BARD Final Scientific Report

BARD Project Number: US-3746-05 R

Date of Submission of Report: September, 1, 2009

ProjectTitle: Structural and Functional Genomic Approaches for Marking and Identifying Genes that Control Chilling Requirement in Apricot and Peach Trees

Investigators:

Albert G. Abbott

Doron Holland

ARO

Affiliated Institutions:

Clemson University

Newe Ya'ar Research Center,

Collaborating Investigators

Douglas Bielenberg

Gregory Reighard

Clemson University

Clemson University

Start Date of Project: September 1, 2005 with one year extension.

Key words: endodormancy, QTL, candidate genes, peach and apricot genomics

LIST OF ABBREVIATIONS

CR, chilling requirement
CH, chilling hours
BD, bloom date
EST, expressed sequence tagged
QTL, quantitative trait loci
BAC, bacterial artificial chromosome
AFLP, amplified fragment length polymorphism
SSR, simple sequence repeat
LG1, LG2, etc., Linkage Group 1, Linkage Group 2 etc.
cM, centiMorgan
MAS, marker assisted selection
SNP, single nucleotide polymorphism
JGI, Joint Genome Institute
PCR, Polymerase Chain Reaction
3'UTR, three prime untranslated region
GDR, Genome Database for Rosaceae, www.rosaceae.org

Budget: IS:\$155,000

US:\$155,000

Signature Principal Investigator

Signing for

Signature authorizing Official
Principal Institution

**Christian E. G. Przirembel
Vice President for Research
and Economic Development**

Abstract: Structural and functional genomic approaches for marking and identifying genes that control chilling requirement in apricot and peach trees.

Specific aims: 1) Identify and characterize the genetic nature of chilling requirement for flowering and dormancy break of vegetative shoots in *Prunus* germplasm through the utilization of existing apricot (Newe Ya'ar Research Center, ARO) and peach (Clemson University) genetic mapping populations; 2) Use molecular genetic mapping techniques to identify markers flanking genomic regions controlling chilling; 3) Comparatively map the regions controlling chilling requirement in apricot and peach and locate important genomic regions influencing chilling requirement on the *Prunus* functional genomic database as an initial step for identification of candidate genes; 4) Develop from the functional genomics database a set of markers facilitating the development of cultivars with optimized chilling requirements for improved and sustained fruit production in warm-winter environments.

Dormant apricot (*Prunus armeniaca* L.) and peach [*Prunus persica* (L.) Batsch] trees require sustained exposure to low, near freezing, temperatures before vigorous floral and vegetative bud break is possible after the resumption of warm temperatures in the spring. The duration of chilling required (the chilling requirement, CR) is determined by the climatic adaptation of the particular cultivar, thus limiting its geographic distribution. This limitation is particularly evident when attempting to introduce superior cultivars to regions with very warm winter temperatures, such as Israel and the coastal southern United States. The physiological mechanism of CR is not understood and although breeding programs deliberately manipulate CR in apricot and peach crosses, robust closely associated markers to the trait are currently not available.

We used segregating populations of apricot (100 F1 individuals, Newe Ya'ar Research Center, ARO) and peach (378 F2 individuals, Clemson University) to discover several discrete genomic loci that regulate CR and blooming date. We used the extensive genomic/genetic resources available for *Prunus* to successfully combine our apricot and peach genetic data and identify five QTL with strong effects that are conserved between species as well as several QTL that are unique to each species. We have identified markers in the key major QTL regions for testing in breeding programs which we are carrying out currently; we have identified an initial set of candidate genes using the peach physical/transcriptome map and whole peach genome sequences and we are testing these currently to identify key target genes for manipulation in breeding programs.

Our collaborative work to date has demonstrated the following: 1) CR in peach and apricot is predominantly controlled by a limited number of QTL loci, seven detected in a peach F2 derived map comprising 65% of the character and 12 in an apricot F1 map comprising 71.6% and 55.6% of the trait in the Perfection and A.1740 parental maps, respectively and that peach and apricot appear in our initial maps to share five genomic intervals containing potentially common QTL. 2) Application of common anchor markers of the *Prunus*/peach, physical/genetic map resources has allowed us not only to identify the shared intervals but also to have immediately available some putative candidate gene information from these intervals, the EVG region on LG1 in peach the TALY 1 region in apricot on LG2 in peach; and several others involved in vernalization pathways (LG1 and LG7). 3) Mapped BAC contigs are easily defined from the complete physical map resources in peach through the common SSR markers that anchor our CR maps in the two species, 4) Sequences of BACs in these regions can be easily mined for additional polymorphic markers to use in MAS applications.

Achievements:

Results of our studies have provided key insights into the fundamental control of endodormancy, a very important character in perennial tree species agriculture, and potentially will provide robust markers for selection of this character in breeding programs utilizing modern translational breeding strategies. To date, our plan to collaboratively map CR QTL in two different species of Rosaceae, (apricot in Israel, peach in the USA) has proven critical in: 1) the definition of a limited set of common genomic regions controlling this important character in these two different fruiting species; 2) identifying potential candidate genes in the major QTL loci of both species; 3) providing markers and sequences of genes for testing in marker assisted selection schemes to predictably breed for chilling requirement in new cultivar development. It is anticipated that our results will likely translate to other important perennial species in Rosaceae and to perennial species in other plant families as well since many of the putative candidate genes are known to respond in vernalization, dormancy, vegetative-floral meristem transitions, and organ development.

Brief overview of the key results from our initial collaborative project:

Our collaborative work to date has demonstrated the following: 1) CR in peach and apricot is predominantly controlled by a limited number of QTL loci, seven detected in a peach F2 derived map comprising 65% of the character and 12 in an apricot F1 map comprising 71.6% and 55.6% of the trait in the Perfection and A.1740 parental maps, respectively and that peach and apricot appear in our initial maps to share five genomic intervals containing potentially common QTL. 2) Application of common anchor markers of the *Prunus*/peach, physical/genetic map resources has allowed us not only to identify the shared intervals but also to have immediately available some putative candidate gene information from these intervals, the EVG region on LG1 in peach the TALY 1 region in apricot on LG2 in peach; and several others involved in vernalization pathways (LG1 and LG7). 3) Mapped BAC contigs are easily defined from the complete physical map resources in peach through the common SSR markers that anchor our CR maps in the two species, 4) Sequences of BACs in these regions can be easily mined for additional polymorphic markers to use in MAS applications.

Collaborative: Through our joint collaboration, we are now positioned to define these CR QTL intervals in high resolution using markers available in the QTL

containing BAC contigs, additional segregating crosses, and association mapping approaches using the available diverse germplasm accessions of both peach and apricot present at each partner institution. Additionally, we have combined both genetic map data and functional genomics data to mine these QTL intervals for genes controlling CR and are now characterizing candidate genes. This degree of progress on this relatively poorly understood character was only possible through the combined collaborative efforts of the participant researchers and their institutions. Unique expertise at Clemson University in mapping and marker development (A. Abbott's lab), plant physiological studies (D. Bielenberg's lab) was coupled with expertise at the Newe Ya'ar Research Center, ARO in gene action studies and basic plant molecular biology (D. Holland's lab) to map and identify genes controlling the extremely important perennial fruit tree character. As the D. Holland's group was focused on apricot, and A. Abbott's group on peach, the cross communication of markers and expertise in each species enabled the rapid accumulation of mapping data for both partners but more importantly the integration of both maps to identify common QTL was dependent on cooperative exchanges of materials and labor efforts among all partners. Thus, this collaboration has been extremely successful and is continuing actively for the further study of the important CR candidate genes that were identified in this initial project.

Select references from collaboration:

Olukolu B A, Trainen T, Fan S, Kole C, Bielenberg D, Reighard G, Abbott A, and D Holland. 2009. Genetic linkage mapping for molecular dissection of CR and dormancy bud break in apricot (*Prunus armeniaca* L.) Genome In press.

Shenghua Fan¹, Douglas G. Bielenberg², Tetyana N. Zhebentyayeva¹, Gregory L. Reighard², William R. Okie³, Doron Holland⁴, Albert G. Abbott¹, 2009. Mapping quantitative trait loci associated with chilling requirement, heat requirement and bloom date in peach. Reviewed for publication in New Phytologist, currently in revision for possible publication in this journal.

Appendixes:

Publication Summary:

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1 in press			1
Submitted, in review, in preparation	1 in review 1 in preparation			2
Invited review papers			1	
Book chapters		1		1
Books				
Master theses	1			
Ph.D. theses	2			2
Abstracts	6			6
Not refereed (proceedings, reports, etc.)				

Cooperation summaries (number):

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		2	2	4
Longer Visits (Sabbaticals)				

Postdoctoral Training: None were supported.

Description cooperation:

The laboratories of A. Abbott and D. Holland are continuing to cooperatively map chilling requirement genes in segregating populations of peach and apricot, respectively, as a critical step in the identification and characterization of the genes controlling these traits in these closely related species. In this project, marked gene loci associated with the traits are comparatively mapped in the peach and apricot to determine the number of shared loci and those unique to each species. Markers were developed in each population for marker assisted selection of this character and used to commence genomic approaches to identify the corresponding candidate genes in each species. The apricot breeding material at Newe Ya'ar and the peach material at Clemson University are central to this effort. The comparative genomic approaches in this cooperation and the genomic resources of the peach and apricot genome databases housed at Clemson and publicly available at Genome Database for Rosaceae (www.genome.clemson.edu/gdr) have contributed significantly to this work.

Patent Summary:

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted		1		
Issued (allowed)				
Licensed				

Additional Unpublished Data:

Specific aims 1 and 2: 1) Identify and characterize the genetic nature of chilling requirement for flowering and dormancy break of vegetative shoots in *Prunus* germplasm through the utilization of existing apricot (Newe Ya'ar Research Center, ARO) and peach (Clemson University) genetic mapping populations; 2) Use molecular genetic mapping techniques to identify markers flanking genomic regions controlling chilling.

1. Establishment of apricot Perfection x A.1740 population in pots and measuring chilling requirements for the population in forcing experiments. Volcani Institute/D. Holland

We established a copy of the field population of Perfection x A.1740 in potted plants. For each individual (total of 94 + parents) we established 10 replicas in winter 2005-2006 (Overall about 1000 grafted plants). The plants were exposed to 200-900 chilling hours. Controls included a) forcing conditions without chilling and b) Exposing the potted plants to natural field conditions without forcing. The following parameters for chilling requirements were measured: 1st leaf, 1st flower, number of sprouting vegetative buds, and percentage of leaf coverage.

Our data indicate that there is a very high variation in chilling requirements among the progenies. The distribution of CR phenotype (for summary see Figure 1) suggests that the trait is probably controlled by several genes. The data obtained was used for further statistical analysis.

2. Isolation of a MADS box gene from apricot, expression study and allele segregation analysis within the population. We isolated an *agl*-like gene homolog (ApMADS) from apricot and analyzed its cDNA and genomic sequences, as well as, its expression in various apricot cultivars. Our data (Northern blot analysis and RT-PCR analysis) indicates that the gene is expressed in leaves and other vegetative parts of the apricot tree but is not expressed in developing fruit. In addition, the gene is expressed in many plants of the Rosaceae (Fig.2).

3. Linking the agl-like gene with chilling requirements. We found an SSR that was located within the sequence of the gene that could distinguish between the parental

alleles of the *agl*-like gene. We found that both parents were heterozygous for the *agl*-like gene (Figure 3). By comparing the SSRs from genomic DNA and from cDNA we found that both alleles of ApMADS were expressed in leaves and that the two parents have different alleles (Fig. 3). Using the SSR described above, we were able to screen a self-crossed population recently established from "Tarog" cultivar, a new Israeli apricot cultivar that has very low chilling requirements (about 200 CR, Fig. 3). Our data indicates that certain combinations of the *agl*-like alleles display a significant difference with respect to their contribution to chilling requirements (Fig.4). We conclude that the gene we isolated is within a locus influencing chilling requirements in apricot.

Figure 1:

Figure 2: RT-PCR expression analysis of a MADS *agl*-like type gene isolated from apricot in various Rosaceae members: 1. Water; 2. 'Oded' peach cultivar; 3. 'Roayl Zee' Japanese plum; 4. 'Sunmist' nectarine cultivar; 5. 'Tarog' apricot cultivar; 6. 'Um El-Fahem' almond; 7.

Figure 3:

Figure 4: One way Anova analysis, Summary of Fit. Allele composition versus the number of days until the appearance of 1st leaf in forcing (DIF) when plants were exposed to 600 chilling hours. A1P1 allele combination is significantly different from the A2P2 combination using Turkey-Kramer HSD test or the student's t test. P=0.05

4. Construction of a High-density Linkage Map and Detection of QTLs Controlling Chilling Requirement in Apricot (*Prunus armeniaca* L.). Volcani/D. Holland and Clemson University/A. Abbott

Apricot cultivars selected for low and high CR (A.1740 and Perfection, respectively) were exploited for the genetic dissection of the CR trait using a two-way pseudo-testcross mapping strategy based on SSR and AFLP markers. Two high-density parental maps were developed using 655 and 592 markers, spanning 550.6 cM and 454.9 cM with average marker intervals of 0.84 cM and 0.77 cM, respectively. Marker colinearity between both parental maps was validated with 177 bridge-markers. To further validate marker order, synteny and marker intervals, a comparative study was performed among the two parental maps, the

Prunus reference map (Joobeur *et al.* 1998, Jáuregui *et al.* 2001, Bliss *et al.* 2002, Aranzana *et al.* 2003) and two other apricot maps (Vilanova *et al.* 2003, Messina *et al.* 2004, Dondini *et al.* 2007) based on common SSR markers. All eight homologous linkage groups between our apricot maps and other published apricot maps show perfect co-linearity. Comparison of all apricot maps against the *Prunus* reference map was also predominantly co-linear except for minor inversions within small genetic distances.

Composite interval mapping generated seven QTLs explaining 21.5%, 15.4%, 16.5%, 20.2%, 19.3%, 14.5% and 19.8% of the phenotypic variation (total of 71.6% and 55.6% in the Perfection and A.1740 parental maps, respectively) on linkage groups 1, 2, 3, 5, 6, 7 and 8, respectively. Following a simultaneous multiple regression fit (to eliminate possible false positives and false negatives) and a general linear model for analysis of variance, a total of 12 putative CR QTLs and their interactions were established on six linkage groups. QTL main effects of additive and additive-by-additive interactions accounted for 58.5 ± 6.7 % and 66.1 ± 5.8 % of the total phenotypic variance in the Perfection and A.1740 maps, respectively.

Two of the QTLs detected were common to both maps on linkage group 1 and 8, while QTLs specific to each map were found on LG 2, 6 and 7 for the Perfection map and LG 1, 2, 5 and 7 on the A1740 map. QTLs detected on LG 1, 5, 6, 7 and 8 were also validated to a similar map position in the CR study in peach. An interesting comparison of common QTLs between the apricot vegetative bud-break and peach floral bud-break studies relates to the difference in the size of the contribution of the major QTL on LG 1 (21% in apricot and 40-45% in peach) suggesting that a gene common to both pathways is present at this location, however contribution in the pathways differs.

We also report two apricot high-density maps and QTLs (Olukolu *et al.* 2009) corresponding to map positions of differentially expressed transcripts and suggested candidate genes controlling CR. Some of the candidate genes have been sequenced and do show mutations (indels and point mutations) within their promoter region, introns, intron-exon junctions as well as synonymous and non-synonymous mutations within the exons. Expression of their transcripts is currently being examined from the two parents over the duration of dormancy initiation, maintenance and release.

Table 1: QTLs for chilling requirement in apricot.

Map	LG	Position	SI ^a	LOD	Additive effect	SSR Markers within or close to SI
Perfection	1	82	80-84	61.29	117.19	BPPCT28-76.1
	2	15	13-17	63.56	72.42	BPPCT04-23.9
	6	29	27-31	64.61	602.62	BPPCT25-43.2
	6	66	64-68	50.80	165.93	UDP98-412-58.8
	7	8	6-10	46.15	-205.37	-
	8	44	42-46	56.44	-221.19	EPDCU3454-42.2
A.1740	1	69	67-71	14.57	400.50	EPDCU2862-64
	1	86	81-89	12.04	265.75	-
	2	4	2-6	11.78	-301.04	-
	2	36	34-38	17.29	247.04	Taly-36.7
	5	1	0-3	12.86	-595.14	BPPCT7-0.0
	5	27	25-29	15.54	112.82	EPDCU3083-21.2
	7	26	26-34	11.63	180.82	Pchgms044b-30.5, CPSC042-33.1
	8	35	33-38	10.52	75.63	-

LG: linkage group. Position: location of the maximum LOD score of the QTL on LG in centiMorgans. Only QTLs above empirical threshold of 9.44 and 8.46, respectively, are listed. LOD threshold computed by 1,000 permutations. ^a2-LOD support interval in the fit. The + and - additive effect signs indicate that the allele which increases the trait values is in the Perfection and A.1740 parent, respectively.

5. Mapping of chilling requirement QTL in Peach: a. Construction of the peach CR linkage

map: Using an F₂ population issued from an F₁ self-cross derived from a cross of the high chill cv. Contender (1050 chill hours) and the low chill genotype Fla.92-2C (300 chill hours) a linkage map consisting eight linkage groups with 127 markers, including 96 SSRs (of which 6 are dominant), 30 AFLPs (of which 4 are codominant) and one phenotypic marker, was constructed. A total map distance of 535cM was covered, corresponding to an average interval of 4.2 cM between adjacent markers. Marker orders in each linkage group are generally in agreement with that in *Prunus* reference map (Dirlewanger, *et al.*, 2004) and “bin map” (Howad, *et al.*, 2005). There is one gap of 24-29cM in each of linkage groups of LG2, LG4 and LG5. LG1 covers the longest genetic distance of 96.4cM, while LG3 covers the shortest genetic distance of 51.7cM.

The majority of loci (77.8%) showed genotypic ratios for a segregating F₂ population of 1:2:1. Among the 28 markers with significantly skewed ratios ($P < 0.01$), 17 mapped to LG1 between 68 and 96.4 cM with an overrepresentation of low chilling parent alleles.

b. Mapping QTLs for Chilling Requirement (CR) and Bloom Date (BD): Chilling accumulation calculated by different models (<7.2 °C, 0-7.2 °C, LC, Utah, and dynamic models) were used for QTL analysis yielding similar results, only LOD score and explained variance differed slightly. A major QTL for CR on the end of LG1 accounted for 40-44.8% of phenotypic

variance. Another QTL on LG7 also accounted for 17.8-24.9% of phenotypic variance. The other 6 QTL for CR on LG4, LG5, LG6 and LG8 accounted for 4.1-9.7% of phenotypic variance.

Table 2: Quantitative trait loci (QTLs) detected for chilling requirement (CR, <7.2°C model) with peach F₂ mapping population

Year	QTL	LG/Pos	CI	Co-factor	LOD	Part R ² %	Add	R ² %
2007/2008	qCR1a-2008	G1/87	86-88	Pchgms29	44.52	44.8	-0.83	55.7
	qCR4a-2008	G4/7	4-19	ssrPaCITA6	9.77	9.7	-0.32	
	qCR4b-2008	G4/54	41-62	AMPA103	2.95	4.1	-0.20	
	qCR5-2008	G5/32	24-38	ssrPaCITA21	3.79	4.5	-0.20	
	qCR6-2008	G6/41	35-43	EPPISF002	3.29	4.2	0.19	
	qCR7-2008	G7/48	43-59	UDAp-409A	16.95	17.8	-0.46	
	qCR8-2008	G8/51	36-54	PacC13	3.61	4.5	-0.20	
2008/2009	qCR1d-2009	G1/6	0-13	UDA-053	6.71	7.7	-0.29	54.0
	qCR1a-2009	G1/87	86-88	pchgms40	17.97	40.0	-0.77	
	qCR4b-2009	G4/46	40-55	M12a	2.97	6.0	-0.26	
	qCR5-2009	G5/33	24-38	ssrPaCITA21	3.91	4.5	-0.20	
	qCR7-2009	G7/47	43-51	CPPCT033	25.47	24.9	-0.58	

LG/Pos, linkage group/QTL position in linkage group in cM; CI, 2-LOD or approximately 99% confidence interval; Part R²%, percentage of phenotypic variance explained by one QTL when other QTLs effects are fixed; Add, additive QTL effect divided by standard deviation of the trait value, male parent is assumed to carry the favorable QTL allele; R²%, percentage of the phenotypic variance explained by all QTLs with the adjustment for the number of QTL terms in the full regression model.

The numbers of detected QTLs for Blooming date (BD) were different in different years, suggesting a stronger effect of the environment on this character. However, 3 QTLs on LG1, LG4 and LG7 have fairly consistent or overlapping confidence interval in different years, although their peak position varied. Of these 3, 2 QTLs on LG1 and LG7 each accounted for about 40% of phenotypic variance (averagely in year 2006-2009). Other QTLs for BD were distributed onto LG1, LG2, LG4, LG5 and LG6, which accounted for 3.5-12.8% of phenotypic variance. Interestingly, all QTLs for CR (except two minor ones) co-localize or partially overlap with BD QTLs, suggesting that CR and BD may share part of their controlling mechanism.

6. *Comparative genomics of CR in peach and apricot*: Clemson /Volcani Institute. One advantage of the design of our collaborative project is that both maps (apricot and peach) used common SSR anchor markers from the general *Prunus* map (Aranzana *et al.*, 2002). The *Prunus* map is anchored on the peach physical map (Zhebentyayeva *et al.*, 2008) thus, we gain two critical pieces of information in our study 1) we can quickly determine the presence of common CR QTL between our maps and 2) once QTL are located, we can cross reference these regions to the peach physical map for high resolution QTL analysis and candidate gene searches.

Comparison of our mapping results for chilling in peach and apricot demonstrates the importance of this common marker approach. The current low resolution data display potentially significant conservation of CR QTL positions on the two species maps (Tables 1 and 2) underscoring the significance of these QTL positions and enabling us to focus attention on these regions to search for candidate genes and for the development of robust markers for MAS.

Candidate gene studies: The major QTL on LG1 maps to a region corresponding to the location of the Evergrowing locus (*EVG*) in peach comprised of MADs-box transcription factors (DAM genes 1-6, Bielenberg *et al.*, 2008). The location of this QTL region provides us with potential gene candidates that have already been extensively characterized in peach (Bielenberg *et al.*, 2008). Also, an SSR marker (TALY1) designed from apricot genes homologous to the AGAMOUS-like 20 (AGL20) in *Arabidopsis* was shown to co-segregate with the CR trait. The SOC1/AGL20 (SUPPRESSOR OF OVEREXPRESSION OF CO) is involved in the mechanisms required to promote flowering including those influenced by vernalization, photoperiod and autonomous pathway (Lee *et al.*, 2000, Samach *et al.*, 2000). We are currently investigating some extremely relevant potential gene candidates identified in sequences from our QTLs on LG1 and LG7 both major loci contributing to the chilling trait (see Table 3).

Table 3. List predicted candidate genes in the peach genomic regions underlying major QTLs for chilling requirement and blooming date on LG1 and LG7 (genes involved in chromatin remodeling and in cell division highlighted in green and yellow color respectively).

Prunus Linkage	Closest homologs in	Name and brief description	Number developed SSRs for association study

Groups	<i>Arabidopsis</i>		
LG1	AT4G24540 (cassette of six DAM genes in EVG region)	AGL24 (AGAMOUS-LIKE 24) INVOLVED IN: maintenance of floral meristem identity, regulation of timing of transition from vegetative to reproductive phase; in peach DAM5 and DAM6 associated with response to cold.	8
	AT4G24560	UBP16 (UBIQUITIN-SPECIFIC PROTEASE 16) INVOLVED IN: flower development, cell proliferation, shoot development, leaf development, root development There is evidence on the role for UB16 in plant development and cell proliferation	
	AT4G30200*	VEL1, VERNALIZATIONS/VIN3-LIKE a part of a polycomb repressive complex (PRC2). INVOLVED IN: epigenetic silencing of the FLC flowering locus	9
	AT5G10940	transducin family protein / WD-40 repeat family protein INVOLVED IN: CUL4 RING ubiquitin ligase complex	
	AT5G49980	AFB5 (Auxin F-Box protein 5); ubiquitin-protein ligase CONTAINS InterPro DOMAIN/s: Cyclin-like F-box	
	AT5G60930	chromosome-associated kinesin LOCATED IN: microtubule associated complex; INVOLVED IN: microtubule-based movement	2
	AT5G33300	chromosome-associated kinesin-related	2
	AT2G22475 (truncated)	GEM (GL2-EXPRESSION MODULATOR) INVOLVED IN: in the maintenance of the repressor histone H3K9 methylation status	3
	AT2G22490	CYCD2;1 (Cyclin D2;1); cyclin-dependent protein kinase regulator INVOLVED IN: regulation of cell cycle	3
LG7	AT3G56150	EIF3C (EUKARYOTIC TRANSLATION INITIATION FACTOR 3C) eIF3 binds to the 40 S ribosome in an early step of translation initiation and promotes the binding of methionyl-tRNAi and mRNA	
	AT2G18290	ANAPHASE PROMOTING COMPLEX, APC10; INVOLVED IN: regulation of mitotic metaphase/anaphase transition	5
	AT2G18350	AtHB24 (ARABIDOPSIS THALIANA HOMEODOMAIN PROTEIN 24) <i>Arabidopsis</i> zinc finger-homeodomain genes encode proteins with unique biochemical properties that are coordinately expressed during floral development	

AT2G23380	CLF (CURLY LEAF); INCURVATA 1 INVOLVED IN: genetic imprinting, DNA mediated transformation, histone methylation, vernalization response, leaf morphogenesis	3
AT4G36650	ATPBRP; PLANT-SPECIFIC TFIIIB-RELATED PROTEIN; RNA polymerase II transcription factor/ rDNA binding INVOLVED IN: translational initiation, regulation of transcription	3
AT5G48330	regulator of chromosome condensation (RCC1) family protein CD: ATS1; Alpha-tubulin suppressor and related RCC1 domain-containing proteins [Cell division and chromosome partitioning]	3
AT5G66750	DDM1, DECREASED DNA METHYLATION 1 SWI2/SNF2 chromatin remodeling ATPase/ helicase; INVOLVED IN: methylation-dependent chromatin silencing	3
AT5G66770	scarecrow transcription factor family protein REPORTED: auxin-responsive SCARECROW-like transcriptional activators can interact with histone deacetylase	
AT5G66870	ASL1, ASYMMETRIC LEAVES2-LIKE 1; LOB domain protein whose overexpression results in KNOX gene repression.	
AT5G66880	SNRK2.3 (SUCROSE NONFERMENTING 1(SNF1)-RELATED PROTEIN KINASE 2.3) One of key protein kinases that mediate a major part of abscisic acid signaling	
AT5G67100	ICU2 (INCURVATA2); DNA-directed DNA polymerase Interacts with genes involved in chromatin-mediated cellular memory, particularly with the Polycomb group (PcG) gene CURLY LEAF	2

*Genes coded in the same color denote components in similar processes. Green= genes in pathways of chromatin remodeling for regulation of specific gene expression, some have been shown to work in the process of vernalization in *Arabidopsis* winter annuals. Yellow= genes involved in cell division and cell cycling which current models predict may play a significant role in cell fate determination and vernalization phenomena (Caro *et al.*, 2007, He *et al.*, 2009)