

How to awaken a sleeping giant: Antagonistic Expression of Flowering Locus T Homologs and Elements of the Age Related Pathway are Associated with the Flowering Transition in *Agave tequilana*

Plant Reproduction

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Online Resource 1 AtqFT primer sequences

Primer	Sequence
FwattB- AtqFT1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCAAAGAGGGAGGGATCCT
RvattB- AtqFT1	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAGCTAATCCTTCTTCCTCCGGA
FwattB1- AtqFT2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAGTAGGGATCCTTTGGT
RvattB2- AtqFT2	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCACAGGTACATCCTTCGCC
FwattB1- AtqFT4	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTCTGCACAATCTTTAGT
RvattB2- AtqFT4	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAGCCAGCTCTGAACCTTC

Online Resource 2 Cis-element analysis of AtqFT gene promoter regions

AtqFT1	AtqFT2	AtqFT3	AtqFT4	AtqFT5	AtqFT8	AtqFT9	AtqFT10
AACACOREOSGLUB1	10PEHVPSBD	10PEHVPSBD	10PEHVPSBD	ARR1AT	10PEHVPSBD	10PEHVPSBD	ARR1AT
ARR1AT	AACACOREOSGLUB1	ARR1AT	2SSEEDPROTBANAPA	BOXIINTPATPB	ARR1AT	ARR1AT	CACTFTPPCA1
ATHB2ATCONSENSUS	ARR1AT	BOXIINTPATPB	ARR1AT	CACTFTPPCA1	BOXIINTPATPB	BOXIINTPATPB	CANBNNAPA
BOXIINTPATPB	CACTFTPPCA1	CACTFTPPCA1	BOXIINTPATPB	CANBNNAPA	CACTFTPPCA1	CACTFTPPCA1	CBFHV
CACTFTPPCA1	CANBNNAPA	CANBNNAPA	CACTFTPPCA1	CCAATBOX1	CANBNNAPA	CBFHV	CCAATBOX1
CANBNNAPA	CBFHV	CCAATBOX1	CANBNNAPA	CGCGBOXAT	CGCGBOXAT	CCAATBOX1	CRTDREHVCBF2
CBFHV	CCAATBOX1	DOFCOREZM	CCAATBOX1	CIACADIANLELHC	CCAATBOX1	DOFCOREZM	DOFCOREZM
CCAATBOX1	CGCGBOXAT	DPBFCOREDCDC3	DOFCOREZM	DOFCOREZM	CIACADIANLELHC	DRE2COREZMRAB17	DRE2COREZMRAB17
CGCGBOXAT	DOFCOREZM	EBOXBNNAPA	DPBFCOREDCDC3	DPBFCOREDCDC3	DOFCOREZM	DRECRTCOREAT	ECCRCRAH1
CIACADIANLELHC	DPBFCOREDCDC3	ECCRCRAH1	EBOXBNNAPA	EBOXBNNAPA	DPBFCOREDCDC3	EBOXBNNAPA	ELRECOREPCRP1
CRTDREHVCBF2	DRE2COREZMRAB17	ELRECOREPCRP1	ECCRCRAH1	EBOXBNNAPA	DRE1COREZMRAB17	ECCRCRAH1	GATABOX
DOFCOREZM	DRECRTCOREAT	GAGA8HVBKN3	ELRECOREPCRP1	ECCRCRAH1	EBOXBNNAPA	GAGA8HVBKN3	GT1CONSENSUS
DPBFCOREDCDC3	EBOXBNNAPA	GAGAGMGSA1	GAGA8HVBKN3	ELRECOREPCRP1	ECCRCRAH1	GAGAGMGSA1	GT1MSCAM4
DRE1COREZMRAB17	ECCRCRAH1	GAREAT	GAGAGMGSA1	GARE1OSREP1	ELRECOREPCRP1	GATABOX	GTGANTG10
DRE2COREZMRAB17	ELRECOREPCRP1	GATABOX	GATABOX	GAREAT	GAREAT	GCCORE	IBOXCORE
DRECRTCOREAT	GAGA8HVBKN3	GT1CONSENSUS	GCCORE	GATABOX	GT1CONSENSUS	GCN4OSGLUB1	INRNTPSADB
EBOXBNNAPA	GAGAGMGSA1	GT1GMSCAM4	GCN4OSGLUB1	GT1CONSENSUS	GT1GMSCAM4	GT1CONSENSUS	LTREATLT178
ECCRCRAH1	GARE1OSREP1	GTGANTG10	GT1CONSENSUS	GT1GMSCAM4	GTGANTG10	GT1GMSCAM4	LTRECREATCOR15
ELRECOREPCRP1	GAREAT	IBOXCORE	GT1GMSCAM4	GTGANTG10	IBOXCORE	GTGANTG10	MYB1AT
GAREAT	GATABOX	INRNTPSADB	GTGANTG10	IBOXCORE	INRNTPSADB	IBOXCORE	MYBCORE
GATABOX	GT1CONSENSUS	MYB1AT	IBOXCORE	INRNTPSADB	LTRE1HVBTL49	INRNTPSADB	MYBST1
GT1CONSENSUS	GT1GMSCAM4	MYBCORE	INRNTPSADB	MYB1AT	MYB1AT	LTRE1HVBTL49	MYCATERD1
GT1GMSCAM4	GTGANTG10	MYBGAHV	LTRE1HVBTL49	MYBCORE	MYB2CONSENSUSAT	LTRECREATCOR15	MYCATRD22
GTGANTG10	IBOX	MYBPZM	LTRECREATCOR15	MYBGAHV	MYBCORE	MYB1AT	MYCCONSUSUSAT
IBOXCORE	IBOXCORE	MYBST1	MYB1AT	MYBPLANT	MYBPZM	MYBCORE	NODCON1GM
INRNTPSADB	IBOXCORENT	MYCATERD1	MYB2AT	MYBST1	MYBST1	MYBPZM	NODCON2GM
LTRE1HVBTL49	INRNTPSADB	MYCATRD22	MYB2CONSENSUSAT	MYCATERD1	MYCATERD1	MYBST1	OSE1ROOTNODE
LTREATLT178	LTRE1HVBTL49	MYCCONSUSUSAT	MYBCORE	MYCATRD22	MYCATRD22	MYCCONSUSUSAT	OSE2ROOTNODE
LTRECREATCOR15	LTRECREATCOR15	NODCON1GM	MYBCOREATCYCB1	MYCCONSUSUSAT	MYCCONSUSUSAT	NODCON1GM	POLLEN1LELAT52
MYB1AT	MYB1AT	NODCON2GM	MYBPZM	MYBPZM	NODCON1GM	NODCON2GM	PYRIMIDINEBOXOSRAMY1A
MYBCORE	MYB2CONSENSUSAT	OSE1ROOTNODE	MYBST1	NODCON2GM	NODCON2GM	OSE1ROOTNODE	QELEMENTZM2M13
MYBGAHV	MYBCORE	OSE2ROOTNODE	MYCATERD1	OSE1ROOTNODE	OSE1ROOTNODE	OSE2ROOTNODE	SEF4MOTIFGM75
MYBPLANT	MYBGAHV	POLLEN1LELAT52	MYCATRD22	OSE2ROOTNODE	OSE2ROOTNODE	POLLEN1LELAT52	SORLIP1AT
MYBPZM	MYBPLANT	PRECONSCRHSP70A	MYCCONSUSUSAT	QELEMENTZM2M13	POLLEN1LELAT52	PRECONSCRHSP70A	WBBOXPCWRKY1
MYBST1	MYBPZM	PYRIMIDINEBOXOSRAMY1A	NODCON1GM	RYREPEATLEGUMINBOX	PRECONSCRHSP70A	QELEMENTZM2M13	WBOXATNPR1
MYCATERD1	MYBST1	QELEMENTZM2M13	NODCON2GM	SEBFCONSSTPR10A	PYRIMIDINEBOXOSRAMY1A	RYREPEATLEGUMINBOX	WBOXHVIS01
MYCATRD22	MYCATERD1	RYREPEATLEGUMINBOX	OSE1ROOTNODE	SEF4MOTIFGM75	QELEMENTZM2M13	SEF4MOTIFGM75	WBOXNTCHN48
MYCCONSUSUSAT	MYCATRD22	SEF4MOTIFGM75	OSE2ROOTNODE	SORLIP1AT	SEBFCONSSTPR10A	SORLIP1AT	WBOXNTERF3
NODCON1GM	MYCCONSUSUSAT	SORLIP1AT	POLLEN1LELAT52	SORLIP2AT	SORLIP1AT	SORLIP2AT	WRKY71OS
NODCON2GM	NODCON1GM	SORLIP5AT	PRECONSCRHSP70A	TBOXATGAPB	SORLIP2AT	TBOXATGAPB	
OSE1ROOTNODE	NODCON2GM	TBOXATGAPB	PYRIMIDINEBOXOSRAMY1A	WBBOXPCWRKY1	SORLREP3AT	WBOXHVIS01	
OSE2ROOTNODE	OSE1ROOTNODE	WBBOXPCWRKY1	QELEMENTZM2M13	WBOXATNPR1	TBOXATGAPB	WBOXNTCHN48	
PALBOXAPC	OSE2ROOTNODE	WBOXATNPR1	RYREPEATLEGUMINBOX	WBOXHVIS01	WBOXATNPR1	WBOXNTERF3	
POLLEN1LELAT52	POLLEN1LELAT52	WBOXHVIS01	SEBFCONSSTPR10A	WBOXNTCHN48	WBOXHVIS01	WRKY71OS	
PRECONSCRHSP70A	PRECONSCRHSP70A	WBOXNTCHN48	SEF3MOTIFGM	WBOXNTERF3	WBOXNTCHN48		
PYRIMIDINEBOXOSRAMY1A	QELEMENTZM2M13	WBOXNTERF3	SEF4MOTIFGM75	WRKY71OS	WBOXNTERF3		
QELEMENTZM2M13	QELEMENTZM2M13	WRKY71OS	SORLIP1AT		WRKY71OS		
SEF4MOTIFGM75	RYREPEATLEGUMINBOX		SORLIP2AT				
SORLIP1AT	SEBFCONSSTPR10A		SORLIP5AT				
SORLIP2AT	SEF4MOTIFGM75		SORLREP3AT				
TBOXATGAPB	SORLIP1AT		TBOXATGAPB				
WBBOXPCWRKY1	TBOXATGAPB		WBBOXPCWRKY1				
WBOXATNPR1	WBOXATNPR1		WBOXATNPR1				
WBOXHVIS01	WBOXHVIS01		WBOXHVIS01				
WBOXNTCHN48	WBOXNTCHN48		WBOXNTCHN48				
WBOXNTERF3	WBOXNTERF3		WBOXNTERF3				
WRKY71OS	WRKY71OS		WRKY71OS				

a**Vegetative SAM****VSAM1****VSAM2**

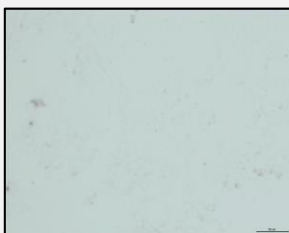
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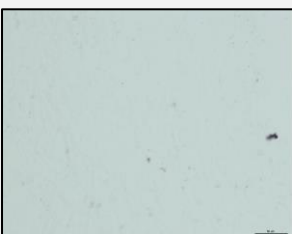
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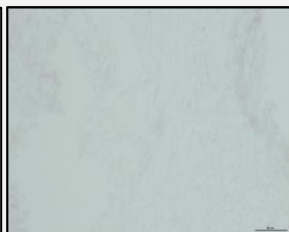
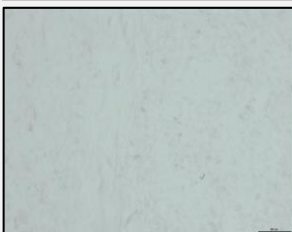
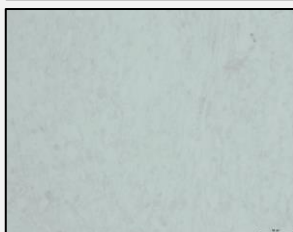
AtqFT1



AtqFT2



AtqFT4

**b****Reproductive SAM****RSAM1****RSAM2**

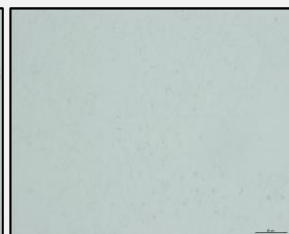
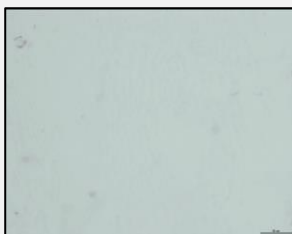
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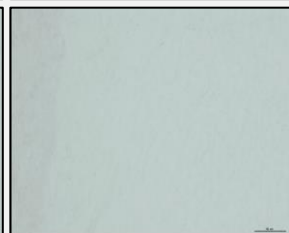
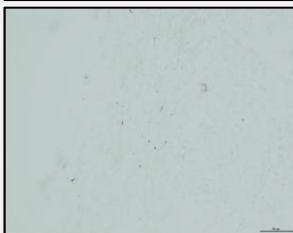
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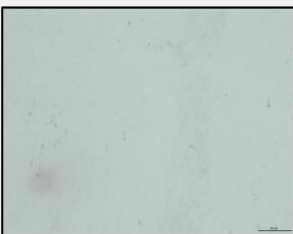
AtqFT1



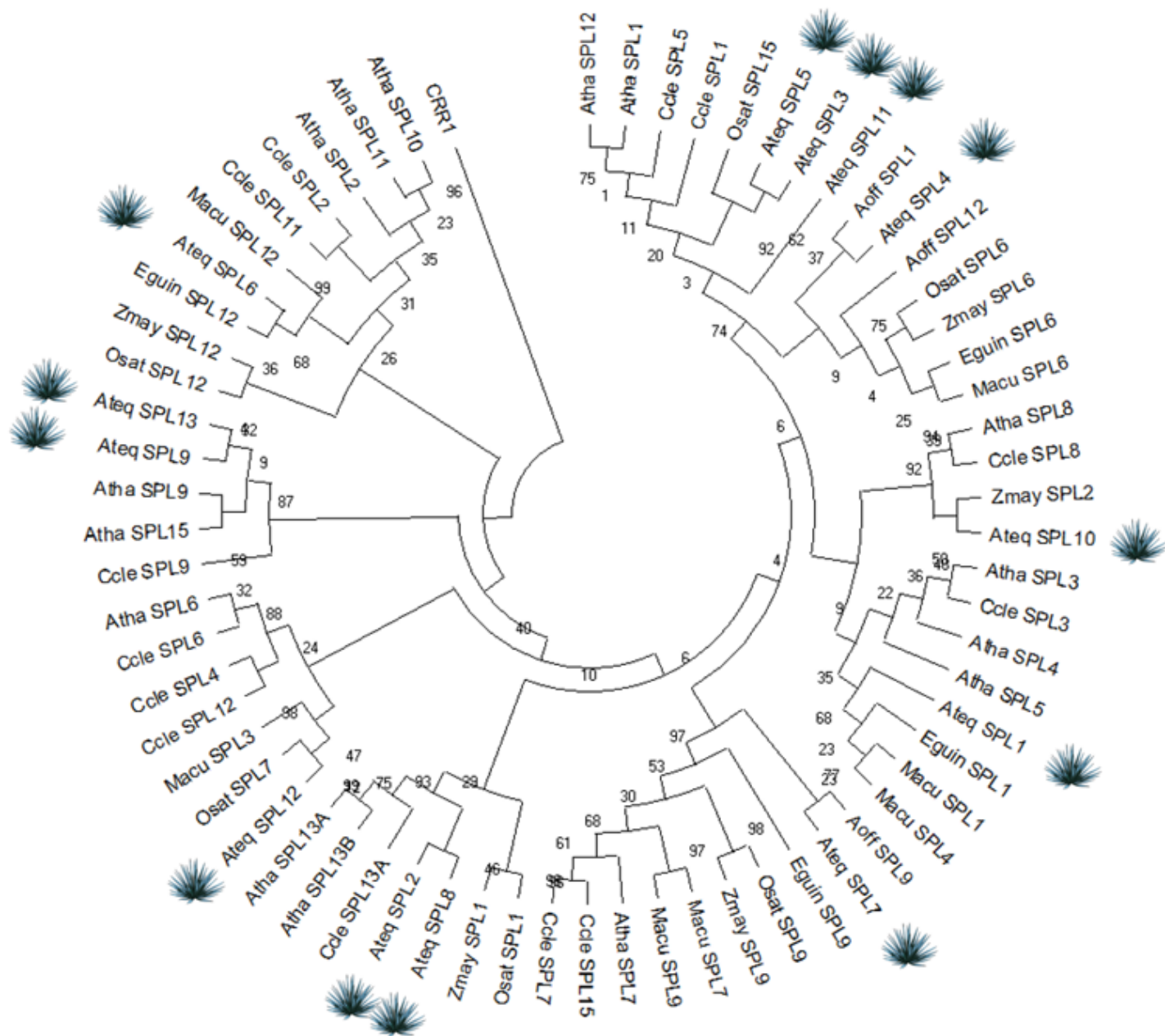
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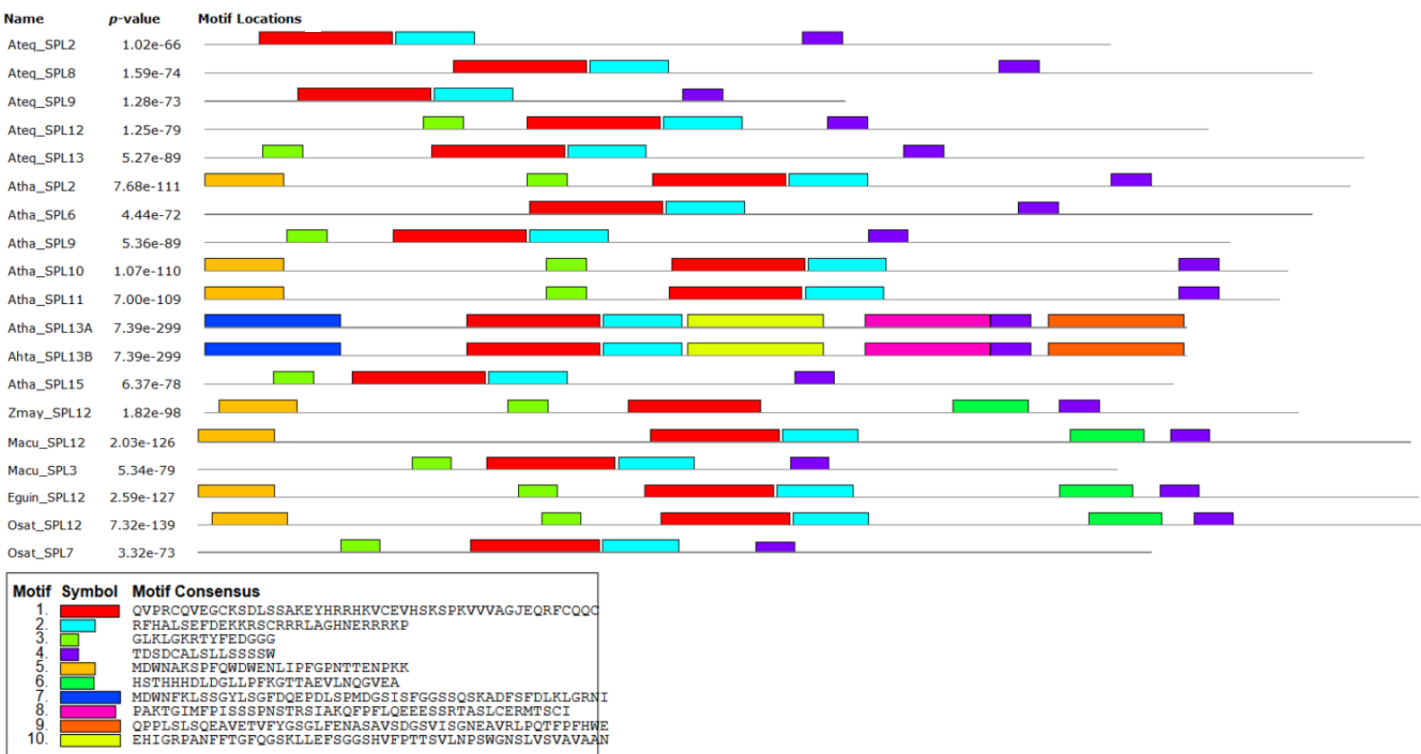
AtqFT4



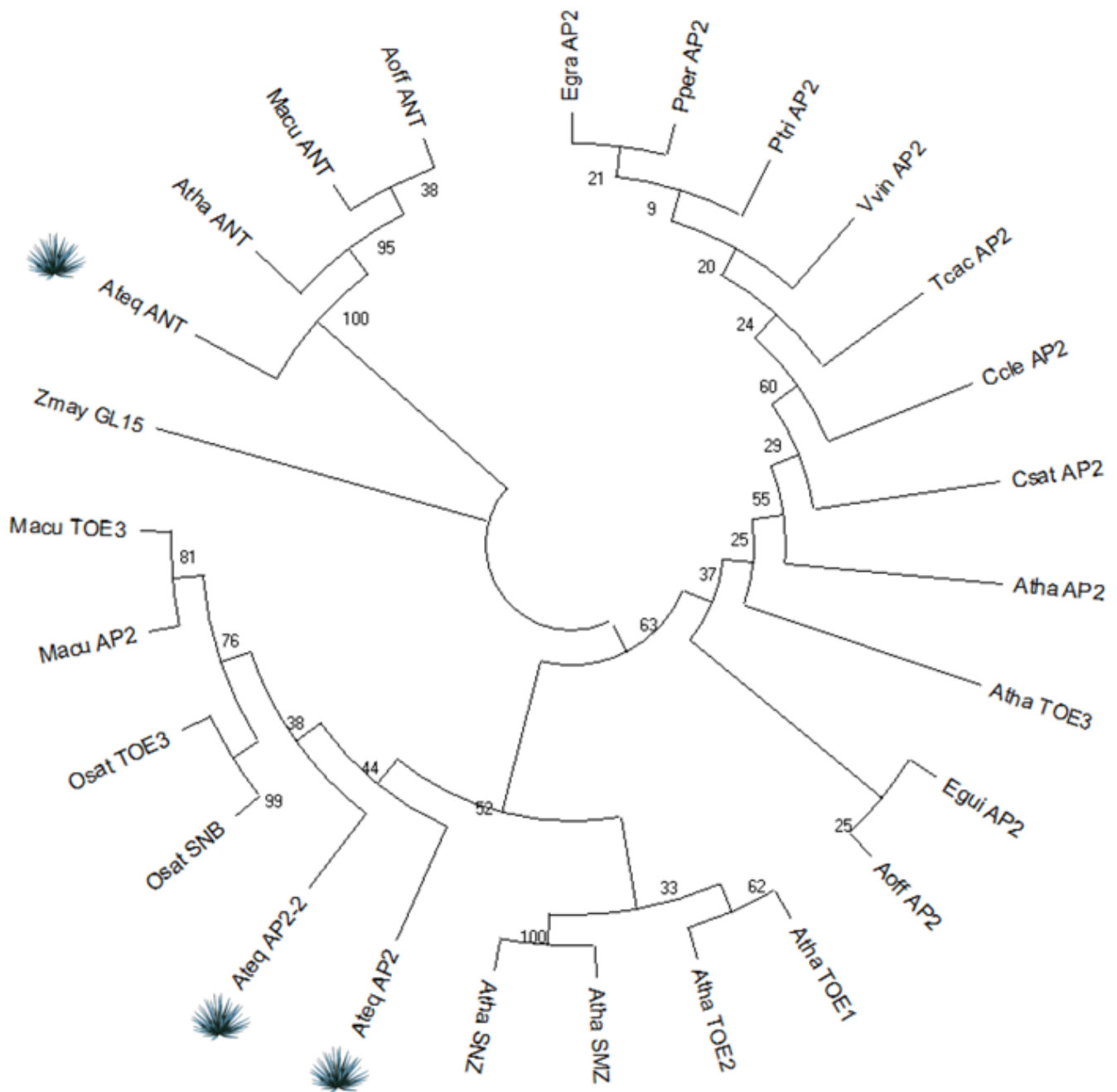
Online Resource 3 *In situ* hybridization analysis meristem samples. (a) Longitudinal section of vegetative meristem tissues was hybridized with AtqFT1, AtqFT2, and AtqFT4 specific antisense and sense probes. (b) Longitudinal sections of reproductive meristem tissues were hybridized with AtqFT1, AtqFT2, and AtqFT4 specific antisense and sense probes. Bars on *In situ* hybridization analysis are 50 um



Online Resource 4 Dendrogram showing relationships between *A. tequilana* SPL genes and those of other plant species. Phylogenetic analysis was carried out by using amino acid sequences and considering all sites including gaps/missing information based on the Maximum likelihood method and the JTT+G substitution model. Agave plants indicate *AtqSPL* genes



Online Resource 5 Conserved motifs between SPL genes from *A. tequilana* and other plant species. Schematic representation of motif locations in *A. tequilana*, *A. thaliana*, *O. sativa*, *E. guineensis*, *M. acuminata* and *Z. mays* SPL sequences. Motif enrichment analysis performed with the MEME suite (version 5.4.1)



Online Resource 6 Dendrogram showing relationships between *A. tequilana* AP2 genes and those of other plant species. Phylogenetic analysis was carried out by using amino acid sequences and considering all sites including gaps/missing information based on the Maximum likelihood method and the JTT+G substitution model. Agave plant figures indicate AteqAP2 genes

Online Resource 7 miRNA families identified in *A. tequilana* transcriptome using miRbase

miR156	miR167	miR394	miR477	miR845	miR5225
miR157	miR168	miR395	miR479	miR858	miR7528
miR159	miR169	miR396	miR482	miR1515	miR7732
miR160	miR171	miR397	miR528	miR2118	miR7767
miR162	miR172	miR398	miR529	miR3711	miR8175
miR164	miR319	miR399	miR535	miR4414	miR8558
miR165	miR390	miR408	miR827	miR5072	
miR166	miR393	miR444	miR828	miR5083	

Online Resource 8 List of microRNAs targeting AtqSPL and AtqAP2 genes

miRNA target	Gene	miRNA target	Gene
miR156	Ateq_SPL12	miR172	Ateq_AP2
	Ateq_SPL13		Ateq_SPL13
	Ateq_SPL2		Ateq_SPL3
	Ateq_SPL6	miR319	Ateq_SPL11
	Ateq_SPL8		Ateq_SPL3
	Ateq_SPL9		Ateq_SPL5
miR159	Ateq_AP2		Ateq_SPL7
	Ateq_AP2-2		Ateq_SPL8
	Ateq_SPL11	miR396	Ateq_SPL10
	Ateq_SPL3		Ateq_SPL13
	Ateq_SPL4		Ateq_SPL3
	Ateq_SPL5		Ateq_SPL5
miR160	Ateq_SPL12		Ateq_SPL6
miR162	Ateq_SPL4		Ateq_SPL9
miR164	Ateq_SPL4	miR408	Ateq_AP2-2
miR166	Ateq_AP2		Ateq_SPL12
	Ateq_SPL10		Ateq_SPL13
	Ateq_SPL2		Ateq_SPL5
	Ateq_SPL3	miR444	Ateq_SPL12
	Ateq_SPL4	miR482	Ateq_SPL11
	Ateq_SPL5	miR535	Ateq_SPL12
	Ateq_SPL6		Ateq_SPL13
	Ateq_SPL7		Ateq_SPL6
	Ateq_SPL8		Ateq_SPL8
miR167	Ateq_SPL4		Ateq_SPL9
	Ateq_SPL8	miR858	Ateq_SPL4
miR168	Ateq_SPL13	miR1515	Ateq_SPL2
	Ateq_SPL9	miR2118	Ateq_AP2
miR171	Ateq_SPL5	miR2118	Ateq_SPL11
		miR8175	Ateq_SPL10
		miR8558	Ateq_AP2

Online Resource 9 List of microRNAs targeting PGHF32 genes. (a) microRNAs targeting AtqPGHF32 genes. (b) microRNAs targeting PGHF32 genes in *A. tequilana* and other plant species

a	miRNA Target	Gene	miRNA target	Gene	miRNA target	Gene
	miR156	Atq1SST-1	miR166	Atq1SST-3	miR396	AtqInv1
		Atq1SST-2		Atq6GFFT-1		AtqInv2
		Atq1SST-3		AtqCwinv-1		AtqVinv1
		AtqCwinv-2		AtqCwinv-2	AtqVinv2	
		AtqInv1		AtqFEH-4	miR397	AtqFEH-3
		AtqVinv1	AtqVinv1	miR444	Atq6GFFT-2	
		AtqVinv2	miR167	AtqVinv2	AtqVinv2	
	miR159	AtqFEH-2	miR168	Atq1SST-1	miR477	AtqInv1
		AtqFEH-3		Atq1SST-3	miR479	AtqFEH-4
		AtqVinv1		AtqInv2	miR529	Atq1SST-1
	miR164	Atq6GFFT-1	miR171	AtqVinv1	miR828	Atq6GFFT-2
		Atq6GFFT-2		Atq6GFFT-1		AtqFEH-3
		AtqFEH-3	miR319	AtqInv1	miR3711	AtqCwinv-2
		AtqInv1	miR395	AtqInv1	miR5225	Atq1SST-1
	miR166	Atq1SST-1	miR396	AtqFEH-1	miR8175	AtqFEH-3
		Atq1SST-2		AtqFEH-3		

b	miRNA target	Gene	miRNA target	Gene	miRNA target	Gene	
	miR156	Atq1SST-1	miR167	Ac1SST	miR396	Ao1SST	
		Atq1SST-2		Ao6GFFT		AoCwinv1-like	
		Atq1SST-3		AtqVinv2		AthCwinv2	
		AtqCwinv-2		BvVinv		AthCwinv4	
		AtqInv1	miR168	Atq1SST-1		BvCwinv	
		AtqVinv1		Atq1SST-3		AtqFEH-1	
		AtqVinv2		Ao1SST		AtqFEH-3	
	miR159	AthCwinv1	miR171	AthCwinv1	miR444	Ao6FEH	
		AthCwinv5		AtqInv2		Bv6FEH	
		BvCwinv		AtqVinv1		AtqInv1	
		AtqFEH-2	miR172	Atq6GFFT-1		miR828	AtqInv2
		AtqFEH-3		AoCwinv1-like			AtqVinv1
		Ac1FEH		AthCwinv5			AtqVinv2
		AtqVinv1	Ao6FEH	AthVinv2			
	miR162	Ao6GFFT	miR172	Ac6GFFT	miR444	Atq6GFFT-2	
	miR164	Atq6GFFT-1	miR172	BvCwinv	miR477	AtqVinv2	
		Atq6GFFT-2		Ac1FEH	miR479	AtqInv1	
		AtqFEH-3		Ac1SST	miR529	AtqFEH-4	
		AtqInv1	Ao1SST	miR827	Atq1SST-1		
	miR165	Ao1FFT1	miR319	Ao6GFFT	miR827	BvVinv	
	miR166	Atq1SST-1		AthCwinv1	miR828	Atq6GFFT-2	
		Atq1SST-2		AthCwinv2	miR1515	AtqFEH-3	
		Atq1SST-3		AthCwinv4		Bv6FEH	
		Ac1SST	AthCwinv5	miR3711		AtqCwinv-2	
		Ao1FFT1	Ac1FEH	miR5072	Ao1FFT1		
		Atq6GFFT-1	AtqInv1	miR5225	Atq1SST-1		
		Ac6GFFT	miR393	Ac6GFFT	miR8175	AtqFEH-3	
		AtqCwinv-1	miR395	Ac1SST			
		AtqCwinv-2		AtqInv1			
AoCwinv2-like		miR397	AtqFEH-3				
AthCwinv1		miR398	Ac1SST				
AtqFEH-4		miR482	AthCwinv4				
Ath61FEH			Ac1FEH				
Ath6FEH			BvVinv				
Bv6FEH		miR8558	Ao1FFT1				
AtqVinv1			AoCwinv2-like				
AthVinv2			BvCwinv				