# 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 genes inhibiting flowering pathway SUPPRESSOR expression including OF**OVEREXPRESSION** OFCONSTANS1 (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 (FLD), D FLOWERING LOCUS PA (FPA), FLOWERING LOCUS VE(FVE), FLOWERING LOCUS Y (FY), (LD)LUMINIDEPENDENS 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 downregulated 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<sup>-8</sup> *E*-

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

**Table 1**. Information on the designed Primers.

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 LIGHT REDUCED1 (SRR1), RED PHYTOCLOCK1 (PCL1), LUX (LUX),ARRHYTHMO 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 recognized. were 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

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



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

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

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	<i>E</i> -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	<i>E</i> -Value	Beta vulgaris TSA	<i>E</i> -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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Genes	AT gene locus	Act/Repr +/-	Beta vulgaris EST	<i>E</i> - 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 6.** List of EST and TSA homologs of other pathway flowering genes.

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

Genes	AT gene locus	Beta vulgaris EST	<i>E</i> -Value	Beta vulgaris TSA	<i>E</i> - 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

Genes	Gene length (bp)	ESTs and TSAs	Sequence length (bp)	Acssesion Number	<i>E</i> -Value (BLASTn)	<i>E</i> -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	998	NM_001036746	7E-84	7E-79
PRR2	2161	JP491094 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 IP537101	826	NM_119166	6E-21	9E-78

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

Accession number	Sequence	<i>E</i> -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	

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

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

## CONCLUSIONS

Bolting tolerance is important an 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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## شناسایی ژنهای کنترل کنندهی گلدهی در گیاه چغندرقند با استفاده از ژن های همولوگ آرابیدوپسیس

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چکیدہ

انتقال از رشد رویشی به دورهی زایشی از تحولات مهم در زندگی گیاهان است. مسیرهای گلدهی شامل فتویریود، 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 کنترل کننده ی گلدهی در گیاه آرابیدویسیس با توالی های EST و TSA گزارش شده در چغندرقند را بررسی و توالیهای چغندرقند را که شباهت معنیداری با ژنهای کنترلکنندهی گلدهی در گیاه آرابیدویسیس داشتند، شناسایی کردیم که احتمالاً بخش هایی از ژنهای کنترلکنندهی گلدهی در چغندرقند می باشند. با سرهم کردن ESTهای شناسایی شده، بخش هایی از توالی ژن های کنترل کنندهی گلدهی در گیاه چغندرقند تعیین شد. بر اساس توالی ESTهای بدست آمده، برای ژنهای SUF4 CDF، CDF 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 Genebankمقايسه شدند. توالي ژنهاي SUF4، CDF، و CDF و CDF مربوط به گياه چغندرقند در بانگاه اطلاعاتی NCBI ثبت شد. مشخص شد که بیشتر ژنهای مسیر گلدهی در آراییدویسیس، 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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