IMPRINTING PATTERN OF TYPE I MADS-BOX TRANSCRIPTION-FACTOR GENES IN ARABIDOPSIS ENDOSPERM

By

Adele Zhou

A Thesis Submitted to THE Honor College
In Partial Fulfillment of the Bachelors of Science Degree
With Honors in
in Biochemistry and Molecular Biophysics
THE UNIVERSITY OF ARIZONA
May 2011

Approved By:

[Signature]
Faculty Director Signature

[Signature]
Undergraduate Committee Member

4/15/2011
Date

4/22/2011
Date
STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for a degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Signed: [Signature]
ABSTRACT

Observations from previous experiments indicate that the Type I MADS-box genes play a role in endosperm development, and previous real-time RT-PCR analysis identified 16 of these genes that are under the control of the Polycomb-group complex after fertilization. In addition, some Type I MADS-box genes are known to be imprinted during early endosperm development. This paper presents experiments using a variation of the Cleaved Amplified Polymorphic Sequences (CAPS) method to determine the imprinting nature of these 16 genes. Results indicate that AGL28, AGL36, AGL90, and AGL96 are maternally expressed and paternally imprinted. AGL23 and AGL37 (PHEI) are paternally expressed and maternally imprinted and AGL59 is biallelically expressed.
1. INTRODUCTION

The plant life cycle alternates between the multicellular diploid sporophyte generation and the multicellular haploid gametophyte generation. In angiosperms, the female gametophyte, which is formed after a megaspore undergoes three rounds of mitosis without cytokinesis, consists of an egg cell, a central cell, two synergid cells, and three antipodal cells. This seven-celled structure plays a critical role in both the reproductive process and the early seed development of the plant. During the process of double fertilization, the pollen tube penetrates one of the synergid cells and releases two sperm cells, one of which fuses with the egg cell giving rise to the embryo, and the other fuses with the central cell giving rise to the endosperm.

The endosperm functions to support the development of the embryo, which forms the next generation, as well as seedling development after germination. In many plants including maize, the endosperm undergoes multiple rounds of mitosis without cytokinesis, forms a multi-nucleated syncytium, becomes fully cellularized during a cellularization phase, and undergoes a period of intense mitosis to produce the bulk of the endosperm in the mature seed. In Arabidopisis, however, most of the endosperm is produced during the syncytial phase and the cellularized endosperm is degraded and absorbed by the embryo as the seed matures. Differentiation of these distinct cell types likely involves coordinated changes in gene expression regulated by transcription factors and their downstream targets [27]. Identification of these transcription factors important for endosperm development will help better understand female gametophyte development.
Few transcription factor genes have been reported in previous studies using expression-based approaches. A study performed previously in our laboratory identified a large number of transcription-factor genes in Arabidopsis using reverse-transcriptase PCR (qRT-PCR). From these experiments, 53 out of 69 Type I MADS-box genes were identified to be expressed in Arabidopsis during early seed development. Type I and Type II MADS-box genes encode transcription factors that control many developmental processes in plants including flower and fruit development [21]. MADS is an acronym that represents the first letter of each of the following genes: MINICHROMOSOME MAINTENANCE 1 (MCM1) in yeast, AGAMOUS (AG) in Arabidopsis, DEFINCIENS (DEFS) in Antirrhinum and serum-response factors in mammalian cells [17]. Observations from previous experiments indicate that the Type I MADS-box genes play a role in endosperm development. Examples include AGL80, a gene that regulates central cell development and the subsequent endosperm cellularization. In agl62 mutants, the nuclear endosperm has fewer nuclei, it undergoes premature cellularization, and it eventually causes collapse of the seed. In addition, expression of AGL37 (PHE1), another Type I gene, has been shown to be regulated by a repressive Polycomb-group (PcG) complex after fertilization.

The polycomb complex is formed of 5 proteins in seed development, FERTILIZATION INDEPENDENT SEED 2 (FIS2), FERTILIZATION-INDEPENDENT ENDOSPERM (FIE/FIS3), MEDEA (MEA/FIS1), MULTICOPY SUPRESSOR OF IRA (MSI1), and SWINGER (SWN) [21]. Mutations in the PcG genes cause premature initiation of endosperm development in response to fertilization and also prolong the syncytial phase of endosperm development and prevent proper endosperm cellularization. PcG complexes are known to be involved in the regulation of imprinted genes by mediating epigenetic modifications [18]. Imprinting has evolved independently in both mammals and flowering plants [12]. One potential
reason for such phenomena is the conflict over resource allocation from mother to offspring. The unequal epigenetic contributions of maternal and paternal genomes are considered necessary to achieve appropriate development [18].

Some Type I MADS-box genes are known to be imprinted, *PHERES 1* for example, is paternally imprinted and a direct target of the FIS PcG complex [12]. In the endosperm, certain genes are expressed differentially depending on their parent of origin and presumably caused by genomic imbalance. Genomic imprinting is controlled epigenetically through histone modifications and DNA methylation [18]. All imprinting genes that have been identified so far have been expressed in the endosperm of Arabidopsis, linking imprinting function with endosperm lineage during female gametogenesis. Of the 53 genes identified previously by the lab, 16 were shown to be under the control of the PcG complex based on their up-regulated expression in the PcG mutants. In this paper, I describe the imprinting patterns of 9 of the 16 AGL genes currently under investigation.
2. MATERIALS AND METHODS

2.1 Plant Material

Two Arabidopsis ecotypes Columbia-0 (Col-0) and Cape Verde Island (Cvi-0, CS22682) were used. Plants were grown under 16-hr light/8-hr dark photoperiod at 22°C. Reciprocal crosses between Col-0 and Cvi-0 were performed at one day after the emasculation of stage 12 flowers [20]. Seeds were collected at 3 DAP from the reciprocal crosses and self-pollinated Col-0 and Cvi-0 for RNA isolation.

2.2 Cleaved Amplified Polymorphic Sequences (CAPS)

Single Nucleotide Polymorphisms (SNPs) between Col-0 and Cvi-0 were identified using TAIR GBrowser (http://gbrowse.arabidopsis.org/cgi-bin/gbrowse/arabidopsis/). SNPs were confirmed by Sanger sequencing of DNA fragments amplified from Cvi-0 genomic DNA. Allele-specific expression based on SNPs was analyzed using the Cleaved Amplified Polymorphic Sequences (CAPS) method. CAPS primers were designed using the Blast2seq to align the two Arabidopsis ecotype sequences. BlastDigester was used to identify regions to be amplified, to select for the regions with the least number of restriction sites, and to use selected restriction enzymes to view Primer3 output for possible primer pairs.

<table>
<thead>
<tr>
<th>Gene</th>
<th>AGI</th>
<th>Amplicon size (bp)</th>
<th>Forward primer (5'-3')</th>
<th>Reverse Primer (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGL23</td>
<td>AT1G65360</td>
<td>504</td>
<td>AACCTTCAAGTCACATTTCACAGAGAGA</td>
<td>AACAGTTGCTGGAGCACAG</td>
</tr>
<tr>
<td>AGL28</td>
<td>AT1G01530</td>
<td>299</td>
<td>CGAAAAATGGCGGGAAGAAATC</td>
<td>GAGCATTTGAATACGGAGCCT</td>
</tr>
<tr>
<td>AGL35</td>
<td>AT5G26630</td>
<td>500</td>
<td>GCTGAAACCGGAGGTGGGC</td>
<td>AGATCAACGATGGCTACACTGT</td>
</tr>
<tr>
<td>AGL36</td>
<td>AT5G26650</td>
<td>776</td>
<td>AGAAGATGATGAGATCAAGAGACTTAC</td>
<td>GGATGTTAGTTGATGTTGATG</td>
</tr>
<tr>
<td>AGL37/PHEI</td>
<td>AT1G65330</td>
<td>684</td>
<td>CGCATGTGCGGTACCGTA</td>
<td>TCAGTGAGGCGGAGTTGAC</td>
</tr>
<tr>
<td>Gene</td>
<td>AGI</td>
<td>Amplicon size (bp)</td>
<td>Forward primer (5’-3’)</td>
<td>Reverse Primer (5’-3’)</td>
</tr>
<tr>
<td>----------</td>
<td>--------------</td>
<td>-------------------</td>
<td>------------------------------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>AGL23</td>
<td>AT1G65360</td>
<td>333</td>
<td>CATCCAAATTTGATGTTCTGCT</td>
<td>GAAGTTTGGGTTTGGTTGATGG</td>
</tr>
<tr>
<td>AGL28</td>
<td>AT1G01530</td>
<td>246</td>
<td>ATGACCAACGAAATCAACCTTCC</td>
<td>CATTGAATACGGAGCTTTGTG</td>
</tr>
<tr>
<td>AGL36</td>
<td>AT5G26650</td>
<td>450</td>
<td>TTCGAGACTCTCCTATTCGCT</td>
<td>TAGTTGACTGAGCTTTGTCGCC</td>
</tr>
<tr>
<td>AGL59</td>
<td>AT1G28460</td>
<td>304</td>
<td>GCAGATCGGTCACATTTCTCTAA</td>
<td>GCTGCCTCTTTGACTTTGACTT</td>
</tr>
<tr>
<td>AGL90</td>
<td>AT5G27960</td>
<td>306</td>
<td>GCTTCAAGATTTCTGAGATGC</td>
<td>AGGAGAGACGGAAGACAACAC</td>
</tr>
<tr>
<td>AGL96</td>
<td>AT5G06500</td>
<td>341</td>
<td>AGTCCAAAATGCTGTAATGTC</td>
<td>TGCTGCTCTCTCTTTATAGGTC</td>
</tr>
<tr>
<td>MEA</td>
<td>AT1G02580</td>
<td>459</td>
<td>GCAGAGGATGATAATGGGAGGAGG</td>
<td>CAGATCATCCAAAACCATTGATCC</td>
</tr>
<tr>
<td>AGL37/PHE1</td>
<td>AT1G65330</td>
<td>489</td>
<td>AGATGTTTGAGTCAAGAGACGGT</td>
<td>CCTCGTCTCTCCATCATTAAAG</td>
</tr>
<tr>
<td>αVPE</td>
<td>At2G25940</td>
<td>397</td>
<td>AGACTTTCAGACGACCTATGA</td>
<td>GAAGGCGCGACTTTATTTAACA</td>
</tr>
</tbody>
</table>

Table 1. CAPS primers for genes

2.2.1 Amplification of AGL genes from CAPS fragment.

Table 2. Gene specific primers

Amplification reactions were performed using a polymerase chain reaction (PCR) mixture containing ~5pg/µL of DNA amplified from cDNA using gene specific primers, 1µL of
each primer, 1.6µL of dNTPs, 1x *Ex Taq* buffer, and 0.5 U of *TaKaRa Ex Taq* DNA polymerase in a 20µL reaction volume. PCR conditions were the same for all genes. PCR was performed with a MJ Research PTC-200 Peltier Thermal Cycler and consisted of a 2 minute denaturing step at 94ºC followed by 16 cycles of 30 second at 94ºC, 45 seconds at 60ºC and 1 minute at 72ºC. Ending with 12 minutes of elongation at 72ºC and held until gel analysis at 4ºC. Fragments were detected using 1% agarose gel electrophoresis stained with ethidium bromide.

2.2.2 Reconditioning PCR.

A reconditioning polymerase chain reaction (Reconditioning PCR) mixture was used containing a 10x dilution of the product of the amplification of AGL gene from CAPS fragment, 1µL of each primer, 1.6µL of dNTPs, 1x *Ex Taq* buffer, and 0.5 U of *TaKaRa Ex Taq* DNA polymerase in a 20µL reaction volume. PCR was performed with a MJ Research PTC-200 Peltier Thermal Cycler and consisted of a 2 minute denaturing step at 94ºC followed by 3 cycles of 30 second at 94ºC, 45 seconds at 60ºC and 1 minute at 72ºC. Ending with 12 minutes of elongation at 72ºC and held until gel analysis at 4ºC. Reconditioned fragments were detected using 1% agarose gel electrophoresis stained with ethidium bromide.

2.2.3 Restriction Enzyme Digestion.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Enzyme</th>
<th>Temperature</th>
<th>Buffer</th>
<th>BSA</th>
<th>Heat Inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGL23</td>
<td>HaeIII</td>
<td>37ºC</td>
<td>4</td>
<td>N</td>
<td>80ºC for 20 minutes</td>
</tr>
<tr>
<td>AGL28</td>
<td>BstUI</td>
<td>60ºC</td>
<td>4</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>AGL36</td>
<td>ScrFI</td>
<td>37ºC</td>
<td>4</td>
<td>N</td>
<td>80ºC for 20 minutes</td>
</tr>
<tr>
<td>AGL37</td>
<td>AfiIII</td>
<td>37ºC</td>
<td>3</td>
<td>Y</td>
<td>80ºC for 20 minutes</td>
</tr>
<tr>
<td>AGL59</td>
<td>StuI</td>
<td>37ºC</td>
<td>4</td>
<td>N</td>
<td>65ºC for 20 minutes</td>
</tr>
<tr>
<td>AGL90</td>
<td>TfiI</td>
<td>65ºC</td>
<td>4</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>AGL96</td>
<td>SmlI</td>
<td>55ºC</td>
<td>4</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>MEA</td>
<td>XbaI</td>
<td>37ºC</td>
<td>4</td>
<td>Y</td>
<td>65ºC for 20 minutes</td>
</tr>
</tbody>
</table>
Table 3. Restriction enzyme.

| αVPE | Tfil | 65°C | 4  | N | N |

Enzyme digestion mixture contained 10µL of the reconditioning PCR product with 1µL of appropriate enzyme, 2µL of appropriate buffer, and 0.2µL of BSA if needed, all in a final volume of 20µL. Enzymes were selected using BLastDigester. All AGLs genes and the associated enzyme conditions are listed in Table 1. Digested fragments were detected using electrophoresis with a 3% MetaPhor Agarose gel, selected for fragment sizes of 100-600 bp, and stained with ethidium bromide.
Amplified Polymorphic Sequences (CAPS) mediated genotyping assay was used for identifying the imprinting patterns of the 16 AGL genes. Single nucleotide polymorphisms (SNP) between Arabidopsis ecotype Col-0 and Cvi-0 were identified for 15 of the 16 genes except for AGL58.

CAPS genotyping to identify imprinting pattern for AGL96, not match the direct sequencing results of RT-PCR products. In Figure 1 (data obtained from the lab), three SNP sites were sequenced from Cvi-0, Cvi-0 as the paternal in Col x Cvi, and Cvi-0 as the maternal in Cvi x Col. The SNP sites in the Cvi x Col sequence matched those of the Cvi-0 sequence, showing that AGL96 is maternally expressed.

Initial CAPS data in Figure 2 showed that both Col-0 and Cvi-0 bands are present in Col x Cvi as well as Cvi x Col, regardless of which ecotype is used as the maternal parent.
Figure 1. Direct sequencing of three SNP sites between Col-0 and Cvi-0 in AGL96 RT-PCR product.

Figure 2. Smll enzyme digestion of AGL96 DNA shows that both Cvi-0 and Col-0 are expressed in both reciprocal crosses.
We hypothesized that the source of the differences between the data obtained from the sequencing of RT-PCR reactions and the CAPS genotyping experiments was the formation of a heteroduplex during mix-template PCR. A combination of two methods were used to eliminate heteroduplex formation, one that used low cycle PCR amplification, the other used reconditioning PCR. In order to select the most optimal cycle number for the PCR, an artificial mixture of 1:1 Col-0 to Cvi-0 AGL96 fragments amplified from cDNA was used to determine which cycle number would give the most accurate 1:1 ratio of PCR products from each ecotype after enzyme digestion. Figure 3 shows the initial PCR of the artificial mixture using AGL96 primers, from 8 cycles up to 32 cycles, with a fragment size of 341 bp. Figure 4 shows the subsequent enzyme digestion of the products with SmlI starting from 16 cycles and up to 32 cycles. Because the template was an artificial 1:1 mixture, digestion should have resulted in an equal concentration of Col-0 and Cvi-0 bands, 341 and 313 bp, respectively. An equal concentration of the two bands was obtained using a16-cycle PCR.

![Figure 3. Varying cycle number PCR product of artificial 1:1 Col-0 to Cvi-0 mixture](image)

![Figure 4. Enzyme digestion of PCR products with SmlI](image)
Figure 4. SmlI enzyme digestion of product from varying cycle number PCR from Figure 3. After digestion Col-0 is 341 bp and Cvi-0 is 313 bp.

The data indicated that the PCR with 16 cycle numbers produced the most accurate 1:1 ratio after enzyme digestion of the artificial 1:1 cDNA mixture (Figure 4). A reconditioning PCR was performed with a 10-fold dilution of the initial PCR product to ensure that the 16 cycle PCR indeed provided optimal results after enzyme digestion using the AGL96 DNA as compared to a 32-cycle PCR. The initial PCR was run for 16 and 32 cycles separately. These PCR products were then reconditioned in a 3-cycle PCR reaction. Figure 5 shows that the products of the reconditioned PCR had a lower concentration than the products of the initial PCR, and produced a higher concentration of DNA in the 32 cycle PCR than the 16 cycle PCR in both the initial PCR and the reconditioned PCR.

![Initial PCR (cycle #) vs Reconditioned 3x](image)

**Figure 5.** 16 vs 32 cycle PCR product alongside reconditioned PCR product

After the reconditioned product of AGL96 was digested by the enzyme SmlI, gel analysis showed that the 16-cycle reconditioned PCR indeed supported maternal expression for AGL96 which matched the results obtained from direct sequencing of RT-PCR reactions. On the other hand, the 32-cycle reconditioned PCR digestion showed an abnormal biallelic expression pattern especially for the Cvi x Col cross (Figure 6).
Figure 6. Comparison of 16 cycle and 32 cycle reconditioned PCR product with SmlI enzyme digestion of *AGL96*. For 16 cycle Col x Cvi only the maternal Col-0 band at 341 bp is present. For 16 cycle Cvi x Col only the maternal Cvi-0 band at 313 bp is present. For 32 cycle Col x Cvi both Col-0 and Cvi-0 are present, though Col-0 is higher in concentration. For 32 cycle Cvi x Col both Col-0 and Cvi-0 bands are present.

This modified CAPS primer genotyping technique of 16 cycle initial PCR followed by a recondition PCR was adopted for the rest of the 16 AGL genes. Figure 7 shows the gel results of 9 these genes, *AGL23, AGL28, AGL36, AGL37, AGL59, AGL90, AGL96, MEA, PHE1*, and *αVPE1*. Table 4 follows the gel image with information on the expected band sizes of each allele in each gene.
Figure 7. CAPS primer genotyping of AGLs.

<table>
<thead>
<tr>
<th>AGL</th>
<th>Col-0</th>
<th>Cvi-0</th>
<th>Imprinting Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>333</td>
<td>167 + 166</td>
<td>Paternally Expressed</td>
</tr>
<tr>
<td>28</td>
<td>91+155</td>
<td>246</td>
<td>Maternally Expressed</td>
</tr>
<tr>
<td>36</td>
<td>194 + 256</td>
<td>450</td>
<td>Maternally Expressed</td>
</tr>
<tr>
<td>37</td>
<td>132 + 357</td>
<td>489</td>
<td>Paternally Expressed</td>
</tr>
<tr>
<td>59</td>
<td>304</td>
<td>132 + 172</td>
<td>Biallelically Expressed</td>
</tr>
<tr>
<td>90</td>
<td>225+81</td>
<td>306</td>
<td>Maternally Expressed</td>
</tr>
<tr>
<td>96</td>
<td>341</td>
<td>313 + 28</td>
<td>Maternally Expressed</td>
</tr>
<tr>
<td>Mea</td>
<td>459</td>
<td>138+321</td>
<td></td>
</tr>
<tr>
<td>αVPE</td>
<td>344+53</td>
<td>397</td>
<td>Biallelically Expressed</td>
</tr>
</tbody>
</table>

Table 4. Imprinting patterns of 9 AGLs
Gel electrophoresis analysis indicated that for AGL23, when Cvi-0 is paternal, there is a stronger level of Cvi-0 mRNA as indicated by the high levels of the band at 167 and 166 bp, whereas and when Cvi-0 is maternal, there is a higher level of the Col-0 mRNA as indicated by the high intensity of the 333 bp bands. For AGL28, when Col-0 is maternal, there is clearer expression for the Col-0 band at 155 bp, and when Cvi-0 is maternal, there is clearer expression for the Cvi-0 band at 246 bp. For AGL36 when Col-0 is maternal, the expected bands for Col-0 at 194 and 256 bp are present and when Cvi-0 is maternal, only the Cvi-0 band at 450 bp is present. For AGL37/PHE1 when Cvi-0 is paternal, there is stronger expression of the Cvi-0 band at 489 bp, and when Col-0 is paternal, there is stronger expression of the Col-0 band at 132 and 357 bp. For AGL90, For AGL96, when Col-0 is maternal the Col-0 band at 341 bp is present, and when Cvi-0 is maternal, the Cvi-0 band at 313 bp is clearly present. For MEA, the positive control for maternal expression and paternal imprinting, the gel shows both Col-0 and Cvi-0 bands, a result that is further discussed below.

Our data indicated that AGL23, AGL28, AGL36, AGL90, and AGL96 are maternally expressed and paternally imprinted. AGL37 (PHE1) is paternally expressed and maternally imprinted, a result in agreement with previous findings [12]. AGL59 is biallelically expressed. MEA was used as a paternal imprinted control that will be discussed in below. And αVPE was presented as the biallelic control, not shown in Figure 7.

3 bio-reps were performed on each of the 9 AGLs and presented the same results, not presented in this paper.
4. DISCUSSION

A variation of the Cleaved Amplified Polymorphic Sequences (CAPS) technique was used in order to identify the imprinting pattern of 16 AGL genes. CAPS allows for sequence alignment for the two genotypes, Col-0 and Cvi-0, containing SNPs that are subjected for the identification of restriction sites for restriction enzyme digestion [9]. The variation to the technique is the addition of a gene specific PCR amplification using the products of the CAPS PCR. This additional step is necessary because some genes in this family have high similarity in sequences and is difficult to design sequence specific CAPS primers. After this gene-specific amplification, a restriction enzyme digestion specifically designed to cut certain SNPs on either Col-0 or Cvi-0 is performed in order to determine allelic expression. The technique was initially only performed on AGL96, and the preliminary results did not validate results from previous direct sequencing data.
The difference between the PCR results and the sequencing data could be due to the possibility of heteroduplex formation. Heteroduplexes are cross-hybridizations of heterologous sequences that form at higher cycle numbers of mix template PCR, where decreasing primer to template ratio no longer favor primer annealing [23]. Heteroduplex formation presents a false ratio of each digested product (an example is displayed in Figure 8). A 1:1 ratio results in the digestion of a homoduplex mixture whereas a 3:1 ratio results in digestion of a heteroduplex mixture. Heteroduplex formation can be detected after the 25th cycle of a reaction, and the fraction of heteroduplex molecules increase with the increasing number of PCR cycles thereafter, with the most rapid increase between cycles 26-31 [16]. The reconditioning PCR method is a low cycle number re-amplification of a 10-fold diluted mixed template PCR product [23]. We started with a 16 low cycle number PCR and combined it with the reconditioning PCR in order to minimize initial heteroduplex formation and to re-establish the proper primer to template ratio respectively. When AGL96 was digested using this method, the results validated the previous direct sequencing data as well as a series of parallel experiments using single base extension with fluorescently labeled ddNTPs (SBE) to look at allelic expression of the 16 AGLs. After the technique was perfected, it was applied to all the AGLs (currently 9 out of the 16).

The data for 8 of the 9 AGL genes using the reconditioning CAPS PCR approach support those of the approach using direct sequencing of the RT-PCR products as well as the SBE experiments. However, the results obtained for MEDEA (MEA) did not match those previously reported in the literature. MEA encodes one of the five Polycomb complex proteins, and only the
maternal wild-type MEA allele is required for proper embryo and endosperm development [11]. MEA, based on our technique and gel imaging, seems to be biallelically expressed instead of maternally expressed and paternally imprinted in the endosperm. This difference in imprinting pattern observed in our hands as compared to the previous findings could be due to the distant phylogeny of Col-0 and Cvi-0 ecotypes of Arabidopsis. It is possible that the epigenetic silencing and imprinting mechanism of MEA by PcG-dependent H3K27me is compromised in a Col-0 and Cvi-0 cross. Further studies using additional pairs of Arabidopsis ecotypes may help to resolve this issue.
5. CONCLUSION

The variation on the CAPS genotyping technique by combining low cycle number and reconditioning PCR was effective in eliminating heteroduplex formation in our mix-template PCR. My data indicates that $AGL_{28}$, $AGL_{36}$, $AGL_{90}$, and $AGL_{96}$ are maternally expressed and paternally imprinted. $AGL_{23}$ and $AGL_{37}$ ($PHEI$) are paternally expressed and maternally imprinted and $AGL_{59}$ is biallelically expressed. Future work will focus on determining the imprinting patterns of $MEA$ using additional ecotypes of Arabidopsis. Further work will also focus on a complete analysis of the 16 AGL genes that may have important roles in endosperm development and understanding the mechanism of imprinting in these genes.
6. REFERENCES


