar was added to the medium. The medium was autoclaved at 121 °C prior to use.

2.1.3. Devices, chemicals and Enzymes

All laboratory devices and chemicals used in this work unless other mentioned, were obtained from the following companies: Roth (Karlsruhe), Sigma-Aldrich (Munich), Bio-Rad (Munich), Fischer Scientific (Düsseldorf), Serva (Heidelberg), Roche (Mannheim) and Duchefa (Haarlem, Netherland). All the restriction endonucleases prepared from New England Biolabs (NEB).

In the case of working with nucleic acids, all the solutions and equipment were autoclaved at 121 °C for 20 minutes.

2.1.4. Bacterial strains and cultivation

For the cloning of DNA fragments, *Escherichia coli* strains XL1-Blue (Bullock et al. 1987) and DH5 α (Hanahan 1983) were used. Both bacterial strains were cultivated on liquid LB medium or LB agar plates (2.1.2) overnight at 37 °C in a floor-standing shaking incubator (Infors HT, eve®) and a benchtop incubator respectively (Thermo Scientific Heraeus®). For the selection of transformants in XL1-Blue and DH5 α strains, following antibiotics with their appropriate final concentration were used:

Antibiotics	Concentration
Ampicillin	50 μ g/ml
Kanamycin	30 μ g/ml
Spectinomycin	50 μ g/ml
Tetracycline	12 μ g/ml

For the aim of the stable transformation of *Arabidopsis thaliana* the bacterial strain, *Agrobacterium tumefaciens* C58C1 (pMP90) was used (Koncz and Schell 1986). *A. tumefaciens* C58C1 (pMP90) cells were cultivated in 2x YT liquid medium or 2x YT agar plates (2.1.2) for 2 days at 28 °C in a floor-standing shaking incubator (Infors HT, eve®) or a benchtop incubator (Thermo Scientific Heraeus®). For the selection of transformants in *A. tumefaciens* C58C1 (pMP90), following antibiotics with their appropriate final concentration were used:

Antibiotics	Concentration
Kanamycin	30 µg/ml
Gentamycin	25 µg/ml
Rifampicin	50 µg/ml

All bacterial strains with their corresponding genotypes and applications are represented in Table 2.

Table 2: The bacterial strains used in this study with their genotypes, applications and references.

Strain	Genotype	Application	Reference
<i>E. coli</i> (XL1-Blue)	F' Tn10 proA+B+lacIq Δ(lacZ)M15/recA1 endA1 gyrA96 (Nalr) thi hsdR17(rk- mk+) glnV44 relA1 lac	Classical cloning	(Bullock et al. 1987)
<i>E. coli</i> (DH5α)	F- 80dlacZ M15 (lacZYA-argF) U169 recA1 endA1 hsdR17(rk-, mk+) phoA supE44 -thi-1 gyrA96 relA1	Gateway™ Cloning	(Hanahan 1983)
<i>A. tumefaciens</i> C58C1 (pMP90)	pMP90 (pTiBo542ΔT-DNA) in C58C1; Rifr, Gentr	Plant transformation	(Koncz and Schell 1986)

2.1.5. Isolation of plasmid-DNA from liquid culture of *E. coli* cells

Plasmid DNA was isolated according to (Bimboim and Doly 1979) method. Plasmid DNA was isolated from 3 ml of the relevant overnight culture, which was grown at 37 °C with the use of NucleoSpin® Plasmid QuickPure-Kits (Macherey-Nagel, Germany).

2.1.6. DNA elution from an agarose gel

To isolate intact DNA from agarose gel during the cloning procedure, the desired DNA fragment was cut from the gel by a sharp blade quickly, without being subjected to the direct UV light. The rest of the elution procedure was done on the base of the NucleoSpin® Gel and PCR clean-up kit (Macherey-Nagel, Germany).

2.1.7. DNA hydrolysis by endonuclease restriction enzymes

To generate specific cohesive or blunt ends on the vector for the cloning approach, restriction endonuclease were utilized with their specific buffers from (NEB) according to the manufacturer's instructions.

2.1.8. DNA ligation

3'-hydroxyl and 5'-phosphate terminus of DNA strands are linked by a phosphodiester bond coupled with ATP hydrolysis. To catalyze the formation of a phosphodiester bond between 3'-hydroxyl and 5'-phosphate terminus, T4 DNA ligase (NEB) was used according to the manufacturer's instructions.

2.1.9. DNA gel-electrophoresis

As it was developed in the 1970s, DNA migrates in an electric field and is separated by their sizes as they move through a gel matrix. The phosphate groups in the DNA backbone give the whole molecule a negative charge, which causes moving towards the anode in an electric current. Smaller fragments migrate easier and quicker than larger fragments through the pores (Sambrook et al. 1989). One percent gel (w/v) was made of agarose suspended in 1x TBE-buffer (2.1.1) and heated in a microwave to be dissolved completely. After cooling the temperature down, 0.006% (v/v) ethidium bromide was added to the mixture to make the DNA visible under the UV light (302 nm wavelength). After solidification of the gel, it was submerged with 1x TBE-buffer. DNA samples were mixed with DNA-loading buffer (2.1.1) as a tracking dye and subsequently loaded into the slots. A voltage between 100-140 V was supplied.

2.1.10. Transformation of *E. coli*

Transformation of electrocompetent *E. coli* cells was done using an electroporator (Bio-Rad, Gene Pulser®) and 0.2 cm electrode gap cuvettes. 3 µl of filter dialyzed plasmid DNA was mixed with the 40 µl of electrocompetent *E. coli* cells and after placing on ice for 30 seconds, the mixture was transferred to the cuvette, which was cooled down to 4 °C. The electroporation condition was adjusted as follow:

Field strength: 2.50 kV/cm, Capacity: 25 µF, Resistance: 200Ω

After doing the pulse, 1 ml of SOC-medium (2.1.1) was immediately added to the cuvette and after 10 minutes for maximizing the recovery of the cells, transformation mixture was plated on the related selective medium.

2.1.11. Transformation of *A. tumefaciens*

Vectors were introduced to the *A. tumefaciens* cells by electroporation. First, 40 µl of frozen electrocompetent *A. tumefaciens* cells were thawed on ice. Then, 50 ng of the appropriate

vector DNA was mixed with *A. tumefaciens* cells and after standing for 30 seconds on the ice, the batch was transferred into a pre-cooled electroporation cuvette and installed in the electroporator (Bio-Rad, Gene Pulser®). The electroporation condition was adjusted as follow:

Field strength: 2.50 kV/cm, Capacity: 25 μ F, Resistance: 200 Ω

After doing the pulse, 1 ml of 2x YT medium (2.1.2) was immediately added to the cuvette and was mixed with electroporated cells. This was followed by transferring to a sterile tube and the incubation for 4 to 6 hours at room temperature. The batch was plated on the selective 2x YT plates.

2.1.12. Polymerase chain reaction (PCR)

To amplify the DNA fragments, polymerase chain reaction (PCR) was used. Depending on the aim, two types of DNA polymerases with different fidelity were used: *Taq* and *PWO* DNA polymerases. A standard PCR reaction contained a 0.1-1 ng Plasmid-DNA or 100-1000 ng genomic DNA, 1x PCR reaction buffer (Molzym), 200 μ M dNTP's (NEB), 1.5 mM MgCl₂, 100 nM forward and reverse oligonucleotides, 1% (v/v) PCR-Enhancer (Molzym) and 0.025 U/ μ l *Taq*-Polymerase (Molzym). In order to achieve high accuracy rate during the replication of the amplicon, PCR was performed using *PWO*-DNA-polymerase (PeqLab). In this case, 300 nM of each oligonucleotide and 0.05 U/ μ l of the *PWO*-DNA-polymerase (PeqLab) was used. Also, the extension temperature of the PCR reaction was set up to 68 °C in order to permit the optimum extension rate (Table 3). Denaturation, annealing and elongation were repeated up to 39 cycles. In this thesis, the amplification of DNA was performed using the Master Cycler Gradient (Eppendorf, Hamburg).

Table 3: Steps and temperature profile of PCR in this study.

Steps	Temperature	Time
Initial denaturation	94 °C	2 min
Denaturation	94 °C	1 min
Annealing	50-65 °C ^a	1 min
Elongation	72 °C	1 min/kb
Final elongation	68 or 72 °C ^b	5 min

^a The appropriate annealing temperature was selected according to oligonucleotides.

^b Depending on the type of DNA polymerase.

2.1.13. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Reverse transcription was performed by OneStep RT-PCR Kit (Qiagen) according to the manufacturer's instructions using isolated RNA treated with RNase-free DNase I (Thermo Scientific) (2.1.18). Possible DNA contaminations in the isolated RNA was monitored by standard PCR (2.1.12) using a positive control prior to the RT-PCR assay.

2.1.14. Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

As there are limitations in the polymerase chain reaction to monitor amplicons at an exponential rate, real-time quantitative PCR is used to quantify the amount of PCR product at the exponential phase (Arya et al. 2005). To quantify the gene expression, the quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was used (Holzapfel and Wickert 2007). The relative quantification, which eliminates the need for standard curves allows determining differences in the expression level of a specific target gene on the base of an endogenous control as a housekeeping gene. Therefore, quantitation of signals in each sample can be normalized relative to the *housekeeping* gene. In this study, the normalization of gene-specific signals was performed relative to *ACTIN2 (ACT2)* housekeeping gene.

To analyze the expression level of genes, the QuantiTect SYBR Green RT-PCR Kit (Qiagen) on the base of manufacture instructions was used. However, the total volume of the reaction was decreased to 15 µl and 35 ng of the RNase-free DNase I (Thermo Scientific) treated RNA samples (2.1.18) was used for the qRT-PCR assay. As seen in Table 4, the QuantiTect SYBR Green RT-PCR Kit allows first the cDNA synthesis. The amount of emitted fluorescence is quantified as the dye attaches to the double-stranded DNA. Depending on the amount of produced amplicon, the intensity of the emitting fluorescence is higher.

Table 4: Program of the qRT-PCR used in this study.

Steps	Temperature	Time
Reverse transcription (cDNA synthesis)	50 °C	30 min
Reverse transcription inactivation and activation of the HotStar <i>Taq</i> -DNA Polymerase	95 °C	15 min
Denaturation	94 °C	15s
Annealing	54-55 °C	60s
Elongation	72 °C	20s
Dissociation	50 °C 0.2 °C/s 50 → 99 °C	245s

The expression level of different genes was determined with the use of gene-specific oligonucleotides, which are listed in Supplementary Table 1. To check the gene specificity of the designed oligonucleotides, using the program Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990), all oligonucleotides were initially analyzed via blasting against other gene sequences of *Arabidopsis* in the National Center for Biotechnology Information (NCBI) website. Then, the oligonucleotide specificity was checked by RT-PCR (2.1.13). Furthermore, prior to the qRT-PCR experiment, an oligonucleotide efficiency test was performed according to Pfaffl and Riedmaier 2011. Oligonucleotides with the efficiency of 1.8 to 2.1 proceeded to qRT-PCR experiment.

To perform the qRT-PCR, the Rotor-Gene Q Cyclor (Qiagen, Hilden) was used. For carrying out the melting curve the temperature was gradually increased from 50 °C to 99 °C (0.2 °C/s). For the reproducibility of the data, three biological replications, as well as three technical replications were done. The evaluation of the obtained data was done using the Rotor-Gene Q- Pure Detection Software 2.1.0 (Build9) (Qiagen, Hilden). The final relative quantification of the expression rate was performed using $\Delta\Delta CT$ methods according to the Livak and Schmittgen 2001.

2.1.15. RNA editing analysis using RT-PCR

Relevant cDNA fragment covering the editing sites was obtained by RT-PCR amplification (2.1.13). At the RNA editing sites, the cDNA sequence was evaluated for the respective C to T differences by electropherogram. RNA editing levels were estimated by the relative areas of the respective nucleotide peaks in the sequence analysis. The percentage of RNA editing

efficiency was calculated as $\frac{T \text{ peak area}}{T \text{ peak area} + C \text{ peak area}} * 100$ (Brehme et al. 2015). RT-PCR products were sequenced via the Sanger method (Sanger et al. 1977).

2.1.16. Sequencing of DNA fragments

On the base of classic chain termination method, which is known as Sanger Sequencing (Sanger et al. 1977), the DNA fragments were sequenced by the Institute of Clinical Molecular Biology (IKMB) at the Christian-Albrechts-University of Kiel.

2.1.17. Quantification of the nucleic acids

The concentration of the dissolved DNA or RNA was quantified photometrically based on the absorbance at 260 nm and 280 nm with the ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Germany) following the structure of the manufacturer. An extinction of 1 is equal with a concentration of 50 µg of double-stranded DNA/ml and 40 µg of RNA/ml.

2.1.18. Control of RNA quality

Because of the similar characteristics of nucleic acids, it is probable to have traces of genomic DNA during the RNA isolation. This leads to getting false-positive results. Therefore, to eliminate the traces of DNA from RNA an RNase-free DNase I (Thermo Scientific) hydrolysis of the DNA according to the manufacturer instruction in a 20 µl volume was applied. To check that the DNA was completely eliminated from isolated RNA samples, a control PCR using the RNase-free DNase I treated RNA samples, as well as a positive control sample, was carried out. In case of having no PCR amplicon for the RNase-free DNase I treated RNA samples, they were further used for RT-PCR and qRT-PCR assays.

2.2. Protein analysis

2.2.1. Determination of protein concentrations

Protein concentration was determined to compare the BSA standard in the samples, which was first described by Bradford 1976. Using the "Bio-Rad Protein assay" 5 µl of the protein sample was mixed with the 795 µl of distilled H₂O and subsequently, 200 µl of the Bradford reagent was added. After 5 minutes incubation at RT, protein concentration was measured by

photometer Uvikon 930 (Kontron Instruments) at 595 nm wavelength. The extraction buffer, which was used in the protein isolation was used for the blank sample.

2.2.2. GFP pull-down assay

To study *in vivo* protein-protein interaction, a GFP pull-down assay was performed using isolated proteins from mitochondria of the AT1G15480:GFP line. The GFP pull-down assay was done using the μ MACS™ anti-GFP Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) (Figure 5). For this aim, a total of 50 μ l of anti-GFP magnetic Microbeads was added to 3 μ g/ μ l of isolated proteins from mitochondria (2.3.10) and filled up to 1 ml with distilled water and incubated for 30 minutes on ice. The μ column was placed in the μ MACS™ separator (Miltenyi Biotec, Bergisch Gladbach, Germany) and equilibrated with 200 μ l of lysis buffer. The mixture of protein/anti-GFP magnetic Microbeads was added to the column and after 5 times of washing steps, the protein complex was eluted with 50 μ l of 95 °C of elution buffer. The immunoprecipitated proteins were separated in the SDS-PAGE (2.2.3) and following the gel excision analyzed by liquid chromatography-mass spectrometry (LC-MS).

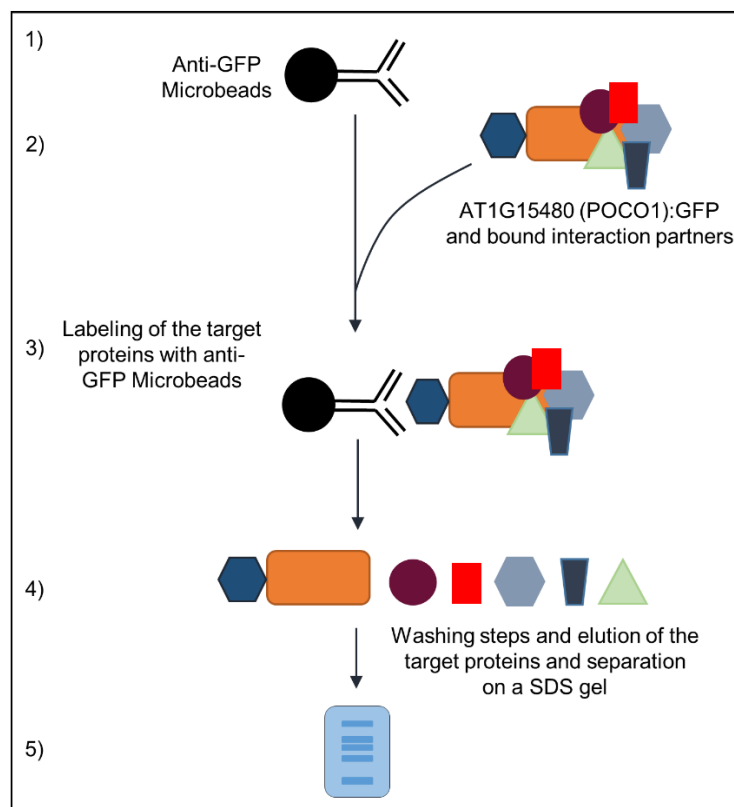


Figure 5: Schematic representation of the GFP pull-down assay. Isolated AT1G15480 (POCO1):GFP protein and associated interaction partners were incubated with anti-GFP magnetic Microbeads. Therefore, AT1G15480 (POCO1):GFP and bound interaction partners bind to the magnetic Microbeads. After several washing steps for removing the unbound non-specific proteins, proteins were eluted and separated in the SDS gel. Finally, protein bands on the SDS gel were analyzed by LC-MS.

2.2.3. SDS polyacrylamide gel electrophoresis (SDS-PAGE)

Protein analysis by sodium dodecyl sulfate (SDS) polyacrylamide gel was done in a small vertical electrophoresis unit (Laemmli 1970; Sambrook and Russell 2001). Stacking gel of 5% and separating-gel of 10-15% were prepared. The components of the separating gel and stacking gel were prepared as follows:

Stacking gel: 125 mM Tris-HCl, 5% (w/v) 37.5:1 acrylamide/bis-acrylamide (AA/BA), 0.1% (w/v) SDS, 0.01% (v/v) N,N,N',N' tetramethylethylenediamine (TEMED), 0.1 (w/v) Ammonium persulfate (APS) (pH 6.8)

Separating gel: 375 mM Tris-HCl, 10-15% (w/v) 37.5:1 acrylamide/bis-acrylamide (AA/BA), 0.1% (w/v) SDS, 0.04% (v/v) N,N,N',N' tetramethylethylenediamine (TEMED), 0.1 (w/v) Ammonium persulfate (APS) (pH 8.8)

Protein samples were then mixed with 0.1% (v/v) of 1 M dithiothreitol (DTT) and 5x SDS-loading buffer. The mixture was incubated for 10 minutes at 95 °C to denature the proteins and avoid the secondary, tertiary and quaternary structure of proteins. After placing the gel in the vertical electrophoresis unit and loading the samples into the stacking gel pockets, it was allowed to proceed at 150 V and 20 mA per gel for 1.5 to 2 h in 1x SDS-electrophoresis buffer. To identify the molecular weight, a protein molecular weight marker (Page Ruler™ Prestained Protein Ladder, Thermo Fisher Scientific) was run alongside samples.

5x loading buffer: 313 mM Tris-HCl, 10% (w/v) SDS, 0.05% (w/v) Bromphenol Blue, 50% (v/v) Glycerin (pH 6.8)

1x SDS-electrophoresis buffer: 25 mM Tris, 192 mM Glycine, 0.1% (w/v) SDS

2.2.4. Staining of proteins in the SDS gel by Coomassie Brilliant-Blue

Separated proteins in the SDS gel (2.2.3) were subsequently stained with the aid of Coomassie Brilliant-Blue dye. For this purpose, after separation of the stacking gel, separating gel was immersed in the Coomassie Brilliant-Blue dye for at least 2 hours at RT on a shaker. Then, the gel was placed in the destaining solution until the clear bands of proteins could be clearly visible without and gel background colour.

Coomassie Brilliant-Blue dye: 45% (v/v) Methanol, 10% (v/v) Acetic acid, 0.5% (w/v) Coomassie Brilliant-Blue R-250

Destaining solution: 45% (v/v) Methanol, 10% (v/v) Acetic acid

2.2.5. Protein identification by LC-MS

After separation of protein samples of GFP pull-down assay (2.2.2) in the SDS gel, following the gel excision, they were analyzed by LC-MS. LC-MS analysis was performed by the department of Plant Proteomics headed by Prof. Dr. Hans Peter Braun at the University of Hannover.

2.3. Experiments with *Arabidopsis*

2.3.1. Culturing of *Arabidopsis thaliana*

In this study, *A. thaliana* ecotype WS-4 (Wassilewskija) as wild-type and the T-DNA insertion line FLAG_465F03 was used as mutant plants. FLAG_465F03 was obtained from Versailles Arabidopsis Stock Center (INRA; <http://publiclines.versailles.inra.fr/>) and has a T-DNA insertion in *AT1G15480* gene (Samson et al. 2002).

A. thaliana plants were grown under controlled conditions in the growth chamber at 22 °C with 65% relative humidity, a light intensity of 110 $\mu\text{Mol m}^{-2} \text{s}^{-1}$ and the photoperiod of 16 hours light and 8 hours dark (Long-day) or 8 hours light and 16 hours dark (short-day). A mixture of sand and peat was used for the cultivation of plants.

Leaf samples for RNA-seq analysis were harvested two and a half hours after the start of the day period.

2.3.2. Flowering time measurements

For analyzing the flowering time differences between wild type and mutants, plants were grown under controlled conditions in growth chambers and either number of days from sowing to flowering was recorded or the number of rosette leaves on the day when the first flower opened was calculated.

2.3.3. Stable transformation of *A. thaliana*

Stable transformation of *A. thaliana* was done by *Agrobacterium*-mediated transformation (Floral dip) method on the base of Clough and Bent 1998 protocol.

A. thaliana plants were grown till the point they had flowers. Subsequently, the flowers were removed to stimulate the formation of secondary shoots. A 10 ml of selective 2x YT pre-culture (2.1.2) with appropriate antibiotics were inoculated with the *Agrobacterium* colony and incubated overnight at 220 rpm at 28 °C. The following, 200 ml the main culture with appropriate antibiotics were inoculated with 1% of the pre-culture and was incubated overnight at 220 rpm at 28 °C. The bacterial culture (OD600: 0.6-0.8) was centrifuged (Beckman Coulter, GS-15R) at 4.000 rpm for 10 minutes and the cell pellet was resuspended in 5% sucrose and 0.05% Silwet L-77 ml infiltration medium. The infiltration medium was applied to the flowers. In order to maintain high humidity, the treated plants with infiltration medium were lying under a plastic cover for the next 16 to 24 hours and then they were transferred to the growth chamber under the controlled condition to complete the life cycle and compose the siliques.

Infiltration medium: 5% (w/v) sucrose, 0.02-0.05% (v/v) Silwet L-77

2.3.4. *Arabidopsis* root culture (ARC)

Arabidopsis root culture was set up according to the modified protocol of Czakó, M, Wilson J, Yu X 1993. The 500 ml Erlenmeyer flasks were filled up with 200 ml ARC-medium and then were autoclaved at 112 °C. After autoclaving the ARC-medium about 20 sterilized 7-day-old *Arabidopsis* seedlings, which were grown on the MS-medium (2.1.2) were dropped into the ARC-medium and were incubated at 80 rpm at 25 °C in the dark. After 2 weeks, Indole-3-acetic acid (IAA) to the end concentration of 0.05 g/l was supplemented to the root culture medium and after 1 week the medium was substituted by a fresh ARC-medium without IAA. After an overall 6 weeks, a dense root system was established, which was used to isolate mitochondria.

Miller's solution: 0.43% (w/v) KH_2PO_4

Vitamin stock solution: 0.05% (w/v) Vitamin B1 (Thiamine hydrochloride), 0.005% (w/v) Vitamin B6 (Pyridoxine hydrochloride), 0.01% (w/v) Glycine, 0.005% (w/v) Vitamin B3 (Nicotinic acid), 0.025% (w/v) Vitamin B9 (Folic acid), 0.005% (w/v) Vitamin H (Biotin)

ARC-Medium: 0.43% (w/v) MS basal salts with minimal organics (Sigma-Aldrich), 0.3% (v/v) Miller's solution, 0.02% (w/v) Myo-inositol, 0.2% (v/v) Vitamin mixture, 3% (w/v) Sucrose, (pH 5.8, KOH)

2.3.5. Isolation of RNA from *A. thaliana*

Standard isolation of total RNA from *A. thaliana* was performed based on the modified protocol of Manickavelu et al. 2007. Frozen plant materials were ground and 2 ml tubes were filled with about 0.5 ml of the ground material and 500 µl of boiling RNA lysis buffer was added. After mixing thoroughly, 0.5 µl of 60 °C Phenol was added to the mixture and the phenolic and aqueous part was separated by centrifugation at 14.000 x g for 10 minutes. The upper phase was transferred into a new tube and mixed two times with 0.5 µl phenol and 0.5 µl chloroform/isoamyl alcohol (24:1). After centrifugation, the upper phase was taken and 0.5 µl of chloroform/isoamyl alcohol (24:1) was added to completely remove the impurities of proteins. Precipitation of nucleic acids was done by adding 1/10 volume 3 M sodium acetate and 2x volume absolute ethanol for 30 min at -80 °C. After centrifugation, the pellet was resuspended in 4M LiCl to precipitate RNA overnight at 4 °C. RNA was pelleted by centrifugation at 14.000 x g for 15 minutes and after washing the pellet twice with 70% (v/v) ethanol, it was dried for 5 minutes and was resuspended in diethyl pyrocarbonate (DEPC)-treated water.

RNA lysis buffer: 0.2 M Boric acid, 30 mM EDTA, 1% SDS (pH 9.0)

For RNA sequencing, RNA was isolated using TriFast™ (Peqlab, Erlangen, Germany) following the manufactures instructions after homogenization of the plant material. Plant material was lysed in 1 ml TriFast™ reagent per 100 mg plant tissue. After vortex and incubation for 5 minutes at RT. 200 µl chloroform was added and the solution was shaken for 15 seconds and incubated for 5 minutes at RT. After centrifugation at 4700 rpm, the aqueous phase containing RNA was separated and transferred to a new tube. Precipitation of RNA was done by adding 500 µl isopropanol. After centrifugation at 4700 rpm at 4 °C, the RNA pellet was washed with 70% ethanol. The isolated RNA was stored in at -80 °C.

2.3.6. Detecting superoxide radicals in leaves

To detect ROS in *Arabidopsis*, nitroblue tetrazolium (NBT) staining according to the method of Xie et al. 2016 was used. NBT staining is utilized to detect superoxide radicals produced in the plant tissues. NBT undergoes reduction by superoxide radicals to form diformazan, which is a highly water-insoluble dark blue precipitate (Flohé and Ötting 1984; Beyer and Fridovich 1987).

Seedlings or detached leaves were first incubated in the reaction buffer for 20 minutes for one hour. Then, the plant materials were washed three times with water. In order to make the NBT visible, they transferred into the acetic acid: ethanol (1:3, v/v) solution to extract chlorophyll for proper visualization under the binocular.

Reaction buffer: 1 mM NBT, 20 mM KH₂PO₄ (pH 6.0)

2.3.7. Measurement of respiration

In order to analyze the respiration rate, oxygen consumption was measured according to (Walker and Walker 1987) using an oxygen electrode system (Oxytherm, Hansatech Instruments, England) at 20 °C. For this purpose, the dark-cultivated ARC (2.3.4) was used. The reaction buffer included dark-cultivated roots in 1.5 ml *Arabidopsis* root culture medium in the measurement chamber. For calibration, the *Arabidopsis* root culture medium was used. The analysis was done under continuous stirring at 20 °C and the amount of oxygen consumption was expressed relative to the dried mass of roots.

2.3.8. ATP level measurement

ATP was measured using the luciferin-luciferase assay. The experiment is based on bioluminescent reactions catalyzed by firefly luciferase, which requires ATP. The firefly luciferase catalyses the oxidation of luciferin to oxyluciferin using ATP, which yields to light emission at 560 nm. Under optimum conditions, the intensity of light is proportional to the concentration of ATP. Therefore, the measurement of the light intensity leads to the quantification of ATP. 50 µl of 10 mg/ml firefly lantern extract (FLE-250; Sigma-Aldrich), which is a crude luciferin and luciferase was added to the isolated mitochondria from the dark-grown root culture (2.3.9) of wild-type and *poco1* in an ATP-reaction buffer. The light emitted from the reaction was measured using a Plate-Reader (Tecan-GENios). A standard curve was generated by different dilutions of ATP, which was used to quantify the ATP produced by mitochondria.

ATP-reaction buffer: 50 mM Tricine-KOH, 6.25 MgCl₂ and 300 mM mannitol (pH 7.4)

2.3.9. Isolation of Mitochondria from ARC

In order to isolate intact mitochondria from root culture, the simplified protocol of (Klein et al. 1998) was used. After homogenization of dark-grown roots with a mixer in the extraction buffer, cells were harvested by filtration over an absorbent gauze (zigzag folded, four-ply, Hartmann).

The harvested cells were then poured into the centrifuge buckets and centrifuged at 2.000 x g (JA25.50-Rotor, Beckman Coulter) for 5 min at 4 °C. The supernatant was filtered over an absorbent gauze (zigzag folded, two-ply, Hartmann) to remove cell debris and was again centrifuged at 16.000 x g (JLA16.250-Rotor, Beckman Coulter) for 10 min at 4 °C. The pellet was slowly resuspended in the wash buffer with a fine brush and the suspension was applied on a four parts sucrose gradient (from down: 8 ml 52%, 10 ml 40%, 10 ml 36% and 4 ml 20%) and centrifuged with a TST28.38-Rotor of Optima TM L-90 K Ultracentrifuge (Beckmann Coulter, Krefeld) for 2 hours at 24.000 x g at 4 °C. The mitochondrial band, which appeared as a yellow-brown band between 52% and 40% gradient was collected and poured into the SS34-tubes. After diluting with the resuspension buffer by centrifugation at 14.000 x g for 10 min at 4 °C, mitochondria were sedimented and were taken up in 1 to 2 ml of resuspension buffer.

Extraction buffer: 450 mM Sucrose, 15 mM MOPS, 1.5 mM EGTA-KOH, 10 mM DTT, 6 g/L Polyvinylpyrrolidon, (pH 7.4)

Mitochondria base buffer: 500 mM Tris-HCL, 63mM EDTA, 1% (w/v) BSA (pH 7.5)

Wash buffer: 300 mM sucrose, 10 mM MOPS, 1 mM EGTA-KOH (pH 5.2)

Resuspension buffer: 400 mM Mannitol, 10 mM Tricine, 1 mM EGTA-KOH (pH 7.2)

2.3.10. Isolation of protein from mitochondria

In order to isolate soluble proteins from mitochondria, the modified method of Takenaka and Brennicke 2007 was used. For this aim, 40 mg (40 mg mitochondria is equal with 4 mg of the mitochondrial protein based on Eubel et al. 2004) of isolated mitochondria from ARC was used. After centrifugation of mitochondria at 14.000 rpm for 10 minutes at 4 °C, the mitochondrial pellet was resuspended in 100 µl of cold extraction buffer, which was followed by 30 minutes of incubation on ice. In order to separate the soluble and insoluble proteins, the mixture was centrifuged for 30 minutes at 18.000 x g at 4 °C. The supernatant was kept as the soluble protein and used for the GFP pull-down assay.

Protein extraction buffer: 30 mM HEPES, 3 mM Magnesium-Acetate, 2 M KCL, 0.2% Triton x-100, 1 mM AEBSF, (pH 7.7)

2.4. Studying the transcriptome with RNA sequencing

2.4.1. Analysis of RNA-Sequencing data

RNA was isolated from wild-type and mutant plant leaves using TriFast™ (2.3.5) and were sequenced by GATC Biotech AG (Konstanz, Germany) using Illumina technology by HiSeq 2000 machine (125 bp paired-end reads). All steps were developed and validated by GATC Biotech AG. Obtained sequences were evaluated by the CLC Genomics Workbench 7.5.1 program from CLC Bio (Qiagen, Hilden) based on the principles of Mortazavi et al. 2008. The raw RNA-seq data were initially trimmed and were undergone a quality control to have a visual inspection of the sequencing data. Then, the trimmed sequences were mapped to the *A. thaliana* reference genome from the 1001 Genome project (<https://1001genomes.org/>). To evaluate gene expression of the mitochondrial genes, the *Arabidopsis* mitochondrial genome reference sequence was obtained from NCBI (accession number NC_037304). The annotated gene model was used according to *Arabidopsis* genome annotation (TAIR10). Expression levels derived from the RNA-seq data Reads per Kilobase per Million mapped reads (RPKM) (Mortazavi et al. 2008) and fold changes were reported using CLC Genomics Workbench 7.5.1. CLC Genomic workbench 7.5.1 follows RNAseq protocol proposed by (Mortazavi et al. 2008). FDR p-value of < 0.05 was considered as the cut-off threshold value for the identification of significant expression differences (Benjamini and Hochberg 1995).

The gene ontology (GO), of differentially expressed genes, were performed using g:profiler online tool (Reimand et al. 2007) and Venn diagrams were made by the online tool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>). Various gene categories involving in flowering, ABA signaling, drought and oxidative stresses, redox state and signaling genes were identified based on the GO described in The Arabidopsis Information Resource (TAIR) website (<http://www.arabidopsis.org/>). Transcription factors were identified from the DAVID bioinformatics resources 6.7 database (Huang et al. 2009a, b).

RNA-seq data have been deposited in the ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-8912 (<http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-8912/>).

2.4.2. RNA editing analysis from RNA-seq data

For RNA editing analysis using RNA-seq data, the *Arabidopsis* mitochondrial genome reference sequence was obtained from NCBI (accession number: NC_037304). The reads were mapped to the reference sequence to navigate RNA editing sites. The editing percentage was calculated by counting the reads containing C or T divided to the sum of total numbers of reads.

2.5. Data banks and software

In order to catch the information about PPR proteins and other gene sequences as well as the structure and localization of the proteins, the following web sites were used:

-**DAVID bioinformatics resources 6.8** (<https://david.ncifcrf.gov/summary.jsp>)

-**TAIR:** The Arabidopsis Information Resource (<http://www.arabidopsis.org/>)

-**NCBI:** National Center for Biotechnology Information (<http://www.arabidopsis.org/>)

-**ExpASY:** Bioinformatics Resource Portal (<http://www.expasy.org/>)

-**Unipro UGENE** (<http://ugene.net/>)

-**1001 Genomes** (<https://1001genomes.org/>).

-**Bioinformatics & Evolutionary Genomics**
(<http://bioinformatics.psb.ugent.be/webtools/Venn/>).

3. Results

In this study the following formats for genes, proteins, mutants and mRNAs are used:

genes such as *AT1G15480*, *POCO1* (capital italic)

proteins such as AT1G15480, POCO1 (capital)

mutants such as *poco1* (small italic)

mRNAs such as *cox2* (small italic)

Prior to this study, searching for editing factors of an editing site in a 40 nt long *cox2* mRNA fragment, protein AT1G15480 was identified (Bolle 2008). The corresponding gene *AT1g15480* possesses an ORF of 1782 bp encoding a protein of 594 amino acids and a molecular weight of 67.5 kDa. The predicted transit peptide constituted of the first 67 amino acid residues at the N-terminus. Using the Unipro UGENE software (<http://ugene.net/>) (Okonechnikov et al. 2012), a structural model of AT1G15480 was obtained (Figure 6A). The mitochondrial localization of AT1G15480 was previously shown in *Arabidopsis* protoplasts transformed with AT1G15480:GFP stained with MitoTracker® Orange CM-H2TMRos (Bruhs 2012), indicating that AT1G15480 is a nucleus-encoded protein, targeted to mitochondria.

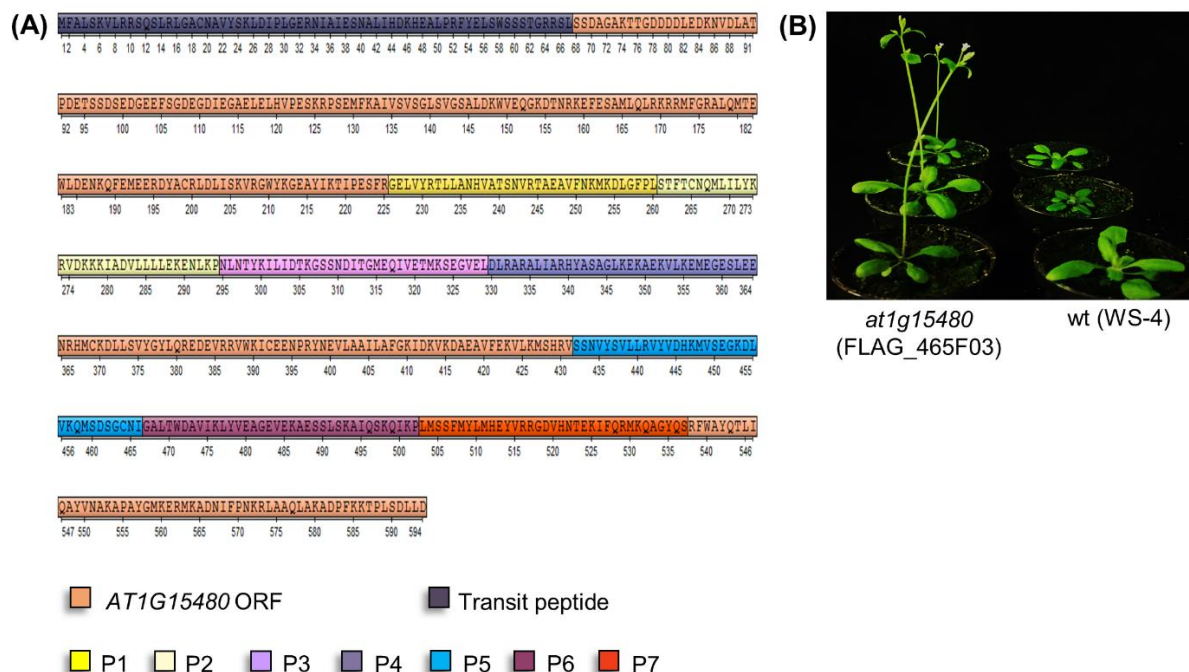


Figure 6: Amino acid sequence of AT1G15480 and position of PPR motifs as well as the phenotype of the T-DNA insertion line (FLAG_465F03). (A): AT1G15480 consists of 594 amino acids and seven P-type PPR motifs: P1 (226-260), P2 (261-294), P3 (295-329), P4 (330-364), P5 (432-466), P6 (467-502) and P7 (503-537). P-type PPR motifs, as well as the N-terminus transit peptide, are marked with different colours. (B): The T-DNA insertion into the *AT1G15480* led to an early-flowering phenotype.

As seen in Figure 6A, *AT1G15480* encodes a PPR protein with tandem arrays of seven predicted PPR motifs and appears to be classified as a P-type member of PPR proteins. *AT1G15480* does not have any C-terminal extension domains like E/E⁺ or DYW domains, which can only be found in the PLS-type of PPR proteins, proposed by Lurin et al. 2004. *AT1G15480* constitutes of three exons and two introns in its coding sequence. A T-DNA insertion line in *AT1G15480* (FLAG_465F03) was acquired. The homozygous T-DNA insertion line showed an early-flowering phenotype compared with wild-type plants (Figure 6B) (Bruhs 2012). In the following sections, the molecular function of *AT1G15480* in *A. thaliana* is analyzed.

3.1. *poco1* displays an early-flowering phenotype under long- and short-day photoperiods

Photoperiod is one of the major pathways of flowering and an important factor among other components of environmental light in *Arabidopsis* (Cerdán and Chory 2003). *Arabidopsis* is a facultative long-day plant, meaning it usually needs long-day conditions to flower (~ 16 hours light; 8 hours dark), but it eventually also flowers under short-day conditions (~ 8 hours light; 16 hours dark). However, the plants take longer to flower under short-day conditions. *poco1* plants were found to exhibit an early-flowering phenotype under long-day conditions (Bruhs 2012). To investigate if the floral transition in *poco1* plants is affected by photoperiod, flowering time was analyzed under the short-day photoperiod. Both, wild-type and *poco1* plants, were simultaneously grown under long- and short-day photoperiods. *poco1* plants displayed a similar phenotype in long- and short-day conditions. In both cases, they exhibited an early-flowering phenotype compared to wild-type plants (Figure 7C). There was a significant difference in the number of days to flowering between wild-type and *poco1* plants in both long- and short-day conditions (Figure 7B and D). Under the long-day condition, wild-type plants took in average 29-30 days to flower, while *poco1* plants took 24-25 days to flower (Figure 7B). Under the short-day condition, both plant lines showed a delay in transition to the flowering of about 20 days in comparison to long-day conditions, but still, *poco1* flowered 9 days earlier than wild-type plants (Figure 7D). The flowering time was also determined by counting the total number of rosette leaves when the first flower opened in both long- and short-day conditions. There is a strong correlation between flowering time and number of rosette leaves (Koornneef et al. 1991). Number of rosette leaves at flowering also confirmed the early-flowering phenotype of *poco1* compared to wild-type plants (Supplementary Figure 1A and B). The rosette leaf number at the time of flowering was lesser in *poco1* compared with wild-type plants under both long- and short-day conditions. The early-flowering phenotype of *poco1* was very

clear and consistent, under both long- and short-day conditions. Due to the observed phenotype, the PPR protein was named PRECOCIOUS1 (POCO1).

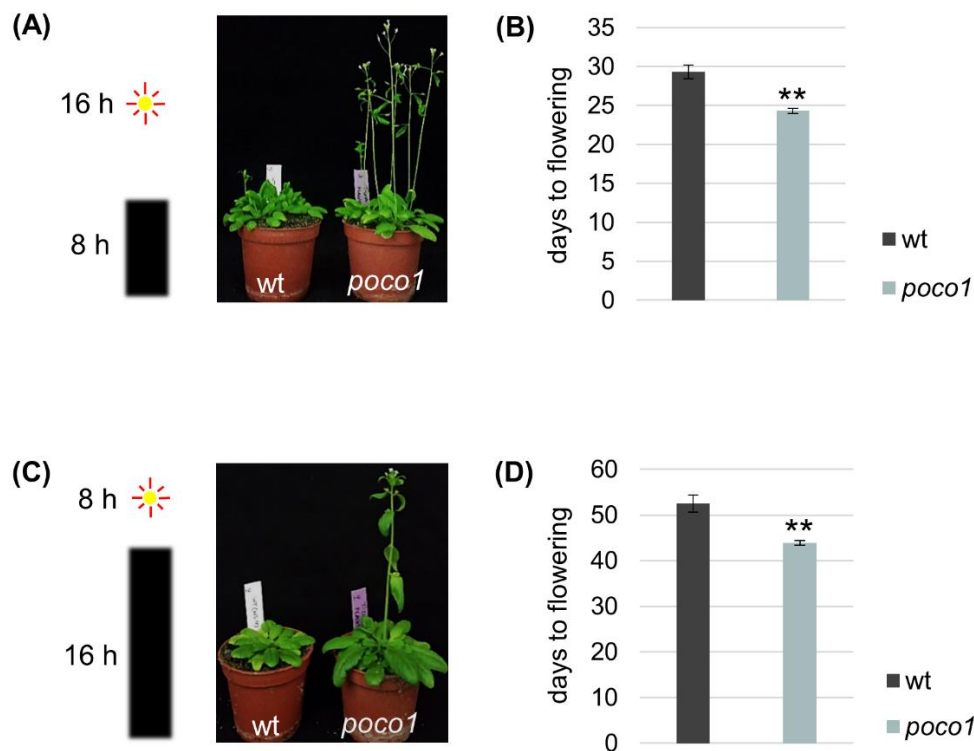


Figure 7: Phenotype of *poco1* plants under long- and short-day conditions. *poco1* plants showed an early-flowering phenotype under both long- and short-day conditions. (A): Wild-type and *poco1* plants grown under the long-day photoperiod. *poco1* plants exhibit an early-flowering phenotype compared to wild-type plants under the long-day condition. The picture was taken 27 days after sowing. (B): Measurement of the flowering time in wild-type and *poco1* plants grown under the long-day photoperiod. *poco1* flowered 5 days earlier than wild-type plant under the long-day condition. Values are means \pm standard errors. (C): Wild-type and *poco1* plants grown under the short-day photoperiod. *poco1* plants exhibit an early-flowering phenotype compared to wild-type plants under the short-day condition. The picture was taken 46 days after sowing. (D): Measurement of the flowering time in wild-type and *poco1* plants grown under the short-day photoperiod. *poco1* flowered 9 days earlier than wild-type plants under the short-day conditions. Values are means \pm standard errors. A significant difference was observed in flowering time between wild-type and *poco1* in both long- and short-day conditions. Asterisks indicate a significant difference at $p \leq 0.01$ by student's *t*-test.

3.2. Complementation of *poco1* restored the normal flowering time

To gain insights into the involvement of POCO1 in the early-flowering phenotype and to confirm the early-flowering phenotype being caused by the T-DNA insertion in *AT1G15480* locus, a complementation analysis by introducing the wild-type *POCO1* gene in the *poco1* knockout-background was performed. For this purpose, a complementation vector (pHE800) was created by using Gateway™ technology.

The Gateway™ technology is an efficient and quick way to clone genes for functional studies on the base of the site-specific recombination features of lambda bacteriophage (Figure 8).

Two recombination reactions according to the manufacturer's instructions were done: The BP reaction was used to facilitate the recombination of an *attB* PCR product (*AT1G15480* flanked by *attB*) with an *attP* substrate as a donor vector, pDONR201 (Supplementary Figure 2), which results in an entry clone containing *attL* recombination site. The LR reaction was used to enable the recombination of an *attL* of the entry clone, pHE879 (Supplementary Figure 3) with an *attR* substrate (destination vector) (pB2GW7) (Supplementary Figure 4). The pB2GW7 vector is an *Agrobacterium*-mediated plant transformation binary vector, which contains cauliflower mosaic virus 35S promoter (p35S), terminator (t35S) and *BaR* selection marker that confers resistance to the herbicide phosphinothricin (Basta™). After performing BP and LR reactions, the new vector, pHE800 (Figure 9A), was created and transformed into the *poco1* plants. Transformants were selected on MS medium containing glufosinate ammonium (50 mg/ml).

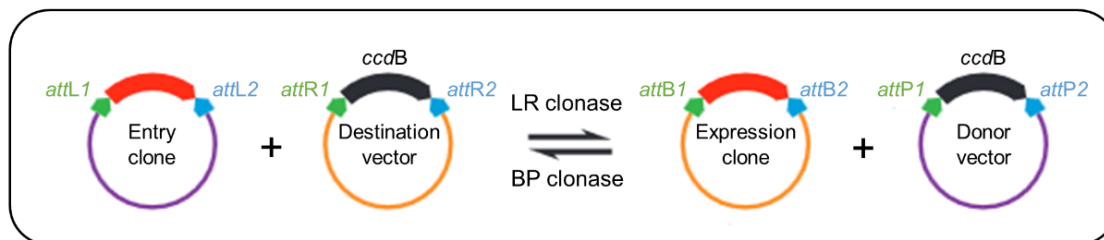


Figure 8: The Gateway reactions. The schematic representation of the Gateway cloning reactions including different plasmids and enzymes. Red arrows show the fragment of interest. A PCR product amplified with oligonucleotides containing *attB* sequence. The gene of interest flanked by *attB* sequences is combined with a donor vector that contains *attP* sequences with the enzyme BP clonase to generate the entry clone containing *attL* sequences. The gene of interest flanked by *attL* sequences is then used to recombine with *attR* sequences with the enzyme LR clonase to create the desired expression clone. From the Gateway cloning technology manual, Invitrogen™.

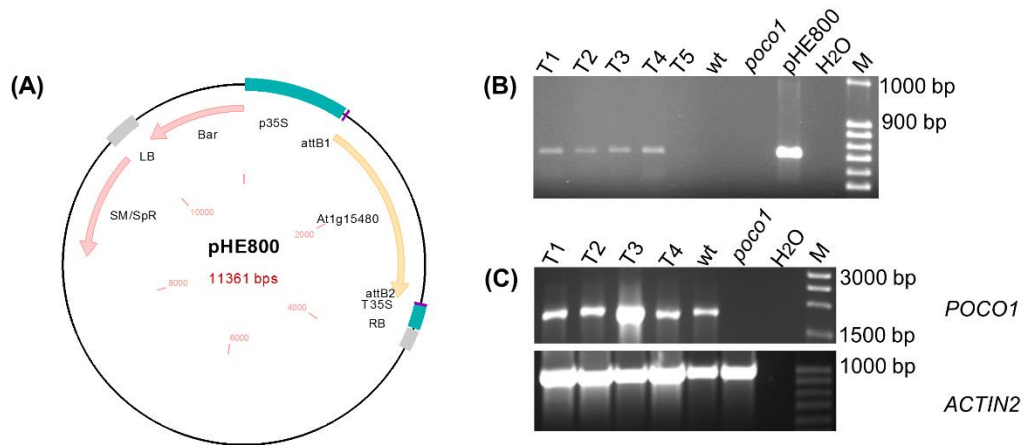


Figure 9: Complementation analysis vector, pHE800 and verification of the successful introduction of wild-type *POCO1* gene to *poco1* plants. The wild-type *POCO1* gene was successfully transferred to *poco1* background and was transcribed. **(A):** pHE800 is a binary vector which is used for *Agrobacterium*-based transformation. Coloured regions represent resistance genes *Sm/SpR* (Streptomycin/Spectinomycin, pink arrows) and *BaR* (Bialaphos, pink arrows), *Left* and *right border* (LB and RB, grey region), promoter and terminator of cauliflower mosaic virus (p35S and t35S, blue boxes), Gateway™ attachment sites (*attB1/attB2*, purple region) and *AT1G15480* gene (yellow arrow). **(B):** After selection with glufosinate ammonium five transformants were verified by the amplification of a 722 bp pHE800 vector-specific fragment using FK589 and AB1451 oligonucleotides. For positive control, the pHE800 vector was used. **(C):** RT-PCR detection of *POCO1* in four transformants as well as wild-type using oligonucleotides AB2060 and AB2061 (upper panel). The integrity of RNAs was tested by the amplification of a 1000 bp fragment of the *ACT2* house-keeping gene (lower panel).

Selected plant transformants were transferred to pots with soil for further molecular and phenotypic analysis. Two oligonucleotides were used: FK589 binding to the 35S promoter region and AB1451 binding to the position 507 in *POCO1* gene. By nature, these oligonucleotides can not amplify a DNA fragment from wild-type as well as *poco1* plants. For five transformants (T1-T5) a 722 bp DNA fragment was amplified using oligonucleotides FK589 and AB1451 (containing the CaMV 35S promoter and the *POCO1* gene) (Figure 9B). There was no amplification of this DNA fragment for both wild-type and *poco1* plants. As a positive control, the complementation analysis vector pHE800 was used and the corresponding DNA fragment, which lies inside of the T-DNA region was successfully amplified. On the base of these results, it was concluded that the expression cassette was successfully transformed into plants with the *poco1* background. To confirm transcription of *POCO1* RT-PCR was performed using transformants T1-T4. The full-length coding sequence of *POCO1* was amplified for T1-T4 and wild-type plants using oligonucleotides AB2060 and AB2061 (Figure 9C, upper panel). The amplified DNA fragment was not detected in *poco1* plants. To proof the integrity of isolated RNAs, another RT-PCR was conducted to amplify a sequence of 1000 bp of the house-keeping gene *ACT2* for transformants T1-T4, wild-type and *poco1* plants (Figure 9C, lower panel).

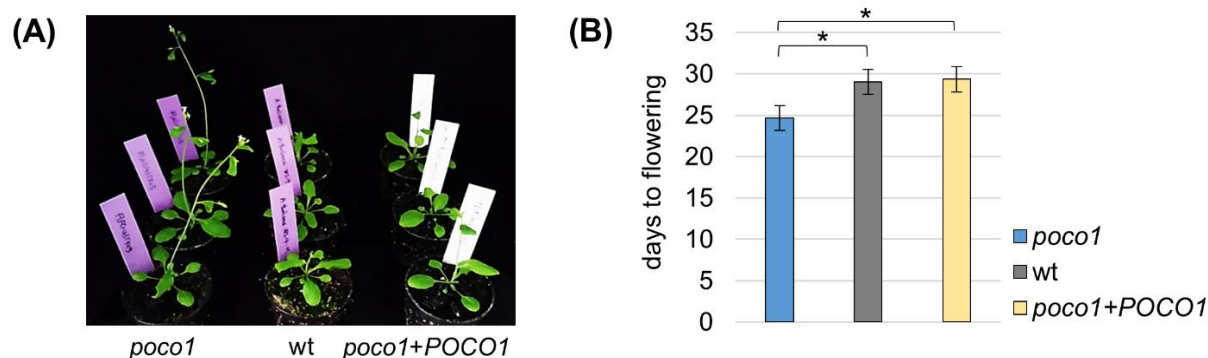


Figure 10: Genetic complementation of the homozygous *poco1* mutant line. The complemented *poco1* plants restored the wild-type phenotype. **(A):** 25-day-old wild-type, *poco1* and complemented mutant plants (*poco1+POCO1*). Complementated mutant plants exhibited the same phenotype as wild-type plants. **(B):** The graph of days to flowering for wild-type, *poco1* and complemented mutant plants (*poco1+POCO1*). The bars represent the means \pm standard errors of each genotype (n=12). No significant difference was observed between wild-type and complemented mutants. A significant difference was observed between *poco1* and wild-type as well as *poco1* and complemented mutants. Asterisks indicate a significant difference of $p \leq 0.05$ by student's *t*-test.

Analyzing the complemented mutants for the restoration of the normal flowering time indicated that the observed early-flowering phenotype in *poco1* plants was reversed in the complemented mutant plants (Figure 10A). As seen in Figure 10B, *poco1* flowered 5 days earlier than wild-type and complemented mutant plants.

3.3. Generating a POCO1:GFP transgenic line

PPR proteins have been suggested to be special factors for effector proteins. Furthermore, several PPR proteins were found to be part of protein complexes. For instance, PPR proteins function in RNA editing occurs by interacting with other PPR proteins and several non-PPR editing factors as a multi-component protein complex (Boussardon et al. 2012; Takenaka 2014; Sun et al. 2016). POCO1 was originally identified in an RNA pull-down assay, using a 40 nt long *cox2* mRNA fragment (Bolle 2008). The identification of native protein interaction partners is a crucial step to decode the molecular mechanisms of biological processes. Putative interaction partners, from which subcellular localization data and functions have been characterized, could give more pieces of evidence about POCO1's function. Therefore, identification of POCO1's interaction partners would be highly desirable.

As there is no antibody available for POCO1, to identify the putative *in vivo* interaction partners of POCO1, a *POCO1:GFP* transgenic line was established to allow for a GFP pulldown with an GFP antibody. For this aim, a fusion of *POCO1* with the *GFP* open reading frame in a plant-specific binary vector was created (Figure 11).

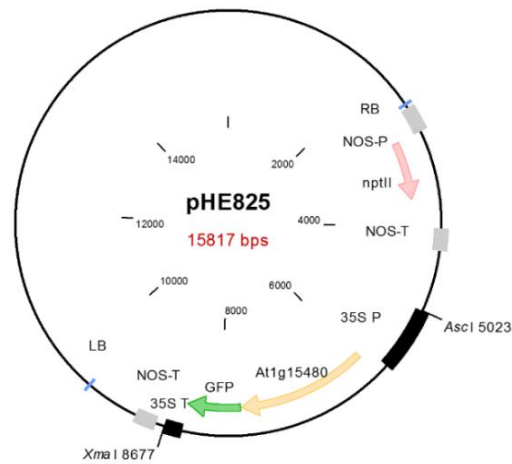


Figure 11: The transformation vector, pHE825, for creating POCO1:GFP fusion protein. pHE825 is a binary vector, which is used for *Agrobacterium*-based transformation. Coloured regions represent resistance gene *nptII* (neomycin-phosphotransferase Typ II, pink arrow), *Left* and *right border* (LB and RB, blue region), promoter and terminator of cauliflower mosaic virus (p35S and t35S, black boxes), *Ascl* and *XmaI* hydrolysis sites, *Nopaline synthase* promoter and terminator (*NOS-P* and *NOS-T*, grey boxes), *POCO1* (*AT1G15480* cDNA, yellow arrow) and Green Fluorescent Protein (GFP, green arrow).

The selectable marker gene, *nptII*, which encodes for kanamycin antibiotic resistance allows the selection of successfully transformed plants. After transformation of pHE825 into the *A. thaliana* wild-type plants by floral-dip, transformants were selected on MS medium containing kanamycin. Putative transformants were verified by PCR as well as analyzed by confocal laser scanning microscopy (CLSM) microscope. To verify the transformant by PCR, the whole expression cassette (3664 bp) was amplified using oligonucleotides KA2297 and KA2298. The selected DNA fragment is not present in the genome of wild-type plants. As seen in Figure 12A no DNA amplification was observed for wild-type. However, a DNA fragment representing the expected size (3664 bp) can be observed for transformants T1-T3 and the positive control, pHE825. Root tips contain rapidly growing cells with a high metabolism rate and also a high number of mitochondria. In Figure 12B, C and D, GFP signals in the root tip of transformants can be observed, which refer to the expression of *POCO1:GFP*.

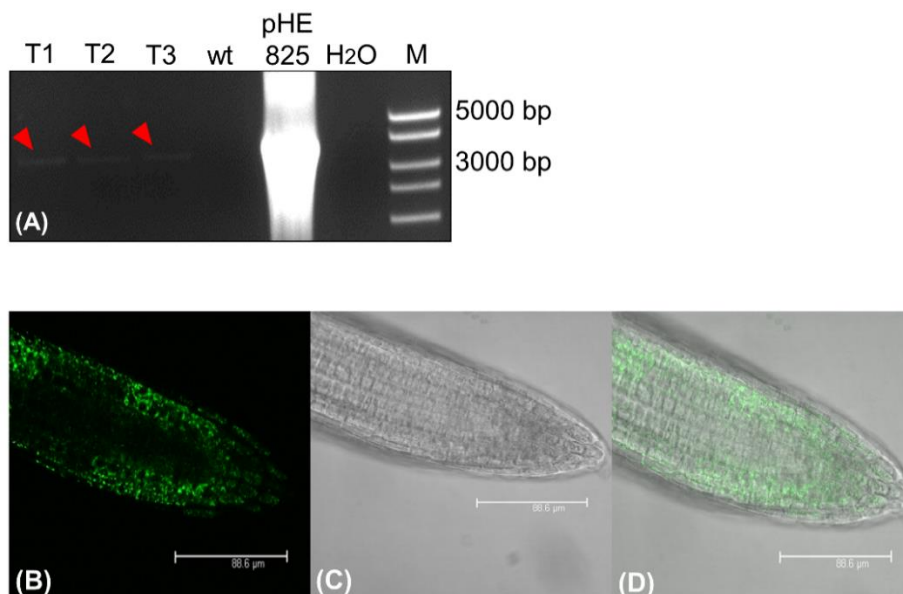


Figure 12: Verification of *POCO1:GFP* expression in root tips of *Arabidopsis* seedlings. The expression of *POCO1:GFP* was verified in transformants. **(A):** PCR amplification of the transformed expression cassette, *POCO1:GFP*, in transgenic plants as well as pHE825 vector as positive control by KA2297 and KA2298 oligonucleotides. KA2297 and KA2298 oligonucleotides amplified *POCO1:GFP* cassette in transformants T1-T3 and pHE825. No *POCO1:GFP* amplificate for the wild-type line was observed. **(B):** GFP signals in the root tip of transgenic *Arabidopsis*, expressing a *POCO1:GFP* a wild-type (WS-4) background. **(C):** Differential interference contrast. **(D):** Merged picture. Images were acquired by the CLSM.

3.4. Identification of putative *in vivo* interaction partners of POCO1

After isolation of the whole proteins from mitochondria of *POCO1:GFP* line under native conditions and using an antibody against GFP, a GFP pull-down assay was performed. Intact mitochondria were isolated from the *POCO1:GFP* line dark-grown root culture and the whole proteins were isolated from mitochondria. Subsequently, the GFP pull-down assay was performed. To distinguish unspecific putative interaction partners, a GFP pull-down assay was carried out with the whole isolated proteins from wild-type mitochondria.

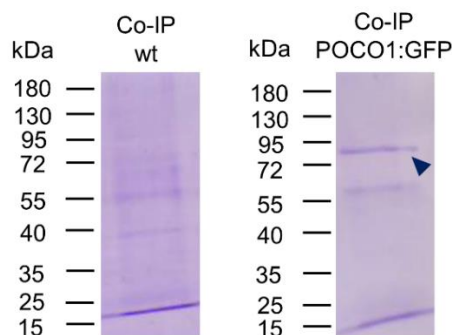


Figure 13: The GFP pull-down assay proteins separated on the SDS gel. A GFP pull-down assay was performed with wild-type and *POCO1:GFP* line and GFP antibodies using μ MACS™ Anti-GFP Kit (Miltenyi Biotec, Bergisch Gladbach). The immunoprecipitates were separated by the SDS gel. The proteins in the gel were stained with Coomassie Brilliant Blue. The band, which was supposed to represent *POCO1:GFP* size is shown with a blue triangle.

The putative *in vivo* interaction partners of POCO1 were identified by LC-MS (Supplementary Table 2). Generally, in pull-down assays, it is expected to observe the immunoprecipitated protein on the SDS gel or to identify it by LC-MS analysis. The mass of POCO1 is 67.5 kDa and the mass of GFP is 26.9 kDa. As it is shown in Figure 13, a protein band, which represents the size of POCO1:GFP was observed. After the LC-MS analysis, POCO1 was also identified by LC-MS analysis (Supplementary Table 2). Among the identified proteins, PPR596, MITOCHONDRIAL HEAT SHOCK PROTEIN 70-1 (mtHsc70-1) and (mtHsc70-2) were experimentally shown to be localized in mitochondria (Herrmann et al. 1994; Doniwa et al. 2010). Based on homology detection and protein sequence alignment, it was shown that PPR596 is the paralogous gene for POCO1 (Emami and Kempken 2019). PPR596 affects the efficiency of an editing site in the mitochondrial *ribosomal protein S3 (rps3)* (Doniwa et al. 2010). Mitochondrial heat shock proteins are components of the stress response and are involved in RNA metabolism and protection (Hsu et al. 2018).

3.5. POCO1 affects the editing efficiency of *rps3*

PPR proteins are involved in post-transcriptional processes and have a range of necessary roles within organelles (Schmitz-Linneberger and Small 2008). RNA editing is crucial for organellar RNA metabolism. The GFP pull-down for POCO1 identified PPR596 as one of the putative POCO1-interacting proteins (3.4). PPR596 is involved in partial editing of the *rps3* transcript (Doniwa et al. 2010). Therefore, it was investigated if POCO1 has a site-specific influence on the same transcript. Using RT-PCR, the involvement of POCO1 in editing efficiency of the editing position: *rps3*-1344 was verified (Figure 14A and B). In the mutant there is a detectable reduction of editing efficiency from 54% to 34% at position 1344. Since this editing site is a silent position, functional proteins are assumed to be translated in *poco1* as they in wild-type plants.

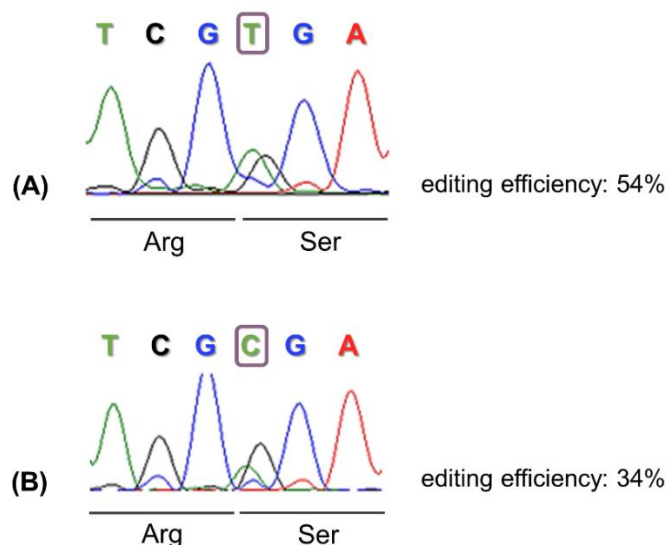


Figure 14: POCO1 influences the editing efficiency of *rps3*. The alteration of the editing extent of *rps3*-1344 in wild-type (A) compared to *poco1* plants (B). Editing status of *rps3*-1344 was obtained by the direct sequencing of RT-PCR products via Sanger sequencing. The editing status is shown by an electropherogram. The 6-nucleotide sequences encompassing the position 1344 (square) are shown and two codons are underlined. The RNA editing efficiencies for wild-type and *poco1* are calculated as 54% and 34% respectively. Analysis of editing in the *rps3*-1344 position reveals that editing efficiency is decreased in *poco1* plants. Arg: Arginine, Ser: Serine.

3.6. POCO1 affects several mitochondrial editing sites

To explore the potential involvement of POCO1 in other mitochondrial editing events, the editing status of mitochondrial transcripts in *poco1* was analyzed using the RNA-seq data of wild-type and *poco1* plants. The editing extent was calculated by the proportion of edited and unedited sites for each mitochondrial mRNA editing site. Whole-transcriptome RNA-seq data show that *poco1* affects several editing sites from eleven mitochondrial transcripts including *cytochrome c maturation subunit F C-terminus (ccmFc)*, *cytochrome c oxidase subunit 3 (cox3)*, *maturase-related (matR)*, *transport membrane protein B (mttB)*, *NADH dehydrogenase subunit 1 (nad1)*, *nad2*, *nad4*, *nad5*, *nad7*, *ribosomal protein small subunit 3 (rps3)* and *rps4* (Table 5). It is noteworthy that the editing efficiency in most of the editing sites is lower in *poco1* except for *ccmFc*-146, *ccmFc*-160, *ccmFc*-415, *nad1*-823, *nad4*-1194 and *nad7*-1124. Besides, RNA editing analysis from whole-transcriptome RNA-sequencing (RNA-seq) data confirm the lower editing efficiency at the site *rps3*-1344, which was previously identified from direct sequencing of RT-PCR product.

Table 5: *poco1* affects multiple mitochondrial RNA editing sites. Several editing sites from eleven mitochondrial transcripts were affected in *poco1*.

mRNA	Position	wt	<i>poco1</i>	AA ^d . Change
<i>ccmFc</i>	146	(3 ^a /17 ^b) 17.6% ^c	(19/24) 79.1%	CCT(P)→CTT(L)
<i>ccmFc</i>	160	(0/13) 0%	(12/19) 63.1%	CCT(P)→TCT(S)
<i>ccmFc</i>	415	(0/11) 0%	(7/21) 33.3%	CTC(L)→TTC(F)
<i>cox3</i>	687	(11/13) 84.6%	(4/9) 44.4%	CCA(P)→TCA(S)
<i>matR</i>	1683	(10/14) 71.4%	(0/6) 0%	Synonymous
<i>mttB</i>	682	(4/4) 100%	(1/4) 25%	CGT(R)→TGT(C)
<i>nad1</i>	823	(7/14) 50%	(28/32) 87.5%	CTT(L)→TTT(F)
<i>nad2</i>	1403	(4/4) 100%	(3/7) 42.8%	TCC(S)→TTC(F)
<i>nad4</i>	1194	(0/19) 0%	(6/11) 54.5%	Synonymous
<i>nad5</i>	1958	(4/4) 100%	(0/4) 0%	TCG(S)→TTG(L)
<i>nad7</i>	24	(5/6) 83.3%	(3/12) 25%	Synonymous
<i>nad7</i>	38	(7/10) 70%	(3/12) 25%	TCG(S)→TTG(L)
<i>nad7</i>	963	(11/17) 64.7%	(19/53) 35.8%	Synonymous
<i>nad7</i>	1124	(4/11) 36.3%	(10/31) 67.7%	CCA(P)→CTA(L)
<i>rps3</i>	64	(11/18) 61.1%	(12/40) 30%	CGG(R)→TGG(W)
<i>rps3</i>	1344	(7/17) 41.1%	(3/30) 10%	Synonymous
<i>rps4</i>	308	(5/6) 83.3%	(4/10) 40%	CCA(P)→CTA(L)
<i>rps4</i>	956	(8/11) 72.7%	(1/4) 25%	TCG(S)→TTG(L)
<i>rps4</i>	967	(8/11) 72.7%	(2/8) 25%	CAT(H)→TAT(Y)
<i>rps4</i>	992	(6/9) 66.6%	(2/7) 28.5%	TCT(S)→TTT(F)
<i>rps4</i>	1042	(5/8) 62.5%	(0/6) 0%	CCA(P)→TTA(L)
<i>rps4</i>	1043	(5/8) 62.5%	(0/6) 0%	CCA(P)→CTA(L)
<i>rps4</i>	1052	(5/8) 62.5%	(0/5) 0%	CCT(P)→CTT(L)
<i>rps4</i>	1057	(5/8) 62.5%	(0/5) 0%	CGG(R)→TGG(W)

^a Number of reads containing T nucleotides.

^b Total number of reads covering the editing site.

^c The editing efficiency was calculated by counting reads containing T divided to the total number of reads for the specific editing position. The differences higher than 30% between wild-type and *poco1* are shown.

^d amino acids.

3.7. Analysis of the expression of flowering marker genes

poco1 plants display an early floral transition. In order to get insight into the molecular mechanism responsible for the phenotype, the expression level of several flowering marker genes was investigated. Many of the known flowering pathways converge on *FLC*, a repressor of flowering, as well as two main integrators of flowering, i.e. *SOC1* and *FT* (Andrés and Coupland 2012). Furthermore, among the repressors of flowering, *FLC* is critical, in that it strongly suppresses *SOC1* and *FT* (Hepworth 2002; Helliwell et al. 2006). Therefore, the transcript level of these three genes was investigated using RT-PCR. As seen in Figure 15A, no *FLC* transcript and a higher transcript level of *FT* could be detected for *poco1*. Also, no detectable difference for *SOC1* could be seen between wild-type and *poco1*. qRT-PCR results validate the results from RT-PCR. Analysing qRT-PCR data, *FLC* was found significantly down-regulated in *poco1* compared to wild-type plants (Figure 15B). Additionally, there was a significant up-regulation of *FT* in the *poco1* compared to that in wild-type plants (Figure 15B). However, no significant difference in *SOC1* expression level could be observed between *poco1* and wild-type plants (Figure 15B).

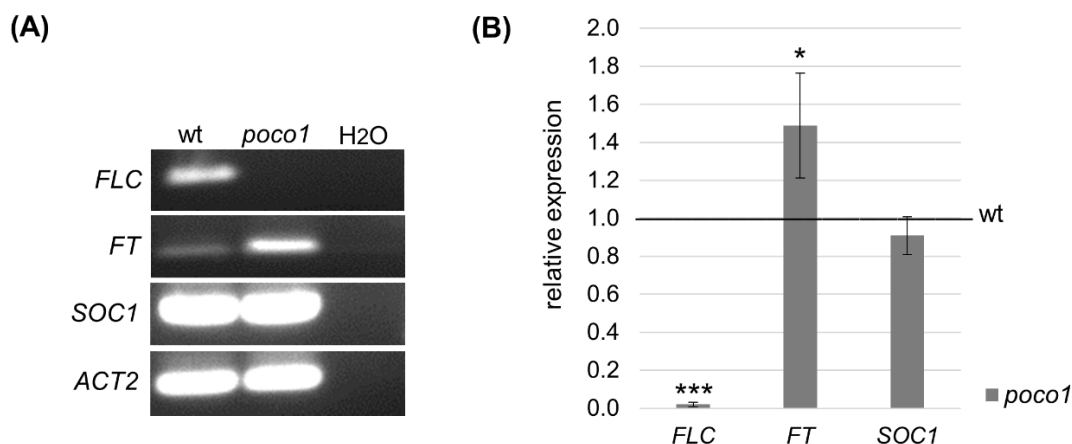


Figure 15: Transcript level and gene expression analysis of *FLC*, *SOC1* and *FT* with qRT-PCR. *FLC* and *FT* were up- and down-regulated respectively in *poco1*. **(A):** The RT-PCR products have been visualized on the gel. No RT-PCR product was detected for *poco1*. A higher *FT* transcript level was detected in *poco1* compared to wild-type. No detectable difference was observed for *SOC1* transcript level in *poco1* and wild-type. **(B):** *FLC* and *FT* are significantly down- and up-regulated respectively. No significant difference was observed for *SOC1* expression between *poco1* and wild-type plants. As a gene control, the *ACT2* housekeeping gene was used. The gene expression quantities in the wild-type were normalized to one value. One and three asterisks indicate a significant difference at $p \leq 0.05$ and $p \leq 0.001$ respectively by student's *t*-test. The standard deviation for each gene was calculated.

3.8. Analyzing the expression level of the *FLC* promoting genes

The *FLC* regulatory pathway is complex and involves multiple factors (Quesada et al. 2005). Analyzing the *FLC* regulatory pathway (Figure 16A), different distinct genetic pathways influence *FLC* expression. Vernalization and autonomous pathways have an inhibitory effect on *FLC* expression. On the other hand, genes of FRIGIDA complex, PAF1 complex and genes such as *HUA2*, *EARLY IN SHORT DAYS 4 (ESD4)*, *PHOTOPERIOD-INDEPENDENT EARLY FLOWERING 1 (PIE1)* (Finnegan et al. 2005) as well as the ABA signaling pathway (Wang et al. 2013) enhance the expression of *FLC*.

To get a hint, how *FLC* is down-regulated in *poco1* plants, we analyzed the expression of those genes, which promote *FLC* expression. Firstly, the transcript level of *FLC* promoting genes was checked by RT-PCR. The results are presented in Figure 16A. The only notable difference could be observed in the case of *ABSCISIC ACID INSENSITIVE 5 (ABI5)*. Also, the expression level of these genes was checked by qRT-PCR. As seen in Figure 16B, qRT-PCR analysis showed the constant expression of the *FRIGIDA (FRI)*, *FRIGIDA-LIKE 1 (FRL1)*, *FRIGIDA-LIKE 2 (FRL2)*, *HUA2*, *EARLY IN SHORT DAYS 4 (ESD4)*, *PHOTOPERIOD-INDEPENDENT EARLY FLOWERING 1 (PIE1)*, *VERNALIZATION INDEPENDENT 3 (VIP3)*, *VERNALIZATION INDEPENDENT 4 (VIP4)*, *EARLY FLOWERING 7 (ELF7)* and *EARLY FLOWERING 8 (ELF8)* between wild-type and *poco1* plants. Solely, a significant down-regulation in *ABI5* was observed for *poco1* plants (Figure 16B). *ABI5* is a basic leucine zipper transcription factor that plays a crucial role in the regulation of growth in the presence of ABA and abiotic stresses. *ABI5* acts as one of the important components of ABA signaling and is robustly associated with the floral transition (Wang et al. 2013a; Skubacz et al. 2016).

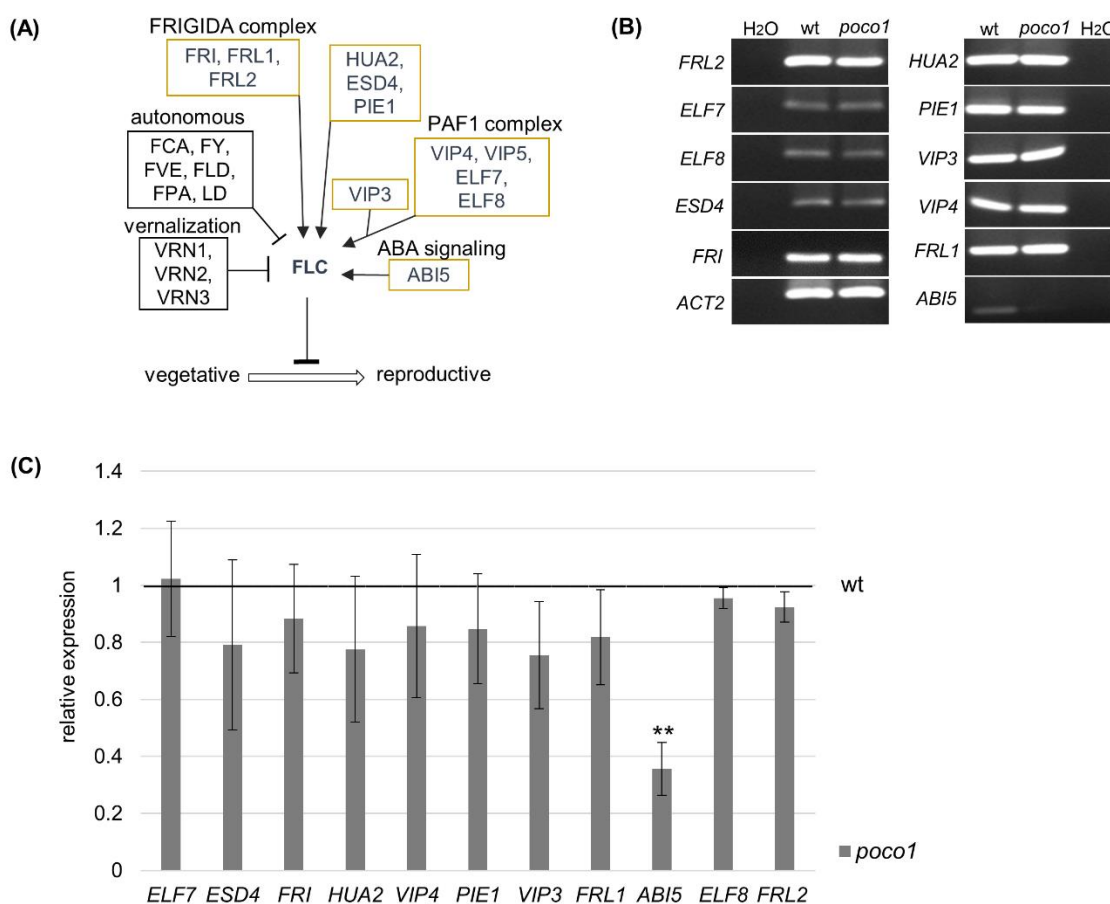


Figure 16: Gene expression analysis of *FRI*, *FRL1*, *FRL2*, *HUA2*, *ESD4*, *PIE1*, *VIP3*, *VIP4*, *ELF7*, *ELF8* and *ABI5* by RT-PCR and qRT-PCR. *ABI5* is significantly down-regulated in *poco1*. (A): Overview of the *FLC* regulatory pathway. The expression of *FLC* promoting genes (yellow boxes) was analyzed. (B): RT-PCR products have been visualized on the gel. There were no detectable differences in transcript levels of all *FLC* promoting genes except for *ABI5*. (C): The expression of *ABI5* was significantly down-regulated in the *poco1* plants, while no significant difference was observed in the expression level of other *FLC* promoting genes between wild-type and *poco1*. As a gene control, the *ACT2* housekeeping gene was used. The gene expression quantities in the wild-type were normalized to one value. Two asterisks indicate a significant difference at $p \leq 0.01$ by student's *t*-test. The standard deviation for each gene was calculated.

In order to determine, if the expression of *ABI5* in *poco1* stably remains constant at a lower level than wild-type plants or it can still be induced, the transcript status of *ABI5* after ABA application was analyzed. As shown in Figure 17, *ABI5* transcripts could be detected in wild-type without ABA treatment, but not for *poco1* plants. After ABA application, *ABI5* transcript abundance rose in both *poco1* and wild-type plants. Interestingly, a significantly higher *ABI5* transcript abundance was observed for treated wild-type plants than treated *poco1* plants (Figure 17). The induction of *ABI5* by ABA seems to be unaffected by the *poco1* mutation. The presented results here indicate that the expression of *ABI5* was sustained at a lower level with or without ABA treatment in *poco1* compared to wild-type plants. Taken together, these data highlight the influence of *poco1* mutation on the activation of *ABI5*, which in turn affect the *FLC* expression.

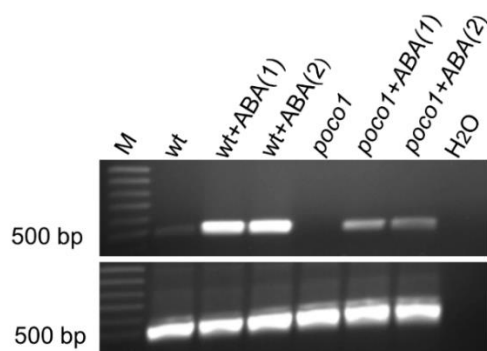


Figure 17: Transcript abundance of *ABI5* was evaluated after application exogenous ABA. After ABA treatment, *ABI5* is induced in both wild-type and *poco1* plants. The transcript abundance of *ABI5* is still lower than wild-type plants (upper panel). A fragment of *ACT2* was amplified as a loading control (downer panel).

3.9. *poco1* plants show altered sensitivity to ABA

The down-regulation of *ABI5* in *poco1* plants may imply a defect in the ABA signaling pathway. The phytohormone ABA plays a major role in plant responses to many stresses and ABA signaling is the most crucial stress signal transduction pathway among all the plant responses to stresses (Zhang et al. 2006a). Given that *ABI5* is one of the key factors in the ABA signaling pathway (Finkelstein and Lynch 2000; Lopez-Molina et al. 2001) and as *ABI5* was found to be down-regulated in *poco1* plants, it is possible that ABA signaling does not function properly. To determine whether the *poco1* plants display altered response to ABA, their sensitivity to ABA was tested. At the higher concentrations, ABA has an inhibitory effect on primary root elongation of the germinated seedlings (Finkelstein et al. 2002). Therefore, the effect of ABA regarding the primary root length in *poco1* plants was analyzed and compared to wild-type plants. To assess the effect of ABA on primary root length of the seedlings, germinated seedlings were transferred 24h after stratification from ABA-free MS medium to MS medium containing 0, 1, 5 and 10 μ M ABA for 5 days. The results showed that there was no significant difference between *poco1* and wild-type seedlings primary root length on ABA-free MS medium. But a stronger ABA inhibition of primary root growth was observed for wild-type than *poco1* seedlings and this inhibition was even more significant in the medium containing the higher concentration of ABA (Figure 18A and B).

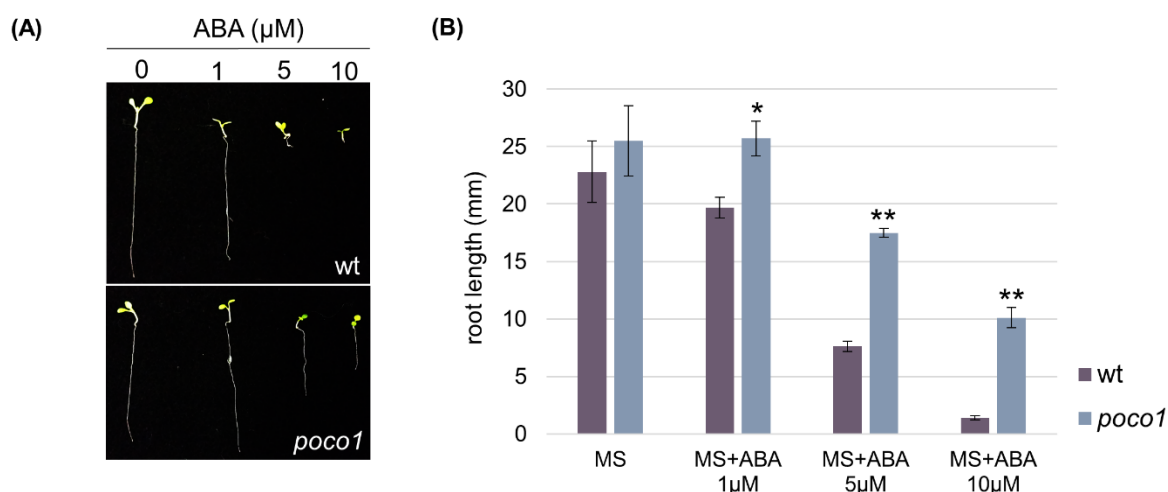


Figure 18: ABA-insensitive phenotype of *poco1* mutants in primary root growth. *poco1* plants revealed a lower sensitivity to ABA than wild-type plants. **(A):** Seedlings grew for 5 days after transfer from MS medium to MS medium supplemented with 0, 1, 5 or 10 μM ABA. Seedlings were transferred from ABA-free medium to ABA-containing medium 24 h after stratification. Primary root growth inhibition was higher in wild-type compared to *poco1* plants. **(B):** Primary root lengths were assayed 5 days after the transfer of seedlings. The difference between *poco1* and wild-type plants was more significant in the higher concentration of ABA. Values are the means ± standard errors of three independent experiments (n=10). One and two asterisks indicate a significant difference at $P \leq 0.05$ and $P \leq 0.01$ respectively by student's *t*-test.

ABA delays floral transition (Martínez-Zapater et al. 1994). Therefore, to assess the effect of ABA on the floral transition of *poco1* plants, which showed down-regulated *ABI5*, 2-week-old plants were sprayed with 100 μM ABA thrice a week until all plants started flowering. In agreement with the result observed in primary root growth assay, the inhibitory effect of ABA on floral transition was much stronger in wild-type compared to *poco1* plants (Figure 19A and B). These results show that the *poco1* mutation leads to decreased ABA sensitivity regarding the primary root growth and floral transition.

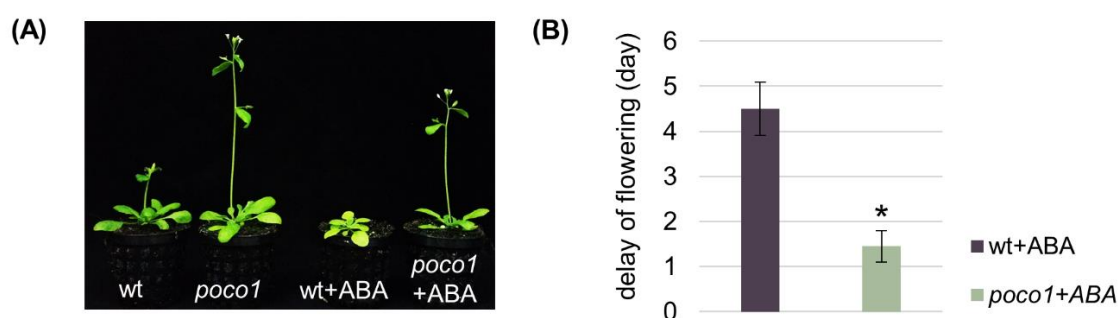


Figure 19: ABA-insensitive phenotype of *poco1* in floral transition. *poco1* plants revealed a lower sensitivity to ABA compared to wild-type plants in floral transition. **(A):** 2-week-old plants were sprayed with 100 μM ABA thrice a week until all plants started flowering. The control treatment was performed with an equal amount of the solvent (ethanol). After exogenous application of ABA, the flowering time in wild-type plants postponed more than *poco1* plants. **(B):** Delay of flowering was analyzed for ABA-treated wild-type and *poco1* plants compared to the control treatment. Values are the means ± standard errors of three independent experiments (n > 20). An asterisk indicates a significant difference between wild-type and *poco1* plants at $p \leq 0.05$ by student's *t*-test.

3.10. *poco1* plants show a higher sensitivity to drought stress

ABA primarily regulates plant tolerance to abiotic stresses such as drought stress (Kazan and Lyons 2016). ABA triggers the induction of numerous stress-related genes, which respond to dehydration and lead to plant tolerance (Yamaguchi-Shinozaki and Shinozaki 2006). Based on these facts and due to the down-regulation of *ABI5* in *poco1* plants, which led to the ABA deficiency, the sensitivity of *poco1* plants to drought stress was analyzed. A drought-tolerance assay on *poco1* and wild-type plants at 14 (Figure 20A) and 20 days (Figure 20B) after stratification was performed. After 6 days of withholding water from 14-day-old plants, all *poco1* plants leaves severely wilted compared to the wild-type plants (Figure 20A). In addition, withholding water from 20-day-old plants caused wilted leaves and ceased the growth of *poco1* plants (Figure 20B).

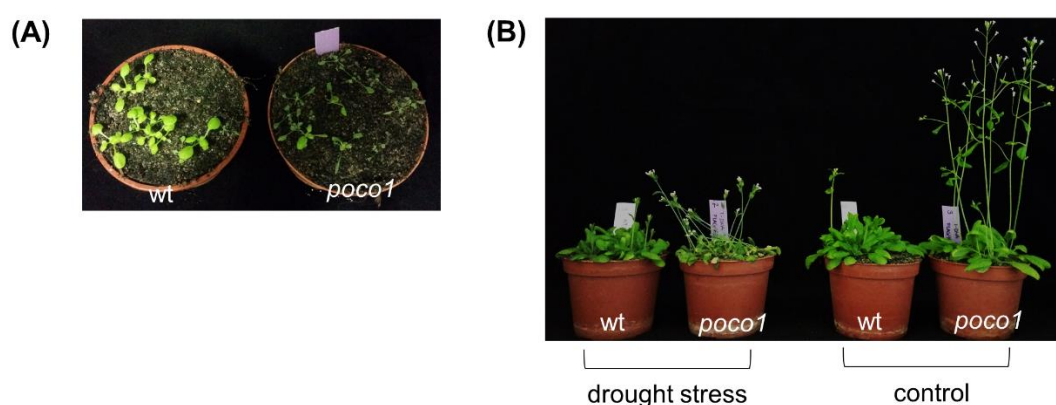


Figure 20: Wild-type and *poco1* plants after application of drought stress. *poco1* plants show enhanced sensitivity to drought stress. (A): Plants were grown under normal conditions for 14 and then withheld from water for six days. (B): Plants were grown under normal conditions for 20 days and then withheld from water for six days. The pictures were taken after 6 days of drought stress.

3.11. *poco1* plants show an altered mitochondrial respiratory activity

To investigate if POCO1 is required for the proper respiratory-associated mitochondrial functions, respiration rate, ATP and ROS generation were analyzed. Respiration is an energy-conserving process, which generates ATP through oxidative phosphorylation to release CO₂ and reduce O₂ to water (Charles et al. 2001; Millar et al. 2011). This process is regulated at different levels, from the transcriptional level in the nucleus to the post-transcriptional level and posttranslational level (Millar et al. 2011). Respiration was analyzed by the measurement of oxygen consumption in wild-type and *poco1* dark-grown root culture using a Clark-type oxygen electrode. The level of oxygen consumption in the *poco1* was significantly lower compared with wild-type and shows a reduction of more than 50% compared to wild-type plants (Figure 21A). The respiration rate in wild-type samples was 26.5 μMol oxygen h⁻¹g⁻¹ dw, while the

respiration rate was drastically reduced in *poco1* samples to $10.4 \mu\text{Mol oxygen h}^{-1} \text{g}^{-1} \text{dw}$. These observations show that the *poco1* mutation leads to the reduction of mitochondrial respiration, which further indicates the involvement of POCO1 in the respiration in mitochondria.

The mitochondrion is the main energy-producing organelle, providing ATP for various biological processes in the cell. The combined action of mETC and ATP production is important for cellular biosynthesis, growth and development. Lower respiration rate affects ATP production. Therefore, ATP content was investigated in wild-type and *poco1* plants and for this aim, the luciferin-luciferase assay was used. In agreement with the lower respiration level in *poco1*, ATP content measured in mitochondria of *poco1* was significantly lower compared to that in wild-type plants (Figure 21B). These results suggest that loss of function of POCO1 results in the decreased respiration and ATP content.

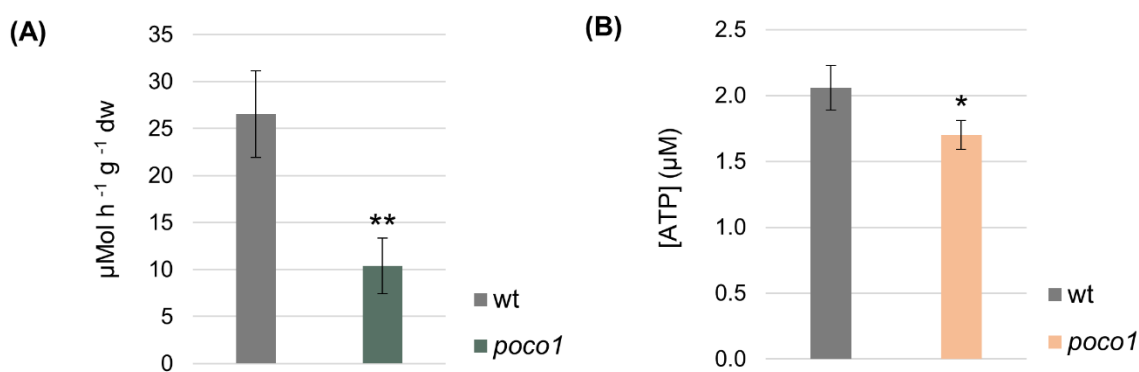


Figure 21: Respiration rate and ATP level in *poco1*. Respiration rate and ATP level were significantly reduced in *poco1*. **(A):** Oxygen consumption in the sliced dark-grown root culture of wild-type and *poco1* was measured. *poco1* significantly reduces the respiration rate. Data are indicated as the mean \pm standard errors ($n=10$) and the asterisk indicates a significant difference between wild-type and *poco1* at $P \leq 0.05$ using Student's *t*-test. **(B):** ATP content is significantly reduced in *poco1* mitochondria. Data are indicated as the mean \pm standard errors ($n=3$) and the asterisk indicates a significant difference between wild-type and *poco1* at $P \leq 0.05$ by Student's *t*-test.

The impairment of mitochondrial respiration affects ROS production (Yang et al. 2014). In different stresses, mETC is the main generator of cellular ROS molecules (Møller 2001). Furthermore, mitochondrial dysfunction links to the ROS accumulation and oxidative stress in plants (Yu et al. 2015). To obtain insight into the function of mitochondria in *poco1* plants, ROS level was analyzed in wild-type and *poco1* plants. Superoxide ions represent one of the most damaging ROS molecules and cause extensive mitochondrial oxidative damage (Zsigmond et al. 2008). Using histochemical NBT-staining superoxide ions can be examined. The NBT staining was performed in two different developmental stages: 11-day-old seedlings and 18-day-old detached leaves. In both developmental stages, an obvious visible difference was found between wild-type and *poco1* plants (Figure 22A and B). Wild-type plants were slightly

stained blue by NBT, while *poco1* plants were stained a darker blue, which refers to the higher accumulation of superoxide compared to wild-type plants (Figure 22A and B).

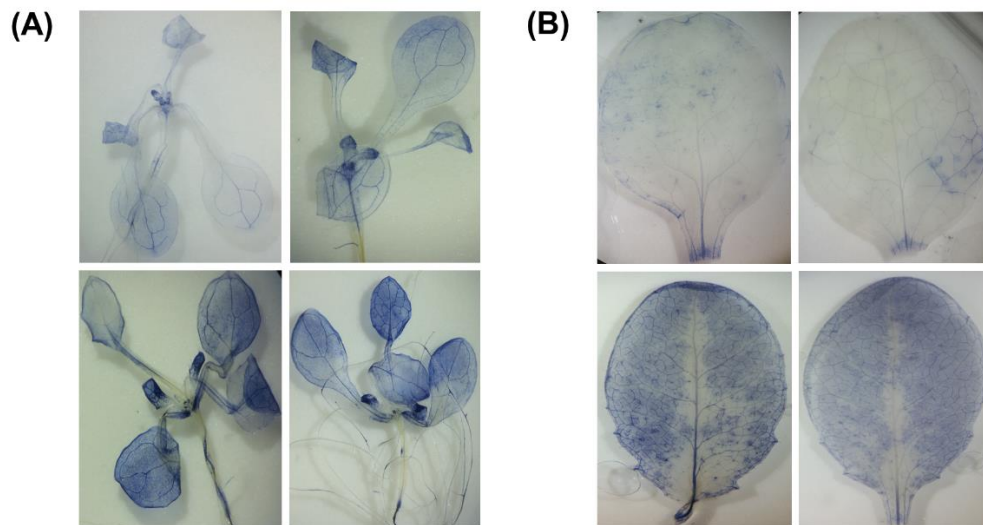


Figure 22: *poco1* accumulate more ROS than wild-type plants. A higher accumulation of superoxide was observed in *poco1* plants in both developmental stages. **(A):** NBT staining for superoxide detection in 11-day-old seedlings for wild-type (upper panel) and *poco1* plants (downer panel). **(B):** NBT staining for superoxide detection in 18-day-old detached leaves for wild-type (upper panel) and *poco1* plants (downer panel).

3.12. Studying the gene expression by RNA sequencing

In the past few years, RNA-seq has become the popular method to analyze whole transcriptome (Mortazavi et al. 2008; Wang et al. 2009), which enables to use several applications for transcriptome analysis. Analysis of the gene expression unravels regulatory mechanisms that control cellular processes. Functional analysis of the *poco1* mutant suggested the possibility of an altered expression level of a wider range of genes. To obtain a global overview of differentially regulated genes in *poco1*, RNA-seq analysis was done. For this aim, RNA was isolated at two different time points including three biological replications. The first stage was on day 20 from sowing, when wild-type plants do not have any inflorescence stem, and *poco1* plants have developed the inflorescence stem (comparison 1: pre-inflorescence-inflorescence). The second stage was on day 25 from sowing, when wild-type plants possess the inflorescence stem, and *poco1* plants flowered (comparison 2: inflorescence-flowering). However, one additional comparison, in which both wild-type and *poco1* plants have developed inflorescence stem was performed. Therefore, wild-type and *poco1* plants of a similar developmental stage were analyzed (comparison 3: inflorescence-inflorescence) (Figure 23).

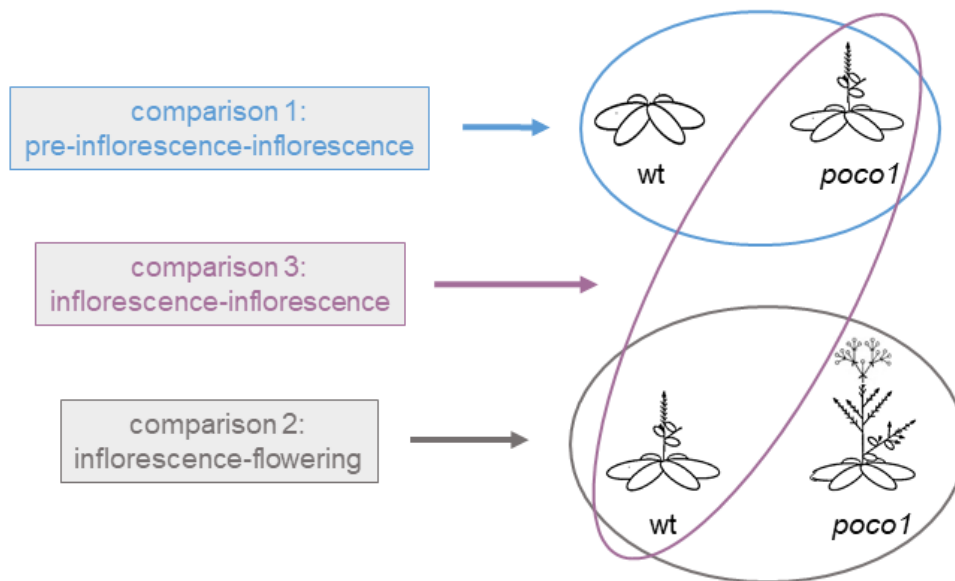


Figure 23: RNA-seq experimental setup. The schematic representation of wild-type and *poco1* developmental stages and performed comparisons for RNA-seq analysis. RNAs were isolated at two different time points. First stage: the wild-type plants without inflorescence stem and *poco1* plants with inflorescence stem. Second stage: the wild-type plants with inflorescence stem and *poco1* plants with flowers. According to existing developmental stages, three different RNA-seq comparisons were performed. Comparisons pre-inflorescence-inflorescence and inflorescence-flowering are affiliated to the first and second stages respectively. Inflorescence-inflorescence is the comparison between wild-type and *poco1* plants of the same developmental stage.

3.12.1. Analysis of differentially expressed genes

The total number of differentially expressed genes in each comparison is represented in Figure 24. The highest number of differentially expressed genes was obtained from inflorescence-inflorescence, which is the comparison of wild-type and *poco1* plants of the same developmental stage. On the contrary, pre-inflorescence-inflorescence represented the fewest number of differentially expressed genes among three comparisons. All differentially expressed genes (fold changes either ≥ 2 or ≤ -2 , FDR p -value < 0.05) dedicated to pre-inflorescence-inflorescence, inflorescence-flowering and inflorescence-inflorescence are presented in Emami et al. 2020 (Additional file 1).

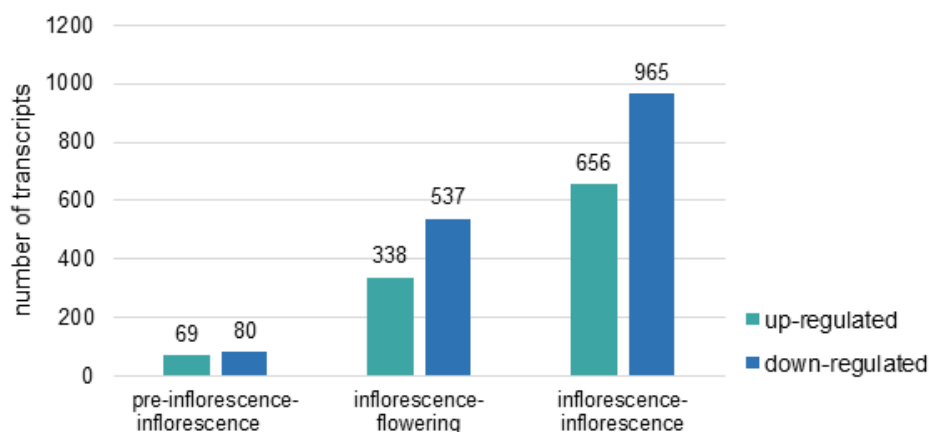


Figure 24: Comparison of differentially expressed genes in three different comparisons of RNA-seq analysis. The number of up-regulated and down-regulated genes is represented for each comparison. The highest number of differentially expressed genes was observed for inflorescence-inflorescence, which refers to the comparison of wild-type and *poco1* from the same developmental stage. Differentially expressed genes were defined as those with a fold change either ≥ 2 or ≤ -2 and an FDR value < 0.05 .

To understand the biological significance of gene expression in *poco1*, a gene ontology (GO) enrichment analysis was performed on all up- and down-regulated genes (fold changes either ≥ 2 or ≤ -2 , FDR p-value < 0.05) for each comparison (Supplementary Table 3, Supplementary Table 4 and Supplementary Table 5). By GO analysis, important enriched biological processes in the regulation of *poco1* were identified. Biological process GO terms related to biotic stresses and defense response such as “glycosyl compound biosynthesis”, “response to biotic stimulus”, “glycosinolate biosynthetic process” and “sulfur compound biosynthesis process” were over-represented for up-regulated genes of pre-inflorescence-inflorescence (69 genes) (Supplementary Table 3). Biological process GO terms of “cell redox homeostasis”, “cellular homeostasis” and “electron transport chain” were enriched for down-regulated genes of pre-inflorescence-inflorescence (80 genes) (Supplementary Table 3). GO enrichment of up- and down-regulated genes of the inflorescence-flowering (338 and 537 genes respectively) (Supplementary Table 4) showed that stress response-related processes are highly over-represented. Also in the same comparison, GO terms related to ageing and cell wall organization were enriched in up- and down-regulated genes respectively. GO enrichment of up-regulated genes of inflorescence-inflorescence (656 genes) (Supplementary Table 5) indicated that terms associated with the “translation” and “peptide biosynthetic process” as well as GO terms related to the biosynthetic and metabolic processes are enriched and biological process GO terms associated with nitrogen compound biosynthesis are over-represented. GO terms mainly related to stresses like “responses to oxygen-containing compound”, “response to chemicals”, “response to chitin”, “response to stress”, “response to biotic stimulus”, “defense response to other organisms”, “response to organonitrogen compound”, “response to water deprivation”, and “response to abscisic acid” were enriched for down-regulated genes of inflorescence-inflorescence (965 genes) (Supplementary Table 5).

Due to the early-flowering phenotype of *poco1* and ABA deficiency, associated genes with these categories were distinguished (3.12.2, 3.12.3). Additionally, because of the drought stress vulnerability and also a higher amount of ROS generation in *poco1* compared to wild-type plants, which is highly correlated with oxidative stress, genes related to these categories in all three comparisons were identified (3.12.4). To improve understanding about the functional classification of the differentially expressed genes in *poco1* in comparison with wild-type, GO enrichment analysis narrowed down to genes of above-mentioned categories. Top ten Significant biological processes have been presented in Table 6. Venn diagrams depicted the number of common and unique up- and down-regulated genes among each category (Figure 25A-C). Besides, to obtain more insight into other possible effects in *poco1*, the expression profile of genes associated with the cellular regulation were studied (3.12.5). Differentially expressed genes associated with redox state (3.12.6), stomatal function (3.12.7) and mitochondrial perturbation (3.12.8), which provide more knowledge about *poco1* were also identified.

Table 6: Gene ontology (GO) enrichment analysis (biological processes). Top ten significant ontologies of up- and down-regulated of flowering-associated genes, ABA-associated genes and drought and oxidative stress-associated genes. GO analysis was done by the g:profiler online tool (Reimand et al. 2007). The adjusted enrichment p-values are in negative log₁₀ scale. GO:BP; gene ontology: biological process.

	Terms name (GO:BP)	Term ID	p-value
flowering-related terms	flower development	GO:0009908	7.031×10 ⁻¹²
	reproductive shoot system development	GO:0090567	9.616×10 ⁻¹²
	post-embryonic development	GO:0009791	1.591×10 ⁻⁹
	reproductive structure development	GO:0048608	5.232×10 ⁻⁹
	reproductive system development	GO:0061458	5.367×10 ⁻⁹
	regulation of flower development	GO:0009909	9.329×10 ⁻⁹
	shoot system development	GO:0048367	1.691×10 ⁻⁸
	regulation of reproductive process	GO:2000241	2.694×10 ⁻⁸
	multicellular organism development	GO:0007275	5.496×10 ⁻⁸
	anatomical structure development	GO:0048856	8.382×10 ⁻⁸
ABA-related terms	response to abscisic acid	GO:0009737	2.951×10 ⁻⁹²
	response to alcohol	GO:0097305	4.791×10 ⁻⁹²
	response to acid chemical	GO:0001101	2.011×10 ⁻⁸⁶
	response to lipid	GO:0033993	3.019×10 ⁻⁸⁶
	response to oxygen-containing compound	GO:1901700	1.555×10 ⁻⁷⁹
	response to hormone	GO:0009725	1.841×10 ⁻⁶⁵
	response to endogenous stimulus	GO:0009719	8.745×10 ⁻⁶⁵
	response to organic substance	GO:0010033	3.096×10 ⁻⁶¹
	response to chemicals	GO:0042221	5.082×10 ⁻⁶¹
	response to stimulus	GO:0050896	4.042×10 ⁻⁴⁷
drought- and oxidative-related terms	Oxidation-reduction process	GO:0055114	1.226×10 ⁻⁷⁷
	response to stress	GO:0006950	3.306×10 ⁻⁴⁷
	response to inorganic substance	GO:0010035	4.264×10 ⁻⁴⁷
	response to chemical	GO:0042221	1.023×10 ⁻⁴²
	response to abiotic stimulus	GO:0009628	3.702×10 ⁻⁴²
	response to oxygen-containing compound	GO:1901700	1.746×10 ⁻³⁵
	response to water deprivation	GO:0009414	2.518×10 ⁻³⁵
	response to water	GO:0009415	7.183×10 ⁻³⁵
	response to stimulus	GO:0050896	2.223×10 ⁻³⁴
	response to oxidative stress	GO:0006979	3.986×10 ⁻²⁷

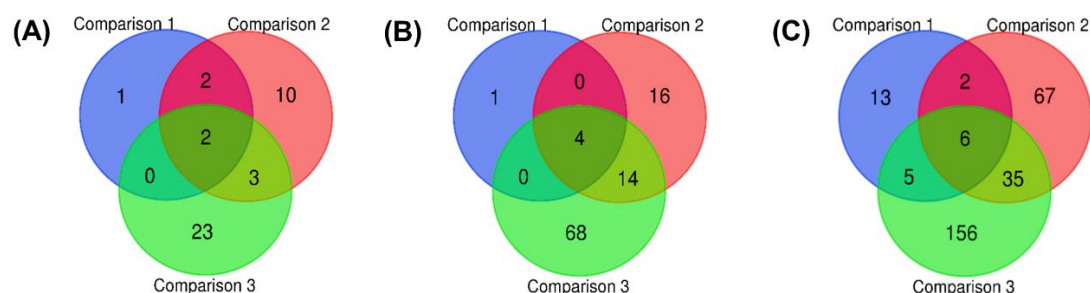


Figure 25: Venn diagrams of the differentially expressed genes of interests. Venn diagrams showing unique or common differentially expressed genes in each category of genes of interest. **(A):** A Venn diagram for up- and down-regulated flowering-associated genes. **(B):** A Venn diagram up- and down-regulated ABA-associated genes **(C):** A Venn diagram up- and down-regulated drought and oxidative stress-associated genes. Venn diagrams were done by an online tool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>).

25 topmost up- and down-regulated genes of each comparison were identified (Supplementary Figure 5A, B and C). Analyzing the 25 topmost up- and down-regulated genes of each

comparison, three genes were found to be commonly up-regulated: *PHOSPHATIDYLINOSITOL 4-KINASE GAMMA 3 (PI4Ky3)*, *Cwf18 PRE-mRNA SPLICING FACTOR* and *PR5-LIKE RECEPTOR KINASE (PR5K)* and four genes were commonly down-regulated in three comparisons: *THIOGLUCOSIDE GLUCOHYDROLASE 2 (TGG2)*, leucine-rich repeat (LRR) family (*AT4G16880*), hypothetical protein (*AT5G22608*), disease resistance protein family (*AT5G43740*). Up-regulation of *PI4Ky3* and down-regulation of *TGG2* were indicated to cause an ABA-insensitive phenotype in *A. thaliana* (Islam et al. 2009; Akhter et al. 2016). *Cwf18 PRE-mRNA SPLICING FACTOR* was previously suggested to have a critical role in gene expression and abiotic stresses (Gehring et al. 2018).

3.12.2. Identification of genes associated with flowering

41 genes associated with flowering were identified among differentially expressed genes (Supplementary Figure 6). Expression analysis of these flowering-associated genes may permit to unravel the mechanism of floral transition in *poco1*. Up- or down-regulation of many of these genes fit the phenotype of *poco1*. GO terms of “flower development” and “reproductive development” were among the highest ranks (Table 6). Flowering-related genes, which are from widely known flowering pathways are as follows: circadian clock and photoperiod pathway genes; *GIGANTEA (GI)*, *CONSTANS-LIKE 1 (COL1)*, *EARLY FLOWERING 4-L1 (ELF4-L1)* and *ELF4-L2*; floral integrator genes; *FT* and *FLOWERING PROMOTING FACTOR 1-LIKE PROTEIN 1 (FLP1)*, Gibberellic acid pathway genes; *GIBBERELLIN 3-OXIDASE 1 (GA3ox1)*, *GIBBERELLIN INSENSITIVE DWARF 1 (GID1B)* and *GID1C*, a photoreceptor gene; *PHYTOCHROME E (PHYE)* and an *FLC* specific regulator; *FRIGIDA-LIKE (FRL)*. In addition to this, other genes associated with the flowering such as *PACLOBUTRAZOL RESISTANCE 1 (PRE1)*, *TEMPRANILLO 1 (TEM1)*, *FLOWERING BHLH2 (FBH2)*, *GLYCIN RICH PROTEIN 7 (GRP7)*, *CYSTEINE-RICH RECEPTOR-LIKE KINASE 6 (CRK6)*, *CRK19*, *RELATED TO ABI3/VP1 1 (RAV1)* and *NAC089* were identified amongst differentially expressed genes. Some examples of identified differentially expressed genes in *poco1* associated with the flowering are represented in Table 7.

Table 7: Some of the differentially regulated genes associated with flowering.

Gene name	Regulation	Description	Reference
<i>ACR4</i>	↑	up-regulated during floral induction in the apical meristem	(Torti et al. 2012)
<i>Ankyrin</i>	↑ ↑	up-regulated during floral induction in the apical meristem	(Torti et al. 2012)
<i>CRK6</i>	↓ ↓	ROS sensing, signaling, mutants flower early	(Idänheimo 2015)
<i>CRK19</i>	↓	abiotic stress tolerance and hypersensitive response, mutants flower early	(Idänheimo 2015)
<i>DGR2</i>	↑	up-regulated during floral induction in the apical meristem	(Torti et al. 2012)
<i>ELF4-L1</i>	↓	circadian clock/photoperiod regulation of flowering, mutants flower early	(Lin et al. 2019)
<i>FLN1</i>	↑	up-regulated during floral induction in the apical meristem	(Torti et al. 2012)
<i>FLP1</i>	↑	floral induction, flowering time control, over-expression line flowers early	(Borner et al. 2000a)
<i>FRL</i>	↓	induction of <i>FLC</i>	(Michaels et al. 2004; Quesada et al. 2005)
<i>FT</i>	↑	positive regulation of floral induction/flower development, mutants flower late	(Koornneef et al. 1991)
<i>GA3ox1</i>	↑	gibberellic acid biosynthetic pathway	(Chiang et al. 1995; Sun 2008)
<i>GI</i>	↑	induction of flowering via the circadian clock/photoperiod, mutants flower late	(Rédei 1962)
<i>GID1B</i>	↑	gibberellic acid signaling pathway	(Griffiths et al. 2006)
<i>GRP7</i>	↑	regulation of flowering time, mutants flower late and over-expression line flowers early	(Streitner et al. 2008)
<i>PHYE</i>	↓	repressor of flowering, phototransduction, mutants flower early	(Halliday et al. 2003; Boss et al. 2004)
<i>ROXY2</i>	↑	anther development	(Xing and Zachgo 2008)
<i>TRM112A</i>	↑	up-regulated during floral induction in the apical meristem	(Torti et al. 2012)
<i>AT1G09390</i>	↑	up-regulated during floral induction in the apical meristem	(Torti et al. 2012)
<i>AT5G56120</i>	↑	up-regulated during floral induction in the apical meristem	(Torti et al. 2012)

Gene regulation symbols from left to right refer to the regulation in pre-inflorescence-inflorescence, inflorescence-flowering and inflorescence-inflorescence respectively. **Gene regulation symbols-** ↑: up-regulation; ↓: down-regulation

3.12.3. *poco1* inactivates ABA signaling and response

Due to the observed ABA-insensitivity phenotype of *poco1* plants with regard to the primary root growth and flowering time, it was concluded that ABA signaling may not function efficiently (3.9). Interestingly, whole-transcriptome RNA-seq results showed that many genes associated with the ABA signaling and response are down-regulated in *poco1*. GO enrichment analysis proved the response to ABA as the top rank biological process (Table 6). Intriguingly, genes, which functions as important components of ABA signaling have been affected and mostly are down-regulated in *poco1* (Supplementary Figure 7). Genes involved in the early perception of ABA like *PYRABACTIN RESISTANCE 1-LIKE 5/REGULATORY COMPONENT OF ABA RECEPTOR 8 (PYL5/RCAR8)* and *PYL9/RCAR1* were identified to be down-regulated in *poco1*. Protein phosphatases (PP2C) like *ABA-INSENSITIVE 1 (ABI1)*, *ABI2*, *ABA-HYPERSENSITIVE GERMINATION 1 (AHG3)*, *HOMOLOGY TO ABI 1 (HAB1)* and *HAB2* are also down-regulated in *poco1*. Protein kinases (SnRK2), *SNF1-RELATED PROTEIN KINASE 2.1 (SnRK2.1)* and *SnRK2.8* exhibited down-regulation in *poco1*. ABA-activated transcription factors, *ABRE BINDING FACTOR 3 (ABF3)* and *ABF4* are master transcription factors in ABA signal transduction, which are down-regulated in *poco1*. An ABA biosynthesis gene, *NCED4* was found differentially expressed in *poco1* (up-regulated in inflorescence-flowering and down-regulated in inflorescence-inflorescence). A gene involved in the ABA catabolism, *ABA 8'-HYDROXYLASE (CYP707A3)* were found down-regulated in *poco1*. Other examples of the differentially expressed genes in *poco1* identified to be associated with the ABA response are shown in Table 8.

Table 8: Some of the differentially regulated genes in *poco1* associated with ABA.

Gene name	Regulation	Description	Reference
<i>ABF3</i>	↓	ABA signaling, ABA and water deprivation response, mutants show ABA-insensitivity	(Kang et al. 2002; Hwang et al. 2019)
<i>ABF4</i>	↓	ABA signaling, ABA and water deprivation response, mutants show ABA-insensitivity	(Kang et al. 2002; Hwang et al. 2019)
<i>ABI1</i>	↓	negative regulator of ABA signaling, mutants show ABA insensitivity	(Leung et al. 1997; Kuhn et al. 2006)
<i>ABI2</i>	↓	negative regulator of ABA signaling, mutants show ABA insensitivity	(Leung et al. 1997; Kuhn et al. 2006)
<i>AHG3</i>	↓	negative regulator of ABA signaling/ water deprivation	(Yoshida et al. 2006)
<i>PI4Ky3</i>	↑ ↑ ↑	response to ABA, over-expression line shows ABA insensitivity and reduced induction of <i>ABI5</i>	(Akhter et al. 2016)
<i>CPK32</i>	↓	ABA signaling and response, over-expression line show ABA-hypersensitivity and enhanced expression of ABA-regulated genes	(Choi et al. 2005)
<i>CYP707A3</i>	↓	ABA catabolic and metabolic processes, involved in dehydration and rehydration	(Umezawa et al. 2006)
<i>HAB1</i>	↓	negative regulator of ABA signaling	(Kuhn et al. 2006)
<i>HAB2</i>	↓	negative regulator of ABA signaling	(Kuhn et al. 2006)
<i>LTP3</i>	↓ ↓ ↓	ABA response	(Guo et al. 2013)
<i>LTP4</i>	↓ ↓ ↓	ABA response	(Deeken et al. 2016)
<i>MARD1</i>	↓	response to ABA, mutants are non-responsive to ABA	(He and Gan 2004)
<i>MLP43</i>	↓ ↓	positive regulator of ABA signaling, involved in drought tolerance	(Wang et al. 2016)
<i>NCED4</i>	↑ ↓	ABA biosynthesis	(Gonzalez-Jorge et al. 2013)
<i>PYL5/RCAR8</i>	↓	ABA signaling and response, drought stress response	(Ma et al. 2009)
<i>PYL9/RCAR1</i>	↓	ABA signaling and response, mutants are ABA-insensitive	(Ma et al. 2009; Zhao et al. 2016)
<i>RAB18</i>	↓	ABA and abiotic stress-responsive	(Wang et al. 2016)
<i>RPK1</i>	↓	ABA signaling pathway, ABA and water deprivation response, altered stress-induced responses in mutants	(Osakabe et al. 2005; Sharma and Pandey 2016)

<i>SnRK2.1</i>	↓	ABA signaling, water deprivation response	(Umezawa et al. 2004)
<i>SnRK2.8</i>	↓	ABA signaling, water deprivation response, over-expression line enhances drought tolerance	(Kang et al. 2002)
<i>SYP121</i>	↓	response to ABA	(Sokolovski et al. 2008)

Gene regulation symbols from left to right refer to the regulation in pre-inflorescence-inflorescence, inflorescence-flowering and inflorescence-inflorescence respectively. **Gene regulation symbols-** ↑: up-regulation; ↓: down-regulation

3.12.4. Identification of genes associated with drought and oxidative stresses

In this thesis, it was shown by experimental evidence that *poco1* plants are more susceptible to drought stress (3.10) and accumulate a higher amount of ROS compared to wild-type plants (Figure 22A and B). The higher level of ROS generation is tightly linked to oxidative stress. RNA-seq data identified several genes associated with drought and oxidative stresses to be differentially regulated in *poco1*. GO enrichment analysis revealed the biological processes assigned to oxidative reduction and response to stress were on the top range with a high p-value (Table 6). Response to water deprivation and response to water, which are regulatory and functional features of drought stress responses were over-presented among top biological process categories (Table 6). The whole-transcriptome RNA-seq study has identified many drought stress-associated and oxidative genes in *poco1* to be differentially regulated compared to wild-type (Supplementary Figure 8). Genes like heat shock proteins (*HSPs*), responsive to desiccation (*RD*), *RAB18*, cold-regulated (*COR*), low-temperature-induced (*LTI*), drought-induced (*DI*), early responsive to dehydration (*ERDs*), glutathion s-transferases (*GSTs*) and thioredoxins (*TRXs*) were among differentially expressed genes. These genes have important molecular roles in adaptive responses to abiotic stresses. Some examples of differentially regulated genes in *poco1* associated with the drought and oxidative stresses identified in RNA-seq are represented in Table 9.

Many of ABA-regulated genes are induced or repressed upon ABA treatment or by various stresses such as drought, cold and high salinity (Huang et al. 2008). Several ABA-inducible stress genes such as, *early response to dehydration (ERDs)*, *lipid transfer protein (LTPs)*, *COLD REGULATED 314 INNER MEMBRANE 1 (COR413IM1)*, *RDs*, *COLD-REGULATED 47 (COR47)*, *COLD-REGULATED 413-PLASMA MEMBRANE 2 (COR413-PM2)*, *RESPONSIVE TO ABA 18 (RAB18)*, which generally are up-regulated by ABA and drought stress, showed down-regulation in *poco1*. Additionally, master regulators of ABA signal transduction such as *PYL5/RCAR8*, *PYL9/RCAR1*, *ABI1*, *ABI2*, *SnRK2.1*, *SnRK2.8*, *ABF3* and *ABF4*, which are

required for ABA-mediated responses to abiotic stresses (Luan 2003; Yoshida et al. 2010) are down-regulated. Also, the expression level of authentic drought-induced genes such as *RAB18* (Lang and Palva 1992) and *RESPONSIVE TO DESICCATION 29A (LT178/RD29A)* (Yamaguchi-Shinozaki and Shinozaki 1994), which are stress-response known marker genes are down-regulated in *poco1*. The majority of these genes have been found mostly in inflorescence-inflorescence (Supplementary Figure 8).

Table 9: Some of the differentially regulated drought and oxidative stress genes in *poco1*.

Gene name	Regulation	Description	Reference
<i>COR47</i>	↓	response to water deprivation	(Guo et al. 1992)
<i>COR413-PM2</i>	↓	cellular response to water deprivation	(Breton et al. 2003)
<i>ERD1</i>	↓	drought stress tolerance	(Tran et al. 2004)
<i>ERD10</i>	↓	response to water deprivation	(Kim and Nam 2010)
<i>LTP3</i>	↓ ↓ ↓	response to water deprivation	(Arondel et al. 2000)
<i>LTP4</i>	↓ ↓ ↓	response to water deprivation	(Arondel et al. 2000)
<i>FRO4</i>	↑	oxidation reduction process	(Jain et al. 2014)
<i>FRO7</i>	↓	oxidation reduction process	(Jain et al. 2014)
<i>LT178/RD29A</i>	↓	Response to water deprivation, response to ROS	(Yamaguchi-Shinozaki and Shinozaki 1993)
<i>PRXQ</i>	↓ ↑	cell redox homeostasis, cellular response to oxidative stress	(Ådén et al. 2011)
<i>PRXR1</i>	↓	response to oxidative stress	(Kiddle et al. 2003)
<i>RD28</i>	↑	response to desiccation	(Alexandersson et al. 2005)

Gene regulation symbols from left to right refer to the regulation in pre-inflorescence-inflorescence, inflorescence-flowering and inflorescence-inflorescence respectively. **Gene regulation symbols-** ↑: up-regulation; ↓: down-regulation

3.12.5. Altered expression of genes associated with cellular regulation in *poco1*.

To provide an understanding of the regulatory network controlling *poco1* cellular metabolism, different classes of transcription factors as well as genes associated with the cellular signaling were identified. RNA-seq analysis has identified 152 transcription factors from different classes; bHLH, bZIP, CCCH zinc finger, C2H2 zinc finger, CO-like, ERF, GATA, GRAS, HMG, Homeobox, HSF, mTERF, MYB, MYB-like, NAC, NF-Y, PLATZ, RWP-RK, RAV, Sigma 70-like, TCP and WRKY (Supplementary Figure 9). The lowest number of differentially expressed genes encoding transcription factors belong to pre-inflorescence-inflorescence and the highest

number refer to inflorescence-inflorescence. Most of the differentially regulated transcription factors in inflorescence-flowering were up-regulated. Inversely, the majority of transcription factors showed down-regulation in inflorescence-inflorescence. All differentially regulated genes from bHLH, MYB-like and NAC transcription factors family showed up-regulation in inflorescence-flowering. Conversely, all differentially expressed genes of MYB-like and NAC transcription factors revealed down-regulation in inflorescence-inflorescence. This expression pattern can be observed for the majority of genes encoding Homeobox, MYB transcription factors. The greatest number of regulated genes encoding transcription factors belong to the ERF transcription factor family. Some examples of differentially regulated genes encoding transcription factors are represented in Table 10.

Table 10: Some of the differentially regulated genes encoding transcription factors in *poco1*.

Gene name	Regulation	TF	Description	Reference
<i>FBH2</i>	↑ ↑	bHLH	photoperiod-independent effect on flowering, over-expression line with an early-flowering phenotype	(Ito et al. 2012)
<i>PRE1</i>	↑	bHLH	over-expression line with an early-flowering phenotype, gibberellic acid-dependent response	(Lee et al. 2006b)
<i>MYC2</i>	↓	bHLH	positive regulator of ABA signaling	(Abe et al. 2003)
<i>MYB2</i>	↑	MYB	response to ABA, response to water deprivation	(Lindemose et al. 2013)
<i>MYB20</i>	↑	MYB	positive regulator of ABA signaling	(Cui et al. 2013)
<i>MYB32</i>	↓	MYB	response to ABA	(Preston et al. 2004; Jung et al. 2008)
<i>MYB51</i>	↓	MYB	response to ABA	(Frerigmann and Gigolashvili 2014)
<i>MYB73</i>	↓	MYB	interaction with ABA signaling components	(Zhao et al. 2014)
<i>NAC089</i>	↓	NAC	negative regulation of flower development	(Li et al. 2010)
<i>RAV1</i>	↓	ERF	negative regulation of flower development	(Matías-Hernández et al. 2014)
<i>TEM1</i>	↓	RAV	<i>FT</i> repressor, mutants flower early, overexpression line flowers late	(Matías-Hernández et al. 2014)
<i>WRKY15</i>	↓ ↓	WRKY	early H ₂ O ₂ responsive, over-expression line disrupts ROS and mitochondrial retrograde signaling	(Eulgem et al. 2000; Vanderauwera et al. 2012)
<i>WRKY25</i>	↓	WRKY	response to various abiotic stresses, ABA response, over-expression line shows ABA sensitivity	(Jiang and Deyholos 2009; Rushton et al. 2012; Bakshi and Oelmüller 2014)

<i>WRKY33</i>	↓	WRKY	response to various abiotic stresses, ABA response, over-expression line shows ABA sensitivity	(Jiang and Deyholos 2009; Rushton et al. 2012; Bakshi and Oelmüller 2014)
<i>WRKY46</i>	↓	WRKY	regulation of ABA signaling and response to water deprivation	(Ding et al. 2015)

Gene regulation symbols from left to right refer to the regulation in pre-inflorescence-inflorescence, inflorescence-flowering and inflorescence-inflorescence respectively. **TF- transcription factor; Gene regulation symbols-** ↑: up-regulation; ↓: down-regulation.

The whole-transcriptome RNA-seq analysis also identified differentially expressed genes encoding proteins associated with the cellular signaling such as cysteine-rich receptor-like kinases (CRKs), receptor-like kinases (RLKs), receptor-like proteins (RLPs), mitogen-activated protein kinases (MAPKs) and leucine-rich repeat receptor kinases (LRR-RKs) (Supplementary Figure 10). In pre-inflorescence-inflorescence, these signaling genes are mostly up-regulated. However, in inflorescence-flowering and inflorescence-inflorescence, a down-regulated expression profile for most of the identified differentially expressed signaling molecules is observed (Supplementary Figure 10).

3.12.6. *poco1* affects the expression of genes associated with cellular redox state

The redox signals derived from mETC as well as ROS and thiol-containing compounds contribute to the cellular redox state (Apel and Hirt 2004; Dietz 2008). Analysis of RNA-seq results showed that redox related genes were differentially regulated in *poco1*. Redox related genes such as glutaredoxins (*GRXs*), glutathione s-transferases (*GSTs*), thioredoxins (*TRXs*) and rotamase cyclophilins (*ROCs*) were found amongst differentially expressed genes in *poco1* (Supplementary Figure 11). *GSTs* function mainly in oxidative stress metabolisms and maintain cellular redox homeostasis. Except one, all significantly regulated *GSTs* identified in inflorescence-inflorescence, are down-regulated. However, regulated *GSTs* in inflorescence-flowering show a different regulation pattern than in inflorescence-inflorescence, in which four of *GSTs* show up-regulation and other three show down-regulation. Some examples of differentially regulated genes in *poco1* associated with the cellular redox state are represented in Table 11.

Table 11: Some of the differentially regulated genes in *poco1* associated with the cellular redox state.

Gene name	Regulation		Description	Reference
<i>GSTU4</i>	↑	↓	degradation of H ₂ O ₂ , cellular redox homeostasis	(Dixon et al. 2009)
<i>GSTF9</i>	↑		glutathione metabolic process	(Horváth et al. 2015)
<i>GSTF12</i>	↑		glutathione metabolic process	(Kitamura et al. 2012)
<i>GSTU16</i>		↓	glutathione metabolic process	(Dixon et al. 2009)
<i>ROC2</i>		↑	protein folding, connecting hormone signals to redox homeostasis in stresses	(Park et al. 2013)
<i>ROC4</i>	↓	↑	protein folding, connecting hormone signals to redox homeostasis in stresses	(Park et al. 2013)
<i>ROXY3</i>		↑	cell redox homeostasis	(Gutsche et al. 2015)
<i>ROXY8</i>		↓	cell redox homeostasis	(Li et al. 2019)
<i>ROXY9</i>	↑	↓	cell redox homeostasis	(Ota et al. 2020)
<i>ROXY12</i>	↓	↑	cell redox homeostasis	(Rouhier 2006)
<i>ROXY13</i>	↓	↑	cell redox homeostasis	(Rouhier 2006)
<i>TRXz</i>		↑	cell redox homeostasis	(Wimmelbacher and Börnke 2014)
<i>TRX5</i>		↓	cell redox homeostasis, oxidation-reduction process	(Laloi et al. 2004)

Gene regulation symbols from left to right refer to the regulation in pre-inflorescence-inflorescence, inflorescence-flowering and inflorescence-inflorescence respectively. **Gene regulation symbols-** ↑: up-regulation; ↓: down-regulation

3.12.7. *poco1* altered expression of genes associated with Stomatal function

It is widely accepted that the phytohormone ABA function to adapt plants against stress conditions. Particularly in water stress, ABA accumulates in plant cells and regulates the expression of many genes, the products of which protect plants from dehydration and advance stomatal closure. RNA-seq results showed that several genes associated with stomatal performance are differentially expressed in *poco1* (Supplementary Figure 12). Some examples of differentially expressed genes in *poco1* associated with stomatal functions are represented in Table 12.

Table 12: Some of the differentially regulated genes in *poco1* associated with stomatal function.

Gene name	Regulation	Description	Reference
<i>ABI1</i>	↓	regulation of stomatal movement, mutants failed to activate anion channels in guard cells	(Roelfsema and Prins 1995; Pei et al. 1997)
<i>ABI2</i>	↓	regulation of stomatal movement, mutants failed to activate anion channels in guard cells	(Roelfsema and Prins 1995; Pei et al. 1997)
<i>CNGC1</i>	↓	highly expressed in guard cells, ion channel	(Wang et al. 2013b)
<i>CNGC6</i>	↓	highly expressed in guard cells, ion channel	(Wang et al. 2013b)
<i>GRP7</i>	↑	enhancement of stomatal opening	(Kim et al. 2008)
<i>MYB44</i>	↓	over-expression line shows enhanced stomatal closure	(Jung et al. 2008)
<i>RBOHD</i>	↓	increasing cytosolic Ca^{2+} , induced by ABA	(Kwak et al. 2006)
<i>RPK1</i>	↓	mutants show insensitivity in ABA-induced stomatal closure	(Osakabe et al. 2010)
<i>TGG1</i>	↓ ↑	regulation of stomatal movement, ABA-mediated stomatal closure	(Barth and Jander 2006; Zhao et al. 2008)
<i>TGG2</i>	↓ ↓ ↓	regulation of stomatal movement, ABA-mediated stomatal closure	(Barth and Jander 2006; Zhao et al. 2008)

Gene regulation symbols from left to right refer to the regulation in pre-inflorescence-inflorescence, inflorescence-flowering and inflorescence-inflorescence respectively. **Gene regulation symbols-** ↑: up-regulation; ↓: down-regulation

3.12.8. Altered expression of marker genes associated with the mitochondrial perturbation

mitochondrion acts as a sensory organelle, which intensely responding to stresses and enhances the plant adaptation (Li et al. 2013; Vanlerberghe 2013). Mitochondrial function is impaired by stresses and retrograde mechanisms will be activated to regulate gene expression, maintaining mitochondrial and cellular operations (Taylor et al. 2002; Rhoads and Subbaiah 2007). Signals are transmitted from mitochondria to the nucleus (retrograde signal) and the appropriate responses as any changes in the gene expression will be made. POCO1 is localized to mitochondria, and its loss of function led to mitochondrial dysfunction (3.11). Therefore, in *poco1*, the impact of retrograde signals on the expression of nuclear genes encoding mitochondrial proteins (NGEMPs) is plausible. The whole transcriptome RNA-seq analysis identified many nuclear genes encoding mitochondrial proteins (NGEMPs), which are targets of mitochondrial perturbations (Supplementary Figure 13). 37 genes associated with

the mitochondria perturbation were identified to be differentially regulated in *poco1* and some of them are shown in Table 13.

Table 13: Some of the differentially regulated NGEMPs in *poco1*.

Gene name	Regulation	Description	Reference
<i>AOX1a</i>	↓	mitochondria-nucleus signaling, alternative respiration	(Clifton et al. 2006)
<i>AOX1d</i>	↑	mitochondria-nucleus signaling, alternative respiration	(Clifton et al. 2006)
<i>ATDOA11</i>	↑	mitochondrial dysfunctions	(Lama et al. 2019)
<i>CRF6</i>	↑	mitochondrial retrograde response	(De Clercq et al. 2013)
<i>ERD5</i>	↑	mitochondria proline catabolic pathway, water deprivation response	(Nakashima et al. 1998)
<i>HSP60</i>	↑	protein import into mitochondrial intermembrane space, involved in mitochondrial dysfunctions as part of retrograde signals	(Van Aken et al. 2009a)
<i>mtHsc70-1</i>	↑	response to unfolded proteins, involved in mitochondrial dysfunctions as part of retrograde signals	(Van Aken et al. 2009a)

Gene regulation symbols from left to right refer to the regulation in pre-inflorescence-inflorescence, inflorescence-flowering and inflorescence-inflorescence respectively. **Gene regulation symbols-** ↑: up-regulation; ↓: down-regulation

3.12.9. Analysis of the mitochondrial gene expression by RNA-seq

Using RNA-seq data the expression changes of the mitochondrial genes were investigated. For this aim, RNA-seq reads were mapped to the mitochondrial genome of *A. thaliana* (NCBI; accession number NC_037304). The analysis revealed that the expression of all mitochondrial genes was not affected in *poco1* and there were no significant changes in the expression of all mitochondrial genes in all three comparisons (data are not shown).

4. Discussion

A pivotal challenge in the era of plant biology is to understand molecular mechanisms, by which organelles coordinate plant growth. The onset of flowering, which is orchestrated by endogenous and external factors indicates the plant reproduction success and is a vital step for viability and persistence of plants facing different environmental conditions. Therefore, this developmental phase is closely connected to productivity, especially in crop plants and thus is important regarding the ecological and economical aspects (Simpson and Dean 2009). PPR proteins are one of the largest protein families in land plants, containing approximately 450 members in *Arabidopsis*, which are targeted to mitochondria and chloroplasts (Lurin et al. 2004). Among the diverse phenotypes of PPR protein mutants (Barkan and Small 2014), the early-flowering phenotype referring to the *POCO1* mutation is rather uncommon. In this study, genetic approaches were used to reveal an unexpected role of a mitochondrial localized PPR protein in the flowering and molecular framework that links the functional state of the endosymbiotic organelle to the nuclear transcriptional regulation.

4.1. Flowering time is promoted by *POCO1* mutation

PPR proteins affect vital biological processes such as plant growth, development and reproduction. Mutations in the PPR proteins often lead to a severe phenotype (Schmitz-Linneberger and Small 2008; Barkan and Small 2014). The seed germination rate, fertility and root morphology in *poco1* seedlings were not affected (Bruhs 2012). This suggests that the function of *POCO1* may be dominant later in development. Upon the transition from vegetative to the reproductive phase, leaves cease to form and flowers emerge instead (Weigel and Glazebrook 2002), meaning that a strong correlation exists between days to flower and the number of leaves produced under certain conditions in *A. thaliana* (Koornneef et al. 1991). Determination of flowering time by analyzing both days to flower and the number of rosette leaves produced at the time of flowering, the dominant early-flowering phenotype of *poco1* plants was confirmed, which indicates that *POCO1* plays an important role in flowering.

Due to the possibility of the presence of T-DNA insertion in the other locus in the genome, it is important to assign the early-flowering phenotype to the *POCO1* gene mutation. Based on the results of the genetic complementation analysis, it was concluded that the introduction of *POCO1* gene to *poco1* background was functional. The genetic complementation analysis endorsed that the T-DNA insertion in *POCO1* gene and the lack of functional *POCO1* was indeed responsible for the early-flowering phenotype of *poco1* plants. Therefore, additional

mutations are not involved in the altered phenotype in this case, connecting POCO1 to floral transition in *A. thaliana*.

Light is one of the most prominent factors in the regulation of flowering at different levels such as photoperiod (Jiao et al. 2007). In *A. thaliana*, the photoperiodic pathway promotes flowering particularly under long-day conditions, so that flowering occurs much faster under these conditions than under short-day conditions. Based on the phenotypic analysis under long- and short-day conditions, *poco1* advanced flowering in both conditions and the difference between wild-type and *poco1* plants in days to flowering was nearly identical. In this view, It is concluded that POCO1's function is not related to the photoperiodic regulation of the flowering time and POCO1 most likely is associated with the other genetic pathways. These results also suggest that POCO1 negatively regulates flowering time under both long- and short-day conditions in *A. thaliana*. There are only two PPR protein-encoding genes, which showed a link to flowering. *AtC401*, a clock-controlled gene, that encodes a protein containing twelve PPR motifs. *AtC401* shows protein kinase activity and influences the photoperiodic formation of flowers (Oguchi et al. 2004). No further illustration of the *AtC401* function, as well as subcellular localization, have been reported. *EFS1* was reported to be involved in the control of flowering. *EFS1* belongs to the P-subfamily of PPR proteins with eight PPR motifs and on the base of prediction analysis is localized to mitochondria. The flowering time of *efs1* and RNAi transgenic plants was 10 days earlier only in the short-day conditions (Zhang et al. 2011). However, there was no further information regarding the mechanism, by which *EFS1* regulates the flowering time. Taking all results of genetic complementation analysis as well as phenotypic analysis of the *poco1* plants under different photoperiods together, it can be concluded that POCO1 has a remarkable impact on flowering time.

4.2. *FLC* and its positive regulator, *ABI5*, are down-regulated in *poco1*

In *A. thaliana* distinct signaling pathways affect flowering converge on the activation of the same flowering-time genes such as floral repressor *FLC* and two floral activators *FT* and *SOC1*. This may enable plants to integrate different responses to generate a coordinated flowering response under ever variable environmental conditions (Mouradov et al. 2002; Andrés and Coupland 2012). *FLC* is an important regulatory gene for the floral initiation, which encodes a repressor of flowering (Michaels and Amasino 1999). *FLC* binds directly to *FT* and *SOC1* and represses their transcription, thereby strongly suppressing the flowering time (Helliwell et al. 2006; Searle 2006). *flc* mutants show an early-flowering phenotype, supporting the repressing role of *FLC* in flowering time (Michaels and Amasino 1999). Studies

demonstrated that *FLC* binds to the intron region of *FT* and prevents its transcriptional activation (Helliwell et al. 2006; Dennis and Peacock 2007). Knowing that *FLC* acts upstream of *FT* in the flowering pathway and promotes its expression, it can be postulated that the down-regulation of *FLC* most likely is one possible cause for the up-regulation of *FT* in *poco1*. In support of the importance of *FLC* on the expression of *FT*, it was shown that in mutants, in which *FLC* was down-regulated, an increased level of *FT* was observed (Mockler et al. 2004). In such a scenario, an up-regulation of *FT* was observed in *poco1* plants, in which *FLC* was down-regulated. The unchanged expression of *SOC1* may indicate that it is circumvented by the other regulatory pathways. It was indicated that *SOC1* can still be up-regulated through other pathways despite the repression of *FLC* (Rouse et al. 2002). Candidate pathways, which can bypass the *FLC*-mediated *SOC1* repression are the photoperiod (Samach et al. 2000) and gibberellic acid (Borner et al. 2000b) pathways. Together, these data suggest that *POCO1* mutation causes an alteration in the expression of *FLC* and *FT* flowering genes. Inclusive, the down-regulation of the *FLC* on one hand and the up-regulation of the *FT*, on the other hand, may ideally explain the early-flowering phenotype of *poco1*.

ABI5 encodes a basic leucine zipper (bZIP) transcription factor that functions in the core of ABA signaling (Finkelstein and Lynch 2000). bZIP family is a large group of transcription factors in eukaryotes and *Arabidopsis*. Most of the abscisic acid-responsive element (ABRE)-binding factors (ABFs) are classified as bZIP transcription factors, which are main elements in ABA signal transduction (Choi et al. 2000). In plants, bZIP transcription factors are involved in the regulation of different biological processes including flower and seed development, seed germination, light signaling, hormone and sugar signaling as well as the involvement in responses to abiotic and biotic stresses (Jakoby et al. 2002; Skubacz et al. 2016; Sornaraj et al. 2016). Regulations of phytohormones in control of the floral transition in *A. thaliana* has been reported (Davis 2009; Domagalska et al. 2010). Additionally, inhibition of floral transition by ABA, using ABA-deficient mutants has been documented (Martínez-Zapater et al. 1994), which led to the conclusion that ABA acts as an inhibitor of the flowering time. Interestingly, *ABI5* was demonstrated to mediate the inhibitory role of ABA on floral transition (Wang et al. 2013a). The study by Wang et al. 2013 showed a connection between floral transition and ABA signal transduction, in which *ABI5* promotes the expression of *FLC* via directly binding to the *FLC* promoter elements. Furthermore, it has been reported that an *abi5* mutant exhibits an early-flowering phenotype (Finkelstein and Lynch 2000; Wang et al. 2013a). Based on these data and the results in this study, it can be concluded that the down-regulation of *ABI5* in *poco1* plants can be one scenario for the lack of *FLC* expression, which also asserts the direct correlation between *FLC* and *ABI5* on the base of previous studies.

In *Arabidopsis* PPR mutants, changes in the expression of ABA-responsive genes including *ABI5* was reported. In the *soar1-1* (Mei et al. 2014) and *grs1-1* (Xie et al. 2016), the expression of *ABI5* has been up-regulated compared to corresponding wild-type plants. However, similar to *poco1*, in *ppr96* (Liu et al. 2016) the expression of *ABI5* has been down-regulated. Based on these data, a correlation between the expression of PPR proteins and ABA-responsive genes is plausible. The action of PPR proteins has significant effects on organelle biogenesis and function and, as a result, on photosynthesis, respiration, plant development, and environmental responses (Barkan and Small 2014). *ABI5* and other ABFs are involved in plant development and environmental responses (Skubacz et al. 2016). This further supports the idea that *poco1*, as well as other above-mentioned PPR proteins, might act upstream of the ABA-responsive genes such as *ABI5*, thereby strongly influencing the ABA signaling. Nevertheless, there is no evidence of a direct regulatory pathway or interaction between *POCO1*, *FLC* and *FT* and *ABI5*. As *POCO1* appeared to be localized in mitochondria (Emami and Kempken 2019), there is presumably no direct regulatory pathway between these genes. In this sense, an indirect effect of *POCO1* on the regulation of these genes is expected. These findings suggest the existence of a signaling cascade from mitochondria, where *POCO1* is localized, to the nucleus, where the changes in the gene expression occur. The regulation of flowering through retrograde signals from chloroplast during the high light stress was reported, in which *FLC* was repressed (Feng et al. 2016). However, any effect on *FLC* through retrograde signals from mitochondria has not yet been reported.

4.3. *poco1* plants show altered sensitivity to ABA and drought stress

ABA has an inhibitory effect on primary root growth (Finkelstein et al. 2002). Furthermore, the inhibitory effect of exogenous application of ABA on the flowering time has been demonstrated (Jiang et al. 2012; Wang et al. 2013a). It has been reported that an *abi5* mutant exhibits an early-flowering phenotype as well as pleiotropic defects in ABA response (Finkelstein and Lynch 2000; Wang et al. 2013a). *ABI5* functions as a positive regulator of ABA signaling, thereby is involved in many biological processes including growth and development (Skubacz et al. 2016). Also, *ABI5* was indicated to be involved in floral transition control via the ABA signaling transduction pathway (Wang et al. 2013a; Shu et al. 2016). Moreover, ABA-insensitive/deficient mutants such as *aba1* and *aba2* showed an early-flowering phenotype (Barrero et al. 2005; Domagalska et al. 2010). The reduced expression level of *ABI5* can confer an impaired ABA signal transduction in *poco1*, which was shown by the reduced sensitivity to ABA compared to wild-type plants in the context of primary root growth and floral transition. These results also show that the *poco1* mutation leads to a decreased exogenous ABA

sensitivity regarding the primary root growth and floral transition, suggesting that POCO1 acts as a positive regulator of ABA response and may adjust ABA responses in development and stress responses.

Any changes in ABA signaling components leads to the alteration of plant response and tolerance, which may contribute to the adaptation mechanisms to cope with stresses. Therefore, the proper plant response to abiotic stresses such as drought stress requires a fine-tuned ABA signaling pathway (Shinozaki et al. 2003; Ma and Qin 2014). The phytohormone ABA plays an important role in molecular and physiological adaptation and tolerance of plants to abiotic environmental stresses such as drought and salinity stresses. One strategy during the drought tolerance taken by the plants is to inhibit the root growth to ensure more water maintenance (Lopez-Molina et al. 2001; Brocard et al. 2002). Therefore, the conclusion that *poco1* reduced the sensitivity to ABA regarding the primary root growth may explain the higher sensitivity to drought stress. Furthermore, *ABI5* was reported to confer adaptation to plants under unfavourable environmental conditions (Skubacz et al. 2016). Thus, *ABI5* is considered as a stress-response marker gene (Brocard et al. 2002). It has been shown that there is a correlation between ABA sensitivity, *ABI5* expression and the reorganization of the dehydration tolerance (Maia et al. 2014). Also from this perspective, the altered sensitivity to the drought stress in *poco1* most likely arises from the ABA-deficiency in these plants (Skubacz et al. 2016), which was supported by the reduced expression of *ABI5* in *poco1* plants and their decreased responsiveness to ABA. This, together with the above-discussed evidence further suggests the positive role of POCO1 in drought stress tolerance. Furthermore, it can be postulated that other ABA-associated genes, as well as stress-responsive genes especially drought stress, are affected in *poco1* plants.

4.4. POCO1 is required for the mitochondrial RNA editing

PPR proteins are involved in diverse RNA maturation functions mostly in mitochondria and chloroplasts (Andrés et al. 2007; Schmitz-Linneweber and Small 2008). RNA editing modifies a C originally encoded at the DNA level to an U in the mature RNA thereby changing the amino acid codons originally encoded by the DNA, affecting the final structure of proteins. Therefore, RNA editing is a considerably essential process for the proper function of organelles. P-type PPR proteins predominantly participate in organellar posttranscriptional processes such as RNA stabilization, splicing and translation (Barkan and Small 2014). For instance, P-type PPR proteins can bind to specific RNA sequences and protect it from endo- or exonucleases and can recruit RNA maturation factors by modifying the secondary structure of RNAs (Takenaka et al. 2013b). PLS-type PPR proteins, on the other hand, are plant-specific and function

typically in RNA editing. The DYW domain in PLS-type PPR proteins is a strong candidate for association with C deaminase activity (Salone et al. 2007; Shikanai 2015) and recently it was demonstrated that only the DYW domain of a PPR protein in *P. patens* was sufficient to perform RNA editing *in vitro* (Hayes and Santibanez 2020). However, a number of exceptions have been found, which highlight the association of P-type PPR proteins with RNA editing. For instance, PPR596, PPR-MODULATING EDITING (PPME) and NUWA are P-type PPR protein, which are implicated in RNA editing (Doniwa et al. 2010; Leu et al. 2016; Guillaumot et al. 2017). PPR596 is involved in the editing efficiency of mitochondrial *rps3* (Doniwa et al. 2010). PPME acts in mitochondrial *nad1* RNA editing via RNA binding (Leu et al. 2016). NUWA was suggested to have complex stabilization role between E⁺-type PPR and DYW2 proteins in the process of RNA editing (Andrés-Colás et al. 2017).

The exact mechanism of the paralogous gene for POCO1, PPR596, is still not clear as the editing efficiency alteration happens in a silent editing position (*rps3-1344*) and can hardly explain the growth defect in *ppr596* mutants (Doniwa et al. 2010). Therefore, it was postulated that the editing alteration may be a direct or an indirect effect of other RNA processing events (Fujii and Small 2011). It was suggested that POCO1 also is involved in the RNA editing of *rps3-1344* position (Doniwa et al. 2010). Using RT-PCR the involvement of POCO1 in editing efficiency of the *rps3-1344* was verified, in which a reduction of 20% in the editing efficiency was observed. However, merely this reduction in the editing efficiency is weak and has little chance to impact mitochondrial functions. In *rps3-1344*, the efficiency of editing was shown to be increased in *ppr596* plants but decreased in *poco1* plants. This antagonistic effect may likely be explained by the different nature of similar PPR proteins, which may bind to or compete for the same RNA target sites (Andrés et al. 2007). For instance, CHLOROPLAST RNA PROCESSING 1 (CRP1) is needed for the translation of its target RNAs (Schmitz-Linneweber et al. 2005), whereas two other similar PPR proteins, RFO and RFK, hinder translation of their target transcripts (Koizuka et al. 2003; Brown et al. 2003).

It is noteworthy that the editing efficiency in most of the editing sites is lower in *poco1* except *ccmFc-146*, *ccmFc-160*, *ccmFc-415*, *nad1-823*, *nad4-1194* and *nad7-1124*. Most of the affected sites with altered editing efficiency are non-silent editing sites and change the nature of the amino acid sequence of proteins encoded by these transcripts. Therefore, the alteration of amino acids might be expected to have an impact on the function of these proteins, subsequently affecting mitochondrial function in *poco1*. A drastic reduction of editing in the editing sites of *rps4*, *nad2*, *nad5*, *nad7*, *matR* and *mttB* may indicate a dominant role for POCO1 at these editing positions. Notably, RNA editing has been severely abolished at all sites of the 3' end of *rps4*, which may severely impair RPS4 function. These data may be interpreted as loss of an and/or some editing site have a global effect on the other editing sites,

in accord with the findings of Grüttner et al. 2013. These results may also lead to speculate that POCO1 has a general function for several mitochondrial transcript, preserving the secondary structure of transcripts. In *ccmFc*-160 and *cox3*-687 amino acid conversions alter hydrophobic to hydrophilic amino acid residues. In complex I subunits (*nad2*-1403, *nad5*-1958 and *nad7*-1124), *mttB*-682 and *rps4*-956, *rps4*-992 and *rps4*-1057 editing alterations lead to changes in the nature of amino acid from hydrophilic to hydrophobic residues. Therefore, editing impairment modifies the overall biochemical nature of the affected mitochondrial proteins in these editing sites.

The involvement of P-type PPR proteins in RNA editing suggests the formation of an editing complex with PLS-type PPR proteins including DYW catalytic domain and other editing factors such as MORFs and RIPs (Bentolila et al. 2012; Takenaka et al. 2014; Zehrmann et al. 2015). In support of this, it is evident that PPR proteins interact with RNA editing factors to perform the editing process (Sun et al. 2016; Ichinose and Sugita 2017) and it was demonstrated that multiple PPR interactions are required for RNA editing in mitochondria and chloroplasts (Andrés-Colás et al. 2017). A robust interaction for directing RNA editing was found between P-type PPR protein NUWA, E⁺ PPR protein SLO2 and a DYW containing PPR protein DYW2 (Andrés-Colás et al. 2017). POCO1, which is a P-type PPR protein, appears to be involved in multiple RNA editing events in mitochondria. Therefore, it could likely be expected that POCO1 may interact with other PPR proteins or other components of RNA editing to affect RNA editing (Boussardon et al. 2012). Hence, it was reported that RNA editing PPR proteins recognize *cis*-elements in the vicinity of the editing sites, they are expected to bind RNA (Zehrmann et al. 2011). Nevertheless, the expected RNA binding site for POCO1 could not be predicted using the previously predicted PPR code (Barkan et al. 2012; Yagi et al. 2013; Yan et al. 2019). The lack of canonical residues in crucial positions in POCO1 and the effect on several editing sites permit the suggestion that POCO1 may have less specific RNA binding features making transcripts accessible for the specific editing factors. Moreover, although most of the *poco1* mutation-affected editing sites had lower editing efficiency, higher editing efficiencies compared to wild-type were also identified, suggesting that additional unknown components are required to determine the editing activity at different sites. A similar observation has been reported from RNA editing factors such as PROTOPORPHYRINOGEN IX OXIDASE 1 (PPO1) (Zhang et al. 2014) and GENOMES UNCOUPLED 1 (GUN1) (Zhao et al. 2019) in plastid RNA editing. POCO1 may raise a level of regulation that refine mitochondrial RNA editing efficiency, leading to the proper mitochondrial function. Besides, it should be considered that POCO1 was initially found in an RNA pull-down experiment using a fragment of *cox2* containing editing sites. Therefore, being as a component of an editing complex would explain the reason for the identification of POCO1 as an editing factor. However, our RNA editing analysis did not show

any editing alteration in *cox2* transcript. Taken all together, it can be proposed that POCO1 may have a direct role in RNA editing as a component of an editing complex.

A number of mitochondrial PPR proteins in *A. thaliana*, which are involved in mitochondrial post-transcriptional gene expression, were shown associated with altered growth phenotype and ABA responses. ABO5, required for the splicing of *nad2* (Liu et al. 2010), AHG11, involved in editing of *nad4* (Murayama et al. 2012), SLG1, involved in editing of *nad3* (Yuan and Liu 2012), ABA OVERLY SENSITIVE 6 (ABO6) involved in splicing of complex I genes (He et al. 2012), AHG11, involved in editing of *nad4* (Murayama et al. 2012), SLO2, involved in editing three complex I genes (Zhu et al. 2014), HAS2/LOI1/MEF11 involved in the RNA editing of *cytochrome c maturation FN2 (ccmFN2)* (Sechet et al. 2015). Therefore, it can be suggested that there is a connection between the RNA editing defects and physiological effects it causes. However, further investigation can potentially shed light on the physiological consequences of RNA editing defects in the affected individual transcripts in *poco1*.

4.5. Mitochondrial respiratory activity is disturbed in *poco1* plants

Mitochondria are responsible for the energy supply of eukaryotic cells through the aerobic respiration (Gray 1999). Mitochondria have their own genome, which encodes a small number of essential factors for the gene expression and functional respiration. The majority of factors involved in the regulation of gene expression have been transferred to the nucleus during the course of evolution (Burger et al. 2003). Therefore, translocation of these factors back to mitochondria and their functionality is crucial for the regulation of gene expression and thus the proper function of mitochondria. It is conceivable that the alteration of multiple mitochondrial editing sites most likely has an impact on the mitochondrial function in *poco1* plants. Furthermore, alterations in ABA signaling and stress sensitivity are correlated to defects in mitochondrial respiration and ROS homeostasis (He et al. 2012). The dysfunction of mitochondria in *poco1* was supported by the reduced respiration rate and ATP generation and increased accumulation of ROS. The process of respiration is regulated on different levels like RNA editing, which can affect the gene activity (Millar et al. 2011). This suggests that the lower rate of respiration in *poco1* plants may be due to the alterations in the editing of mitochondrial transcripts especially editing defects in the subunits of complex I, which is the major electron source of the mitochondrial respiration chain (Fromm et al. 2016). The editing defects were observed in five subunits of the complex I (*nad1*, *nad2*, *nad4*, *nad5* and *nad7*) of the mitochondrial respiration chain. Impairment of the mETC and particularly complex I usually hinders the NADH dehydrogenase activity, which affects cellular ATP generation and increases ROS accumulation (Yang et al. 2014). Alteration of the nature of amino acids in

complex I subunits (especially *nad2-1403*, *nad5-1958* and *nad7-1124* that editing alterations lead to changes in the nature of amino acid) most likely leads to the alteration of structure and characteristic of the complex I and thus the higher generation of ROS in *poco1* plants can be explained. Consistent with the lower respiration rate in *poco1*, the ATP content in *poco1* mitochondria was lower compared to wild-type plants. Mitochondria have a central role in cellular energy production, contributing to ATP synthesis. Energy deficits have pleiotropic effects on plant metabolism. One of the consequences of energy deficiency includes alteration in the ABA signaling pathway (Liu et al. 2010). Furthermore, lower energy supply causes plants to be vulnerable to abiotic stresses (Ghosh and Xu 2014). Also from this perspective apart from ABA deficiency, *poco1* plants exhibited a higher sensitivity to drought stress.

Mitochondria are targets of several various stresses (Bartoli et al. 2004; Giraud et al. 2008). A general theme in many stress responses is the production of ROS (Fujita et al. 2006). Although ROS formation in mitochondria happens under normal respiratory conditions, it can be highly elevated by a wide range of biotic and abiotic stresses (Rhoads et al. 2006). Impairment of mitochondrial respiratory activity leads to ROS accumulation and can be recognized as an unfavourable condition for plants. The higher ROS accumulation in *poco1* suggests that the defective respiration in *poco1* may thus affect the homeostasis of ROS and further indicates that the loss of function of POCO1 contributes to the defected mitochondrial respiratory activity. The major sites of ROS production are complex I and III, where superoxide anions are formed (Raha and Robinson 2000; Møller 2001). Given that the complex I is one of the major sites for ROS production, it can be also proposed that the higher ROS production in *poco1* plants may be due to changes in the editing of mitochondrial transcripts of complex I subunits. Enhanced ROS production has been reported from several mutants affected in mETC RNA metabolism (Zsigmond et al. 2008; He et al. 2012; Zhu et al. 2014). When ROS accumulation exceeds the normal level, there are further consequences for the cell such as alteration of gene expression or even programmed cell death (PCD). Therefore, apart from being a damaging agent, ROS also act as signaling molecules (retrograde signal). This further leads to the modification of the gene expression in the nucleus (Woodson and Chory 2008; Kmiecik et al. 2016; Mignolet-Spruyt et al. 2016). The alteration in RNA editing levels for *poco1* mutation-affected transcripts may result in abnormal mitochondrial proteins, which leads to the mitochondrial defects and an enormous ROS production, triggering the ROS-dependent retrograde signaling. This can be considered as one possible link between RNA editing and retrograde signaling. ROS were reported to penetrate membranes and leave the mitochondrion, thereby are implicated in the control of the plant development and adaptation to the stresses (Desikan et al. 2001; Gechev et al. 2006). Moreover, it was reported that ROS cause changes in gene expression, thereby inducing flowering (Ionescu et al. 2017), which is the strategy taken by the plants to cope with different stress conditions to ensure the reproduction and seed set (Kazan and Lyons 2016).

Therefore, ROS produced in mitochondria can be regarded as a candidate signaling molecule in *poco1*, which can lead to the further expression alteration of genes in the nucleus and permits promotion of floral transition. Another potential link between RNA editing and retrograde signaling is, that the improper editing of mitochondrial transcripts may lead to the misfolding and aggregation of the corresponding proteins, which have to be eliminated by mitochondrial protein quality control (mtPQC) pathway. This pathway is likely to be linked to retrograde signaling, as defective mitochondrial proteins lead to the mtPQC-specific retrograde response (Wang and Auwerx 2017). A possible link between RNA editing in chloroplasts and retrograde signaling was proposed (Zhao et al. 2019), in which GUN1 affects the editing during retrograde signaling. Therefore, a similar link between RNA editing in mitochondria may most likely also be possible.

4.6. Putative interaction partners of POCO1

Identification of accurate interacting partners is a rather complex task, which requires knowledge about different protein properties. PPR proteins have been reported to have various functions especially in RNA editing, in which they interact with modular RNA editing factors to accomplish the editing process (Sun et al. 2016; Ichinose and Sugita 2017). Among the increasing amount of data regarding protein interactions of non-PPR RNA editing factors, minor PPR protein interactions have been reported. This is mainly due to the low expression levels and highly unstable feature of the PPR proteins (Andrés-Colás and Van Der Straeten 2017).

Taking into consideration that POCO1 is localized to mitochondria (Emami and Kempken 2019), it would be anticipated that the mitochondrial localized putative interaction partners have a higher probability of being real interaction partners and no false-positive results. PPR596, as mentioned in the previous sections, is involved in the RNA editing efficiency of mitochondrial *rps3* transcript (Doniwa et al. 2010). Both PPR596 and POCO1 affect the same mitochondrial editing site, *rps3*-1344. However, besides *rps3*-1344, POCO1 affects several other editing positions in mitochondria. PPR596 and POCO1 may thus cooperate as a part of an editing complex in mitochondrial editing sites, particularly at *rps3*-1344. This hypothesis is supported by another report, in which PPR596 was found in a protein complex along with POCO1 in the *A. thaliana* mitochondrial complexome of the same size (Senkler et al. 2017). Furthermore, since PPR596 is the paralogous gene for POCO1 and shows high sequence similarity to POCO1 (Emami and Kempken 2019), it may be expected that both proteins will interact with the same partners. Therefore, it is a promising candidate for being an interacting partner for POCO1 to be further investigated. mtHsp70 facilitates mitochondrial protein

homeostasis by involvement in the import and folding of proteins for the matrix compartment (Voos 2013). To perform its various functions, mtHsp70 interacts with several interaction partners (Böttinger et al. 2015). Interestingly, the interactions between mitochondrial HSPs and editing factors were previously reported (Law et al. 2015; Andrés-Colás et al. 2017). Therefore, identified mitochondrial HSPs can be considered as likely potential interaction candidates for POCO1. Since the investigation of interacting proteins was carried out via GFP pull-down assay, interaction partners can also be identified that do not interact directly with POCO1 but are linked to POCO1 via other proteins or nucleic acids (Lalonde et al. 2008). However, the interaction between POCO1 and these candidates should be tested by further analysis such as the direct observation of the interaction by bimolecular fluorescence complementation (BiFC) assay.

4.7. Differential gene expression analysis by RNA-seq

Gaining a comprehensive knowledge of differentially expressed genes in *poco1* and the specific contribution of POCO1 in flowering and ABA signaling is the key to understanding its function. Identification of a large number of differentially expressed genes in inflorescence-inflorescence compared to pre-inflorescence-inflorescence and inflorescence-flowering may suggest a high variation in metabolic processes in each same developmental stage between wild-type and *poco1* plants. Also, it may be due to the developmental reprogramming before sexual reproduction, which may have occurred more intensively in *poco1*. Besides, although being in the same developmental stage, they are not the same age. The high number of differentially expressed genes in inflorescence-inflorescence compared to other two comparisons, including many relevant genes to this study, suggests that the comparison in the same developmental stage may be highly informative for analyzing differentially expressed genes. Therefore, this asserts the necessity of biological analysis in different developmental stages.

The GO enrichment for the up- and down-regulated genes of each comparison suggests the crucial roles of those biological processes, in particular, the over-representation of many processes associated with response to stresses in regulating the developmental processes in *poco1*. The highly enriched defense response-related biological processes for the up-regulated genes of pre-inflorescence-inflorescence may highlight the fact that Plant defense process is linked with the transition from the vegetative to the reproductive phase. A significant correlation between the regulation of glucosinolate content and flowering time in *Aethionema arabicum* (Brassicaceae) has been identified. FLC was suggested to be the potential regulator of glucosinolate content (Halkier and Gershenzon 2006). Furthermore, the glucosinolate and

glycosinolate biosynthetic processes, which are enriched for the up-regulated genes in pre-inflorescence-inflorescence, are considered as principal regulators in the floral transition phase and enable the protection of plants against pathogens during flowering (Deshpande et al. 2017). These compounds are composed of sulfur and nitrogen. Interestingly, *TGG2*, which is strongly down-regulated in *poco1* in all comparisons (the most down-regulated gene in pre-inflorescence-inflorescence and inflorescence-flowering and the sixth most down-regulated in inflorescence-inflorescence) (Supplementary Figure 5A, B and C), was demonstrated to be required for glucosinolate breakdown (Barth and Jander 2006). Biological process GO terms of “cell redox homeostasis” and “electron transport chain” enriched for down-regulated genes of pre-inflorescence-inflorescence (Supplementary Table 3), may be indicative of the perturbation of mitochondrial function, the higher generation of ROS and altered redox state in *poco1*.

The presence of biological processes GO terms associated with response to various stresses for up-regulated genes in inflorescence-flowering may indicate that different stresses allocate some identical pathways. GO terms associated with ageing for up-regulated genes in inflorescence-flowering may pronounce the forefront developmental maturation of *poco1* compared to wild-type plants. As a natural outcome of ageing-related processes, cell wall organization and biogenesis were enriched for down-regulated genes of inflorescence-flowering.

highly enriched GO terms “translation” and “peptide biosynthetic process” along with terms associated with “ribosome assembly” for the up-regulated genes of inflorescence-inflorescence, may indicate control of ribosomes. Hence, *de novo* protein synthesis is essential for the floral transition in *poco1*. Modification of the translational machinery has been reported in *A. thaliana* under stress conditions (Harb et al. 2010). These data may emphasize the significance of the translation apparatus in *poco1*, which bears with adverse conditions, due to the mitochondrial dysfunction. Accordingly, *Cwf18* pre-mRNA splicing factor (among the top 10 most up-regulated genes in all three comparisons) (Supplementary Figure 5A, B and C) was reported to function in the early response to abiotic stresses. *Cwf18* pre-mRNA splicing factor was found to function in the gene expression process and acts along with proteins that function as part of the ribosome (Gehring et al. 2018). Therefore, its over-expression may further support the rescheduling of stress-induced transcriptional events in *poco1*. GO biological process terms related to nitrogen compound biosynthesis processes are over-represented for the up-regulated genes of inflorescence-inflorescence. Studies have reported that plants can utilize the required nitrogen from organic compounds such as proteins and amides (Näsholm et al. 2007; Rentsch et al. 2007). Consistently, protein and amide biosynthesis processes are highly enriched for the up-regulated genes of inflorescence-

inflorescence. Availability of nitrogen is a limiting factor for plant growth and development, which controls developmental phase change (Vidal et al. 2014; Weber and Burow 2018). Some studies showed that higher nitrogen conditions promoted flowering in *Arabidopsis* (Pigliucci and Schlichting 1995; Marín et al. 2011; Yuan et al. 2016; Teng et al. 2019). The GO terms “response to abscisic acid” and “response to water deprivation” enriched for the down-regulated genes of inflorescence-inflorescence, may explain the impaired ABA signaling and susceptibility of *poco1* to drought stress.

4.7.1. Effect on genes involved in flowering

Flowering is a critical step in the plant life cycle as it ensures plant species survival. In many plant species, various stress factors can induce or inhibit flowering. Evidence that stresses act as a flowering-inducing factor is increasing (Yaish et al. 2011; Pieterse 2013; Riboni et al. 2014; Kazan and Lyons 2016). The mitochondrial respiratory activity impairment can be recognized as an adverse condition by *poco1* plants, which may ultimately influence floral transition.

Induction of *FT* and the *FT* integrator, *GI*, in *poco1* may suggest the promotion of flowering in a circadian-clock manner (Martin-Tryon et al. 2007). *GI* allows the activation of *FT* by either directly binding to its promoter or inhibition of its repressor (Sawa and Kay 2011). Expression of *GI* was previously reported to be a stress escape response and it is required for the early flowering of *Arabidopsis* in response to stress. Further studies proposed that, except *GI*, *FT* is also involved in stress-induced flowering (Riboni et al. 2013; Takeno 2016). These data strengthen the idea that the early flowering in *poco1* may be related to stress-induced flowering. Another intriguing note is the effect of *TEM1* and *FRL* on the expression of *FT* and *FLC*. *TEM1* is induced by *FLC* and was indicated to repress *FT* expression and its loss of function mutation showed an early-flowering phenotype (Matías-Hernández et al. 2014). *FRL* promotes the expression of *FLC* (Quesada et al. 2005; Wang et al. 2006; Choi et al. 2011). Both *TEM1* and *FRL* were shown to be down-regulated in *poco1*, which may consequently lead to the release of the *FT* suppression and lessen *FLC* expression in *poco1* respectively. Besides, loss of function of *GRP7* (up-regulated in *poco1*) was reported to increase the total functional sense *FLC* transcript and delays flowering time (Xiao et al. 2015). These results suggest that the early flowering of *poco1* is affected through repression and promotion of *FLC* and *FT* respectively. Apart from that, RNA-seq analysis identified several genes in all three comparisons, which allow early floral transition and their up- or down-regulation leads to the early-flowering phenotype and fits *poco1*'s phenotype. For instance, up-regulation of *FBH2*, *PRE1*, *FLP1* and down-regulation of *PHYE*, *ELF4-L1*, *RAV1*, *NAC089*, *CRK6* and *CRK19* allow an early-flowering phenotype. *FLC* was not found among differentially expressed genes

in *poco1*, which may be due to different methods of RNA isolation for qRT-PCR and RNA-seq analysis. It also may be addressed by the different sampling time for both analyses. However, taking qRT-PCR data and whole transcriptomic RNA-seq analysis together it can be postulated that *FLC* down-regulation, *G1* up-regulation and *TEM1* down-regulation may be potential causes leading to *FT* up-regulation in *poco1* plants, promoting the floral transition in *poco1* plants.

FT is known to be induced by gibberellic acid (Hisamatsu and King 2008). FLP1 (up-regulated in *poco1*) has 92% amino acid identity to the FPF1 protein. It can be suggested that FLP1 may likely act in a gibberellic acid-dependent manner similar to FPF1 (Kania et al. 1997). Together these data with the differential regulation of two gibberellic acid receptors *GID1B* and *GID1C* as well as the gibberellic acid biosynthesis gene, *GA3ox1*, may highlight a role for gibberellic acid in the floral transition of *poco1*.

A study demonstrated that besides the suppression of flowering, *FLC* has regulatory roles and binds to more than 500 target sites in the *Arabidopsis* genome, implying that *FLC* potentially is involved in the regulation of many genes that act in developmental pathways. Except for *FT* and *TEM1*, *COL1* from circadian clock pathway and *GID1C* from gibberellic acid pathway are other examples of identified *FLC* target genes (Deng et al. 2011). This may explain the regulatory effect of *FLC* in circadian rhythm and a link with the gibberellic acid pathway to ensure the appropriate initiation of flowering. This further may imply that the observed change in the expression of *FT*, *TEM1*, *COL1* and *GID1C* may be due to the change in the *FLC* expression level in *poco1*. By analysis of regulated genes associated with the flowering, it is concluded that *poco1* apparently determined a route to accomplish the life cycle earlier and *POCO1* mutation leads to the alteration of expression of genes that outcomes in the acceleration of flowering.

4.7.2. ABA signaling and response is impaired in *poco1*

The phytohormone ABA regulates many aspects of plant growth and development and has a crucial role in adaptive stress responses, especially stresses occurring by water deficit (Cutler et al. 2010; Bartoli et al. 2013). Mutant plants with altered ABA biosynthesis are not able to decode ABA components (Jaradat et al. 2013). *POCO1* mutation was shown to confer altered sensitivity to ABA. In accordance, the data from the whole-transcriptome analysis identified many ABA-associated genes down-regulated in *poco1*.

Pyrabactin resistance 1/Pyrabactin resistance 1-like/regulatory components of ABA receptors (PYR/PYL/RCAR) proteins are intracellular receptors of ABA either in the cytosol or nucleus,

which form a complex with negative regulators of ABA signaling, type 2C protein phosphatases (PP2Cs). As a result, PP2Cs are inactivated, which allows the activation of downstream targets, snf1-related protein kinases 2 (SnRK2s). After activation of SnRK2s by upstream activating kinases (UAKs), nuclear targets such as *ABI5*, which is an ABF/AREB will be induced. Eventually, ABF/AREBs bind to ABRE sequences to activate ABA-responsive gene expression (Figure 26) (Uno et al. 2000; Finkelstein et al. 2002; Umezawa et al. 2010; Yoshida et al. 2014). Identification of several factors, which act as main components of ABA signaling such as PYR/PYL/RCARs, PP2Cs, SnRK2s and ABF/AREBs coupled with the experimental results (3.9) highly endorse the impaired ABA signaling pathways from the early perception to the impaired expression of many ABA-responsive genes in *poco1*. Differential expression of genes, which function as both positive and negative regulators of ABA signaling in *poco1* (such as PP2Cs and SnRK2s) may suggest the presence of a complicated feed-forward and feedback mechanism that try to balance positive and negative regulation of gene expression to optimize ABA signaling. Calcium-dependent protein kinases (CDPKs) have been revealed to mediate phosphorylation signaling cascades, ion flux and transcriptional regulation response to ABA. CDPKs are Ca^{2+} binding sensory proteins and have been indicated to play roles in ABA/stress signaling (Cheng et al. 2002; Antoni et al. 2011). *CALCIUM-DEPENDENT PROTEIN KINASE 32 (CPK32)* (down-regulated in *poco1*) forms a part of the ABA signaling component that regulates ABA-responsive gene expression via ABF4 phosphorylation. Over-expression of *CPK32* leads to hypersensitivity to ABA and enhanced expression of ABA-regulated genes in *Arabidopsis* (Choi et al. 2005). The up-regulated expression of an ABA biosynthesis gene, *NCED4*, and the down-regulated expression of an ABA catabolism gene, *CYP707A3* both in inflorescence-flowering may suggest an imbalance in ABA biosynthesis and catabolism in *poco1*. This imbalance is perceivable in inflorescence-inflorescence, where *NCED4* is down-regulated, but no genes with ABA catabolism function were detected.

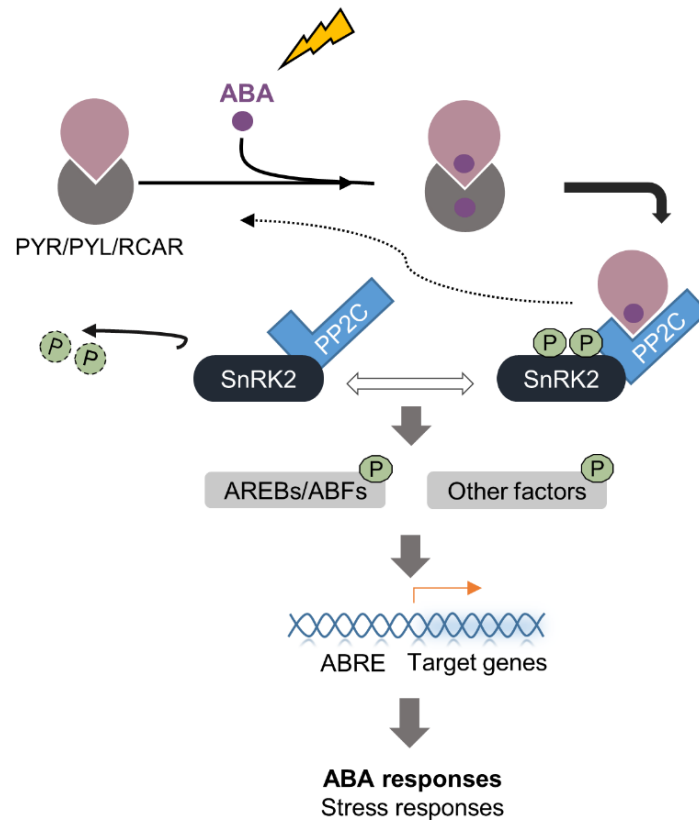


Figure 26: A simplified model of the ABA signaling pathway. PYR/PYL/RCAR receptors perceive ABA and form a complex with PP2Cs. Thus phosphatases are inactivated, which allows the activation of downstream targets, SnRK2s. Phosphorylation of these kinases leads to ABA-induced regulation of nuclear targets such as ABF/AREBs, which recognize the ABA-responsive elements in the promoters of ABA-inducible genes. Adapted from Umezawa et al. 2010.

Exogenous application of ABA significantly inhibits floral transition (Martínez-Zapater et al. 1994; Wang et al. 2013a). The role of ABA-responsive ABFs, ABF3 and ABF4 (both down-regulated in *poco1*), on floral transition has to be taken into consideration. In the promoter region of *FLC* six ABRE-binding sites were identified (Wang et al. 2013a). The activation of genes containing ABRE element is mainly reliant on ABFs (Yoshida et al. 2010). Based on the previous study, except ABI5, other ABFs can distinctly promote the expression of *FLC*, by the function of which ABA affects floral transition (Wang et al. 2013a). Therefore, a likely but not the only scenario is that except ABI5, ABF3 and ABF4 have a direct effect on the repression of *FLC* in *poco1* (Figure 27). The inhibitory role of ABI5 and other ABFs may be modulated through SnRK2s. ABI5 and other ABFs are phosphorylated by SnRK2s, which effects transactivation of *FLC* by ABI5 and other ABFs. This modulation can directly affect floral transition (Wang et al. 2013a). Regulation of *ABI5* includes an intricate mechanism, in which many transcription factors and enzymes are involved. The expression of *ABI5* is resultant of WRKY and MYB transcription factors functions and epigenetic events (Skubacz et al. 2016). Studies suggested that MYB7 is involved in the suppression of *ABI5* expression under stress (Kim et al. 2015). Up-regulation of MYB7 (Supplementary Figure 9) highlights its potential role to suppress *ABI5* in *poco1*.

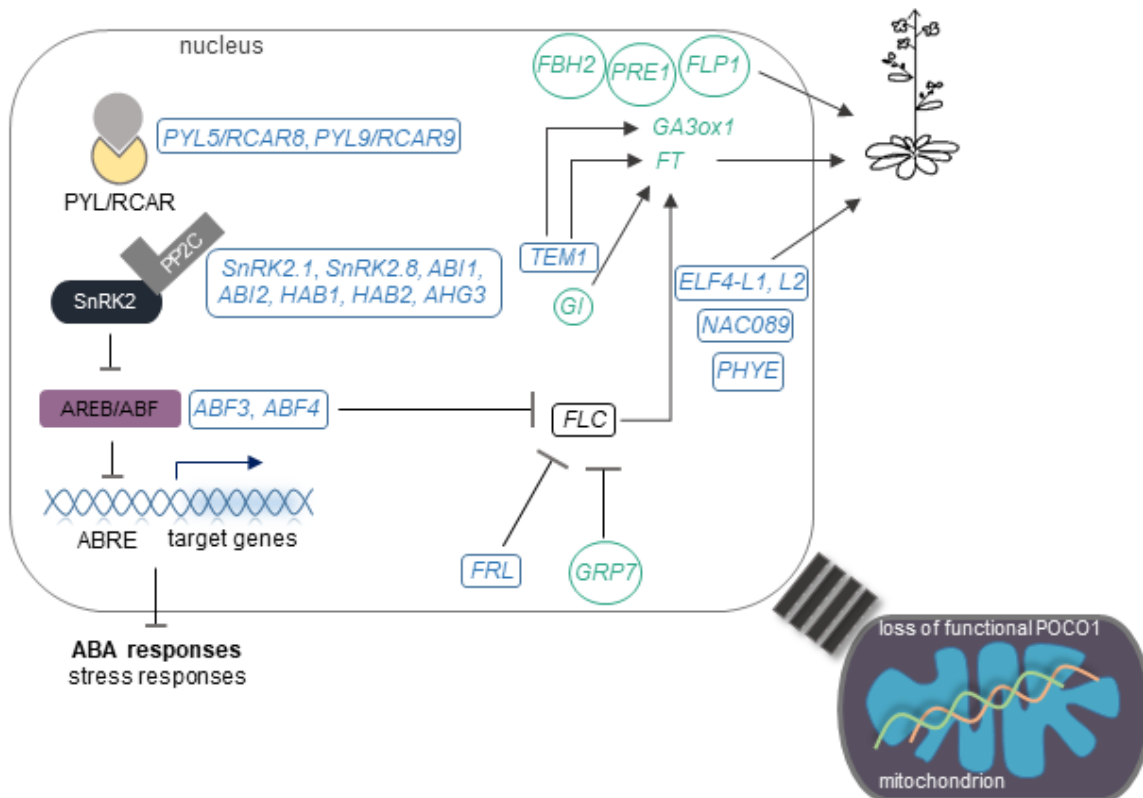


Figure 27: Affected genes in core components of ABA signaling and flowering in *poco1* in a schematic representation. In *Arabidopsis*, multiple factors affect flowering time to adapt the unfavourable conditions. In *poco1* mutants, the core ABA signaling genes (*PYL/RCARs*, *PP2Cs*, *SnRK2s* and *ABF/AREBs*) are down-regulated, which may lead to an impaired ABA and stress response. Down-regulation of *ABF3* and *ABF4*, *FRL* and up-regulation of *GRP7* may have an inhibitory effect on *FLC* expression. Besides, down-regulation of *TEM1* and up-regulation of *G1* may induce *FT* expression. Thus, the early flowering of *poco1* may be the consequence of repression and promotion of *FLC* and *FT* respectively. Down-regulation of *TEM1* also induces the gibberellic acid biosynthesis gene, *GA3ox1*. Down-regulation of *ELF4-L1*, *ELF4-L2*, *NAC089* and *PHYE* and up-regulation of *FLP1*, *PRE1* and *FBH2* are consistent with the early-flowering phenotype. Green and blue genes indicate up- and down-regulated genes respectively. Arrows and dashed arrows are indicative of inducing and inhibiting effects respectively.

ABA-mediated stress responses involve reprogramming of gene expression, which finally affect adaptive responses such as stomatal closure to regulate water loss (Chandrasekar et al. 2000). A specific role of ABA in drought stress is to modulate stomatal closure, preventing less water loss via transpiration (Himmelbach et al. 2003). The transcriptomic data support the idea that the stomatal closure in *poco1* may be impaired. SnRK2s are important regulators of stomatal movements (Yoshida et al. 2002). Myrosinases were demonstrated to be crucial components of ABA signaling in guard cells (Zhao et al. 2008). *TGG2* is an abundant protein in guard cells (lowest fold change in pre-inflorescence-inflorescence and inflorescence-flowering and the sixth-lowest fold change in inflorescence-inflorescence) (Supplementary Figure 5A, B and C). It was indicated that *TGG2* has a critical role in ABA-mediated stomatal closure and ABA failed to induce stomatal closure in *tgg2.1* plants (Islam et al. 2009). *TGG1* and *TGG2* were reported to have functional redundancy (Islam et al. 2009). *GRP7* (up-regulated in *poco1*) was demonstrated to promote the stomatal opening and causes lowered

dehydration tolerance (Kim et al. 2008; Askari-Khorsgani et al. 2018). *CNGCs*, a family of plant ion channels act to respond to abiotic stresses, which eventually leads to the tolerance mechanism (K. Jha et al. 2016). Among the down-regulated *CNGCs*, *CNGC1*, *CNGC6* and *CNGC16* were shown to be highly expressed in guard cells (Wang et al. 2013b). *RBOHD* was demonstrated to be involved in increasing cytosolic Ca^{2+} , which leads to stomatal closure (Kwak et al. 2006). This function was reported to be impaired in an ABA-insensitive mutant (*gca2*) (Pei et al. 2000). *RBOHD* is induced by ABA (Kwak et al. 2006) and therefore, might be under the impact of impaired ABA signaling in *poco1*. RECEPTOR-LIKE PROTEIN KINASE 1 (*RPK1*) functions in the early perception of ABA and thus its function is important regarding the regulation of downstream genes in ABA signal transduction. *rpk1* plants have insensitivity in ABA-induced stomatal closure, which results in an increased water loss rate. Conversely, *RPK1* over-expression line showed the opposite phenotype (Osakabe et al. 2010). In two ABA insensitive mutants, *abi1* and *abi2* the ABA activation of anion channels in guard cells is impaired, which support their important role in stomatal closure (Roelfsema and Prins 1995; Pei et al. 1997). *MYB44* is a member of the MYB family of transcription factors and was shown to be involved in responses to abiotic stress. Over-expression of *MYB44* promotes stomatal closure and stress tolerance in *A. thaliana* (Jung et al. 2008). Alteration of expression of above-mentioned genes, which are associated with the stomatal regulation suggests that stomatal closure may not operate effectively in *poco1* and further propose that *POCO1* mutation may lead to stomatal failure. This conclusion most likely can be explained by ABA deficiency in *poco1*.

4.7.3. Effects on drought and oxidative stresses-associated genes

The acclimation of plants to the ever-variable environmental conditions is acquired by a complex signaling network. The role of plant mitochondria in stress resistance is becoming more and more of interest. Furthermore, the role of ABA in a range of physiological functions and tolerance of plants to various stresses is demonstrated. ABA is involved in diverse cellular responses by long term alteration of gene expression, among which well-knowns are the stress-related genes and especially drought stress genes. Therefore, ABA signaling is essential for stress tolerance particularly drought stress (Finkelstein et al. 2002; Hirayama and Shinozaki 2007; Verslues and Zhu 2007). ABA content limitation, as well as a malfunction of ABA signaling pathways, change the gene expression and affect plant growth, development and tolerance to stress conditions.

Higher generation of ROS and failure to control their accumulation leads to the oxidative stress (Bartosz 1997). The mitochondrial dysfunction observed in *poco1* can conceivably generate oxidative stress by the accumulation of ROS. Accordingly, a gene involved in ROS metabolism,

CHITINASE A (CHIA) (Lee et al. 2015), revealed a significant up-regulation in *poco1*. Differentially expressed genes associated with the oxidative stress most likely refer to the elevated levels of ROS observed in *poco1*. Differentially expressed genes such as *GRXs*, *GSTs* and *TRXs* have an oxidoreductase activity, which may be also associated with the higher accumulation of ROS in *poco1*. Changes in the expression of oxidative stress genes can further cause the alteration in antioxidants level such as glutathione (GSH) (Busi et al. 2011). Presence of oxidative stress-related genes was previously reported in the transcriptomic data induced by mitochondrial dysfunction (Busi et al. 2011).

Upon the stress, ABA induces the expression of multiple stress response genes and allows adaptation to maintain during stress. Different studies showed that higher expression of ABA-induced stress-related genes confers resistance against drought stress (Kusano et al. 2009; Atkinson et al. 2013). ABA-induced stress genes have been shown to regulate the response to the stresses especially drought stress (Kim et al. 2011). In agreement, many of stress-associated genes, which are strongly induced by ABA showing down-regulation in *poco1*. Down-regulated expression of ABA-induced stress genes in *poco1* strengthens the view about the importance of a fine-tuned ABA signaling in inducing these genes. Consequently, it can be concluded that alteration in stress-associated genes especially the suppression of ABA-induced stress genes expression confers susceptibility to *poco1* plants to drought stress. It can further be concluded that POCO1 may act positively to regulate the plant response to drought stress. There are different signal transduction pathways involved in abiotic stresses such as drought, high salinity, and cold stress, which include ABA-dependent and ABA-independent pathways (Shinozaki and Yamaguchi-Shinozaki 2007). However, the effect of ABA-dependent pathways is superior in plant responses to drought stress (Yamaguchi-Shinozaki and Shinozaki 2006; Huang et al. 2008). The conserved ABRE elements in the promoter regions of ABA-dependent drought-related genes are crucial in ABA-responsive gene expression (Uno et al. 2000; Shinozaki and Yamaguchi-Shinozaki 2007). ABRE *cis*-acting elements are predominantly regulated by ABF/AREBs. Therefore, down-regulation of these ABFs in *poco1* may likely be one important possible cause for the down-regulation of many ABA-induced stress-responsive genes (Uno et al. 2000; Liu et al. 2018). *abf3* and *abf4* showed an ABA-insensitive phenotype. Both ABF3 and ABF4 are involved in water deprivation response (Kang et al. 2002; Hwang et al. 2019). Previous studies showed that in *abf3* and *abf4*, an ABA-mediated signal for the activation of *ABF3* and *ABF4* is required. This was confirmed by their lower expression in *abi1* ABA-insensitive mutants (Uno et al. 2000). On the other hand, over-expression of *ABF3* or *ABF4* caused ABA hypersensitivity and enhanced drought tolerance in transgenic *Arabidopsis* plants and their corresponding mutants showed ABA-insensitivity and reduced drought tolerance (Kang et al. 2002; Yoshida et al. 2015). Regulators of ABA signaling such as *PYL5/RCAR8*, *PYL9/RCAR1*, *ABI1*, *ABI2*, *SnRK2.1*, *SnRK2.8*, *ABF3* and *ABF4* were

reported to function in ABA-mediated responses in abiotic stresses (Luan 2003; Yoshida et al. 2010). Many of the ABA- and drought-regulated genes have previously been shown to be induced or repressed by ABA treatment or by various stresses such as drought, cold, and high salinity. Several well-known ABA- and drought stress-related genes *DI19*, *DR4*, *LTP3*, *LTP4*, *ERDs*, *RDs*, *COR47*, *COR413IM1*, *COR413-PM2*, *RAB18*, *MYC2*, which are generally up-regulated by ABA and drought stress, are down-regulated in *poco1* (Supplementary Figure 8). up-regulation of genuine drought-induced genes such as *RAB18* (Lang and Palva 1992) and *LT178/RD29A* (Yamaguchi-Shinozaki and Shinozaki 1994), which are marker genes of ABA signaling is generally coincidence by the elevated tolerance to drought stress, which is not the case in *poco1*. *ERDs* are described as one of the members of the dehydrin family, which are rapidly induced by ABA and during drought stress (Alves et al. 2011). Several *ERDs* like *ERD1*, *ERD6*, *ERD7*, *ERD10*, *ERD14* and *ERD15* are significantly down-regulated in RNA-seq results (Supplementary Figure 8), which may also contribute to the reduced drought tolerance in *poco1*.

Although ABA initially demonstrated to orchestrate abiotic responses, further studies reported additional involvement of ABA in biotic stresses. It was reported that ABA confers resistance to pathogens and diseases (Yasuda et al. 2008; Ton et al. 2009). In other cases, ABA was shown to act as a negative regulator of abiotic stress tolerance (Audenaert et al. 2002; Thaler and Bostock 2004). Enriched biological processes associated with abiotic stresses such as “glycosyl compound biosynthesis”, “response to biotic stimulus”, “glycosinolate biosynthetic”, “sulfur compound biosynthesis process”, “defense response” and “defense response to other organisms” thus may allegedly be due to the ABA signaling impairment in *poco1*, which may lead to the alteration in the expression of biotic stress-associated genes.

Molecular studies revealed the role of PPR proteins in biotic and abiotic stresses, a number of which affect ABA signaling and response. SLG1, a mitochondrial PPR protein in *Arabidopsis*, affects growth phenotype and negatively regulated ABA and drought stress response (Yuan and Liu 2012). Another mitochondrial PPR protein in *Arabidopsis*, PPR40, was determined to function as a link between mETC and regulation of stress and hormonal response. *ppr40* plants showed enhanced accumulation of ROS and disruption of stress-responsive genes, suggesting a correlation between cellular respiration and stress adaptation in *Arabidopsis* (Zsigmond et al. 2008). A mitochondrial PPR protein in *Arabidopsis*, PGN, positively regulates biotic and abiotic response. *pgn* mutant plants exhibited a lower resistance to necrotrophic fungal infections as well as toward ABA, glucose and high salinity (Laluk et al. 2011). The *Arabidopsis* PPR protein ABO5 was identified to be required for the splicing of *nad2* intron 3 in mitochondria. *abo5* plants revealed a decreased expression of stress-related genes such as *RD29A*, *COR47*, and *ABF2*, together with the higher generation of ROS than wild-type plants.

The ROS accumulation was enhanced on exposure to ABA (Liu et al. 2010). This may imply the redox imbalance due to the mitochondrial defects, which activates ABA signaling, further causing the growth retardation and abiotic stress sensitivity in plants. AHG11 is a PPR protein in *Arabidopsis* that is involved in the mitochondrial *nad4* RNA editing. *ahg11* plants were shown to have impaired ABA signaling and mitochondrial function (Murayama et al. 2012). *SLO2* is involved in several mitochondrial editing sites in *Arabidopsis* and regulates plant growth and was linked with the stress response. *slo2* mutants displayed hypersensitivity to ABA during seed germination (Zhu et al. 2012). From the above-mentioned information, important roles of mitochondrial activity, as well as mitochondrial RNA editing in stress responses, are evident. In accordance with these data, *poco1* plants showed an impaired mitochondrial activity, RNA editing and lower sensitivity to drought stress, the latest most likely due to the altered expression of stress response genes.

4.7.4. Cellular regulation genes are affected by *poco1*

Organelles are firmly linked to the network of cellular processes and alteration in their biochemistry will have an impact at the cellular level. Therefore, in response to organelle's dysfunctionality not only nuclear-encoded organellar genes can be readjusted, but also genes associated with any cellular regulation. The identification and functional characterization of key regulatory transcription factors involved in the control of flowering, ABA and stress responses may be helpful to reveal the regulatory mechanisms in *poco1*. ERF, MYB and NAC are three highly regulated transcription factors identified, implying that the POCO1 is likely to be associated with the modulation of several transcription factors that regulate critical biological processes and stress responses. The identification of various differentially expressed genes encoding transcription factors in different comparisons in *poco1* indicates that the development in *poco1* may be controlled by a complex transcriptional regulation. Notably, the majority of differentially expressed genes encoding transcription factors in inflorescence-flowering reveal up-regulation in *poco1* compared with wild-type plants, which may be indicative of a different level of regulation at the reproductive phase compared with the earlier developmental phase.

Of all identified transcription factors, The bHLH (bHLH81 and bHLH136), NAC (NAC089), RAV (TEM1), and ERF (RAV1) transcription factors may play a role in the regulation of flowering time in *poco1*. Studies also reported the involvement of NAC, bHLH, MYB, C2H2, CCCH zinc-finger and WRKY transcription factors in orchestrating the stress response and flowering (Lee et al. 2006b; Li et al. 2010; Ito et al. 2012; Du et al. 2013; Chao et al. 2014; Zhang et al. 2018). bHLH, MYB, TCP and WRKY transcription factors may be involved in the transcriptional regulation of flowering genes (Zhou et al. 2019).

The effect of ABA on the induction of several genes encoding transcription factors has been proven (Shinozaki et al. 2003). In this context, several genes encoding transcription factors associated with the ABA signaling were recognized to be differentially regulated in *poco1*. A bHLH transcription factor MYC2 is a positive regulator of ABA signaling (Abe et al. 2003). The involvement of the largest plant transcription family, WRKY, in the regulation of biotic and abiotic responses and ABA signaling has been demonstrated, which suggests some connections to PYR/PYL/RCAR-PP2C-SnRK2 pathway (Jiang and Yu 2009; Shang et al. 2010; Antoni et al. 2011). WRKY transcription factors are reported as an early connection to ABA signaling (Rushton et al. 2012; Lindemose et al. 2013). MYB transcription factors are involved in plant development, hormone signal transduction and abiotic stress tolerance (Lindemose et al. 2013). Identification of several numbers of MYB transcription factors, which are one of the central factors regulating different stress signaling pathways (Rizhsky et al. 2004; Mattana et al. 2005; Dubos et al. 2010) may signify the alteration in regulating stress pathways in *poco1*. Many members of MYB transcription factors are involved in ABA signaling (Lindemose et al. 2013). Altered expression of genes encoding WRKY and MYB transcription factors involved in ABA signaling (*WRKY2*, *WRKY25*, *WRKY33*, *WRKY46*, *MYB2*, *MYB20*, *MYB32*, *MYB44*, *MYB51* and *MYB73*) suggests their critical role in the ABA signaling impairment in *poco1*. The WRKY family possesses broad functions in intracellular signaling and are involved in a communication system between the organelles (Kleine and Leister 2016). Furthermore, WRKY transcription factors regulate the expression of genes encoding mitochondrial and chloroplast proteins. It was shown that the presence of the W-box, which is a core binding site for WRKYs, are essential for regulating stress-responsive genes encoding mitochondrial proteins (Van Aken et al. 2013). WRKY2 functions as a transcriptional regulator of ABF/AREBs through binding W-box sequences in the promoter regions of ABF/AREBs (Bakshi and Oelmüller 2014). A study showed that *wrky2* mutants displayed delayed or decreased expression of *ABI5* and *ABI3* (Jiang and Yu 2009). WRKY genes induce ABA signaling and hence are positive regulators of the drought response. ABA-responsive genes such as *ABF4*, *ABI5*, *MYB2* and *RAB18* are target genes of WRKYs. Also, *LT178/RD29A* and *COR47* are downstream of WRKYs (Bakshi and Oelmüller 2014). It can be thus proposed that one cause for the down-regulation of the ABA-responsive genes might be the repression of WRKY genes in *poco1*. NAC transcription factors constitute a rather large family in plants and regulate ABA response as well as plant development and abiotic stress response (Jensen et al. 2009). Except for WRKYs and MYBs, NAC transcription factors function as key components of ABA-mediated stress signaling (Lindemose et al. 2013). The role of WRKY, MYB and NAC transcription factors in regulating stress responses especially drought stress was demonstrated (Abe et al. 1997; Hu et al. 2006). Another group of transcription factors, mTERFs, are involved in the organellar gene expression and regulation of stresses. They have

been proposed to be the ideal factors for coordination of both nuclear and organelle genomes (Kleine 2012; Shevtsov et al. 2018). Besides, the involvement of mTERFs in response to ABA and stresses has been reported (Xu et al. 2017). Other members of various regulatory gene families such as bZIP, CCCH zinc finger, C2H2 zinc finger, ERF, GATA, GRAS, Homeobox, and MYB-like were also differentially expressed in *poco1*. The common biological processes, in which these regulatory proteins are involved, are stress and development regulation. As ABA has a key role in mediating stresses and plant development, it is reasonable to observe an alteration in the expression of many transcription factors possibly due to ABA impairment in *poco1*.

RLKs are a class of transmembrane proteins, which sense and transmit a number of various signals to regulate plant growth and development (Shiu and Bleecker 2001) and are reported to be involved in plant responses to various stresses (Chae et al. 2009; Osakabe et al. 2013). The involvement of CRKs in ROS signaling transduction has been demonstrated (Idänheimo 2015). RLPs were identified as membrane-bound signaling molecules, which include an extracellular receptor domain. They can be transferred into the nucleus, chloroplast or mitochondria. RLPs act to enhance plant responses to biotic and abiotic stresses (Tör et al. 2009). MAPKs are signal transduction kinases, which are involved in many cellular processes like stress signal transduction and are considered as central signal transduction links in plants (Overmyer et al. 2003; Moore et al. 2016). MAPKs frequently regulate a broad spectrum of downstream events. Thus, any change in their expression may lead to changes in other factors (Zhang et al. 2006b; Fujita et al. 2006). MAPK cascades are triggered by a wide range of signals including ABA, auxin, ethylene, ROS and pathogens (Jonak et al. 2002). ROS signaling via MAPK pathways was reported to be critical for plant stress responses (Taj et al. 2010). Mitochondrial proteins are phosphorylated by MAPKs (Singh et al. 2012). It can be postulated that down-regulation of MAPK cascade may be under the effect of the ABA deficiency in *poco1* (Figure 28). *MPK3* is responsive for drought, low temperature, high salinity and touch stresses (Mizoguchi et al. 1996). *MPK3* is down-regulated in *poco1*, which may be one of the important causes affecting drought tolerance in *poco1*. LRR-RKs, as well as LRR-RLKs, comprise transmembrane kinase proteins with a wide range of function in regulation and signal transduction. It was suggested that ROS generated in specific cellular compartments use a common signal to induce and integrate different cellular signaling pathways and that ROS from different sources converge with mitochondrial retrograde regulation, thus affecting plant growth, development and the stress response (Pu et al. 2015). The altered expression of a great number of genes encoding transcription factors that regulate critical biological processes as well as various signaling factors in *poco1* may affect their activity and collectively highlight the alteration in cellular regulation and signaling in *poco1* plants.

4.7.5. Altered expression of genes associated with the mitochondrial perturbation

Mitochondria are critical organelles regarding signaling and responding to stresses (Vanlerberghe 2013). Signals can be transmitted from mitochondria to the nucleus and the corresponding responses in the form of any changes in the gene expression are performed. Physical interaction may exist between mitochondria-nucleus (Smart et al. 1994) and mitochondria-endoplasmic reticulum (Ng et al. 2013), which may facilitate the transmittance of genetic information to and from the mitochondria. However, limited data are available about the underlying regulatory mechanisms of mitochondrial retrograde signaling in plants. It has also become increasingly clear that retrograde signaling exploits factors expanded in other contexts such as signaling factors associated with MAP kinase or ABA signaling (Kleine and Leister 2016). Therefore, a general mitochondrial stress response appears to be linked to ABA signaling (Schwarzländer et al. 2012). The molecular components of retrograde signals regulate stress-associated NGEMPs. Although the exact role of stress-associated NGEMPs is still not completely understood, they are suggested to be involved in the maintenance of mitochondrial functions (Skirycz et al. 2010). Expression alteration of stress-induced NGEMPs is affected by internal and external stimuli (Schwarzländer et al. 2012; Ng et al. 2014).

RNA-seq analysis identified several differentially expressed stress-induced NGEMPs in *poco1*, which can be targets of mitochondria retrograde signals. The expression of NGEMPs is altered to a wide range of mitochondrial perturbations, which causes a discrete expression pattern. Various expression characteristics of NGEMPs by different mitochondrial perturbation points towards the existence of multiple mitochondrial retrograde regulations (Ng et al. 2014). Studies reported that the transcripts encoding mitochondrial HSPs are specifically generated by mitochondrial dysfunctions as a part of retrograde signals (Van Aken et al. 2009a). Mitochondrial stress-responsive genes, which are candidate genes involved in retrograde signaling such as *HSPs* and *AOX1a* share a common feature. They all have *cis*-regulatory elements in their promoter region, which is called mitochondrial dysfunction motif (MDM) (De Clercq et al. 2013). This motif can be recognized by transcription factors such as NAC and WRKY families. Furthermore, transcription factors from different classes have been identified to bind the promoters of NGEMPs as well as to some transcripts from all five respiratory complexes and regulate their expression (Ng et al. 2014). RNA-seq results confirm, that several of these transcription factors were found to be differentially expressed in *poco1* including *WRKY15*, *WRKY30*, *WRKY33*, *ABF4*, *Athb-6*, *bZIP10*, *bZIP25* and *bHLH81*. The RNA-seq data showed that *AOX1a* is down-regulated in inflorescence-inflorescence and *AOX1d* was found up-regulated in inflorescence-flowering in *poco1*. Mitochondrial *AOX1a* was the first nuclear gene that was identified to be retrograde regulated, particularly in the context

of the response to stress (Gray et al. 2004; Dojcinovic et al. 2005). *aox1a* plants were shown to accumulate higher superoxide radicals and were more sensitive to the combined drought and light stresses (Giraud et al. 2008). A study on tobacco showed that the lack of AOX resulted in higher cellular levels of ROS (Amirsadeghi et al. 2006) leading to the conclusion that an important role of AOX is to reduce the high levels of ROS. Plant hormones such as ABA, salicylic acid, ethylene, methyl salicylate and methyl jasmonate can induce AOX expression (Lennon et al. 1997; Fung et al. 2004; Ederli et al. 2006). Repression of *AOX1a* can be reversed by ABA. The *cis*-acting regulatory elements, ABRE, in the promoter region of *AOX1a*, which are potential binding sites for ABA-responsive factors, which affect *AOX1a* expression (Giraud et al. 2009; Ng et al. 2014). This provides a molecular link between the expression of *AOX1a* and ABA response factors. Thus, different ABA response factors may be involved in binding and regulation of *AOX1a*. For instance, ABI4 can act as an activator or repressor depending on binding context (León et al. 2012). Studies reported that many of NGEMPs such as *HSP70*, *AOX1a* and *BCS1* may be truly ABA-responsive, as their transcript abundance is changed after ABA treatment (Berkowitz et al. 2016). Since many ABA regulatory factors are down-regulated in *poco1*, it may be suggested that *AOX1a* repression is affected by the defective ABA signaling in *poco1* (Figure 28). On the other hand, elevated expression of AOX leads to the stress tolerance of many plant species by reducing the amount of ROS accumulation (Van Aken et al. 2009a; Vanlerberghe 2013), which is not the case for *poco1* plants. The altered expression of antioxidant enzymes leads to the changes in the regulation of plant tolerance to biotic and abiotic stresses. Likewise, the reduced expression of *AOX1a* in *A. thaliana* altered the expression of antioxidant defense components and stress-responsive genes, thereby changing the fundamental defense status and tolerance of the plants (Giraud et al. 2008). In consistence, in *poco1* alteration of antioxidant genes such as *GRXs*, *GSTs*, *TRXs* and stress-responsive genes and the higher sensitivity to drought stress was observed. Enhanced induction of stress-responsive *AOX1d* suggests the activation of the compensatory AOX pathway in *poco1* mitochondria. Studies reported that *WRKY15* promotes the expression of genes involved in mitochondrial dysfunction regulon and negatively regulates retrograde signaling (Vanderauwera et al. 2012). Over-expression of *WRKY15* caused disruption of the ROS and mitochondrial retrograde signaling A newly characterized gene family, DUF295, was shown to be localized in the mitochondria and chloroplasts and they were proposed to be integrated into mitochondrial signaling network (Lama et al. 2019) and a member of this gene family, *ATDOA11*, was found up-regulated in *poco1*.

Mitochondria act as signaling hubs, sense and transduce appropriate signals based on growth and stress conditions. Any perturbation of mitochondria makes them adjust the proper function and reprogram the gene expression. Several factors are believed to be involved in retrograde signaling such as redox signaling, tetrapyrrole signaling, sugar signaling and signaling based

on ROS (Leister 2005). ROS are well-known stress factors in plants and are generated by the impaired oxidative phosphorylation. ROS molecules can act as signaling molecules that regulate plant growth, development and adaptation to stresses (Gapper and Dolan 2006; Ślesak et al. 2007; Møller and Sweetlove 2010; Ng et al. 2014). Consistently, higher accumulation of ROS was observed in *poco1*. Furthermore, ABA was demonstrated to be the main phytohormone, which acts as the regulator of mitochondrial function and regulates the expression of mitochondrial genes (Bakshi and Oelmüller 2014). Therefore, alteration in the expression of NGEMPs in *poco1*, which may be conferred to a defected ABA signaling is conceivable (Figure 28). Differential expression pattern of these genes may be an indication of the existence of various pathways and signals, through which mitochondria transmit signals to the nucleus in *poco1*. However, as there is no specific retrograde signal identified, the interpretation of the expression of all these genes is complicated. Detailed studies, which target specific genes and mutants are required to yield insight into the regulation of the stress-induced NGEMPs through POCO1.

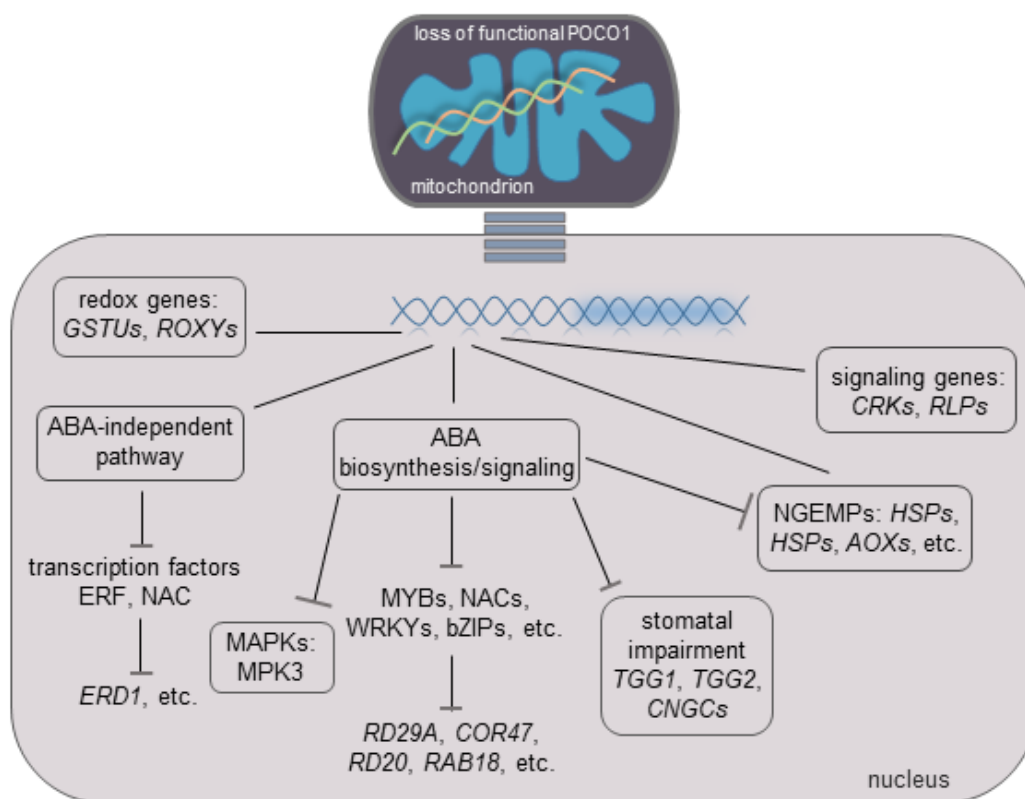


Figure 28: View of the possible regulatory network of gene expression in response to the loss of function of POCO1 in mitochondria. Loss of function of POCO1 is sensed by mitochondria. Subsequently, a retrograde signaling cascade may be activated to launch gene expression changes. Several transcription factors, which control stress-inducible gene expression are affected. Several processes may be under the influence of the defected ABA signaling in *poco1*. Black lines indicate crosstalk and differential regulation. Dashed arrows are indicative of possible inhibiting effects.

4.7.6. Cellular redox genes are differentially regulated in *poco1*

Previous reports on PPR proteins functioning in mitochondria endorsed that the mutation of PPR proteins was correlated with the higher levels of ROS accumulation or the redox imbalance (Zsigmond et al. 2008; Murayama et al. 2012). Any perturbation of mitochondrial respiratory components has a severe effect on the redox balance (Millar et al. 2003). ROS react with various signaling factors such as redox proteins, which leads to initiating communication events to coordinate plant growth, development, and stress responses (Bartoli et al. 2013; Mignolet-Spruyt et al. 2016). Redox homeostasis requires the efficient coordination of reactions, which is directed by a complex network of antioxidant systems. A proper intracellular balance exists between ROS production and scavenging. Accordingly, antioxidant-related genes such as *GRXs*, *GSTs* and *TRXs* were found to be differentially expressed in *poco1*, which may propose a role of POCO1 associated with the redox state. The disturbance of mitochondrial function leads to the perturbation of mitochondrial redox state, which has extreme consequences on cellular redox homeostasis. In *Nicotiana sylvestris* mutants lacking complex I activity, alteration in the expression of antioxidant-related genes was reported (Dutilleul et al. 2003). Also, PPR mutants in *A. thaliana*, which possess impaired mETC due to a defective complex I, have a higher accumulation of ROS and a redox imbalance (Liu et al. 2010; Yang et al. 2014). Different categories of redox processes (redox state of mETC, ROS and the redox-active compounds such as thioredoxins) can be regarded as important candidates for mitochondrial retrograde signaling (Apel and Hirt 2004; Dietz 2008). Several studies reported that the redox-based signaling is a crucial constituent in mitochondria-nucleus communication to modulate gene expression (Rhoads et al. 2006; Noctor et al. 2007; Rhoads and Subbaiah 2007; Schwarzländer and Finkemeier 2013). Redox signals are involved in various aspects of plant biology and are specifically critical in cross-tolerance phenomena, allowing a general acclimation of plants to stressful conditions (Foyer and Noctor 2003). *GRXs* and *TRXs* are crucial components of cellular redox state connected to the ROS regulation (Dietz 2008). Also, these factors were reported to be regulators of many stress signaling pathways (Noctor et al. 2012). The altered expression of genes related to the redox cascade such as *GRXs*, *GSTs*, *TRXs*, and *ROCs* point towards the altered redox state in *poco1*. This hypothesis is supported by the higher accumulation of ROS in *poco1* plants and the fact that change in the ROS level leads to the redox state alteration (Foyer and Noctor 2003).

It was previously proposed that *TRXs* as thiol-containing redox-active compounds can function as important candidates in mitochondrial retrograde signaling (Dutilleul et al. 2003). Moreover, enzymatic antioxidant genes such as *TRXs* have an important role to neutralize the produced ROS to reduce the oxidative state of the cell and maintain the cell redox (He et al. 2017). *TRXs*

regulate protein's functions and may regulate respiratory metabolism, antioxidant activity, and redox signaling in plant mitochondria (Schwarzländer and Finkemeier 2013). ROCs, which can interact with TRXs, are known to connect hormone signals to amino acid biosynthesis and redox homeostasis during stress responses (Park et al. 2013). GRXs have crucial roles in oxidative stresses. They have peroxidase activity and confer resistance to oxidative stresses (Rouhier et al. 2003). Antioxidants such as TRXs and GRXs are central redox regulators of hormone signalling pathways. Differential expression of several members of TRX and also GRX family such as *ROXY2*, *ROXY3*, *ROXY8*, *ROXY9*, *ROXY12*, *ROXY13*, *ROXY14*, *ROXY15*, *ROXY17*, *ROXY20* and *ROXY21* in *poco1* may indicate the alteration of redox signaling hub in order to react with the hormone signaling to control growth. GSTs are major players in oxidative stress metabolisms and maintain cell redox homeostasis and protect cells against oxidative stresses. GSTs are a family of enzymes that catalyze the conjugation of reduced glutathione (GSH) to a wide range of substrates, which usually results in cellular detoxification. GSTs modulates GSH homeostasis in regulating *Arabidopsis* development (Jiang et al. 2010). Furthermore, GSTs can serve as a signaling molecule (Foyer and Noctor 2005; Chen et al. 2012). Higher GSH levels lead to an increase in ABA accumulation (Chen et al. 2012). Previous studies demonstrated that GST transcripts were up-regulated in response to exogenous application of GSH and phytohormones such as ABA, salicylic acid and ethylene in plant tissues (Marrs 1996; Mang et al. 2004; Chen et al. 2012; Sinha et al. 2015). The stress-tolerant phenotypes, especially drought stress, can be well explained by the greater GSH content, ABA accumulation and fine-tuned ABA signaling (Thompson et al. 2007; Chen et al. 2012). Furthermore, a lower glutathione redox led to the early flowering in *Oncidium* orchid (Chin et al. 2016). Except one, all significantly regulated GSTs identified are down-regulated in *poco1* in inflorescence-inflorescence. However, differentially regulated GSTs in inflorescence-flowering show more differentially expressed than inflorescence-inflorescence, in which five of GSTs show up-regulation and three of them show down-regulation. Numerous ABA-related genes are down-regulated in inflorescence-inflorescence compared to the other two comparisons, which highlights considerable ABA signaling deficiency. Thus, the lower induction of GSTs in inflorescence-inflorescence may be caused by the significant ABA signaling and response defect shown in this comparison.

ROS signals interact directly or indirectly with different hormone signaling pathways (Overmyer et al. 2003; Apel and Hirt 2004; Yang et al. 2014), especially are interconnected with responses to ABA (Bartoli et al. 2013). Besides, the increased amount of ROS has a direct impact on the cellular redox status (Foyer and Noctor 2003), which can be another candidate for retrograde signal, through which alteration in gene expression occurs. Presumably, the impairment of mitochondrial function caused by defective RNA editing in different components of mETC complexes in *poco1* induces redox imbalance, which in turn confers an abnormal response to

the phytohormone ABA. In accordance, ABA has been associated with mitochondrial retrograde signaling and it is well documented that ABA signaling components, as well as stress-responsive genes in the nucleus, are targets of organellar signals (Bakshi and Oelmüller 2014). Therefore, the impact on the nuclear gene expression by retrograde signals from mitochondria in *poco1* is strongly relevant. The redox imbalance caused by impaired mitochondrial functions may be involved in the inactivation of ABA signaling in *poco1* plants, in which ROS may play important roles. Altogether, these results may emphasize the impact of POCO1 related to maintaining the cellular redox state.

5. Summary

Pentatricopeptide repeat (PPR) proteins are nuclear-encoded proteins, assigned to various aspects of RNA metabolism in mitochondria and chloroplasts such as RNA splicing, editing, processing, and translation, thereby affecting important biological processes. The main function of mitochondria is cellular respiration. Also, they act as sensory organelles, where signals triggered by a wide range of mitochondrial perturbation stimuli cause changes in the nuclear gene expression (Woodson and Chory 2008). The significant phenotypes such as embryo lethality, reduced fertility and a dwarf phenotype, which are associated with mutations of PPR genes emphasize the important roles of PPR proteins in the regulation of plant growth and development (Lurin et al. 2004). In this thesis, a PPR gene is characterized, which is implicated in mitochondrial RNA editing, respiratory activities, affects floral transition, ABA sensitivity and abiotic stress responses in *Arabidopsis*.

Arabidopsis plants with a T-DNA insertion in the PPR gene, *AT1G15480*, exhibited an early-flowering phenotype. The early-flowering phenotype was observed under both long- and short-days. Genetic complementation analysis reversed the observed phenotype, connecting *AT1G15480* to flowering. Due to the observed phenotype, the PPR protein was named PRECOCIOUS1 (POCO1). In this study, it is demonstrated that *poco1* mutation affects multiple RNA editing sites in 11 mitochondrial transcripts, supporting a broad role for POCO1 in the regulation of RNA editing efficiency. Conceivably, *poco1* showed an impaired respiratory activity, as was shown by a lower respiration rate, ATP level and a higher accumulation of superoxide radicals compared with wild-type plants. To gain insight into the involvement of POCO1 in floral transition, the expression level of *FLOWERING LOCUS C (FLC)*, *FLOWERING LOCUS T (FT)* and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)* in *poco1* and wild-type plants were investigated. Importantly, the quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) analysis showed that in *poco1* the expression level of the potent floral repressor, *FLC*, and an important promoter of flowering, *FT*, were significantly down- and up-regulated respectively. Likewise, the expression level of the *FLC* positive regulator, *ABSCISIC ACID-INSENSITIVE 5 (ABI5)* was reduced in *poco1*. Consistent with the qRT-PCR results, *poco1* plants showed reduced sensitivity to abscisic acid (ABA) compared with wild-type with respect to primary root growth and days to flowering. Furthermore, the *poco1* mutation enhanced the sensitivity to drought stress.

To provide an overview of the transcriptome changes in *poco1*, a RNA-seq analysis was done. Based on the analysis of differentially expressed genes in three comparisons, several biological processes were found to be enriched, which indicates that complex regulatory

mechanisms underlie *poco1* development. RNA-seq analysis identified many transcripts associated with flowering such as *FT*, which most likely is involved in the early-flowering phenotype of *poco1*. Numerous ABA-associated genes, including the core components of ABA signaling along with the important genes for stomatal function, were mostly down-regulated in *poco1*. Drought and oxidative stress-related genes, including ABA-induced stress genes, were differentially regulated. The RNA-seq analysis identified candidate genes that would be interesting in relation to stress regulation, such as those from the stress-induced nuclear genes encoding mitochondrial proteins (NGEMPs) and drought- and oxidative stress-related genes as well as different families of transcription factors such as ERF, WRKY, MYB, and NAC etc. RNA-seq analysis also uncovered differentially regulated genes encoding various classes of transcription factors and genes involved in cellular signaling. Redox-related genes were affected, suggesting that the redox state in *poco1* might be altered. Overall, the data generated in this study can be used to facilitate further dissection of the underlying molecular mechanisms connecting mitochondrial influence and ABA signaling as well as floral transition.

6. Zusammenfassung

Pentatricopeptide Repeat (PPR) Proteine sind kernkodierte Proteine, die verschiedenen Prozessen des RNA-Metabolismus, wie dem Spleißen, Edierung, Prozessierung und Translation von RNA, in Mitochondrien und Chloroplasten zugeordnet sind und dadurch wichtige biologische Prozesse beeinflussen. Die Hauptfunktion der Mitochondrien ist die Zellatmung. Mitochondrien sind sensorische Organellen, bei denen Signale, die durch eine Vielzahl von mitochondrialen Störungsstimuli ausgelöst werden, Veränderungen in der nuklearen Genexpression verursachen (Woodson and Chory 2008). Die Phänotypen wie Embryoletalität, verminderte Fruchtbarkeit und ein Zwergenwachstum, die mit Mutationen von PPR-Genen assoziiert sind, unterstreichen die wichtige Rolle von PPR-Proteinen bei der Regulation des Pflanzenwachstums und der Pflanzenentwicklung (Lurin et al. 2004). In dieser Arbeit werden die Funktionen eines PPR Proteins beschrieben, das an der mitochondrialen RNA Prozessierung und Atmungsaktivität, am Blühzeitpunkt, der ABA-Empfindlichkeit und der abiotischen Stressreaktionen bei Arabidopsis beteiligt ist und diese beeinflusst.

Arabidopsis Pflanzen mit einer T-DNA-Insertion in dem PPR-Gen AT1G15480 zeigten einen früh blühenden Phänotyp. Der früh blühende Phänotyp wurde sowohl unter lang-Tag als auch kurz-Tag Bedingungen beobachtet. Die genetische Komplementation der Mutante mit dem Wild-Typ Gen stellte den Wild-Typ Phänotyp wieder her und bestätigte somit eine Beteiligung von AT1G15480 an dem Blühzeitpunkt. Aufgrund des beobachteten Phänotyps wurde das PPR-Protein PRECOCIOUS1 (POCO1) genannt. In dieser Studie wird gezeigt, dass die poco1 Mutation mehrere RNA-Edierungspositionen in 11 mitochondrialen Transkripten beeinflusst, was eine ausgeweitete Rolle von POCO1 bei der Regulation der RNA-Edierungseffizienz unterstützt. In poco1 Pflanzen wurde eine geringere Atmungsrate, ein geringeres ATP-Level und eine erhöhte Akkumulation von Superoxidradikalen nachgewiesen: Dies weist auf eine beeinträchtigte Atmungsaktivität hin. Um einen Einblick in die Beteiligung von POCO1 am Blühzeitpunkt zu erhalten, wurde das Expressionsniveau von FLOWERING LOCUS C (FLC), FLOWERING LOCUS T (FT) und SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) in poco1 und Wildtyppflanzen untersucht. Die Analyse der quantitativen reverse Transkriptase-Polymerase-Kettenreaktion (qRT-PCR) zeigte, eine Runterregulierung von FLC, einem Repressor des Blühzeitpunktes und eine Hochregulierung von FT, einem positiven Regulator des Blühzeitpunktes. Ebenso war das Expressionsniveau von ABSCISIC ACID-INSENSITIVE 5 (ABI5) in poco1 reduziert, einem positiven Regulator der FLC-Expression. In Übereinstimmung mit den qRT-PCR-Ergebnissen zeigten poco1 Pflanzen im Vergleich zum Wildtyp eine geringere Empfindlichkeit gegenüber Abscisinsäure (ABA) in Bezug auf das

primäre Wurzelwachstum und die Tage bis zum Blühzeitpunkt. Darüber hinaus zeigen die *poco1* Mutanten eine erhöhte Empfindlichkeit gegenüber Trockenstress.

Um einen Überblick über die Transkriptomveränderungen in *poco1* zu erhalten, wurde eine RNA-seq-Analyse durchgeführt. Basierend auf der Analyse differentiell exprimierter Gene in drei Vergleichen wurde festgestellt, dass die differentiell exprimierten Gene vielen biologischen Prozessen zugewiesen werden konnten, was darauf hinweist, dass der *poco1* Entwicklung komplexe Regulationsmechanismen zugrunde liegen. Viele der differentiell exprimierten Gene sind an der Regulierung des Blühzeitpunktes beteiligt, wie FT, die höchstwahrscheinlich zum früh blühenden Phänotyp von *poco1* führen. Zahlreiche ABA-assoziierte Gene, einschließlich der Kernkomponenten der ABA-Signalübertragung, sowie wichtige Gene für die stomatale Funktion, liegen in *poco1* meist runterreguliert vor. Gene in Zusammenhang mit Trockenheit und oxidativem Stress, einschließlich ABA-induzierter Stressgene, liegen unterschiedlich reguliert vor. Durch die RNA-seq-Analyse konnten Kandidatengene identifiziert werden, die in Bezug auf die Stressregulation interessant wären, wie z. B. stressinduzierte Gene im Zellkern, die für mitochondriale Proteine (NGEMPs) kodieren, Gene, die mit Trockenheit und oxidativem Stress zusammenhängen, sowie verschiedene Familien von Transkriptionsfaktoren (ERF, WRKY, MYB und NAC usw.). Auch verschiedene Klassen von Transkriptionsfaktoren und Gene, die an der zellulären Signalübertragung beteiligt sind, wurden als differentiell reguliert identifiziert. Redox-verwandte Gene sind betroffen, was darauf hindeutet, dass der Redoxzustand in *poco1* verändert sein könnte. Insgesamt können die in dieser Studie generierten Daten verwendet werden, um die zugrunde liegenden molekularen Mechanismen besser zu verstehen, die den mitochondrialen Einfluss, die ABA-Signalübertragung sowie den floralen Übergang miteinander verbinden.

7. Abbreviations

2YT	2 x yeast extract and tryptone
ABA	Abscisic acid
ATP	Adenosine triphosphate
bp	Base pairs
BSA	Bovine serum albumin
C	Cytidine
CaMV	Cauliflower Mosaic Virus
CLSM	Confocal laser scanning microscopy
°C/s	Degree centigrade per second
DNA	Desoxyribonucleic Acid
DNaseI	DeoxyribonucleaseI
dNTP	Desoxynukleosidtriphosphat
dw	Dried weight
EDTA	Ethylenediaminetetraacetic acid
et al.	<i>et alii</i>
etc.	<i>Et cetera</i>
mETC	Mitochondrial electron transport chain
GO	Gene ontology
g/l	Gram per liter
h	Hour
kb	Kilo base pairs
kDa	Kilodalton
LB	Luria-Bertani (medium)
LB	Left border (T-DNA)
LC-MS	Liquid chromatography-mass spectrometry
MCS	Multiple cloning site
MES	2-(N-morpholino) ethanesulfonic acid
mETC	Mitochondrial electron transport chain
mM	Mili molar
mRNA	Messenger ribonucleic acid

MS	Murashige and Skoog medium
μMol	Micromolar
NBT	Nitroblue tetrazolium
nM	Nano molar
nt	Nucleotide
ORF	Open reading frame
PCR	Polymerase chain reaction
qRT-PCR	Quantitative reverse transcriptase-polymerase chain reaction
RB	Right border
RNA	Ribonucleic acid
RNA-seq	RNA-sequencing
ROS	Reactive oxygen species
rpm	Rounds per minute
rRNA	Ribosomal RNA
RT	Room temperature
RT-PCR	Reverse transcriptase-polymerase chain reaction
SDS	Sodium dodecyl sulphate
SE	Standard error
Taq	<i>Thermus aquaticus</i>
T-DNA	Transfer-DNA
tRNA	Transfer-RNA
U	Uridine
U/μl	Unit/microliter
UTR	Untranslated region
V	Volt
v/v	Volume percent
w/v	Mass fraction
WS-4	Wassilewskija-4
Wt	Wild-type

Abbreviation of frequently used genes and proteins:

<i>ABI5</i>	<i>ABA INSENSITIVE 5</i>
<i>ACT2</i>	<i>ACTIN2</i>
<i>FLC</i>	<i>FLOWERING LOCUS C</i>
<i>FT</i>	<i>FLOWERING LOCUS T</i>
<i>GFP</i>	Green fluorescent protein
<i>POCO1</i>	<i>PRECOCIOUS1</i>
<i>PPR</i>	Pentatricopeptide repeat protein
<i>SOC1</i>	<i>SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1</i>

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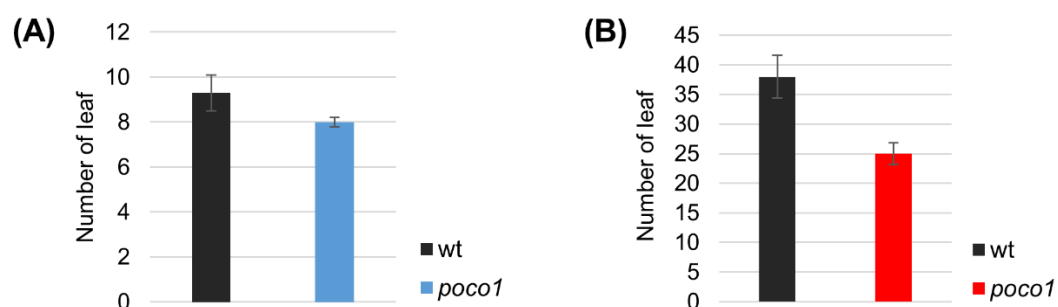
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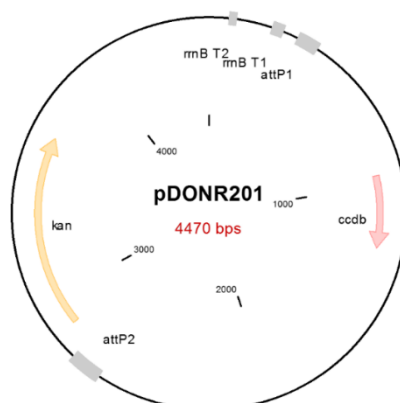
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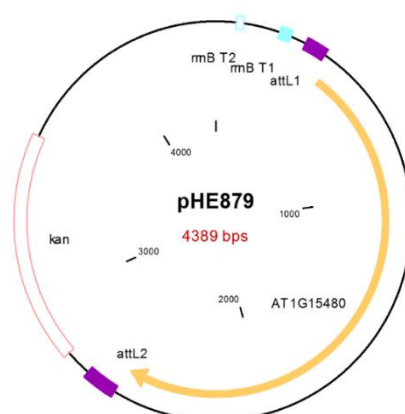
9. Appendix



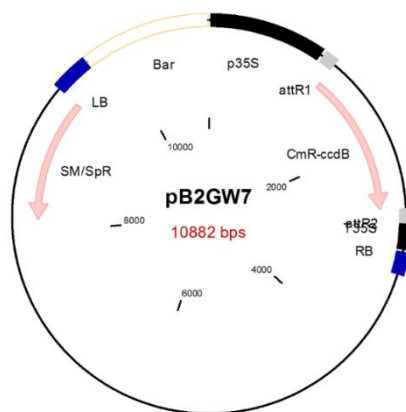
Supplementary Figure 1: The effect of long- and short-day conditions on flowering on the flowering time of *poco1*. Under both long- (A) and short-day (B) conditions *poco1* plant have less number of rosette leaves at the time of flowering than wild-type plants. The standard deviations for each data were calculated. Flowering time was measured as the number of rosette leaves. The rosette leaf number was reduced in *poco1*, showing the dominant early-flowering phenotype of *poco1* when compared with wild-type plants.



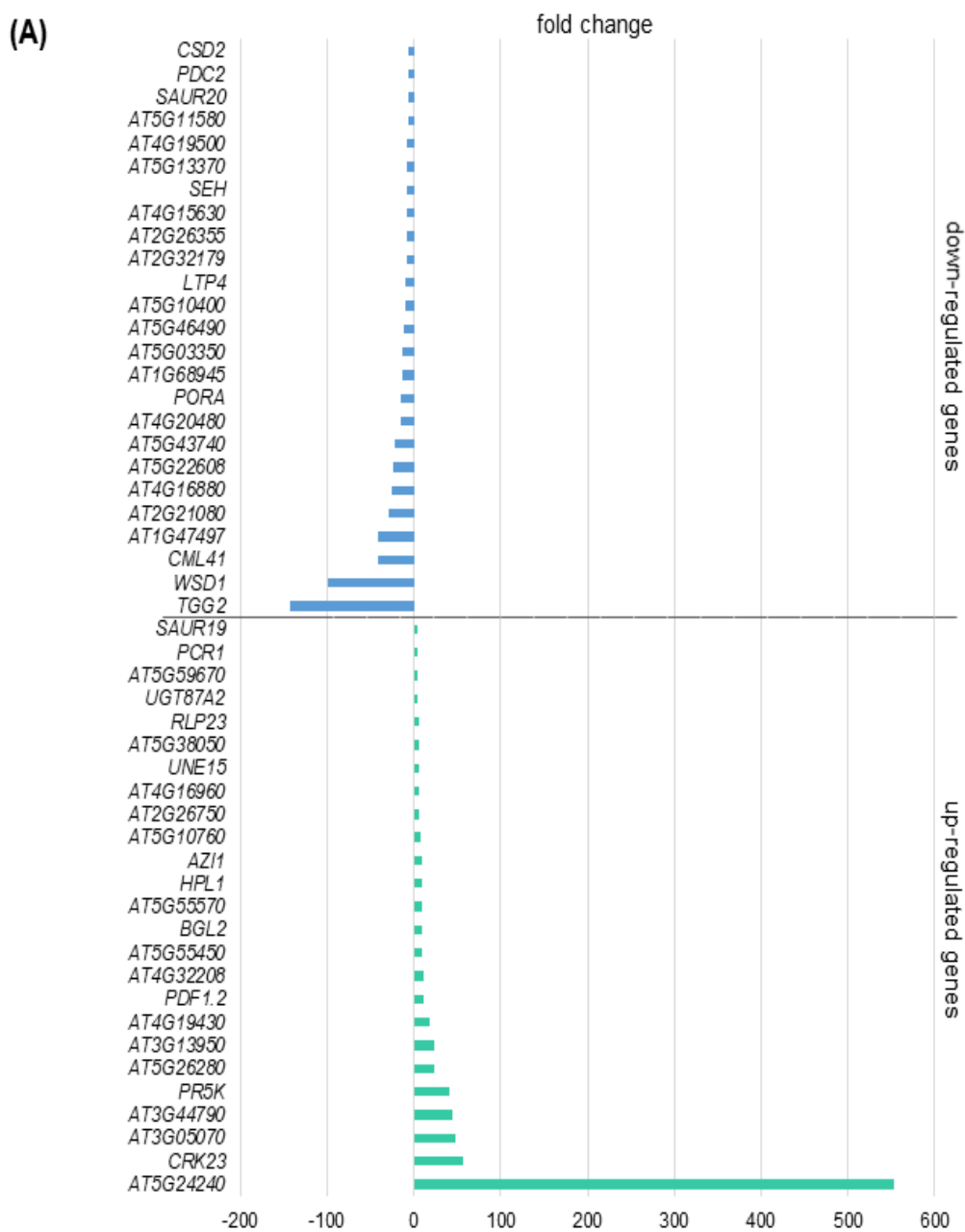
Supplementary Figure 2: Vector map of pDONR201.

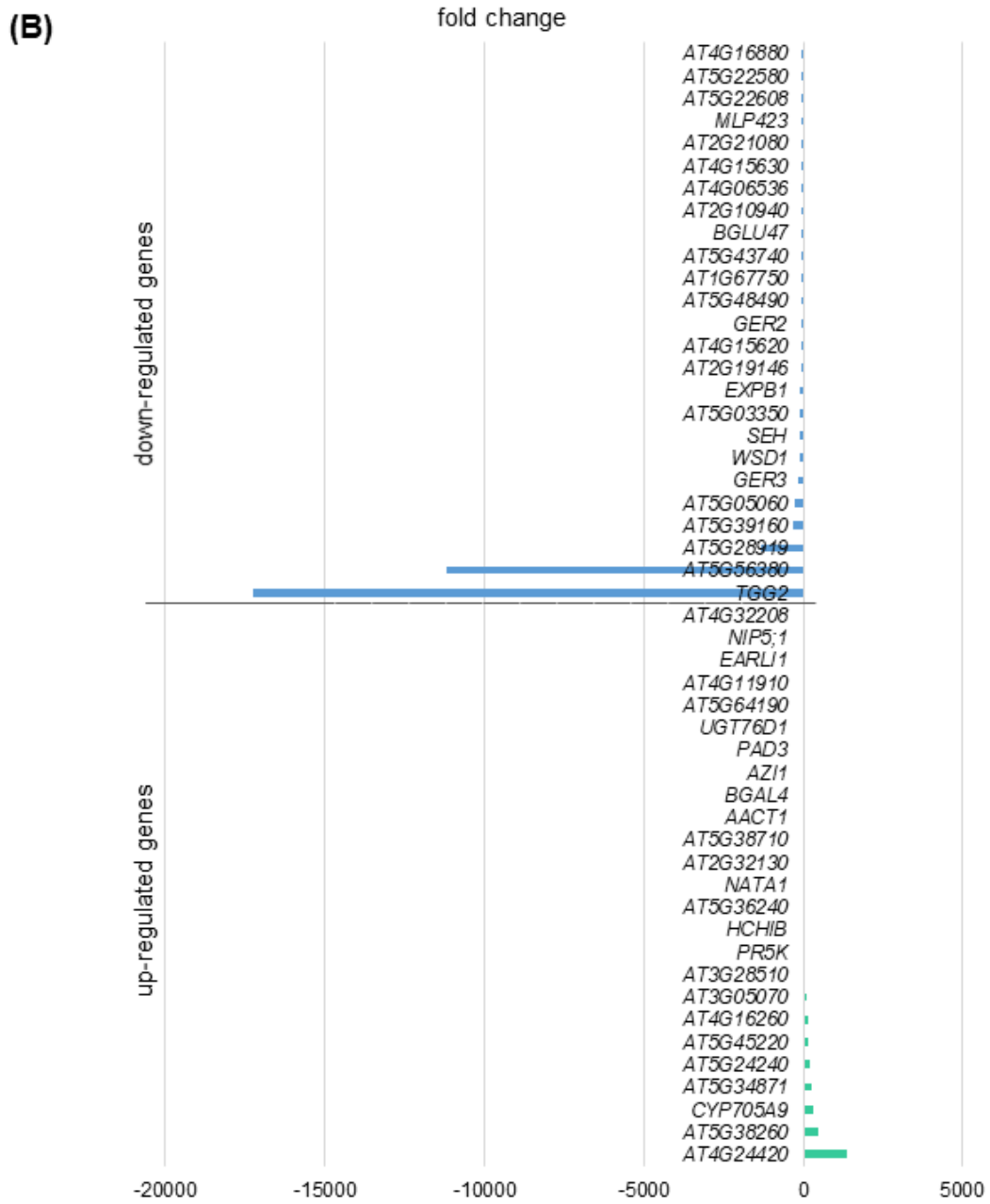


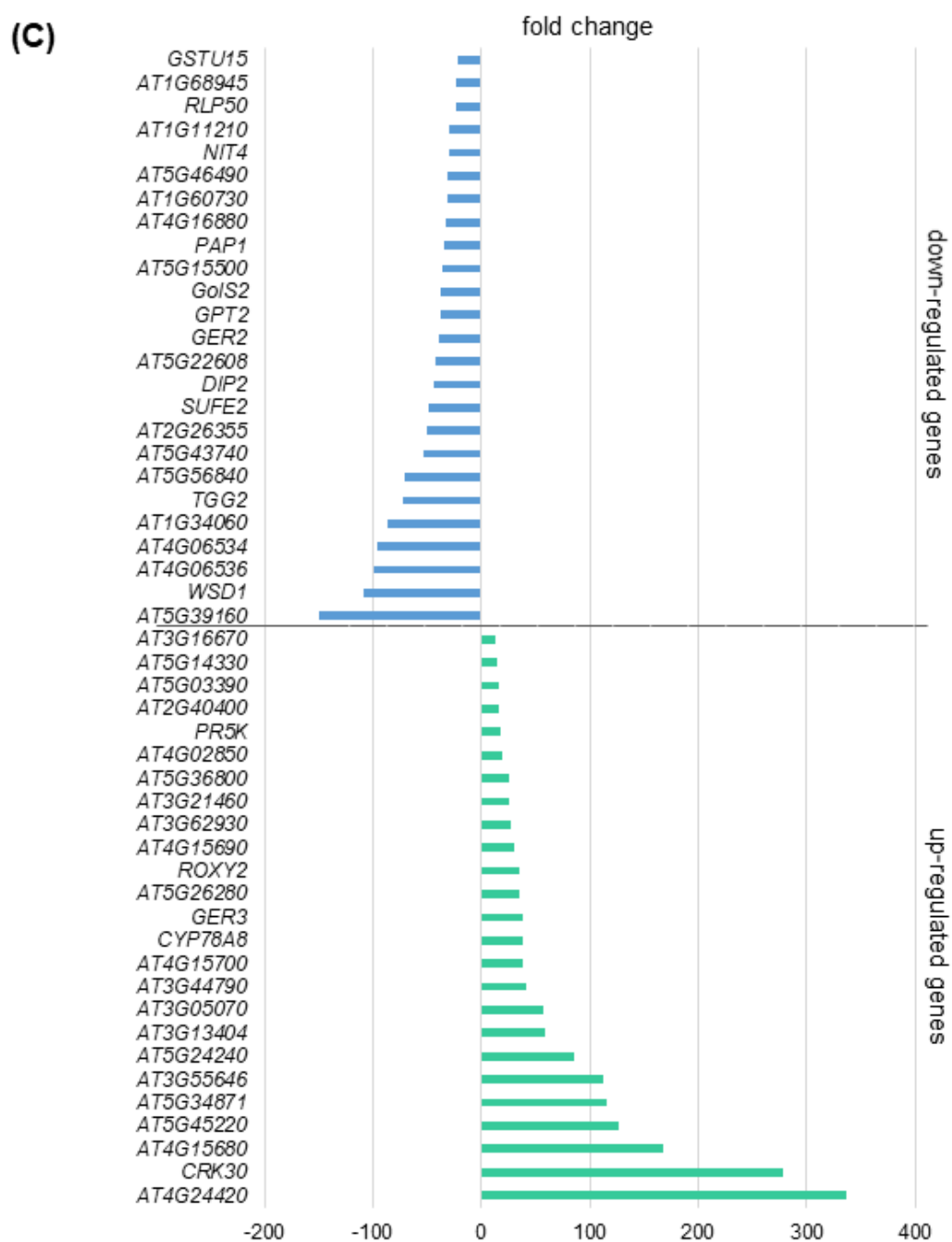
Supplementary Figure 3: Vector map of pHE879.



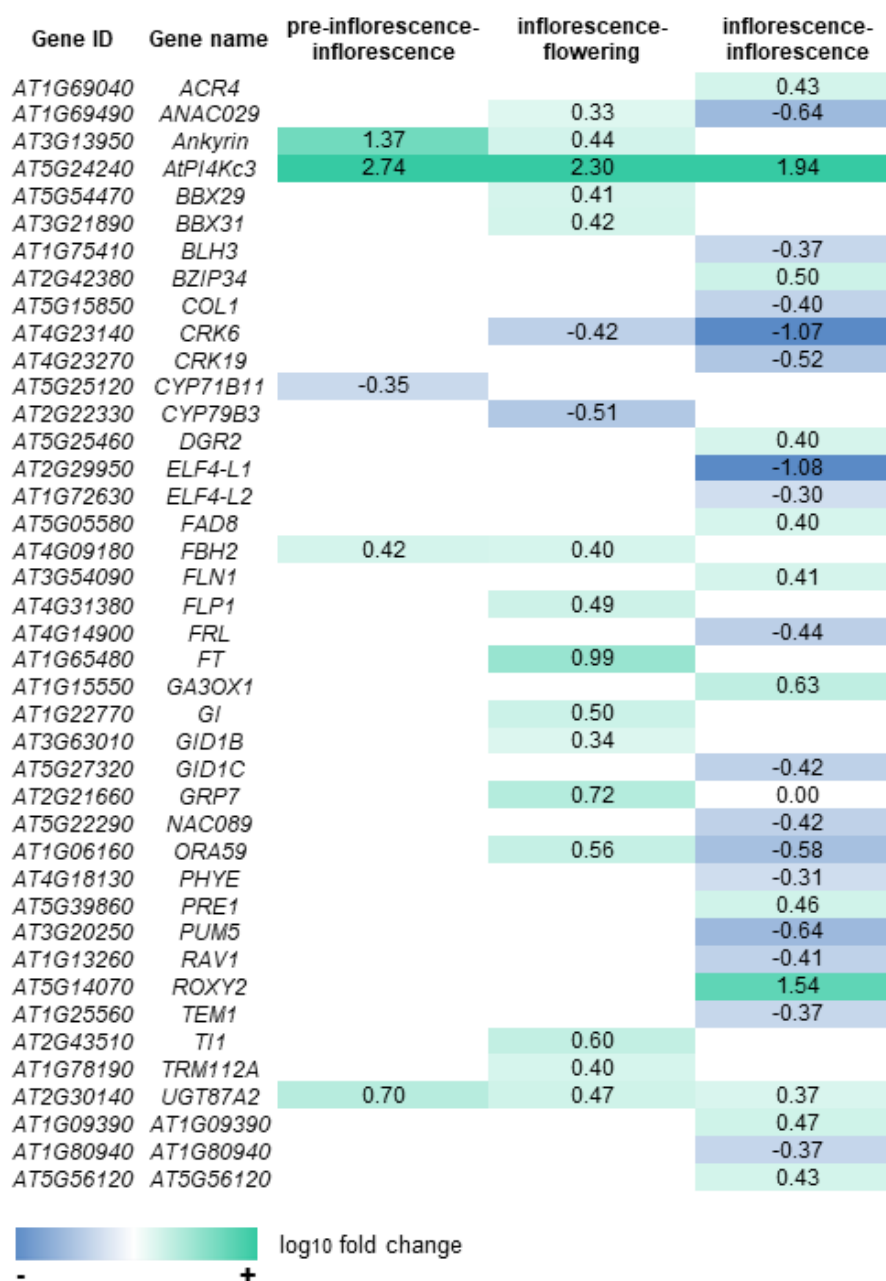
Supplementary Figure 4: Vector map of pB2GW7.





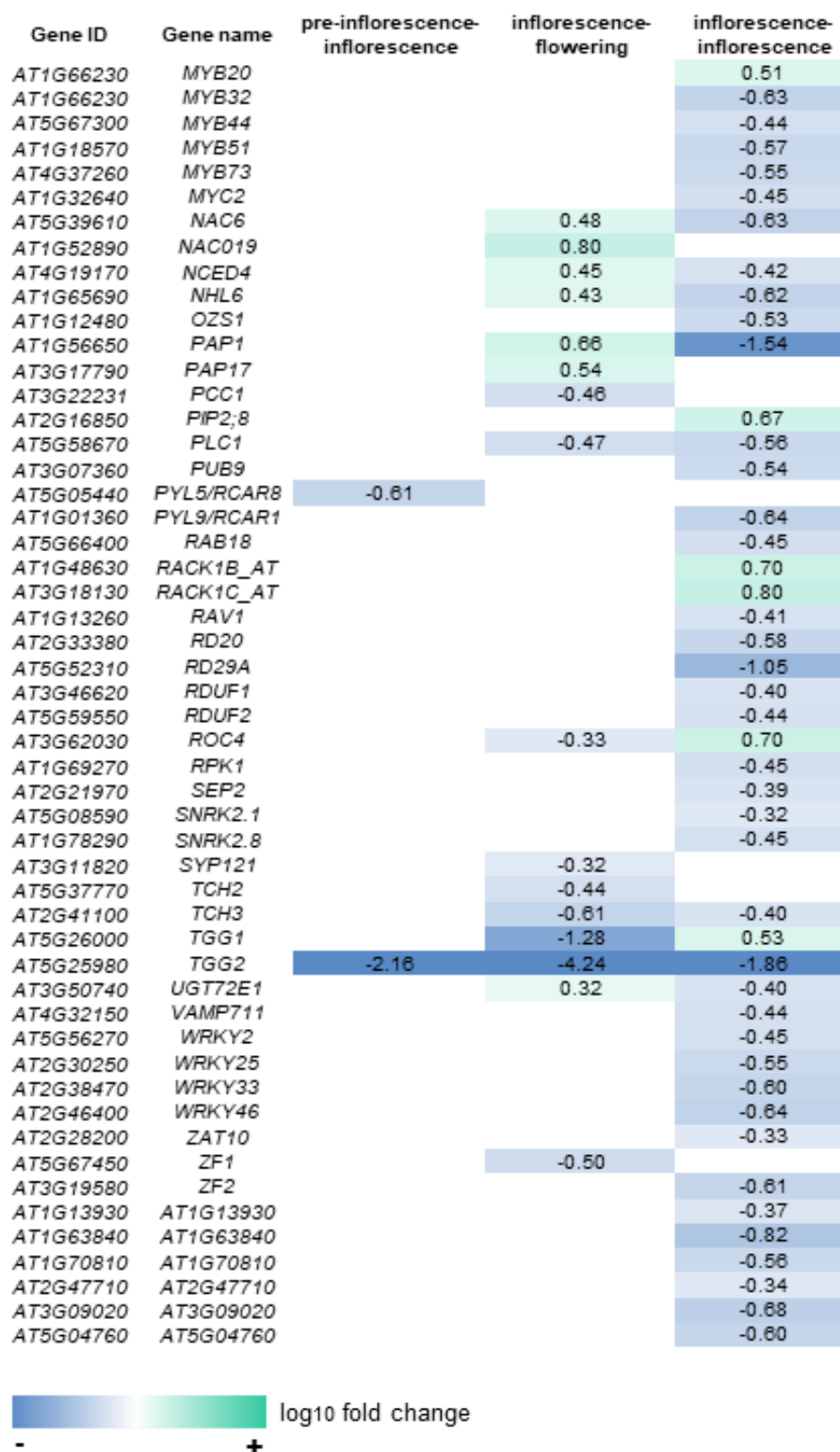


Supplementary Figure 5: Overview of top 25 genes up- and down-regulated in different RNA-seq comparisons. Differentially expressed genes in pre-inflorescence- inflorescence (A) inflorescence-flowering (B) and inflorescence- inflorescence (C). The differentially expressed genes were ranked on their fold change and the 25 with the highest or lowest fold changes are shown here.



Supplementary Figure 6: Heat map of affected flowering-associated genes. Heat map of differential expressed flowering-associated genes in *poco1* versus wild-type plants. Differentially expressed genes associated with the various known flowering pathways were identified. Fold changes (log₁₀) were used for representing in the heat map. Fold changes comparison is relative to wild-type. Green and blue represent up- and down-regulated genes respectively. White represents that a fold change either ≥ 2 or ≤ -2 with an FDR $p < 0.05$ were not detected.

Gene ID	Gene name	pre-inflorescence- inflorescence	inflorescence- flowering	inflorescence- inflorescence
AT4G26080	ABI1			-0.41
AT5G57050	ABI2			-0.39
AT4G34000	ABF3			-0.45
AT3G19290	ABF4			-0.36
AT3G11410	AHG3			-0.41
AT4G25000	AMY1		0.76	
AT1G01720	ATAF1			-0.41
AT5G24240	AtPI4Kc3	2.74	2.30	1.94
AT4G16830	ATRGGGA			-0.31
AT2G35940	BLH1			-0.36
AT5G27420	CNI1			-0.72
AT4G23600	COR13		-0.52	-0.38
AT1G20440	COR47			-0.66
AT3G50830	COR413-PM2			-0.34
AT1G29395	COR413-IM1			-0.85
AT4G35310	CPK5			-0.31
AT2G17290	CPK6			-0.44
AT4G23260	CRK18			-0.75
AT5G66210	CPK28		-0.31	
AT3G57530	CPK32			-0.40
AT4G04490	CRK36			-0.80
AT5G45340	CYP707A3		-0.76	
AT1G19570	DHAR1			-0.49
AT1G56280	DI19			-0.44
AT1G73330	DR4			-0.99
AT3G15210	ERF4			-0.39
AT1G08930	ERD6		-0.37	
AT2G17840	ERD7			-0.60
AT1G20450	ERD10			-0.53
AT1G76180	ERD14			-0.50
AT2G41430	ERD15			-0.41
AT4G08980	FBW2			-0.35
AT5G13200	GER5			-0.37
AT1G56600	GoIS2		0.59	
AT3G20470	GRP5		-0.50	0.36
AT2G32690	GRP23		-0.39	
AT1G07430	HAI2		0.65	
AT1G72770	HAB1			-0.42
AT1G17550	HAB2			-0.42
AT2G22430	HB6			-0.40
AT3G61890	HB-12			-0.56
AT1G74520	HVA22A			-0.31
AT1G01470	LEA14			-0.31
AT5G59320	LTP3	-0.72	-0.47	-0.98
AT5G59310	LTP4	-0.97	-0.43	-1.35
AT5G52900	MAKR6		0.36	
AT3G63210	MARD1			-0.54
AT1G70890	MLP43		-0.32	-0.48
AT1G70000	MYBD		0.31	
AT5G67300	MYBR1			-0.44
AT2G47190	MYB2		1.01	
AT1G71030	MYBL2		0.30	



Supplementary Figure 7: Heat map of the expression of ABA-associated genes. Heat map of differentially expressed ABA-associated genes in *poco1* versus wild-type plants in three experiments. Many important genes associated with the ABA signaling and response are down-regulated in *poco1*. Fold changes (log₁₀) were used for representing in the heat map. Fold changes comparison is relative to wild-type. Green and blue represent up- and down-regulated genes respectively. White represents that a fold change either ≥ 2 or ≤ -2 with an FDR $p < 0.05$ were not detected.

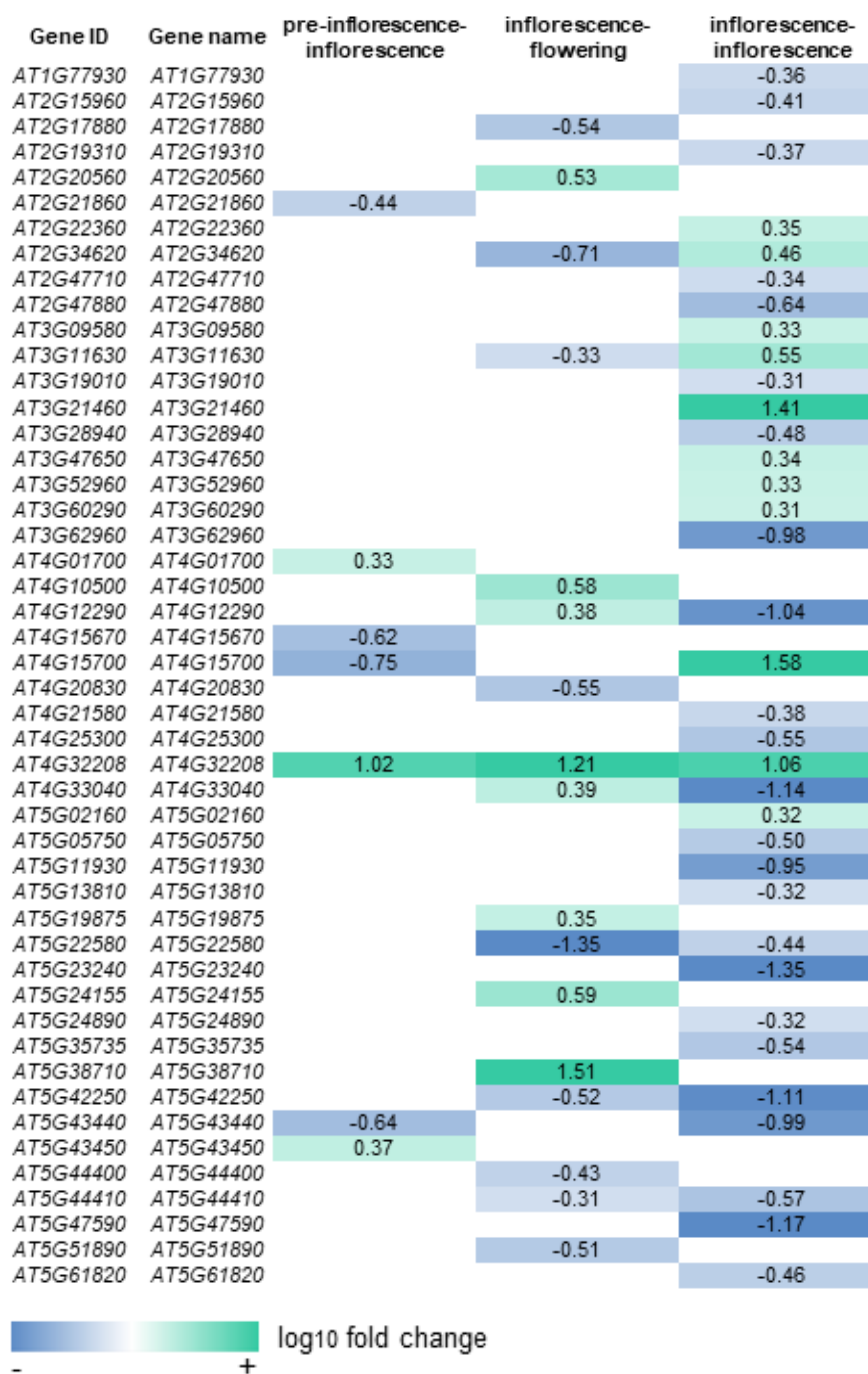
Gene ID	Gene name	pre-inflorescence- inflorescence	inflorescence- flowering	inflorescence- inflorescence
AT4G34000	ABF3			-0.45
AT3G19290	ABF4			-0.36
AT5G06530	ABCG22			-0.46
AT4G34710	ADC2			0.34
AT4G19710	AK-HSDH II			0.36
AT3G29035	ANAC059		0.72	-0.96
AT5G65020	ANNAT2		-0.54	
AT1G35720	ANNAT1		-0.48	
AT2G32800	AP4.3A			-0.34
AT5G43850	ARD4		0.32	
AT5G03630	ATMDAR2			-0.47
AT5G28540	BIP1	0.30		
AT5G42020	BIP2			0.30
AT5G20230	BCB			-0.43
AT3G50930	BCS1			-0.53
AT4G25700	BETA-OHASE 1			0.35
AT2G35940	BLH1			-0.36
AT5G44070	CAD1			-0.36
AT4G34230	CAD5		0.37	
AT4G39330	CAD9		-0.58	
AT1G20620	CAT3		0.40	
AT5G48810	CB5-D			-0.31
AT5G53560	CB5-E			-0.33
AT1G12520	CCS	-0.56		
AT4G33790	CER4			0.73
AT5G24090	CHIA		0.92	
AT1G20440	COR47			-0.66
AT3G50830	COR413-PM2			-0.34
AT4G24280	cpHsc70-1			0.42
AT5G49910	cpHsc70-2			0.47
AT5G20720	CPN20			0.45
AT2G28000	CPN60A			0.70
AT3G13470	Cpn60beta2			0.72
AT1G55490	CPN60B			0.90
AT4G23190	CRK11		-0.48	
AT4G23220	CRK14		-0.49	
AT2G04030	CR88			0.34
AT2G28190	CSD2	-0.79	-0.71	-0.47
AT2G45510	CYP704A2			-0.53
AT2G27010	CYP705A9		2.50	
AT3G20100	CYP705A19		0.36	
AT3G20130	CYP705A22		-0.44	
AT4G22710	CYP706A2		-0.63	-1.30
AT4G12300	CYP706A4		0.48	
AT4G12310	CYP706A5		0.39	
AT3G26220	CYP71B3		0.32	-0.58
AT3G26280	CYP71B4		0.31	-0.46

Gene ID	Gene name	pre-inflorescence- inflorescence	inflorescence- flowering	inflorescence- inflorescence
AT1G13110	CYP71B7			-0.32
AT5G25120	CYP71B11			-0.54
AT5G25140	CYP71B13			-0.38
AT3G26170	CYP71B19		0.56	-0.70
AT3G26180	CYP71B20		0.32	
AT3G26210	CYP71B23			-0.44
AT3G26290	CYP71B26			-0.97
AT3G26230	CYP71B24	0.50		
AT3G14620	CYP72A8		-0.32	
AT3G10570	CYP77A6		-0.56	
AT2G46660	CYP78A6		0.45	
AT3G61880	CYP78A9		0.52	
AT2G22330	CYP79B3		-0.51	
AT1G16410	CYP79F1	0.41		
AT5G36220	CYP81D1			-0.49
AT5G57220	CYP81F2		-0.67	-0.53
AT5G10610	CYP81K1			-0.44
AT4G00360	CYP86A2		-0.33	
AT2G45970	CYP86A8		-0.36	
AT1G64900	CYP89A2			-0.32
AT1G64950	CYP89A5		0.43	
AT3G03470	CYP89A9		0.64	-0.81
AT5G52320	CYP96A4		-0.35	-0.47
AT1G19570	DHAR1			-0.49
AT1G56280	DI19			-0.44
AT5G24530	DMR6	0.63		
AT1G73330	DR4			-0.99
AT5G17710	EMB1241			0.51
AT5G51070	ERD1			-0.53
AT3G30775	ERD5		0.56	
AT1G10370	ERD9		-0.39	0.32
AT1G20450	ERD10			-0.53
AT1G76180	ERD14			-0.50
AT2G41430	ERD15			-0.41
AT4G17490	ERF6		-0.93	-0.86
AT3G56090	FER3			0.83
AT5G23980	FRO4		1.00	
AT5G49730	FRO6	0.31	0.47	
AT5G49740	FRO7			-0.52
AT3G26650	GAPA			0.37
AT1G12900	GAPA-2		-0.35	0.39
AT1G42970	GAPB			0.38
AT1G15550	GA3OX1			0.63
AT4G33010	GLDP1		0.34	
AT1G11840	GLX1		-0.31	
AT1G56600	GoIS2		0.59	-1.58
AT2G41540	GPDHC1		-0.53	

Gene ID	Gene name	pre-inflorescence- inflorescence	inflorescence- flowering	inflorescence- inflorescence
AT2G21660	GRP7		0.72	
AT2G30860	GSTF9	0.35		
AT3G03190	GSTF11		-0.37	-0.32
AT5G17220	GSTF12		1.01	
AT2G29460	GSTU4		0.58	-1.22
AT2G29420	GSTU7			-0.39
AT1G27130	GSTU13			-0.35
AT1G59670	GSTU15		0.40	-1.35
AT1G59700	GSTU16			-0.45
AT1G78370	GSTU20		-0.53	
AT1G17170	GSTU24			-1.20
AT2G46680	HB-7		0.36	
AT3G61890	HB-12		0.32	-0.56
AT1G58290	HEMA1			-0.32
AT4G15440	HPL1	0.95	0.36	
AT4G36990	HSF4			-0.69
AT4G18880	HSF A4A			-0.60
AT5G62020	HSFB2A			-0.41
AT5G59720	HSP18.2			0.44
AT3G23990	HSP60			0.41
AT3G12580	HSP70		0.40	-0.37
AT5G52640	HSP90.1			-0.53
AT1G72970	HTH		-0.56	
AT1G74520	HVA22A			-0.31
AT5G14200	IMD1		-0.44	
AT1G31180	IMD3			0.36
AT1G80920	J8			-0.53
AT4G36040	J11		0.53	-0.52
AT5G03260	LAC11			0.55
AT4G15093	LigB			-0.66
AT1G75690	LQY1			0.37
AT5G52310	LT178			-1.05
AT1G09970	LRR XI-23			-0.48
AT5G59320	LTP3	-0.72	-0.47	-0.98
AT5G59310	LTP4	-0.97	-0.43	-1.35
AT5G23010	MAM1		-0.42	-0.54
AT1G63940	MDAR6			0.55
AT2G22240	MIPS2			-0.79
AT1G70890	MLP43		-0.32	-0.48
AT1G24020	MLP423		-1.36	
AT3G45640	MPK3			-0.67
AT1G65290	mtACP2			0.31
AT4G37910	mtHsc70-1			0.35
AT5G67300	MYBR1			-0.44
AT2G47190	MYB2		1.01	
AT1G66230	MYB20			0.51
AT5G07690	MYB29		-0.75	

Gene ID	Gene name	pre-inflorescence- inflorescence	inflorescence- flowering	inflorescence- inflorescence
AT1G18710	MYB47		0.74	
AT4G34990	MYB32			-0.63
AT1G18570	MYB51			-0.57
AT1G71030	MYBL2		0.30	
AT2G46810	MYC70			0.43
AT4G04840	MSRB6			0.41
AT3G59970	MTHFR1		-0.48	
AT2G44160	MTHFR2		-0.33	
AT1G56010	NAC1			-1.14
AT5G39610	NAC6		0.48	-0.63
AT1G34180	NAC016			-0.75
AT1G52890	NAC019		0.80	
AT4G19170	NCED4		0.45	-0.42
AT5G63310	NDPK2			0.80
AT2G15620	NIR1			0.34
AT4G12720	NUDT7		-0.73	-0.75
AT1G76680	OPR1			-0.44
AT2G19810	OZF1			-0.77
AT3G26830	PAD3		1.37	
AT2G43020	PAO2			-0.54
AT3G17790	PAP17		0.54	
AT5G60640	PDIL1-4			-0.40
AT1G17745	PGDH			-0.65
AT1G01620	PIP1C		-0.68	0.44
AT2G39010	PIP2E		-0.38	
AT4G23400	PIP1;5		-0.33	
AT2G16850	PIP2;8			0.67
AT2G22170	PLAT2		-1.20	
AT5G61640	PMSR1			-0.56
AT5G07460	PMSR2	0.38		
AT1G05850	POM1		-0.40	
AT5G54190	PORA	-1.17		
AT4G27440	PORB		-0.31	
AT1G03630	PORC			0.32
AT3G26060	PRXQ		-0.34	0.37
AT4G21960	PRXR1			-0.59
AT3G52150	PSRP2			0.57
AT4G20960	PyrD		-0.33	
AT5G66400	RAB18			-0.45
AT1G13260	RAV1			-0.41
AT5G38430	RBCS1B			0.78
AT5G47910	RBOHD			-0.39
AT2G21620	RD2			-0.46
AT4G39090	RD19			-0.37
AT2G33380	RD20			-0.58
AT1G47128	RD21A			-0.38
AT2G37180	RD28			0.35

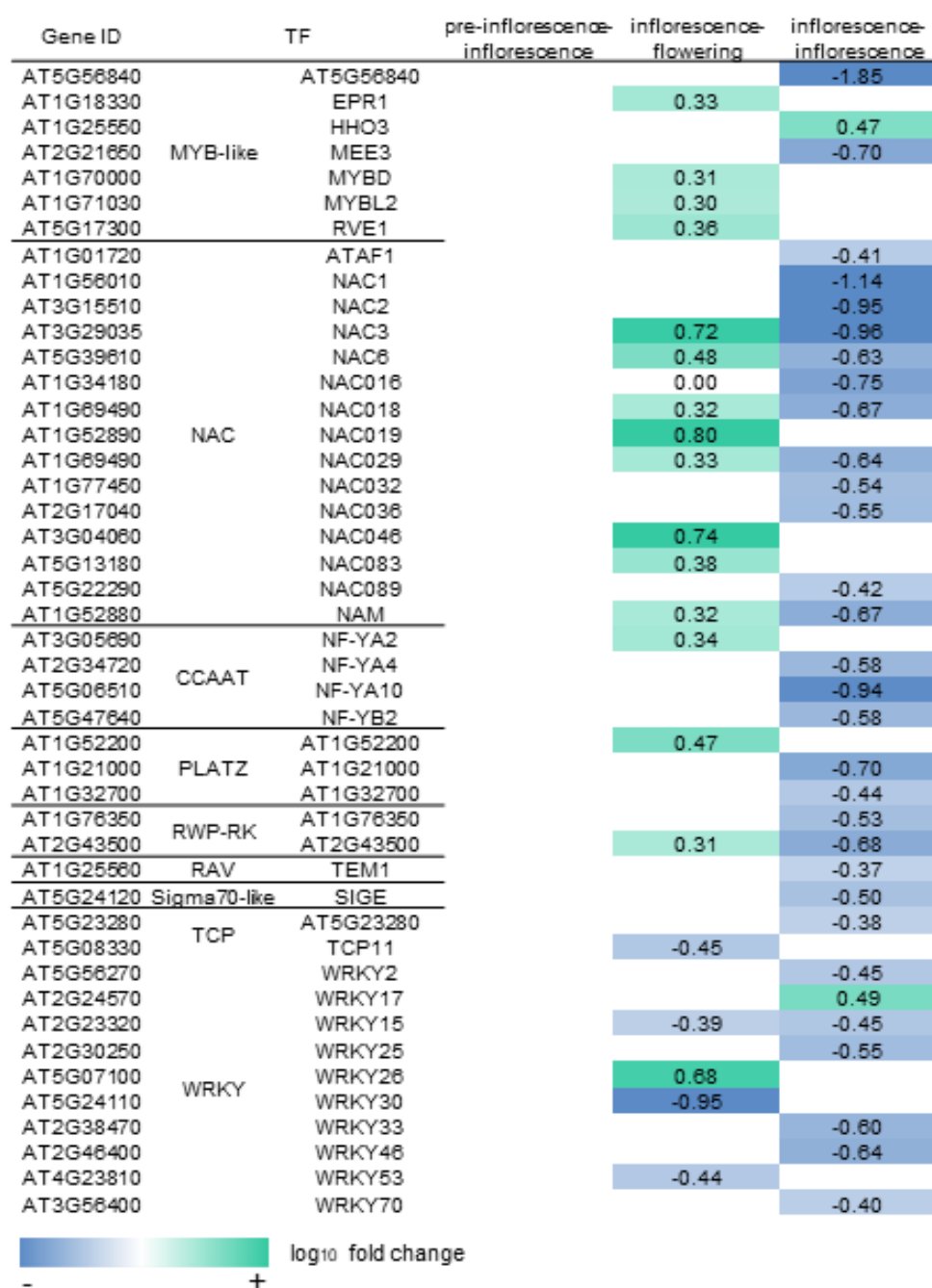
Gene ID	Gene name	pre-inflorescence- inflorescence	inflorescence- flowering	inflorescence- inflorescence
AT3G46620	<i>RDUF1</i>			-0.40
AT5G59550	<i>RDUF2</i>			-0.44
AT3G62030	<i>ROC4</i>		-0.33	0.70
AT1G69270	<i>RPK1</i>			-0.45
AT5G14070	<i>ROXY2</i>			1.54
AT4G15690	<i>ROXY12</i>	-0.58		1.47
AT4G15680	<i>ROXY13</i>	-0.51		2.23
AT4G15660	<i>ROXY15</i>	-0.56		
AT3G62930	<i>ROXY17</i>	-0.42		1.44
AT2G29350	<i>SAG13</i>		0.63	-1.25
AT4G12040	<i>SAP7</i>			-0.56
AT2G26740	<i>SEH</i>	-0.88	-2.06	-1.12
AT2G21970	<i>SEP2</i>			-0.39
AT4G12420	<i>SKU5</i>		-0.71	0.38
AT5G08590	<i>SNRK2.1</i>			-0.32
AT1G78290	<i>SNRK2-8</i>			-0.45
AT5G24160	<i>SQE6</i>		0.59	
AT1G27730	<i>STZ</i>			-0.77
AT5G55220	<i>TIG1</i>			0.45
AT4G09010	<i>TL29</i>			0.48
AT3G27060	<i>TSO2</i>			0.36
AT1G65980	<i>TPX1</i>			-0.35
AT3G06730	<i>TRXZ</i>			0.34
AT1G45145	<i>TRX5</i>			-0.47
AT2G30250	<i>WRKY25</i>			-0.55
AT5G07100	<i>WRKY26</i>		0.68	
AT5G24110	<i>WRKY30</i>		-0.95	
AT2G38470	<i>WRKY33</i>			-0.60
AT2G46400	<i>WRKY46</i>			-0.64
AT3G56400	<i>WRKY70</i>			-0.40
AT5G65730	<i>XTH6</i>		-0.46	-0.31
AT5G04340	<i>ZAT6</i>			-0.94
AT3G19580	<i>ZF2</i>			-0.61
AT1G03410	<i>2A6</i>	-0.59	-0.45	-0.60
AT5G06290	<i>2-Cys Prx B</i>			0.52
AT1G02360	<i>AT1G02360</i>		-0.87	
AT1G04350	<i>AT1G04350</i>			-0.42
AT1G06640	<i>AT1G06640</i>		-0.36	0.42
AT1G13340	<i>AT1G13340</i>		0.61	
AT1G14810	<i>AT1G14810</i>			-0.33
AT1G21400	<i>AT1G21400</i>			-0.72
AT1G22190	<i>AT1G22190</i>			-2.12
AT1G26390	<i>AT1G26390</i>		0.86	-1.51
AT1G60730	<i>AT1G60730</i>			-0.50
AT1G64710	<i>AT1G64710</i>			-0.35
AT1G67360	<i>AT1G67360</i>			-0.92
AT1G69450	<i>AT1G69450</i>			0.32
AT1G74470	<i>AT1G74470</i>			2.09



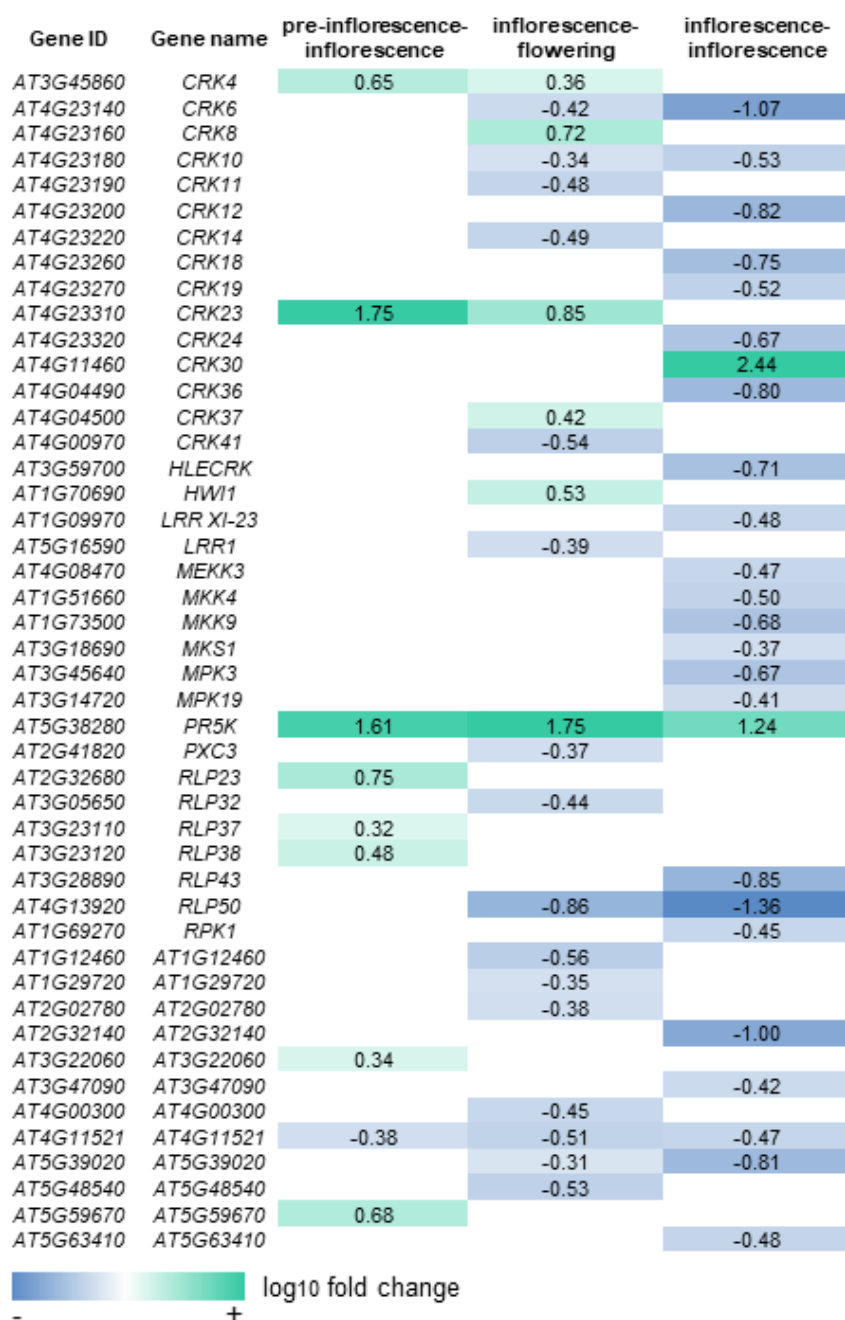
Supplementary Figure 8: Heat map of the expression of drought and oxidative-associated stresses genes. Heat map of differential expressed oxidative and drought stress-associated genes in *poco1* versus wild-type plants in three experiments. Fold changes (\log_{10}) were used for representing in the heat map. Fold changes comparison is relative to wild-type. Green and blue represent up- and down-regulated genes respectively. White represents that a fold change ≥ 2 or ≤ -2 with an FDR $p < 0.05$ was not detected.

Gene ID	TF	pre-inflorescence- inflorescence	inflorescence- flowering	inflorescence- inflorescence
AT4G09180	bHLH81	0.42	0.40	
AT5G39860	bHLH136			0.46
AT2G18300	HB11			0.58
AT2G43060	IBH1		0.39	-0.31
AT1G32640	MYC2			-0.45
AT5G46760	MYC3			-0.39
AT4G17880	MYC4			-0.30
AT2G46810	MYC70			0.43
AT4G00050	UNE10			-0.47
AT4G01460	AT4G01460			0.39
AT4G00870	AT4G00870			-0.66
AT4G34000	ABF3			-0.45
AT3G19290	ABF4			-0.36
AT3G54620	BZIP25			-0.37
AT2G42380	BZIP34			0.50
AT1G42990	BZIP60			-0.44
AT3G58120	BZIP61			0.62
AT4G02640	BZO2H1			-0.41
AT1G03970	GBF4			-0.43
AT1G77920	TGA7			-0.96
AT5G44260	AT5G44260		-0.49	
AT2G25900	ATCTH		-0.32	
AT2G40140	CZF1			-0.55
AT2G19810	OZF1			-0.77
AT4G29190	OZF2			-0.66
AT3G55980	SZF1		-0.34	-0.49
AT1G75710	AT1G75710		-0.37	
AT3G46080	AT3G46080			-1.19
AT4G17810	AT4G17810			0.32
AT3G58070	GIS			-0.43
AT1G55110	IDD7			-0.41
AT3G13810	IDD11			-0.49
AT1G27730	STZ			-0.77
AT5G04340	ZAT6			-0.94
AT2G28200	ZAT10			-0.33
AT5G67450	ZF1		-0.50	
AT3G19580	ZF2			-0.61
AT5G15850	CO-like COL1			-0.40
AT1G01250	AT1G01250			-0.69
AT2G22200	AT2G22200			0.74
AT4G16750	AT4G16750			-0.50
AT4G32800	AT4G32800		-0.47	
AT5G07580	AT5G07580			-0.57
AT5G51190	AT5G51190		-0.77	-0.87
AT5G61590	AT5G61590	-0.33		-0.45
AT4G23750	CRF2			0.73
AT3G61630	CRF6		0.35	
AT1G22985	CRF7			-0.42
AT1G71130	CRF8			-0.34
AT2G23340	DEAR3			-0.34
AT3G16770	EBP		0.47	
AT5G47220	ERF2			-0.49
AT3G15210	ERF4			-0.39
AT5G47230	ERF5		-0.44	-0.58
AT1G28370	ERF11			-0.66
AT4G17490	ERF6		-0.93	-0.86
AT2G31230	ERF15			-0.61
AT5G61600	ERF104		-0.48	-0.90
AT5G25190	ESE3		-0.57	0.72
AT1G06160	ORA59		0.56	-0.58
AT4G36900	RAP2.10			-0.77
AT1G53910	RAP2.12			-0.34
AT1G13260	RAV1			-0.41
AT5G11590	TINY2		-0.56	-0.76

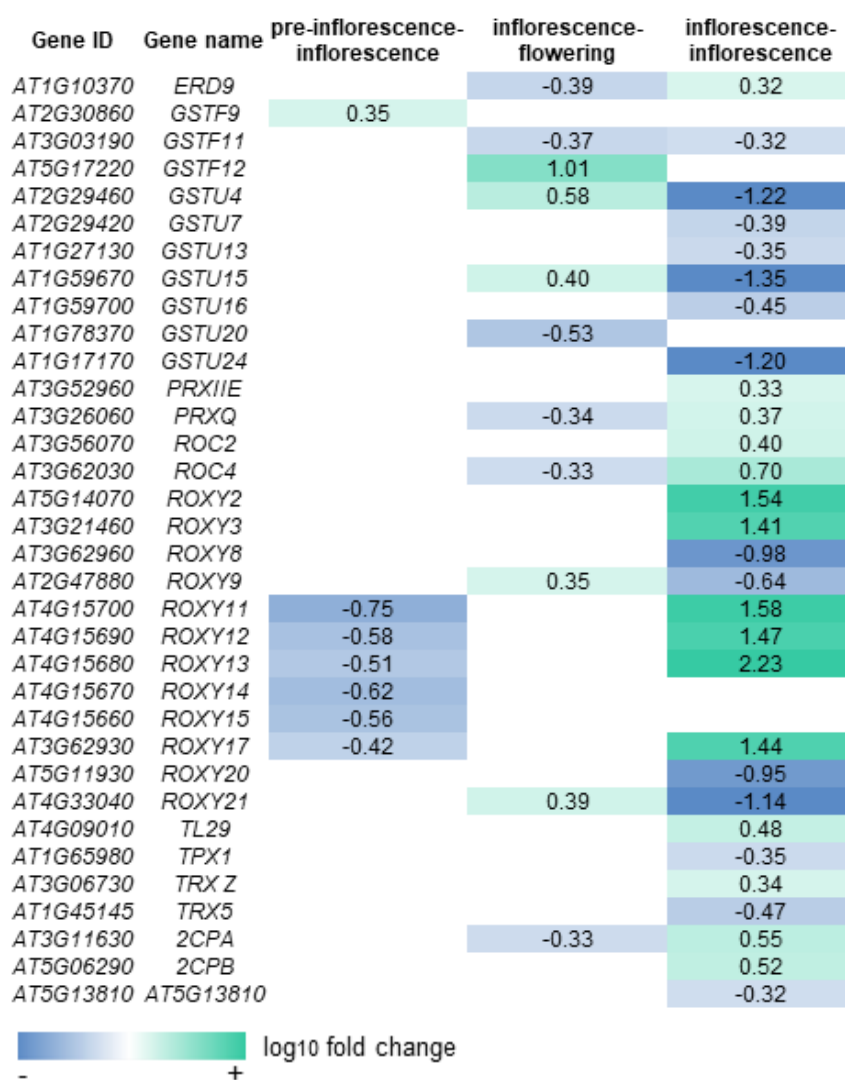
Gene ID	TF		pre-inflorescence- inflorescence	inflorescence- flowering	inflorescence- inflorescence	
AT4G16141	GATA	AT4G16141			0.62	
AT4G36240		GATA7		-0.53		
AT1G07520	GRAS	AT1G07520			-0.45	
AT3G46600		AT3G46600			-0.37	
AT4G17230		SCL13			-0.55	
AT1G07530		SCL14			-0.34	
AT1G20893	HMG	HMGB2		0.41		
AT2G35940	Homeobox	BLH1			-0.36	
AT1G75410		BLH3			-0.37	
AT1G19700		BEL10			-0.36	
AT4G16780		HB-2			-0.33	
AT5G47370		HB-4		0.31		
AT2G22430		HB6			-0.40	
AT2G46680		HB-7		0.36		
AT3G61890		HB-12		0.32		
AT1G26960		HB23			-0.51	
AT3G50890		HB28		-0.49		
AT4G36990		HSF	HSF4			-0.69
AT4G18880			HSFA4A			-0.60
AT5G62020		HSFB2A			-0.41	
AT2G36000	mTERF	mTERF3			0.39	
AT4G38160		mTERF6			0.42	
AT5G55580		mTERF9			0.48	
AT2G34620		mTERF10		-0.71	0.46	
AT5G23930		mTERF34	0.58	0.84	0.54	
AT5G04760		AT5G04760			-0.60	
AT2G40970	MYB	MYB1			-0.58	
AT2G47190		MYB2		1.01		
AT2G16720		MYB7		0.55		
AT1G66230		MYB20		0.51		
AT5G07690		MYB29		-0.75	-0.37	
AT1G74650		MYB31			-1.06	
AT4G34990		MYB32			-0.63	
AT5G60890		MYB34		-0.60		
AT5G67300		MYB44			-0.44	
AT1G18710		MYB47		0.74		
AT1G18570		MYB51			-0.57	
AT5G59780		MYB59		0.38	-0.58	
AT2G23290		MYB70	-0.50		-0.60	
AT4G37260		MYB73			-0.55	
AT3G50060		MYB77			-0.35	
AT1G66390		MYB90		1.10		
AT1G56650		PAP1		0.66	-1.54	



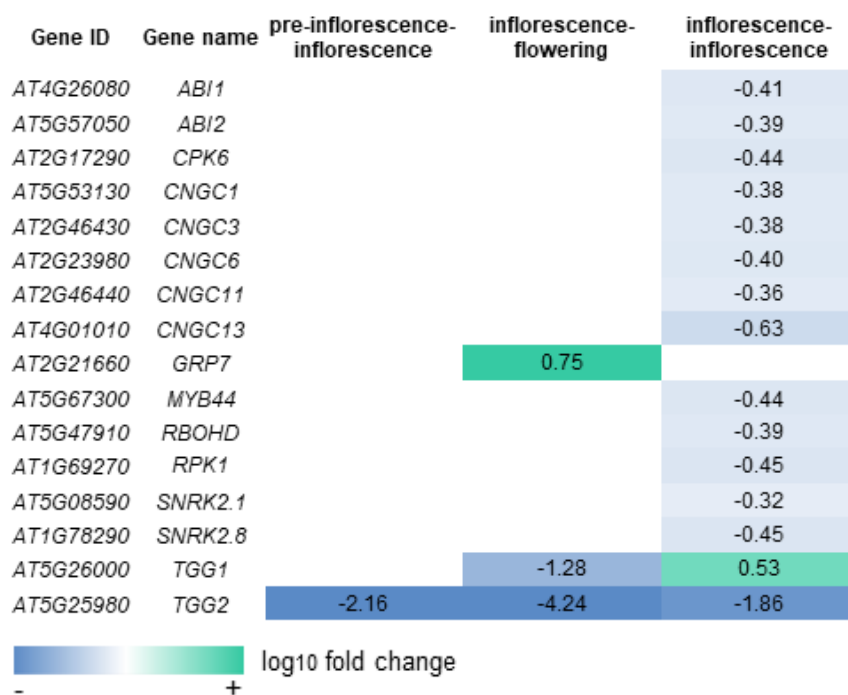
Supplementary Figure 9: Heat map of differential expressed genes encoding transcription factors. Heat map of differential expressed genes encoding transcription factors in *poco1* versus wild-type plants in three comparisons. A high number of genes encoding transcription factors are differentially regulated in *poco1*, suggesting a complex regulatory network in *poco1*. Fold changes (log₁₀) were used for representing in the heat map. Fold changes comparison is relative to wild-type. Green and blue represent up- and down-regulated genes respectively. White represents that a fold change either ≥ 2 or ≤ -2 with an FDR $p < 0.05$ were not detected.



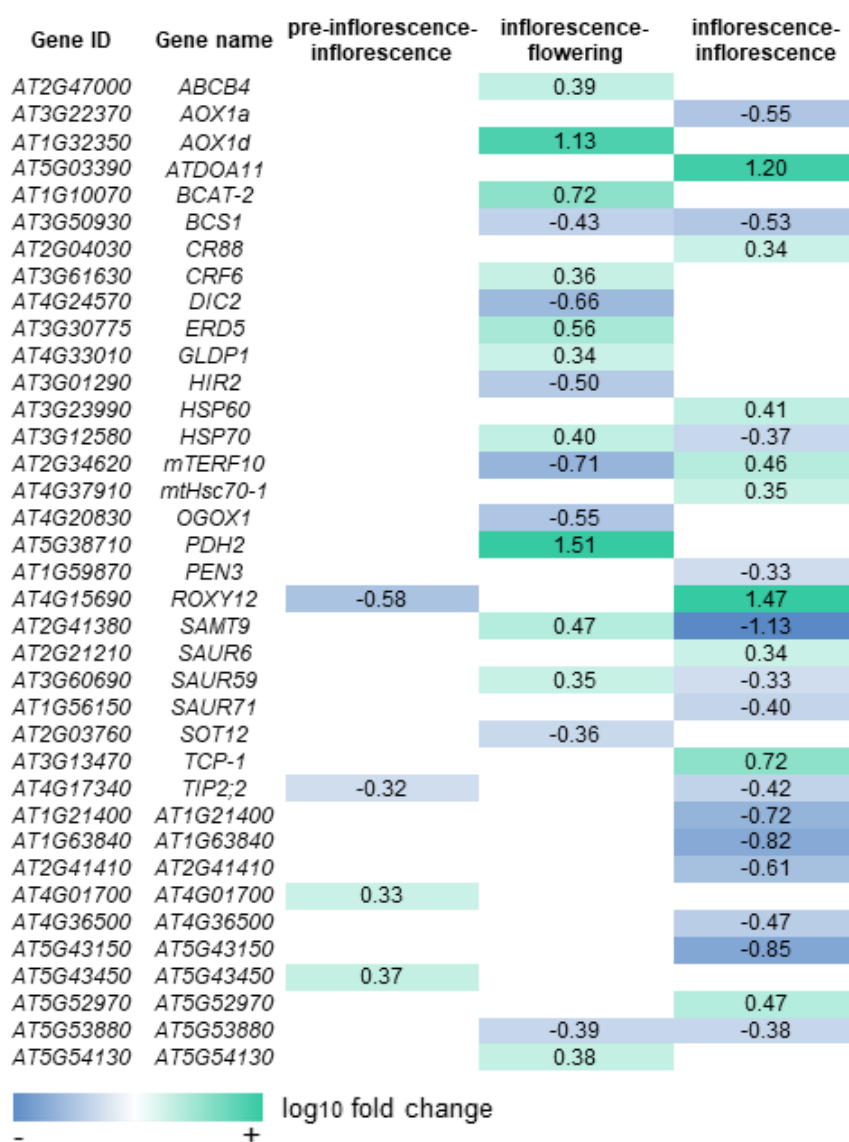
Supplementary Figure 10: Heat map the differentially regulated signaling genes. Genes associated with the cellular signaling were found to be differentially regulated in *poco1*. Fold changes (log₁₀) were used for representing in the heat map. Fold changes comparison is relative to wild-type. Green and blue represent up- and down-regulated genes respectively. White represents that a fold change either ≥ 2 or ≤ -2 with an FDR $p < 0.05$ were not detected.



Supplementary Figure 11: Expression alteration in redox-related genes. Heat map represents different genes involved in the redox state. Differential expression of redox-related genes in *poco1* suggests an imbalance of proper redox state of the cells. Fold changes (log₁₀) were used for representing in a heat map. Fold changes comparison is relative to wild-type. Green and blue represent up- and down-regulated genes respectively. White represents that a fold change either ≥ 2 or ≤ -2 with an FDR $p < 0.05$ were not detected.



Supplementary Figure 12: Altered expression of genes associated with the stomatal function. A heat map for genes involved in stomatal function is represented. As genes associated with the stomatal function are mostly down-regulated, stomatal closure may be failed in *poco1*. Fold changes (log₁₀) were used for representing in heat maps. Fold changes comparison is relative to wild-type. Green and blue represent up- and down-regulated genes respectively. White represents that a fold change either ≥ 2 or ≤ -2 with an FDR $p < 0.05$ were not detected.



Supplementary Figure 13: Altered expression of mitochondrial perturbation targets. Heat map represents the fold change (log₁₀) values of differential expressed mitochondrial perturbation targets in *poco1* versus wild-type plants in three comparisons. Fold changes comparison is relative to wild-type. Green and blue represent up- and down-regulated genes respectively. White represents that a fold change either ≥ 2 or ≤ -2 with an FDR $p < 0.05$ were not detected.

Supplementary Table 1: DNA oligonucleotides used in this thesis. DNA oligonucleotides were ordered as HPLC purified from Eurofins MWG. The sequences are shown in 5' to 3' direction.


Name	Sequence	Remarks
FK589	GGATGACGCACAATCCCCTACTA	PCR- CaMV35S 3' end, Forward
AB1451	CCTCTAGAATATCCCCTTCATCACCGG	PCR- <i>POCO1</i> , Reverse
AB2060	GCTAGCATGTTTGCTCTTTCCAAGGT	PCR- <i>POCO1</i> , Forward
AB2061	GGTACCAATCAAGGAGATCCGAGAGAG	PCR- <i>POCO1</i> , Reverse
KK2297	TAGGCGCGCCAAGCTTGCATGCCTGCAG	CaMV 35S Promoter, Forward
KK2298	AGCCCGGGGTCACTGGATTTTGGTTTTAG	CaMV 35S Terminator, Reverse
HE2583	CGGGCGCGCCCTCAATCAAGGAGATCCGAGAGAG	PCR- <i>POCO1</i> , Forward
HE2584	CGGGCGCGCCAATGTTTGCTCTTTCCAAAGTTTTG	PCR- <i>POCO1</i> , Reverse
HE2592	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGTTTGCTCTTTCCAAGGT	Gateway cloning with <i>attB</i> site, Forward
HE2593	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAATCAAGGAGATCCGAGAGAGG	Gateway cloning with <i>attB</i> site, Reverse
HE2696	GGTACCGGATGGGTAAAGGAGAAGAAGT	GFP and CaMV terminator, Forward
HE2697	GGTACCGTCACTGGATTTTGGTTTTAGG	GFP and CaMV terminator, Reverse
HE2710	GGTATGGTGAAGGCTGGATTTGCAGG	RT-PCR of Actin2, Forward
HE2711	AAGCAAGAATGGAACCACCGATCCAGAC	RT-PCR of Actin2, Reverse
HE3165	GCCACCGTACTATCCTCAG	RT-PCR, <i>FRL2</i> , 100 bp, forward
HE3166	GAGCATAGACCTGTTTCGAGC	RT-PCR, <i>FRL2</i> , 100 bp, Reverse
RS3137	TCTCGACCAGTTCATGCCAC	RT-PCR, <i>ELF7</i> , 100 bp, forward
RS3138	TCGCTACCACAACTGCTCA	RT-PCR, <i>ELF7</i> , 100 bp, Reverse
HE3163	TTCACCGTGAAGCTGCTGAG	RT-PCR, <i>ELF8</i> , 100 bp, forward
HE3164	TCAGCTTTACGGCGTGCTTC	RT-PCR, <i>ELF8</i> , 100 bp, Reverse
RS3127	TTGGCTAGCCAGATGGAGAA	RT-PCR, <i>FLC</i> , 100 bp, forward
RS3128	GGAGAGTCACCGGAAGATTGT	RT-PCR, <i>FLC</i> , 100 bp, Reverse
RS3129	TTGTTTCGACAGCTTGGCAG	RT-PCR, <i>FT</i> , 100 bp, forward
RS3130	CGGGAAGGCCGAGATTGTAG	RT-PCR, <i>FT</i> , 100 bp, Reverse
RS3131	AGCAACAGCTTGAGAAAAGTGT	RT-PCR, <i>SOC1</i> , 100 bp, forward
RS3132	GCTAGAGCTTTCTCCTTTTGCT	RT-PCR, <i>SOC1</i> , 100 bp, Reverse
RS3141	TCAGTCTCGCCCAAAGACTG	RT-PCR, <i>ESD4</i> , 100 bp, forward
RS3142	AAAGGCACGGTAGACTTCAGC	RT-PCR, <i>ESD4</i> , 100 bp, Reverse
RS3143	TGGCATTAAAGAAGCGGCT	RT-PCR, <i>FRI</i> , 100 bp, forward

RS3144	CCTGGTAGTTCTTTTCGCAGG	RT-PCR, <i>FRI</i> , 100 bp, Reverse
RS3145	TGGTGAGGAGAGAAAGGCTG	RT-PCR, <i>HUA2</i> , 100 bp, forward
RS3146	ATGCTCCAGTGA CTGCTAA	RT-PCR, <i>HUA2</i> , 100 bp, Reverse
RS3147	TTACAGAGGACGGTATCGGC	RT-PCR, <i>PIE1</i> , 100 bp, forward
RS3148	CTCGTTCACTGCACTGTCG	RT-PCR, <i>PIE1</i> , 100 bp, Reverse
RS3149	GTCCTTGTGTTCTCCCCTG	RT-PCR, <i>VIP3</i> , 100 bp, forward
RS3150	CCCCAACAGCGTTTTCCCT	RT-PCR, <i>VIP3</i> , 100 bp, Reverse
RS3151	AGGGCAGAGAAGGATCACC	RT-PCR, <i>VIP4</i> , 100 bp, forward
RS3152	TACGACGATGAGTCGGAGC	RT-PCR, <i>VIP4</i> , 100 bp, Reverse
RS3153	AGGTTGGTGTCAAGCCTTTC	RT-PCR, <i>FRL1</i> , 100 bp, forward
RS3154	ACGTAATCAGAAAGCTCCTCTGT	RT-PCR, <i>FRL1</i> , 100 bp, Reverse
HE3167	GGA ACTCGTCAAGGGTCAATG	RT-PCR, <i>ABI5</i> , 100 bp, forward
HE3168	GCGCAAGCGAGACATAATGG	RT-PCR, <i>ABI5</i> , 100 bp, Reverse
RS3133	TACCGAGGCTCCTCTTAACC	RT-PCR, <i>ACT2</i> , 100 bp, forward
RS3134	AGCTTGGATGGCGACATACA	RT-PCR, <i>ACT2</i> , 100 bp, Reverse


Supplementary Table 2: Potential POCO1-interacting candidates identified by LC-MS in GFP pull-down experiment.


Accession number	Protein
AT1G80270	PPR596
AT1G16300	glyceraldehyde-3-phosphate dehydrogenase of plastid 2
AT2G26900	Sodium Bile acid symporter family
AT1G15480	Pentatricopeptide repeat protein, POCO1
AT1G12900	glyceraldehyde 3-phosphate dehydrogenase A subunit 2
AT1G74030	Enolase 1
AT1G43800	Plant stearyl-acyl-carrier-protein desaturase family protein
AT5G43780	Pseudouridine synthase/archaeosine transglycosylase-like
AT2G01140	Aldolase superfamily protein
AT1G80480	plastid transcriptionally active 17
AT3G14415	Aldolase-type TIM barrel family protein
AT5G46290	3-ketoacyl-acyl carrier protein synthase I
AT3G29410	Protein prenyltransferases superfamily protein
AT2G35490	Plastid-lipid associated protein PAP/fibrillin family protein
AT3G52960	Thioredoxin superfamily protein
AT2G33150	peroxisomal 3-ketoacyl-CoA thiolase 3
AT1G79530	glyceraldehyde-3-phosphate dehydrogenase of plastid 1
AT4G20360	RAB GTPase homolog E1B
AT1G24360	NAD(P)-binding Rossmann-fold superfamily protein
AT3G48870	Clp ATPase
AT5G09590	mitochondrial HSO70 2
AT5G50920	CLPC homologue 1
AT4G34200	D-3-phosphoglycerate dehydrogenase
AT3G47520	malate dehydrogenase
AT1G13440	glyceraldehyde-3-phosphate dehydrogenase C2
AT3G04120	glyceraldehyde-3-phosphate dehydrogenase C subunit 1
AT4G37910	mitochondrial heat shock protein 70-1
AT5G25940	early nodulin-related
AT3G03590	SWIB/MDM2 domain superfamily protein
AT3G62530	ARM repeat superfamily protein
AT2G07696	Ribosomal protein S7p/S5e family protein
AT4G35630	phosphoserine aminotransferase
AT4G20260	plasma-membrane associated cation-binding protein 1

Supplementary Table 3: GO enrichment analysis (Top 5 molecular functions and top thirty biological processes) for up- (A) and down-regulated genes (B) of pre-inflorescence-inflorescence.



(A) GO:BP		stats	
Term name	Term ID		P _{adj}
glycosyl compound biosynthetic process	GO:1901659		1.488×10 ⁻⁶
response to external biotic stimulus	GO:0043207		1.568×10 ⁻⁵
response to other organism	GO:0051707		1.568×10 ⁻⁵
response to biotic stimulus	GO:0009607		1.802×10 ⁻⁵
sulfur compound biosynthetic process	GO:0044272		2.499×10 ⁻⁴
S-glycoside biosynthetic process	GO:0016144		3.158×10 ⁻⁴
glucosinolate biosynthetic process	GO:0019761		3.158×10 ⁻⁴
glycosinolate biosynthetic process	GO:0019758		3.158×10 ⁻⁴
multi-organism process	GO:0051704		4.542×10 ⁻⁴
response to external stimulus	GO:0009605		8.617×10 ⁻⁴
systemic acquired resistance	GO:0009627		1.257×10 ⁻³
glycosyl compound metabolic process	GO:1901657		1.868×10 ⁻³
defense response to other organism	GO:0098542		2.525×10 ⁻³
innate immune response	GO:0045087		4.104×10 ⁻³
secondary metabolite biosynthetic process	GO:0044550		4.382×10 ⁻³
immune response	GO:0006955		4.764×10 ⁻³
defense response, incompatible interaction	GO:0009814		6.746×10 ⁻³
sulfur compound metabolic process	GO:0006790		7.435×10 ⁻³
immune system process	GO:0002376		9.111×10 ⁻³
defense response	GO:0006952		1.347×10 ⁻²
response to stimulus	GO:0050896		2.644×10 ⁻²
glycosinolate metabolic process	GO:0019757		3.377×10 ⁻²
S-glycoside metabolic process	GO:0016143		3.377×10 ⁻²
glucosinolate metabolic process	GO:0019760		3.377×10 ⁻²
secondary metabolic process	GO:0019748		4.994×10 ⁻²

(B)


GO:MF				stats
Term name	Term ID		P _{adj}	
protein disulfide oxidoreductase activity	GO:0015035		1.176×10 ⁻⁶	
disulfide oxidoreductase activity	GO:0015036		3.694×10 ⁻⁶	
2 iron, 2 sulfur cluster binding	GO:0051537		6.148×10 ⁻⁶	
oxidoreductase activity, acting on a sulfur group of donors	GO:0016667		2.539×10 ⁻⁵	
iron-sulfur cluster binding	GO:0051536		1.735×10 ⁻⁴	
metal cluster binding	GO:0051540		1.735×10 ⁻⁴	
electron transfer activity	GO:0009055		3.016×10 ⁻⁴	
superoxide dismutase copper chaperone activity	GO:0016532		1.435×10 ⁻²	
oxidoreductase activity	GO:0016491		4.629×10 ⁻²	


GO:BP				stats
Term name	Term ID		P _{adj}	
cell redox homeostasis	GO:0045454		3.216×10 ⁻⁴	
cellular homeostasis	GO:0019725		6.826×10 ⁻⁴	
electron transport chain	GO:0022900		9.937×10 ⁻⁴	

Supplementary Table 4: GO enrichment analysis (Top 5 molecular functions and top thirty biological processes) for up- (A) and down-regulated genes (B) of inflorescence-flowering.

GO:MF			stats
Term name	Term ID		P _{adj}
oxidoreductase activity, acting on paired donors, with incor...	GO:0016709		1.404×10 ⁻⁵
monooxygenase activity	GO:0004497		1.949×10 ⁻⁴
iron ion binding	GO:0005506		9.154×10 ⁻⁴
oxidoreductase activity, acting on paired donors, with incor...	GO:0016705		3.066×10 ⁻³
glucosyltransferase activity	GO:0046527		6.345×10 ⁻³
GO:BP			stats
Term name	Term ID		P _{adj}
response to stimulus	GO:0050896		1.587×10 ⁻¹¹
response to chemical	GO:0042221		1.153×10 ⁻⁸
response to external stimulus	GO:0009605		6.676×10 ⁻⁸
response to stress	GO:0006950		2.770×10 ⁻⁶
response to acid chemical	GO:0001101		3.316×10 ⁻⁵
leaf senescence	GO:0010150		5.848×10 ⁻⁵
response to external biotic stimulus	GO:0043207		6.128×10 ⁻⁵
response to other organism	GO:0051707		6.128×10 ⁻⁵
aging	GO:0007568		6.384×10 ⁻⁵
response to biotic stimulus	GO:0009607		7.942×10 ⁻⁵
plant organ senescence	GO:0090693		9.759×10 ⁻⁵
innate immune response	GO:0045087		9.854×10 ⁻⁵
immune system process	GO:0002376		1.309×10 ⁻⁴
immune response	GO:0006955		1.367×10 ⁻⁴
response to oxygen-containing compound	GO:1901700		1.384×10 ⁻⁴
response to inorganic substance	GO:0010035		3.351×10 ⁻⁴
response to abiotic stimulus	GO:0009628		5.961×10 ⁻⁴
defense response to other organism	GO:0098542		7.403×10 ⁻⁴
response to organic substance	GO:0010033		1.077×10 ⁻³
response to hormone	GO:0009725		1.083×10 ⁻³
response to endogenous stimulus	GO:0009719		1.876×10 ⁻³
response to antibiotic	GO:0046677		4.887×10 ⁻³
defense response, incompatible interaction	GO:0009814		5.132×10 ⁻³
systemic acquired resistance	GO:0009627		5.410×10 ⁻³
pigment metabolic process	GO:0042440		5.410×10 ⁻³
multi-organism process	GO:0051704		8.037×10 ⁻³
response to drug	GO:0042493		8.529×10 ⁻³
response to extracellular stimulus	GO:0009991		1.083×10 ⁻²
cellular response to extracellular stimulus	GO:0031668		1.747×10 ⁻²
cellular response to external stimulus	GO:0071496		2.283×10 ⁻²


(B)


GO:MF			stats
Term name	Term ID		P _{adj}
xyloglucan:xyloglucosyl transferase activity	GO:0016762		7.548×10^{-4}
manganese ion binding	GO:0030145		2.521×10^{-2}
catalytic activity	GO:0003824		2.686×10^{-2}
nutrient reservoir activity	GO:0045735		3.560×10^{-2}
hydrolase activity, hydrolyzing O-glycosyl compounds	GO:0004553		3.859×10^{-2}


GO:BP			stats
Term name	Term ID		P _{adj}
cell wall organization or biogenesis	GO:0071554		1.779×10^{-11}
cell wall organization	GO:0071555		3.055×10^{-11}
external encapsulating structure organization	GO:0045229		2.543×10^{-10}
response to external biotic stimulus	GO:0043207		3.787×10^{-6}
response to other organism	GO:0051707		3.787×10^{-6}
response to biotic stimulus	GO:0009607		5.395×10^{-6}
defense response	GO:0006952		7.908×10^{-6}
response to abiotic stimulus	GO:0009628		1.143×10^{-5}
polysaccharide metabolic process	GO:0005976		1.310×10^{-5}
defense response to other organism	GO:0098542		2.441×10^{-5}
response to stimulus	GO:0050896		2.650×10^{-5}
systemic acquired resistance	GO:0009627		5.349×10^{-5}
pectin metabolic process	GO:0045488		5.667×10^{-5}
galacturonan metabolic process	GO:0010393		6.143×10^{-5}
response to stress	GO:0006950		2.753×10^{-4}
defense response, incompatible interaction	GO:0009814		3.762×10^{-4}
response to external stimulus	GO:0009605		7.299×10^{-4}
response to oxygen-containing compound	GO:1901700		9.473×10^{-4}
innate immune response	GO:0045087		1.263×10^{-3}
response to bacterium	GO:0009617		1.341×10^{-3}
drug metabolic process	GO:0017144		1.751×10^{-3}
immune response	GO:0006955		1.803×10^{-3}
multi-organism process	GO:0051704		2.096×10^{-3}
syncytium formation	GO:0006949		2.150×10^{-3}
response to salt stress	GO:0009651		2.891×10^{-3}
S-glycoside metabolic process	GO:0016143		2.962×10^{-3}
glucosinolate metabolic process	GO:0019760		2.962×10^{-3}
glycosinolate metabolic process	GO:0019757		2.962×10^{-3}
response to chemical	GO:0042221		3.041×10^{-3}
carbohydrate metabolic process	GO:0005975		4.658×10^{-3}


Supplementary Table 5: GO enrichment analysis (Top 5 molecular functions and top thirty biological processes) for up- (A) and down-regulated genes (B) of inflorescence-inflorescence.

(A)

GO:MF			stats
Term name	Term ID		P _{adj}
structural constituent of ribosome	GO:0003735		1.601×10^{-188}
structural molecule activity	GO:0005198		5.291×10^{-165}
RNA binding	GO:0003723		1.080×10^{-59}
rRNA binding	GO:0019843		1.799×10^{-47}
mRNA binding	GO:0003729		3.032×10^{-43}

GO:BP			stats
Term name	Term ID		P _{adj}
translation	GO:0006412		5.699×10^{-152}
peptide biosynthetic process	GO:0043043		1.738×10^{-151}
amide biosynthetic process	GO:0043604		2.152×10^{-147}
peptide metabolic process	GO:0006518		3.901×10^{-144}
cellular amide metabolic process	GO:0043603		9.961×10^{-135}
organonitrogen compound biosynthetic process	GO:1901566		1.565×10^{-126}
cellular nitrogen compound biosynthetic process	GO:0044271		1.586×10^{-58}
organic substance biosynthetic process	GO:1901576		2.243×10^{-53}
biosynthetic process	GO:0009058		4.209×10^{-52}
cellular biosynthetic process	GO:0044249		2.561×10^{-48}
ribosome biogenesis	GO:0042254		5.711×10^{-45}
gene expression	GO:0010467		5.236×10^{-42}
cellular nitrogen compound metabolic process	GO:0034641		1.740×10^{-39}
cellular macromolecule biosynthetic process	GO:0034645		2.413×10^{-38}
macromolecule biosynthetic process	GO:0009059		3.609×10^{-37}
ribonucleoprotein complex biogenesis	GO:0022613		1.397×10^{-36}
organonitrogen compound metabolic process	GO:1901564		1.599×10^{-34}
ribosomal large subunit biogenesis	GO:0042273		2.614×10^{-30}
cellular protein metabolic process	GO:0044267		4.131×10^{-29}
ribosome assembly	GO:0042255		6.410×10^{-28}
protein metabolic process	GO:0019538		3.842×10^{-25}
cytoplasmic translation	GO:0002181		1.204×10^{-19}
ribosomal large subunit assembly	GO:0000027		3.385×10^{-18}
cellular process	GO:0009987		5.624×10^{-17}
organelle assembly	GO:0070925		2.017×10^{-16}
cellular component biogenesis	GO:0044085		6.008×10^{-16}
ribonucleoprotein complex subunit organization	GO:0071826		6.569×10^{-16}
cellular metabolic process	GO:0044237		8.132×10^{-16}
response to cytokinin	GO:0009735		1.116×10^{-15}
ribonucleoprotein complex assembly	GO:0022618		1.266×10^{-15}

GO:MF			stats
Term name	Term ID		Padj
DNA-binding transcription factor activity	GO:0003700		2.937×10^{-6}
omega peptidase activity	GO:0008242		3.741×10^{-5}
transcription regulator activity	GO:0140110		1.563×10^{-4}
ion binding	GO:0043167		1.200×10^{-2}
nucleobase transmembrane transporter activity	GO:0015205		1.251×10^{-2}

GO:BP			stats
Term name	Term ID		Padj
response to oxygen-containing compound	GO:1901700		5.180×10^{-32}
response to chemical	GO:0042221		1.409×10^{-28}
response to drug	GO:0042493		5.735×10^{-28}
response to stimulus	GO:0050896		3.319×10^{-27}
response to chitin	GO:0010200		6.590×10^{-27}
response to organic substance	GO:0010033		5.457×10^{-25}
response to stress	GO:0006950		3.048×10^{-24}
response to acid chemical	GO:0001101		3.582×10^{-21}
response to organonitrogen compound	GO:0010243		1.538×10^{-20}
response to external stimulus	GO:0009605		2.517×10^{-18}
response to other organism	GO:0051707		6.424×10^{-18}
response to external biotic stimulus	GO:0043207		6.424×10^{-18}
response to biotic stimulus	GO:0009607		1.406×10^{-17}
response to nitrogen compound	GO:1901698		1.054×10^{-16}
response to endogenous stimulus	GO:0009719		3.065×10^{-15}
response to hormone	GO:0009725		8.353×10^{-15}
defense response	GO:0006952		2.225×10^{-14}
defense response to other organism	GO:0098542		5.690×10^{-13}
response to bacterium	GO:0009617		1.461×10^{-12}
immune system process	GO:0002376		1.848×10^{-12}
response to water	GO:0009415		1.855×10^{-12}
response to water deprivation	GO:0009414		3.417×10^{-12}
innate immune response	GO:0045087		5.756×10^{-12}
response to antibiotic	GO:0046677		6.805×10^{-12}
immune response	GO:0006955		1.300×10^{-11}
response to abiotic stimulus	GO:0009628		1.778×10^{-11}
defense response to bacterium	GO:0042742		2.664×10^{-11}
response to inorganic substance	GO:0010035		4.640×10^{-11}
response to abscisic acid	GO:0009737		5.247×10^{-11}
response to alcohol	GO:0097305		7.015×10^{-11}

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Lebenslauf

Persönliche Daten

Name	Hossein Emami
Geburtsdatum	20.07.1985
Geburtsort	Mahshahr
Staatsangehörigkeit	Iranisch

Studium

- 2013-heute** Doktorand, Christian-Albrecht Universität zu Kiel (CAU), Germany, im Fachgebiet Molekularbiologie von Pflanzen, Titel der Arbeit: "Charakterisierung ein mitochondrial PPR Protein aus *Arabidopsis thaliana*". Betreuer: Prof. Dr. Frank Kempken
- 2007-2009** M.Sc. Guilan Universität, Iran, im Fachgebiet Zierpflanzen, Titel der Arbeit: "The effect of gibberellic acid and benzyladenine on growth and flowering of Lily (*Lilium longiflorum*)". Betreuer: Prof. Dr. Abdollah Hatamzadeh
- 2003-2007** B.Sc. Guilan Universität, Iran, im Fachgebiet Agrarwissenschaft

Lehre

- 2019-Heute** Botany, Christian-Albrecht Universität zu Kiel (CAU)
- 2015-Heute** Genetik (Mutation), Christian-Albrecht Universität zu Kiel (CAU)

Publikationsliste

- Emami H**, Kumar A and Kempken F (2020). Transcriptomic analysis of *POCO1*, a mitochondrial PPR protein mutant in *Arabidopsis thaliana*. BMC Plant Biology.
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- Emami H** and Kempken F (2020). POCO1, A Mitochondrial PPR Protein Involved in ABA Signaling and Flowering Time Regulation in *Arabidopsis*. 33rd Tagung Molekularbiologie der Pflanzen. Dabringhausen. University of Hohenheim. (Vortrag)
- Emami H** and Kempken F (2018). A mitochondrial PPR protein negatively regulates flowering time and affects drought tolerance in *Arabidopsis thaliana*. International Plant molecular biology (IPMB), Montpellier, France (Poster)
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Emami H and Kempken F (2017). A mitochondrial PPR protein delays floral transition by affecting flowering locus c (*flc*) expression in *Arabidopsis thaliana*. Botanikertagung, Kiel, Germany (Poster)

Emami H, Grüttner S and Kempken F (2016). Characterization of two mitochondrial PPR proteins: DWEORG1 and AT1G15480. Mitochondria at the crossroad symposium. University of Strasbourg. France (Vortrag)

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Emami H and Kempken F (2015). Towards characterization of the mitochondrial pentatricopeptide repeat protein: AT1G15480. 28rd Tagung Molekularbiologie der Pflanzen. Dabringhausen. University of Hohenheim (Poster)

Auszeichnungen

2018 DAAD Reisestipendium

2016 Mitocross Reisestipendium

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Der Zulassung von Zuhörern/Zuhörerinnen bei der mündlichen Prüfung wird nicht widersprochen.

Kiel, den

.....

Hossein Emami