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Identification of Sugar Beet Flowering Genes Based on *Arabidopsis* Homologous Genes

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ABSTRACT

Transition from vegetative to reproductive growth is an important stage in plant's life. Flowering pathways including photoperiod, vernalization, gibberellins, and autonomous pathway are regulated by different genes. Identification of flowering genes is essential for the development of bolting-resistant sugar beet cultivars. In this study, a set of 118 *Arabidopsis thaliana* genes involved in flowering time control were used as a reference to identify homologous counterparts in Expressed Sequence Tags (ESTs) and Transcriptome Shotgun Assembly (TSA) sequence of sugar beet. Based on obtained ESTs, primers were designed for Suppressor of Frigida 4 (*SUF4*), Curly Leaf (CLF), Constitutive Photomorphogenesis1 (COP1), and Cycling Dof Factor (CDF) genes. *SUF4* and *CLF* are components of vernalization pathway and *COP1* and *CDF* are in photoperiod pathway. The sequence regions of these genes were amplified using cDNA PCR technique, and compared with other identified sequences in Gene Bank. Four genes namely *CLF, COP1, CDF* and *SUF4* were deposited in Gene Bank. Results showed that most of the flowering pathway genes in *Arabidopsis* are detectable in sugar beet which can be contributed to the understanding of the genetic control of bolting resistance.

Keywords: Bioinformatic, Expressed sequence tags, Flowering genes, Shotgun Assembly sequences, Sugar beet, Transcriptome.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is a biennial root crop that provides~ 25% of the world's sugar. Sugar beet grows vegetatively in the first year and starts shoot elongation (bolting) and flowering after exposure to cold temperatures (Abbasi *et al.*, 2014). Early bolting in the first year is an undesirable feature which results in yield reduction. The characterized flowering genes in sugar beet can be used as molecular markers in breeding programs or in genetic manipulation of the flowering time control (Kim *et al.*, 2010; Bakooie *et al.*, 2015). In 2004, the *Beta vulgaris* EST (Expressed Sequence Tags)_project with PRJNA12549 number was performed in Michigan University and identified EST sequences were deposited in the database of NCBI (National Center for Biotechnology Information). In 2011, in *B. vulgaris* transcriptome project with PRJNA73561 number in Cambridge University, total mRNA from terminal bud tissues were sequenced and 56,737 TSA sequences were deposited. In 2013, about 4.9 billion bases of sugar beet genome were sequenced and deposited in Genebank (transcriptome project PRJNA219421). Therefore, many sugar beet sequences are deposited in NCBI without identified annotations. TSAs and ESTs are valuable resources for gene discovery (Jung and Main, 2014) which can be used for identification of sugar beet flowering genes. Through constructing a set

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of Unigene cDNA clone, Herwig *et al*. (2002) showed that 89% of the sugar beet ESTs are similar to other plants such as *Arabidopsis*. Flowering pathways are well characterized in *Arabidopsis*. *FLOWERING LOCUS C* (FLC) is a key gene in vernalization pathway and is expressed in stem and root apex (Michaels and Amasino, 2000). *FLC* suppresses flowering through inhibiting flowering pathway genes expression including *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (SOC1), *FLOWERING LOCUS T* (FT), and *LEAFY* (LFY) (Michaels and Amasino, 1999; Sheldon *et al.*, 1999). However, vernalization may repress *FLC* expression. *FRIGIDA* (FRI) is the main activator of *FLC* expression (Johanson *et al.*, 2000). The key gene in photoperiod pathway is *CONSTANS* (CO) which has a significant role in light absorption with temperature susceptibility (Boss *et al.*, 2004). *CO* protein is a transcription factor which directly activates *FT*. Autonomous pathway is another flowering pathway which decreases the *FLC* expression. Different pathway genes such as *FLOWERING LOCUS CA* (FCA), *FLOWERING LOCUS D (FLD),* *FLOWERING LOCUS PA* (FPA), *FLOWERING LOCUS VE* (FVE), *FLOWERING LOCUS Y* (FY), *LUMINIDEPENDENS* (LD) and *FLOWERING LOCUS K* (FLK) repress *FLC* expression (Jordan, 2006). Based on both physiological and genetic studies, gibberellins accelerate *Arabidopsis* flowering (Langridge, 1957) and *LFY* is one of the main targets in gibberellins signalling pathway (Blázquez *et al.*, 1998). Some flowering genes have been characterized in sugar beet. Pairs of *FT* homologs (BvFT1 and BvFT2) encode phosphatidylethanolamine protein which acts differently in transition to flowering; *BvFT1* prevents flowering and *BvFT2* induces flowering. The expression of *BvFL1* in sugar beet was shown to be down-regulated during vernalization. *BvFT1* repression continues which indicates similar activity of *BVFT1* and *FLC* in *Arabidopsis* (Pin *et al.*, 2010). Also four homologs of autonomous pathway genes in sugar beet including *BvFLK*, *BvFVE*, *BvLD* and *BvLDL1* were identified and mapped by Abou-Elwafa *et al.* (2010). Reeves *et al.* (2007) reported that sugar beet genes are similar to their homologs for intron-exon structure and domain organization (Reeves *et al.*, 2007). Three *CO* homologous genes *CONSTANS-Like 1* (COL1), *COL2* and *COL3* are involved in the photoperiod pathway, and are reported in sugar beet (Chia *et al.*, 2008). A part of *SHORT VEGETATIVE PHASE* (SVP1) and *APETALA1* (AP1) have been identified in sugar beet. *SVP1* acts as a flowering repressor in autonomous and gibberellins pathway (Li *et al.*, 2008). In 2010, *CENTRORADIALIS1* (CEN1) was reported in sugar beet (Pin *et al.*, 2010).

BOLTING TIME CONTROL 1 (BvBTC1) is a master switch which distinguishes annual plants from biennials. This gene regulates the *FLOWERING LOCUS T* genes and is necessary for flowering (Pin *et al.*, 2012). A new bolting locus B2 was identified as a transcription factor that is diurnally regulated and acts like *BvTC1* upstream of *BvFT1* and *BvFT2* (Dally *et al.* 2014). The aim of this study was to identify homologs for *Arabidopsis* flowering time genes from sugar beet by ESTs and TSAs by bioinformatic analysis. The most homologs of *Arabidopsis* flowering genes were found in sugar beet.

MATERIALS AND METHODS

Bioinformatic Analysis

The sequence of the flowering genes in *Arabidopsis* and their proteins were obtained from Gene Bank. The obtained sequences were queried against sugar beet ESTs and TSAs. However, previously identified sugar beet flowering genes were removed from this study. Using BLASTn and tBLASTx software, for most *Arabidopsis* flowering genes, EST and TSA homologs with 10^{-8} *E-*

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value were identified. All identified flowering sequences were checked by VecScreen (NCBI tools) to identify vector sequence contamination. *Beta vulgaris* ESTs and TSAs that bear homology to *Arabidopsis* flowering genes were assembled using SeqMan software (version 7.1.0(44.1), Lasergene).

Plant Materials

Otype 7,112 seeds received from Sugar Beet Seed Institute were planted in pots in a greenhouse at 25°C. Total RNA was extracted from young leaves using CinnaGen kit (RNX-Plus solution). The cDNA was synthesized using the Oligo (dt) primer and CinnaGen kit.

Primer Design

The Primer 3 software was used to design four gene-specific primers named *CDF*, *COP1*, *CLF*, *SUF4* (Table 1).

PCR Amplification

PCR was performed in a volume of 25 µl containing 4 µl of cDNA template. The cycle parameters in the PCR program were as follows at 94°C for 5 minutess, 35 cycles of denaturing at 94°C for 1 minute, annealing at 58°C for 1 minute for all primer pairs, extension at 72°C for 2 minutes and a final step at 72°C for 7 minutes. Qualitative assessment of four *B. vulgaris* transcripts was performed by examining PCR products on 1% agarose gels. PCR products were sent to Bioneer Company for sequencing.

RESULTS AND DISCUSSION

Bioiformatic Analysis

In this study, 137 significantly similar ESTs were characterized for 24 photoperiod pathway genes. Using BLASTN algorithm, no EST was identified for *SENSITIVITY TO RED LIGHT REDUCED1* (SRR1), PHYTOCLOCK1 (PCL1), LUX ARRHYTHMO (*LUX)*, *EARLY FLOWERING4* (ELF4), *TERMINAL FLOWER1* (TFL1) and *RED AND FAR-RED INSENSITIVE 2* (RFI2) (Table 2). Except *ELF3*, some TSAs with significant similarities were also identified for photoperiod genes by tBLASTn algorithm. For each gene, only one EST and TSA with significant value for both BLASTn and tBLASTx analyses were recognized. Activation and repression of flowering by each gene is indicated as + and –, respectively (Mouhu *et al.*, 2009).

FLC gene has been identified in vernalization pathway, therefore its homologous sequence, *MAF1*, was examined. No sequence was identified for *FRI* and BLASTn algorithm results also showed no homologs for it. However, using Tblastx algorithm, similar ESTs were identified for *FRI1* and *FRI2*. Using tBLASTn algorithm, TSA homologs were identified for all 22 vernalization pathway genes (Table 3).

Using BLASTn algorithm, for some

Table 1. Information on the designed Primers.

Genes	EST	Primer	Primer sequence
CDF	BQ488386	Forward	*GGTGCAGGTAGACGGAAGAA*
CDF	BQ593385	Reverse	*GCCTCATCTGGGTCATCAAT*
COP1	BQ587440	Forward	*CTTCCCCAAAATTATGGCCT*
COP1	EX956277	Reverse	*TTGGCTGAATGAAAAGGGTC*
CLF	CF543355	Forward	*GCCGGTGTTACGTTTTTGAT*
CLF	BQ585470	Reverse	*TTTTCCCAGTCACGACCTTC*
SUF4	BQ591620	Forward	*AAACACTTTAAATGCCATGTTTG*
SUF4	BQ589067	Reverse	*TTGAATTCATCTGGCTGGTTT*

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Table2. List of EST and TSA homologs of photoperiod pathway genes in sugar beet.

Genes	AT gene locus	Act/Repr+/-	*Beta vulgaris* EST	*E*-Value	*Beta vulgaris* TSA	*E*-Value
CCA1	AT2G46830	-	BQ591669	5E-25	JP516307	3E-30
CDF	AT5G62430	-	BQ589119	1E-44	JP528059	8E-42
CO	AT5G15840	+	BQ589119	7E-50	JP495583	3E-47
COP1	AT2G32950	-	CV301332	6E-113	JP532831	2E-123
ELF3	AT2G25920	-	BQ582323	1E-132	NONE	
ELF4	AT2G40080	-	BQ588775	2E-09	JP491819	2E-27
ELF6	AT5G04240	-	EG551058	1E-38	JP521395	9E-46
FD	AT4G35900	+	BQ584903	4E-15	JP488489	5E-16
FKF1	AT1G68050	+	FG343952	1E-92	JP503688	6E-53
FYPP3	AT1G50370	-	BQ588603	2E-70	JP502472	1E-128
GI	AT1G22770	+	FG345154	4E-88	JP513392	0
HAP3b	AT5G47640	+	BQ592365	2E-64	JP486242	3E-67
LHY	AT1G01060	-	BQ591669	2E-26	JP516307	7E-30
LUX	AT3G46640	-	BQ490630	7E-17	JP535165	3E-41
PRR3	AT5G02810	+	BQ488991	1E-25	JP483216	2E-52
PRR5	AT5G24470	+	BQ488991	1E-45	JP483216	3E-84
REF6	AT5G04240	-	BQ488255	1E-19	JP521395	2E-67
SPA1	AT2G46340	-	BQ489531	2E-68	JP524349	2E-163
SRR1	AT5G59560	-	BI543444	3E-08	JP523485	1E-69
TOC1	AT5G61380	-	FG344833	7E-58	JP506937	3E-150
TSF	AT1G65480	+	FG343952	6E-40	JP524752	3E-48
ZTL	AT5G57360	+	BQ584903	5E-109	JP503688	9E-61

Table 3. List of EST and TSA homologs of vernalization pathway genes.

Genes	AT gene locus	Act/Repr +/-	*Beta vulgaris* EST	*E*-Value	*Beta vulgaris* TSA	*E*-Value
ARP6	AT3G33520	-	AW063023	1E-40	JP532887	1E-62
ATX1	AT2G31650	-	BQ594945	2E-67	JP521302	0
CLF	AT2G23380	-	BQ585470	1E-63	JP525541	0
EFS	AT1G77300	-	BQ587534	2E-31	JP535847	2E-47
ELF7	AT1G79730	-	BQ592749	6E-32	JP523257	7E-160
ELF8	AT2G06210	-	BQ583923	8E-102	JP513375	9E-167
EMF2	AT4G16845	+	FG345541	5E-35	JP493912	0
FIE	AT3G20740	+	BI543337	2E-13	JP512818	0
FRI	AT4G00650	-	NONE		JP514309	5E-04
FRL1	AT5G16320	-	EG552056	2E-27	JP513340	1E-16
LHP1	AT5G17690	+	BQ584695	1E-09	JP487767	9E-29
MAF1	AT1G77080	-	BQ595637	1E-26	JP512496	7E-23
PIE	AT3G12810	-	BQ585682	4E-116	JP529895	0
SEF1	AT5G37055	-	CV301292	4E-51	JP530923	4E-68
SETD2	AT1G77300	-	BQ587534	8E-32	JP521302	3E-31
SUF4	AT1G30970	-	BQ591620	2E-55	JP494761	3E-60
SWN1	AT4G02020	+	BQ585470	2E-48	JP511273	0
VIN3	AT5G57380	+	BQ593505	3E-44	JP509920	6E-109
VIP3	AT4G29830	-	BQ490245	2E-52	JP485693	5E-37
VIP4	AT5G61150	-	BQ587025	2E-95	JP496115	4E-138
VRN1	AT3G18990	+	BQ594447	8E-17	JP530325	1E-27
VRN2	AT4G16845	+	EG552056	5E-29	JP493907	5E-62

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autonomous pathway genes including *FCA, FY, FPA* and *LDL2*, no similar EST was identified. Nevertheless, tBLASTx algorithm results showed homologs for all genes in this pathway. Using tBLASTn algorithm, protein translation for 8 genes in autonomous pathway was performed and TSA homologs were identified for all genes (Table 4).

No EST and TSA homologs were identified for *FLOWERING PROMOTIVE FACTOR1* (FPF1) in gibberellins pathway. Other genes had similar EST and TSA (Table 5).

In addition to flowering pathway genes, several genes that are not present in any particular direction were also identified. For *PHYTOCHROME AND FLOWERING TIME1* (PFT1), no EST homologs were identified but results were satisfactory for other flowering pathway genes (Table 6).

No EST homologs were identified for floral integrator *LEAFY* (LFY). However, EST homologs were identified for another floral integrator *SOC1*. Based on BLASTn and tBLASTn results, 5 significant EST homologs were identified for *AP1* (Table 7).

Assembling the Flowering Genes Sequences

Large sequences were constructed through joining homologous regions of small sequences (ESTs and TSAs). The constructed sequences were subjected to blast analysis using BLASTn and tBLASTx algorithm against several EST and TSA data (Table 8).

Identification of Flowering Genes Using PCR Technique

Results of PCR product amplification for *CLF, COP1, CDF* and *SUF4* genes was almost as expected based on related *Arabidopsis* genes.

The similarities between these sequences and their homologs in other plants were analysed in NCBI Reference RNA Sequence Database using BLASTn algorithm (Table 9). All four genes had significant correspondence with their homologs in other plants. In this study, *CDF* with accession number JQ911665.1 and 1017 bp length,

Table 4. List of EST and TSA homologs of autonomous pathway genes.

Genes	AT gene locus	Act/Repr +/-	*Beta vulgaris* EST	*E*-Value	*Beta vulgaris* TSA	*E*-Value
FCA	AT4G16280	+	BQ595139	3E-22	JP512707	6E-31
FLD	AT3G10390	+	CV301493	4E-89	JP497669	0
FPA	AT2G43410	+	BQ586740	1E-16	JP526592	2E-55
FY	AT5G13480	+	BQ588779	3E-17	JP494345	0
LDL2	AT3G13682	+	CV301493	2E-95	JP497669	0
PEP	AT3G04610	+	BQ586739	3E-66	JP511866	7E-62
RBBP4	AT2G19520	+	EG550040	2E-99	JP519422	2E-31
SKB1	AT4G31120	+	BQ589933	6E-76	JP519223	0

Table 5. List of EST and TSA homologs of gibberellin pathway genes.

Genes	AT gene locus	Act/Repr +/-	*Beta vulgaris* EST	*E*-Value	*Beta vulgaris* TSA	*E*-Value
AtMYB33	AT5G06100	+	EG551357	6E-57	JP485273	2E-42
DDF1	AT1G12610	+	BQ594833	2E-29	JP519293	2E-47
GA1	AT1G14920	-	BQ594788	1E-28	JP521140	0
RGA	AT2G01570	-	BQ594875	5E-32	JP521140	0
SPY	AT3G11540	-	BQ593497	3E-96	JP487268	0

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Table 6. List of EST and TSA homologs of other pathway flowering genes.

Genes	AT gene locus	Act/Repr +/-	*Beta vulgaris* EST	*E*-Value	*Beta vulgaris* TSA	*E*-Value
AGL24	AT2G22540	-	FG343252	7E-40	JP484672	2E-43
AP2	AT4G36920	-	FG344915	6E-35	JP493583	6E-102
HRB1	AT5G49230	+	BQ594772	3E-39	JP515307	3E-32
PFT1	AT1G25540	+	None		JP521069	9E-45

Table 7. List of EST and TSA homologs of floral integrators.

Genes	AT gene locus	*Beta vulgaris* EST	*E*-Value	*Beta vulgaris* TSA	*E*-Value
SOC1	AT2G45660	BQ488304	4E-33	JP513580	1E-67
AP1	AT1G69120	BQ584393	1E-39	JP498298	2E-71

COP1 with accession number JQ714253.1 and 1422 bp length, *CLF* with accession number JQ678603.1 and 1458 bp length, and *SUF4* with accession number JQ911666.1 and 1106 bp length were deposited in Gene bank database.

For identification of flowering pathway genes, EST homologs of 118 *Arabidopsis* flowering time genes were analysed and 236 EST homologs were identified for 70 genes. 118 flowering time genes in *Arabidopsis* were also identified in sugar beet TSA database using tBLASTx algorithm. At this stage, 230 TSAs with significant similarities were identified for 103 flowering time genes. Through joining overlapped EST and TSA sequences, flowering gene regions could be identified in sugar beet. These ESTs and TSAs homologous sequences made 17 longer sequences which showed significant similarities with their homologs in Gene Bank database (Table 8). It is suggested that these 17 long fragments are parts of *B. vulgaris* flowering genes. The sequences of 12 *B. vulgaris* fragments were shorter than their *Arabidopsis* homologs, and for 5 *B. vulgaris* fragments, the sequences were longer than *Arabidopsis* homologs. This may be owing to the differences between *A. thaliana* and *B. vulgaris* in the terms of alternative splicing or sequencing errors. This may also arise due to assembling different copies of sugar beet flowering genes.

We have shown that most of the central genes in *Arabidopsis* flowering pathway can be detected in sugar beet. Although new regulation mechanisms such as the role of *FT1* have been reported in sugar beet (Pin *et al.*, 2010) which illustrate the difference in flowering gene performance in sugar beet and *Arabidopsis*. In this study, genetic components of the flowering pathways in sugar beet were identified. However, for some genes, no homologs were identified which may be due to their low expression.

Transcript sequences of the flowering genes including *CDF*, *COP1*, *CLF*, and *SUF4* were also identified. A BLASTn search with these four genes resulted in several hits with *Expect* values < 10^{-8} (Table 9). These four genes were specifically expressed in sugar beet before flowering (Yanagisawa, 2002; Nakagawa and Komeda, 2004; Kim and Michaels, 2006; Jang *et al.*, 2008). To confirm their own conserved domains, translated protein of these four nucleotide sequences were evaluated. The *CDF* DOF transcription factors are essential for a photoperiodic flowering response. The DOF family has 26 members (Yanagisawa, 2002) in which only 5 members cause a strong delay in flowering under long days and the remaining 21 have no influence on flowering (Fornara *et al.*, 2009). These five proteins belong to a

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Table 8. Specifications of the overlapped ESTs and TSAs for flowering genes in sugar beet.

Genes	Gene length (bp)	ESTs and TSAs	Sequence length (bp)	Acssesion Number	*E*-Value (BLASTn)	*E*-Value (tBLASTx)
ARP6	1474	JP532887 JP528785	1353	NM_114070	1E-96	3E-106
ATX1	1243	BQ594744 BQ594830	643	NM_112335	2E-24	2E-48
CCA1	2268	CF543189 CF543190 FG343382	702	NM_180129	2E-63	5E-42
ELF8	3579	BQ583923 BQ583819 JP529671	773	NM_126631	3E-88	1E-86
FLK	1819	JP511866 BQ586739	2377	AY849999	2E-31	1E-61
FRL1	1413	BQ589554 BQ589570 BQ589767	833	NM_113143	6E-139	2E-111
GI	4136	FG345609 BQ589847 BQ490109	809	NM_102124	1E-167	5E-121
LHY	1377	BQ591669 JP491093 JP491094	998	NM_001036746	7E-84	7E-79
PRR2	2161	BQ594416 JP490185	2350	NM_179073		4E-115
SEC	3337	BQ488277 BQ588706	1072	NM_111295	0	0
SEF1	652	BQ584105 JP530923 CV301292	908	NM_123064	1E-54	2E-49
SPY	3788	BQ593497 JP487268	3124	NM_111987	0	0
SRR1	1402	FG344833 JP523485	1464	NM_125348	3E-34	1E-58
SWN1	1348	BQ583122 BQ488304	593	M55552	3E-67	7E-42
PRR1	2707	BQ589097 BI543444 BI543434 JP506937 JP506938	3081	NM_125531	4E-93	7E-93
VIP3	1176	BQ490245 BQ583526	586	NM_119129	6E-39	1E-56
VRN5	2662	BQ593505 JP537101	826	NM_119166	6E-21	9E-78

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Table 9. Comparison of the identified genes in sugar beet with their homologs in other plants.

Accession number	Sequence	*E*-Value
	CDF	
NM_114618	*CDF3* gene in *Arabidopsis*	3E-52
XM_003565574	*CDF3* gene in *Brachypodium distachyon*	6E-35
	COP1	
NM_001159010	*COP1* gene in *Zea mays*	4E-176
XM_003616828	*COP1* gene in *Medicago truncatula*	3E-146
	CLF	
XM_003611648	*CLF* gene in *Medicago truncatula*	3E-83
NM_127902	*CLF* gene in *Arabidopsis*	9E-65
	SUF4	
NM_102836	*SUF4* gene in *Arabidopsis*	1E-69
NM_001084165	*SUF4* gene in *Arabidopsis*	2E-67

phylogenetic group previously referred to as group II (Yanagisawa, 2002) or subfamily A (Moreno-Risueno *et al.*, 2007). In this group, *CDF2* and *CDF3* are the closest homologs of *CDF1*, and were shown to interact with *FKF1* and *LKP2* in yeast but not to delay flowering when expressed from the *CaMV 35S* promoter (Imaizumi *et al.*, 2005). CDF4 and CDF5 proteins are also located in this group (Fornara *et al.*, 2009). High expression of *CDF1*, *CDF2*, and *CDF3* in plant's phloem cause delay in flowering under long days. These proteins repress *CO* expression through joining *CO* promoter and inhibiting its transcription. In the absence of *CDF1*, *CDF2*, *CDF3*, and *CDF5*, *CO* expression increases dramatically during the day which indicates the role of these four genes in inhibition of *CO* expression during the day (Imaizumi, 2010).

COP1, CLF and *SUF4* are other transcript sequences of flowering genes identified in this study. *COP1* plays an important role in photoperiod pathway. To clarify the role of this gene in flowering, its mutants were analysed in darkness (Nakagawa and Komeda, 2004). COP1 is a negative regulator of the photomorphogenesis reaction since its mutants induce photomorphogenesis in darkness and in the absence of photoreceptors (Deng *et al.*, 1991). Its mutants also induce leaf production in the plant before flowering which highlights its role in flowering inhibition (Nakagawa and Komeda, 2004). COP1 and SPA proteins control CO protein accumulation (Schrader and Uhrig, 2013). CLF was initially known as a homeotic repressor of flowering (e.g., AGAMOUS) (Goodrich *et al.*, 1997). Homeotic genes have a key role in regulation of organism's development and any change in these genes will alter the developmental pattern. The role of *CLF* in repression of *FLC* and *FT* flowering promoter activities is identified in early flowering samples (Jang *et al.*, 2008). It also prevents *FLC* expression through vernalization (Wood *et al.*, 2006; Kim *et al.*, 2010). *SUF4* is located in vernalization pathway and its protein is involved in flowering delay. Even in the absence of this gene and in the presence of *FRI*, *FLC* expression down regulates. Mutation in *SUF4* does not impede *FLC* or *FLC*-like genes expression which illustrates its importance for *FLC* expression. SUF4 protein is a putative Zinc-finger-containing transcription factor which is essential for flowering delay in winter-annual *Arabidopsis*. It encodes a protein of 368 amino acids, the N-terminal end of which contains a BED-finger domain. The

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domain of the LIM family is located in its protein which influences the protein-protein interaction (Kim and Michaels, 2006).

CONCLUSIONS

Bolting tolerance is an important characteristic for autumn planting of sugar beet in Iran. In this study, the sequence region of *CLF, COP1, CDF* and *SUF4* genes was determined experimentally and homologs of *Arabidopsis* flowering genes were identified by bioinformatics analysis. These sequences are important for the identification of complete transcripts of flowering time genes in sugar beet.

Since the performing of this study, the sugar beet genome has been released and the complete genomic sequence of double haploid sugar beet line KWS2320 has been reported as reference genotype (Dohm *et al.,* 2104). The ESTs and TSAs databases are from experimental sequences of mRNAs from different genotypes. This point makes an opportunity to compare our finding with the complete genome of sugar beet.

Identified sequences can contribute to understanding of gene expression and their protein performance. They can also be used in the evaluation of genetic variation among sugar beet genotypes in terms of flowering and bolting resistance

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REFERENCES

1. Abbasi, Z., Arzani, A. and Majidi, M. M. 2014. Evaluation of Genetic Diversity of Sugar Beet (*Beta vulgaris* L.) Crossing Parents Using Agro-Morphological Traits and Molecular Markers. *J. Agr. Sci. Tech.*, **16:** 1397-1411.
2. Abou-Elwafa, S. F., Büttner, B., Chia, T., Schulze-Buxloh, G., Hohmann, U., Mutasa-Göttgens, E., Jung, C. and Müller, A. E. 2010. Conservation and Divergence of Autonomous Pathway Genes in the Flowering Regulatory Network of *Beta vulgaris*. *J. Exp. Bot.,* **62:**3359-3374.
3. Bakooie, M., Pourjam, E., Mahmoudi, S. B., Safaie, N. and Naderpour, M. 2015. Development of an SNP Marker for Sugar Beet Resistance/Susceptible Genotyping to Root-Knot Nematode. *J. Agr. Sci. Tech.,* **17:** 443-454.
4. Blázquez, M. A., Green, R., Nilsson, O., Sussman, M. R. and Weigel, D. 1998. Gibberellins Promote Flowering of Arabidopsis by Activating the LEAFY Promoter. *Plant Cell Online,* **10:** 791-800.
5. Boss, P. K., Bastow, R. M., Mylne, J. S. and Dean, C. 2004. Multiple Pathways in the Decision to Flower: Enabling, Promoting, and Resetting. *Plant Cell Online,* **16**: S18-S31.
6. Chia, T. Y. P., Müller, A., Jung, C. and Mutasa-Göttgens, E. S. 2008. Sugar Beet Contains a Large *CONSTANS-LIKE* Gene Family Including a CO Homologue that Is Independent of the Early-Bolting (B) Gene Locus. *J. Exp. Bot.,* **59:** 2735-2748.
7. Dally, N., Xiao, K., Holtgrawe, D., and Jung, C., 2014. The B2 Flowering Time Locus of Beet Encodes a Zinc Finger Transcription Factor, *Proc. Natl. Acad. Sci. USA 111*, 10365-10370.
8. Deng, X. W., Caspar, T. and Quail, P. H. 1991. COP1: A Regulatory Locus Involved in Light-Controlled Development and Gene Expression in Arabidopsis. *Genes Dev.*, **5:** 1172-1182.
9. Dohm, J. C., Minoche, A. E., Holtgrawe, D., Capella-Gutierrez, S., Zakrzewski, F., Tafer, H., Rupp, O., Sorensen, T. R., Stracke, R., Reinhardt, R., Goesmann, A., Kraft, T., Schulz, B., Stadler, P. F., Schmidt, T., Gabaldon, T., Lehrach, H., Weisshaar, B., and Himmelbauer, H., 2014. The Genome of the Recently Domesticated Crop Plant Sugar Beet (*Beta vulgaris*), *Nature* **505**: 546-549.
10. Fornara, F., Panigrahi, K. C. S., Gissot, L., Sauerbrunn, N., Rühl, M., Jarillo, J. A. and Coupland, G. 2009. Arabidopsis DOF Transcription Factors Act Redundantly to Reduce Constans Expression and Are Essential for a Photoperiodic Flowering Response. *Dev. Cell,* **17:** 75-86.

[DOR: 20.1001.1.16807073.2017.19.3.11.0]

11. Goodrich, J., Puangsomlee, P., Martin, M., Long, D., Meyerowitz, E. M. and Coupland, G. 1997. A *Polycomb-Group* Gene Regulates Homeotic Gene Expression in Arabidopsis. *Nature*, **386**: 44-51.
12. Herwig, R., Schulz, B., Weisshaar, B., Hennig, S., Steinfath, M., Drungowski, M., Stahl, D., Wruck, W., Menze, A., O'Brien, J., Lehrach, H. and Radelof, U., 2002. Construction of a 'Unigene' cDNA Clone Set by Oligonucleotide Fingerprinting Allows Access to 25000 Potential Sugar Beet Genes. *Plant J.,* **32:** 845-857.
13. Imaizumi, T. 2010. Arabidopsis Circadian Clock and Photoperiodism: Time to Think about Location. *Curr. Opinion Plant Biol.,* **13:** 83-89.
14. Imaizumi, T., Schultz, T. F., Harmon, F. G., Ho, L. A. and Kay, S. A. 2005. FKF1 F-Box Protein Mediates Cyclic Degradation of a Repressor of Constans in Arabidopsis. *Sci.,* **309**: 293-297.
15. Jang, S., Marchal, V., Panigrahi, K. C. S., Wenkel, S., Soppe, W., Deng, X. -W., Valverde, F. and Coupland, G. 2008. Arabidopsis COP1 Shapes the Temporal Pattern of CO Accumulation Conferring a Photoperiodic Flowering Response. *EMBO J.,* **27:** 1277-1288.
16. Johanson, U., West, J., Lister, C., Michaels, S., Amasino, R. and Dean, C. 2000. Molecular Analysis of FRIGIDA, a Major Determinant of Natural Variation in Arabidopsis Flowering Time. *Sci.,* **290**: 344-347.
17. Jordan, B. R. 2006. The Molecular Biology and Biotechnology of Flowering. CABI.
18. Jung, C. and Muller, A. 2009. Flowering Time Control and Applications in Plant Breeding. *Trend. Plant Sci .,***14:** 563 - 573.
19. Jung, S. and Main, D. 2014. Genomics and Bioinformatics Resources for Translational Science in Rosaceae. *Plant Biotech. Rep.,* **8:** 49-64.
20. Kim, D. -H., Zografos, B. R. and Sung, S. 2010. Mechanisms Underlying Vernalization-Mediated VIN3 Induction in Arabidopsis. *Plant Signal. Behav.,* **5:** 1457-1459.
21. Kim, S. Y. and Michaels, S. D. 2006. Suppressor of FRI 4 Encodes a Nuclear-Localized Protein that Is Required for Delayed Flowering in Winter-Annual Arabidopsis. *Dev.,* **133:** 4699-4707.
22. Langridge, J. 1957. Effect of Day-Length and Gibberellic Acid on the Flowering of Arabidopsis. *Nature,* **180:** 36-37.
23. Li, D., Liu, C., Shen, L., Wu, Y., Chen, H., Robertson, M., Helliwell, C. A., Ito, T., Meyerowitz, E. and Yu, H. 2008. A Repressor Complex Governs the Integration of Flowering Signals in Arabidopsis. *Dev. Cell,* **15**: 110-120.
24. Michaels, S. D. and Amasino, R. M. 1999. Flowering Locus C Encodes a Novel MADS Domain Protein That Acts as a Repressor of Flowering. *Plant Cell Online*, **11**: 949-956.
25. Michaels, S. D. and Amasino, R. M. 2000. Memories of Winter: Vernalization and the Competence to Flower. *Plant Cell Environ.,* **23:** 1145-1153.
26. Moreno-Risueno, M., Martínez, M., Vicente-Carbajosa, J. and Carbonero, P. 2007. The Family of DOF Transcription Factors: From Green Unicellular Algae to Vascular Plants. *Mol. Genet. Genom.,* **277:** 379-390.
27. Mouhu, K., Hytonen, T., Folta, K., Rantanen, M., Paulin, L., Auvinen, P. and Elomaa, P. 2009. Identification of Flowering Genes in Strawberry, a Perennial SD Plant. *BMC Plant Biol.*, **9:** 122.
28. Nakagawa, M. and Komeda, Y. 2004. Flowering of Arabidopsis COP1 Mutants in Darkness. *Plant Cell Physiol.,* **45:** 398-406.
29. Pin, P. A., Benlloch, R., Bonnet, D., Wremerth-Weich, E., Kraft, T., Gielen, J. J. L. and Nilsson, O. 2010. An Antagonistic Pair of FT Homologs Mediates the Control of Flowering Time in Sugar Beet. *Sci.,* **330**: 1397-1400.
30. Pin, P. A., Zhang, W., Vogt, S. H., Dally, N., Büttner, B., Schulze-Buxloh, G., Jelly, N. S., Chia, T. Y., Mutasa-Göttgens, E. S. and Dohm, J. C. 2012. The Role of a Pseudo-Response Regulator Gene in Life Cycle Adaptation and Domestication of Beet. *Curr. Biol.,* **22:** 1095-1101.
31. Reeves, P. A., He, Y., Schmitz, R. J., Amasino, R. M., Panella, L. W. and Richards, C. M. 2007. Evolutionary Conservation of the Flowering Locus C-Mediated Vernalization Response: Evidence from the Sugar Beet (*Beta vulgaris*). *Gene*., **176:** 295-307.
32. Schrader, A. and Uhrig, J. F. 2013. MIDGET Cooperates with COP1 and SPA1 to Repress Flowering in *Arabidopsis thaliana*. *Plant Signal. Behav*., **8:** e25600.

[DOR: 20.1001.1.16807073.2017.19.3.11.0]

33. Sheldon, C. C., Burn, J. E., Perez, P. P., Metzger, J., Edwards, J. A., Peacock, W. J. and Dennis, E. S. 1999. The FLF MADS Box Gene: A Repressor of Flowering in Arabidopsis Regulated by Vernalization and Methylation. *Plant Cell Online,* **11**: 445-458.
34. Wood, C. C., Robertson, M., Tanner, G., Peacock, W. J., Dennis, E. S. and Helliwell, C. A. 2006. The *Arabidopsis thaliana* Vernalization Response Requires a Polycomb-Like Protein Complex that also Includes Vernalization Insensitive 3. *Proceed. Nation. Acad. Sci.,* **103:** 14631-14636.
35. Yanagisawa, S. 2002. The DOF Family of Plant Transcription Factors. *Trend. Plant Sci.,* **7**: 555-560.

شناسایی ژن‌های کنترل‌کننده‌ی گلدهی در گیاه چغندرقند با استفاده از ژن های همولوگ آرابیدوپسیس

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چکیده

انتقال از رشد رویشی به دوره‌ی زایشی از تحولات مهم در زندگی گیاهان است. مسیرهای گلدهی شامل فتوپریود، ورنالیزاسیون، جیبرلین‌ها و مسیر خود انگیزی، تحت تاثیر ژن‌های مختلفی هستند. شناسایی عوامل ژنتیکی کنترل کننده‌ی گلدهی در گیاه چغندرقند، در تولید ارقام مقاوم به ساقه‌روی در این گیاه مهم است. ما در این تحقیق با استفاده از آنالیزهای بیوانفورماتیکی، شباهت توالی 118 ژن کنترل‌کننده‌ی گلدهی در گیاه آرابیدوپسیس با توالی‌های EST و TSA گزارش شده در چغندرقند را بررسی و توالی‌های چغندرقند را که شباهت معنی‌داری با ژن‌های کنترل‌کننده‌ی گلدهی در گیاه آرابیدوپسیس داشتند، شناسایی کردیم که احتمالاً بخش‌هایی از ژن‌های کنترل‌کننده‌ی گلدهی در چغندرقند می‌باشند. با سرهم کردن ESTهای شناسایی شده، بخش‌هایی از توالی ژن‌های کنترل‌کننده‌ی گلدهی در گیاه چغندرقند تعیین شد. بر اساس توالی ESTهای بدست آمده، برای ژن‌های *SUF4*، *CLF*، *COP1* و *CDF* چغندرقند آغازگرهایی طراحی شد. ژن‌های *SUF4* و *CLF* در مسیر گلدهی بهاره‌سازی و ژن‌های *COP1* و *CDF* در مسیر گلدهی تناوب نوری قرار دارند. توالی این ژن‌ها با استفاده از تکنیک cDNA-PCR تکثیر و با سایر ژن‌های شناسایی شده موجود در پایگاه داده Genebankمقایسه شدند. توالی ژن‌های *SUF4*، *CLF*، *COP1* و *CDF* مربوط به گیاه چغندرقند در پایگاه اطلاعاتی NCBI ثبت شد. مشخص شد که بیشتر ژن‌های مسیر گلدهی در آرابیدوپسیس، در چغندرقند نیز قابل شناسایی هستند. توالی‌های شناسایی شده در این تحقیق ممکن است به فهم کنترل ژنتیکی مقاومت به ساقه روی در چغندرقند کمک کند.

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