

Vernalisation mediated lncRNA-like gene expression in *Beta vulgaris*

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Abstract. Sugar beet (*Beta vulgaris* L.) cannot form reproductive shoots during the first year of their life cycle. Flowering only occurs if plants are vernalised and are subsequently exposed to long days. However, the vernalisation mechanism remains poorly understood in sugar beet. Three putative lncRNAs associated with vernalisation (*AGL15X1*, *AGL15X2* and *CAULIFLOWER A*) were investigated and the hypothesis that their expression occurred in response to vernalisation was experimentally tested. The regulation mechanisms of *BvRAVI-like*, lncRNA-like genes, *BvFT1* and *BvFT2* were also examined. The *BvRAVI-like* gene associated with vernalisation in sugar beet was validated for the first time. Our data confirmed the hypothesis that *AGLX2* was the first candidate lncRNA of sugar beet and the *BvRAVI-like* gene was expressed in response to vernalisation. *BvRAVI-like* and *AGLX2* genes might be coordinated with *BvFT2* to promote reproductive growth by repressing *BvFT1* during cold exposure followed by long day conditions. A new complementary flowering model of sugar beet was proposed. Our findings opened up new possibility for future studies and further illuminated the molecular mechanism of vernalisation in sugar beet.

Additional keywords: *BvRAVI-like*, *COOLAIR*, day length, flowering, sugar beet.

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Introduction

A predominant developmental transition of plant species is the transformation between vegetative and reproductive growth. Vernalisation plays a crucial role in this process. The most clearly cold-responsive model of *Arabidopsis thaliana* (L.) has been well explained, especially the *FLOWERING LOCUS C* (*FLC*) molecular mechanism. Sugar beet (*Beta vulgaris* L.) is a biennial root crop that requires vernalisation to initiate the transition from vegetative to reproductive growth. Sugar beet is a member of the Amaranthaceae family that evolved from the monocot–dicot split ~140 million years ago (Dally *et al.* 2014). Two *FLOWERING LOCUS T* (*FT*) genes such as *BvFT1* and *BvFT2* of *B. vulgaris* had evolved antagonistic functions. *BvFT2* was functional conserved comparing with *FT* in *A. thaliana* that promoted the floral transition, whereas *BvFT1* suppressed floral transition and was downregulated by the vernalisation response (Pin *et al.* 2010). The transcriptome-scale analysis of two sugar beet cultivars found a *RAVI-like* gene that was upregulated by vernalisation, which further suggested that *BvRAVI-like* participated in the vernalisation-induced reproductive growth processes (Mutasa-Göttgens *et al.* 2012); however, this study did not include experimental tests. Several pathways were found to control the flowering transformation and the gene expression of the floral repressor *FLC* (Michaels and Amasino 1999; Sheldon *et al.* 2000). Vernalisation accelerated flowering through the epigenetic silencing of *FLC* that was induced by

cold exposure (Michaels and Amasino 1999; Sheldon *et al.* 1999).

In parallel with vernalisation, the second pathway repressed *FLC* expression has also been proposed (Sheldon *et al.* 2000). Noncoding RNAs (ncRNAs) were commonly regarded as RNAs that included small ncRNAs (<200 nt) and long ncRNAs (lncRNAs, >200 nt) whose functions did not translate into proteins (Li *et al.* 2015). The function of lncRNAs were based on their essential ‘double stem-and-loop’ secondary structure that was formed by less than 100 nucleotides (Zhao *et al.* 2010). lncRNAs were a less studied group of ncRNAs, of which only a small set were known to function in plants (Li *et al.* 2015). Plant lncRNAs played key roles in flowering time, gene silencing and reproduction (Berry and Dean 2015). The first clearly described lncRNA in plants was *COOLAIR*. Antisense transcription of *COOLAIR* in *A. thaliana* was induced by vernalisation to transiently suppress *FLC* sense transcription (Swiezewski *et al.* 2009). The long antisense RNA group *COOLAIR* expressed at the *FLC* locus and played a dominant role in silencing *FLC* expression (Liu *et al.* 2010; Marquardt *et al.* 2014; Wang *et al.* 2014a). *COOLAIR* was alternatively polyadenylated, including a proximal poly(A) (polyadenylic acid) site and a distal poly(A) site, similar to many transcripts in the *A. thaliana* genome (Wu *et al.* 2011). *COOLAIR* transcription was regulated by vernalisation and increased to a peak after 2–3 weeks of cold exposure (Swiezewski *et al.*

2009). *COOLAIR* significantly accelerated *FLC* transcriptional repression and was physically associated with *FLC* chromatin states during cold exposure (Csorba *et al.* 2014).

LncRNAs have been increasingly explored in plant species but little is known about their roles in sugar beet. In this study, we investigated three highly similar lncRNAs of sugar beet and hypothesised that they were lncRNA-like genes associated with vernalisation. We have validated the hypothesis and investigated the regulation mechanisms of these lncRNAs, *BvRAVI-like*, *BvFT1* and *BvFT2* during the development of sugar beet under long day conditions (LDs), which further illuminated the molecular mechanism of vernalisation. We also proposed a new complementary flowering model for sugar beet.

Materials and methods

Plant growth conditions and vernalisation treatment

The cultivated sugar beet (*Beta vulgaris* L.) line DY14-O was used for plant materials throughout this study. Fifty sugar beet roots were cut into two equal sections from the shoot apical meristems. Vernalisation treatment was performed on one section for 16 weeks while the other section was not treated. Cold treatment was at 4°C in darkness for 16 weeks, and then the vernalised and nonvernalised samples were transferred into the soil, where the temperature increased from 18–28°C under LDs. Leaves were harvested from the vernalised samples at five time points: 1 day of vernalisation (V-1D), 16 weeks of vernalisation (V-16W), 1 week of devernalisation (Dv-1W), bolting and flowering, and the corresponding time points for the nonvernalised samples: 1 day of nonvernalisation, 16 weeks

of nonvernalisation (Nonv-16W), 17 weeks of nonvernalisation, 19 weeks of nonvernalisation (equivalent to bolting) and 20 weeks of nonvernalisation (equivalent to flowering).

Quantitative real-time PCR

Total RNA was isolated using the MiniBEST Plant RNA Extraction Kit (Takara). For each biological replicate, materials from five plants were pooled to form a single sample for RNA purification (Cartagena *et al.* 2008). For quantitative real-time PCR, 3 µg of total RNA was used to synthesise cDNA with the PrimeScript™ II cDNA Synthesis Kit (Takara). Three independent biological and three technical replicates of each sample were performed and analysed. Real-time PCR was performed using the SYBR Premix Ex Taq™ Kit (Takara) on a CFX96 Fast Real-Time PCR Amplifier (Bio-Rad) with a final reaction volume of 20 µL. The comparative CT method (Schmittgen and Livak 2008) was used to analyse the results. Relative expression levels were calculated and normalised to the geometric mean of *BvICDH*.

Phylogenetic analysis of *COOLAIR* in *Beta vulgaris*

The entire primary sequence of *A. thaliana COOLAIR* (Swiezewski *et al.* 2009; Heo and Sung 2011) was used for a BLAST search in the *B. vulgaris* database of the National Center for Biotechnology Information (www.ncbi.nih.gov, accessed 11 April 2017). We identified the highly conserved regions (*E-value* < 10⁻⁵) (Wang *et al.* 2014b; Table 1) and then used the ClustalW (Medici *et al.* 2015) alignment of eight sequences to construct a phylogenetic tree (Fig. 1).

Table 1. The results of BLAST analysis in the *B. vulgaris* database using the *COOLAIR* gene of *A. thaliana*. Data are based on *A. thaliana* paralogy information. The last column lists the size of the conserved regions (CRS)

Gene	Chromosome	Identity (%)	E-value	Accession	CRS
<i>CAULIFLOWER A</i>	<i>Bvchr5</i>	70	6.00 × 10 ⁻¹⁰	XM_010680062	140 bp
<i>AGL15X2</i>	<i>Bvchr2</i>	73	4.00 × 10 ⁻⁶	XM_010696453	91 bp
<i>AGL15X1</i>	<i>Bvchr2</i>	73	4.00 × 10 ⁻⁶	XM_010696448	91 bp
<i>CMB1-like</i>	<i>Bvchr6</i>	69	6.00 × 10 ⁻¹⁰	XM_010682823	163 bp
<i>23-like</i>	<i>Bvchr9</i>	70	6.00 × 10 ⁻¹⁰	XM_010693149	121 bp
<i>SEPALLATA1 X3</i>	<i>Bvchr2</i>	70	4.00 × 10 ⁻⁶	XM_010695832	121 bp
<i>SEPALLATA1 X2</i>	<i>Bvchr2</i>	70	4.00 × 10 ⁻⁶	XM_010695823	121 bp
<i>SEPALLATA1 X1</i>	<i>Bvchr2</i>	70	4.00 × 10 ⁻⁶	XM_010695815	121 bp

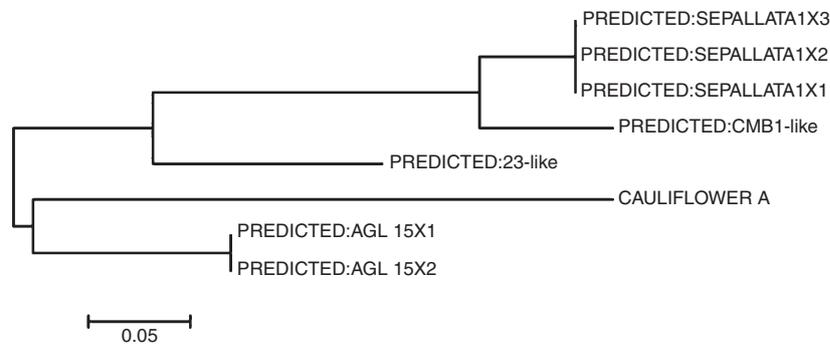


Fig. 1. Phylogenetic tree based on maximum likelihood analysis of eight sequences compared with *Arabidopsis thaliana COOLAIR* in *B. vulgaris*.

Secondary structure prediction and primer design

Three genes were selected on the basis of detailed analysis of the phylogenetic tree (Fig. 1). The secondary structures were predicted by RNAfold (<http://mfold.rna.albany.edu/?q=mfold/RNAFolding-Form>). We selected the most significant sequences that were similar to *A. thaliana* *COOLAIR* (Fig. S1 (HG975389) and Fig. S2, available as Supplementary Material to this paper) and designed primers with Primer ver. 5.0 software (Liang *et al.* 2004) (Table 2).

The vernalisation markers in sugar beet

BvFT2 was repressed by *BvFT1* function as a floral repressor that was downregulated by the vernalisation response (Pin *et al.* 2010). Another gene associated with vernalisation, *BvRAV1-like*, was explored via transcriptome-scale analysis (Mutasa-Göttgens *et al.* 2012), which found that vernalisation induced reproductive growth in sugar beet (Table 3).

Results

Three lncRNA-like genes, *BvRAV1-like*, *BvFT1* and *BvFT2* of *B. vulgaris*, and primers

Table 1 showed the sequence orthologues of *A. thaliana* *COOLAIR* that were explored on the National Center for Biotechnology Information website. We obtained three genes that were predicted to be orthologues of *A. thaliana* *COOLAIR* such as *AGL15X1*, *AGL15X2* and *CAULIFLOWER A* whose conserved regions were 91 bp, 91 bp and 140 bp respectively

and delimited on chromosome 2 and chromosome 5. The functions of the three lncRNA-like genes and *BvRAV1-like*, and their primer sequences designed with Primer ver. 5.0 are listed in Table 2. *BvFT1* and *BvFT2* were reported to regulate flowering in the vernalisation response (Table 3).

Secondary structure prediction and expression analysis of the three lncRNA-like genes

COOLAIR was found to be associated with vernalisation and its cold-sensing mechanism might be associated with the relationship between the antisense transcript-covered domain of *COOLAIR* and the FLC locus in *A. thaliana* (De Lucia and Dean 2011). The 'double stem-and-loop' structure of lncRNA was the key domain in its function and the 'double stem-and-loop' was consisted of less than 100 nucleotides. We predicted the secondary structure of the *AGL15X1*, *AGL15X2* and *CAULIFLOWER A* genes (Fig. S2) in sugar beet by using BLAST analysis and RNAfold (<http://mfold.rna.albany.edu/?q=mfold/RNAFolding-Form>) on the basis of *A. thaliana* *COOLAIR* (Fig. S1). The conserved structural motifs of the lncRNA-like genes are listed in Fig. S2. The structural motifs found in *B. vulgaris* were orthologues of *A. thaliana*, which suggested that *AGL15X1*, *AGL15X2* and *CAULIFLOWER A* genes might be functional lncRNA candidates. We examined the expression levels of the three lncRNA-like genes under different environmental stresses and time points by quantitative real-time PCR. The greatest level of *AGL15X2* expression was observed at time point V-16W, and then

Table 2. Sequences of oligonucleotides synthesised specifically to amplify the genes that were found to be highly similar to *A. thaliana* *COOLAIR* in *B. vulgaris* database and the *BvRAV1-like* gene (Mutasa-Göttgens *et al.* 2012) F, forward; R, reverse; MADS, is an acronym for the four founder proteins MCM1 (from brewer's yeast, *Saccharomyces cerevisiae*), AGAMOUS (from *Arabidopsis*), DEFICIENS (from *Antirrhinum*), and SRF (a human protein); AP2, the homeotic gene APETALA2 (AP2); ERF, ethylene response factor

Gene	Gene product	Primer sequence (5' to 3')
CAULIFLOWER A	Truncated transcription factor	F: GGGAGAGGTAGGGTTCA R: GCTTGCCTTTAGTGGAG
AGL15X2	Agamous-like MADS-box protein AGL15X2 transcript variant X2	F: GTTGAATTGAAATGGGTC R: CAACTTCAGCATCACAAA
AGL15X1	Agamous-like MADS-box protein AGL15X1 transcript variant X1	F: AATGGGTCGTGGTAAGA R: AACAGCAACTTCAGCATC
<i>BvRAV1-like</i>	AP2/ERF and B3 domain-containing transcription factor RAV1-like	F: AGTCGTCCACAGCCT R: GCCCGAGTTCATCCT

Table 3. Sequences of oligonucleotides synthesised specifically to amplify the genes that respond to vernalisation in *B. vulgaris* and the reference gene

BvFT1, *BvFT2* and *BvICDH* were indicated by Pin *et al.* (2010). and *BvICDH* was the reference gene. F, forward; R, reverse; ISORT, GenBank accession number AF173666

Gene	Gene function	Primer sequence (5' to 3')
<i>BvFT1</i>	Flower repressor	F: GCATCATTGGAGAAGAGATTGTTTAC R: GCGTTGTGTGGAGCATTTA
<i>BvFT2</i>	Flower promoter	F: GTGCATCATTTGGCCAAGAAG R: TGAAGTTTTGACGCCACCCT
<i>BvICDH</i>	ISORT isocitrate dehydrogenase	F: CACACCAGATGAAGGCCGT R: CCCTGAAGACCGTGCCAT

decreased sharply during the bolting and flowering periods after they were transferred to soil under LDs. However, the expression level of *AGL15X2* in the nonvernalised samples was not only lower than the vernalised samples but was almost unchanged at time points Nonv-1D, Nonv-16W and 17 weeks of nonvernalisation. Interestingly, *AGL15X2* expression level gradually decreased at the time points 19W and 20W in the nonvernalised samples (Fig. 2). We observed the upregulation of *AGLX1* expression, which reached a peak in both the vernalised and nonvernalised samples at time points V-16W and Nonv-16W, while the expression levels decreased sharply in both samples after they were transferred to soil under LDs (Fig. 3a). However, *CAULIFLOWER A* gene expression gradually decreased throughout the entire periods in the vernalised and nonvernalised samples in contrast to *AGLX1* and *AGLX2* (Fig. 3b).

Experimental validation of the *BvRAV1*-like gene

The *BvRAV1*-like gene's association with the vernalisation response was explored by transcriptome-scale analysis in sugar beet (Mutasa-Göttgens *et al.* 2012). The highest expression level of *BvRAV1*-like was observed at time point V-16W, which then decreased slightly at time point Dv-1W. However, the expression levels of *BvRAV1*-like were almost unchanged throughout all periods in the nonvernalised samples and these results were consistent with the transcriptome-scale analysis (Mutasa-Göttgens *et al.* 2012) (Fig. 4).

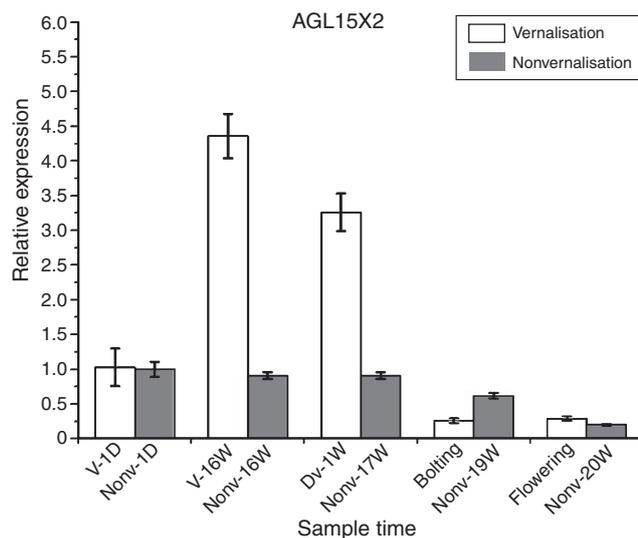


Fig. 2. The expression patterns of *AGL15X2* in sugar beet leaves were analysed by qRT-PCR at five different time points. 1 day of vernalisation (V-1D), equivalent to 1 day of nonvernalisation (Nonv-1D); 16 weeks of vernalisation (V-16W), equivalent to 16 weeks of nonvernalisation (Nonv-16W); 1 week of devernalisation (Dv-1W), equivalent to 17 weeks of nonvernalisation (Nonv-17W); Bolting equivalent to 19 weeks of nonvernalisation (Nonv-19W); Flowering equivalent to 20 weeks of nonvernalisation (Nonv-20W). The white bars denote vernalised samples and the grey bars denote the nonvernalised samples. *BvICDH* was used as the internal control. Error bars, mean \pm s.e. ($n=3$).

Functions of the three lncRNA-like genes, *BvRAV1*-like, *BvFT1* and *BvFT2* in sugar beet

Collectively, the three lncRNA-like genes, *BvRAV1*-like, *BvFT1* and *BvFT2* in the vernalised and nonvernalised samples are presented in Fig. 5a, b. The expression levels of *BvRAV1*-like, *AGLX1* and *AGLX2* reached their highest level at time point V-16W and *BvFT1* also reached a peak at this point. The expression levels of *BvRAV1*-like, *AGLX1* and *AGLX2* then decreased until the bolting period, while *BvFT2* increased

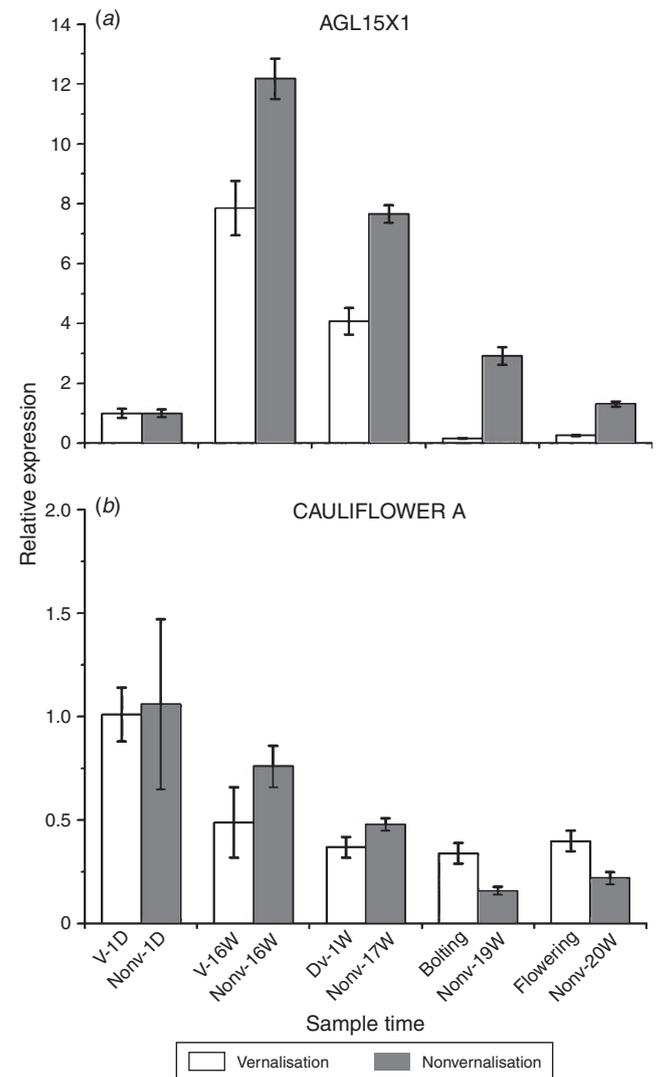


Fig. 3. Expression patterns of (a) *AGL15X1* and (b) *CAULIFLOWER A* in sugar beet leaves were analysed by qRT-PCR at five different time points. 1 day of vernalisation (V-1D), equivalent to 1 day of nonvernalisation (Nonv-1D); 16 weeks of vernalisation (V-16W), equivalent to 16 weeks of nonvernalisation (Nonv-16W); 1 week of devernalisation (Dv-1W), equivalent to 17 weeks of nonvernalisation (Nonv-17W); Bolting equivalent to 19 weeks of nonvernalisation (Nonv-19W); Flowering equivalent to 20 weeks of nonvernalisation (Nonv-20W). The white bars denote vernalised samples and the grey bars denote the nonvernalised samples. *BvICDH* was used as the internal control. Error bars, mean \pm s.e. ($n=3$).

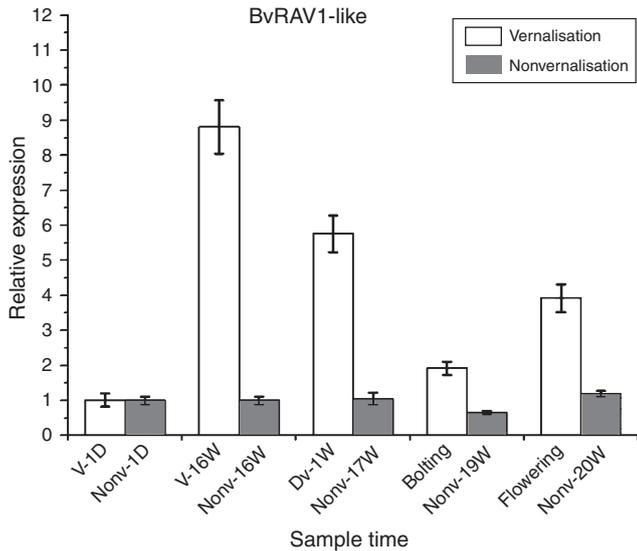


Fig. 4. Expression patterns of *BvRAV1-like* gene were analysed by qRT-PCR at five different time points, which validated the transcriptome-scale analysis. 1 day of vernalisation (V-1D), equivalent to 1 day of nonvernalisation (Nonv-1D); 16 weeks of vernalisation (V-16W), equivalent to 16 weeks of nonvernalisation (Nonv-16W); 1 week of devernalisation (Dv-1W), equivalent to 17 weeks of nonvernalisation (Nonv-17W); Bolting equivalent to 19 weeks of nonvernalisation (Nonv-19W); Flowering equivalent to 20 weeks of nonvernalisation (Nonv-20W). The white bars denote vernalized samples and the grey bars denote the nonvernalised samples. *BvICDH* was used as the internal control. Error bars, mean \pm s.e. ($n = 3$).

consistently in the vernalized samples (Fig. 5a; Fig. S3). The expression levels of *BvFT1* and *BvFT2* increased sharply after they were transferred to warm temperatures under LDs until the bolting period, and then *BvFT1* expression decreased dramatically, whereas *BvFT2* expression increased steadily (Fig. S4). At the same time, the *BvRAV1-like* expression level increased then decreased. We speculated that the *BvRAV1-like* gene initiated the *BvFT1* repression mechanism after bolting and indirectly promoted the upregulation of *BvFT2* expression in the vernalised materials (Fig. 5a). *AGL15X1* increased to its highest expression level and *BvFT1* increased at time point Nonv-16W (Fig. S3). *AGL15X1* decreased gradually in the following periods (Fig. 5b). The highest expression level of *BvFT1* in the nonvernalised samples was observed at time point 19 weeks, then it decreased dramatically alongside a slight increasing of *BvRAV1-like* expression under LDs (Fig. 5b). The expression levels of *AGL15X2* and *CAULIFLOWER A* were lower than the others and remained almost unchanged throughout the entire study periods (Fig. 5b). The lncRNA-like genes *AGL15X1* and *AGL15X2* might be repressed by LDs but vernalisation induced to increase in their transcriptions. The vernalisation gene *BvRAV1-like* combined with *BvFT2* to impair the transcription of *BvFT1* after 19 weeks in both the vernalised and nonvernalised samples. In our study, we found that *BvFT1* expression was induced by LDs, which was contrary to the study by Pin et al. (2010). A new complementary flowering model in sugar beet was proposed (Fig. 6).

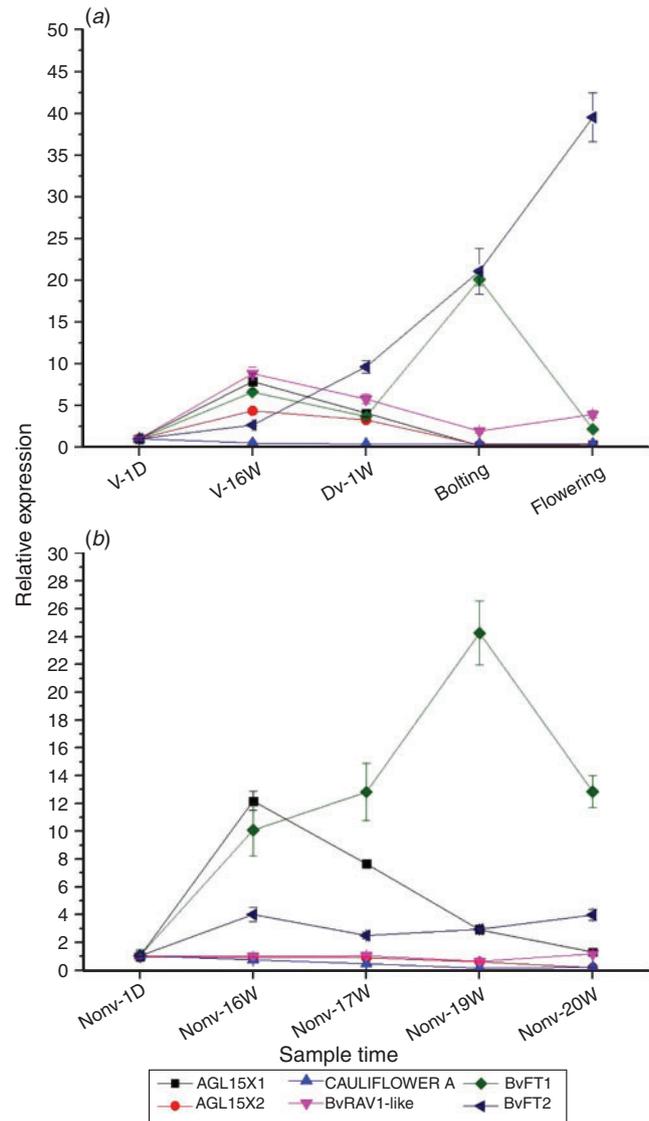


Fig. 5. Expression patterns of the three lncRNA-like genes, *BvRAV1-like*, *BvFT1* and *BvFT2* during the whole developmental processes in (a) vernalised and (b) nonvernalised sugar beet were analysed by qRT-PCR. *BvICDH* was used as the internal control. Error bars indicate the mean \pm s.e. ($n = 3$).

Discussion

In the model plant *A. thaliana*, *COOLAIR* (Fig. S1) upregulated expression across the entire *FLC* locus, in which antisense transcription occurred earlier and independently of other vernalisation markers that reduced the sense transcription of *FLC* (Swiezewski et al. 2009). *COOLAIR* was divided into the proximally polyadenylated Class I and the distally polyadenylated Class II by alternative polyadenylation and was also alternatively spliced in warm conditions (Swiezewski et al. 2009; Hornyik et al. 2010). We used the entire *COOLAIR* nucleotide sequence to query similarly conserved regions in the *B. vulgaris* database. Three lncRNA-like genes (Table 2) were

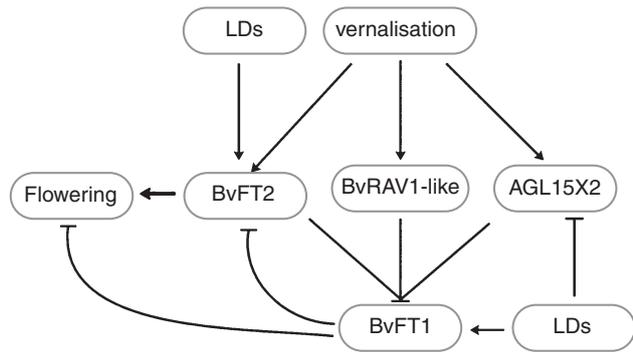


Fig. 6. Complementary flowering model of sugar beet. Lines with arrows indicate the induction of expression. Lines with a bar indicate repression. LDs, long day conditions.

explored by BLAST analysis and their secondary structure was computed (Fig. S2).

Gene expression analysis was conducted in vernalised and nonvernalised samples to identify lncRNA-like genes and to explore other molecular mechanisms. The highest expression level of *AGL15X2* was observed at time point V-16W, which decreased during the bolting and flowering periods after the plants were transferred to soil under LDs. However, the trend of expression in the nonvernalised plants was lower than the vernalised plants and the expression levels were almost unchanged throughout the whole development processes. The long noncoding antisense transcripts transiently accumulated then almost returned to a level similar to that of the nonvernalised plants when the plants were transferred to the warm after cold treatment (Swiezewski *et al.* 2009). Taken together with the secondary structure, our data confirmed the hypothesis that *AGL15X2* was a lncRNA candidate gene in *B. vulgaris*. However, *AGLX1* has been shown to be different from *AGL15X2*, although they have the same secondary structure. There might be other factors that influenced its action. *AGLX1* expression levels increased to a peak in the vernalised and nonvernalised samples at time point 16 weeks but decreased sharply in both samples after they were transferred to soil under LDs (Fig. 3a), which suggested that LDs might be directly affected *AGLX1* expression. However, *CAULIFLOWER A* expression levels gradually decreased throughout the whole periods both in the vernalised and nonvernalised samples in contrast to *AGLX1* and *AGLX2* (Fig. 3b), which indicated that it associated with neither vernalisation nor LDs, although it also had the ‘double stem-and-loop’ structure.

The transcriptome-scale analysis revealed an *AtRAV1* homologue, which was mapped to Locus 29 609 and was designated *BvRAV1-like*, the highly expressed transcripts of which are positively correlated with vernalisation. Further BLASTX analysis of *BvRAV1-like* indicated 49% sequence identity to the *A. thaliana RAV1*, which translated to over 256 amino acids with 66% similarity and an E-value of 4×10^{-68} (Mutasa-Göttgens *et al.* 2012). However, this study was not experimentally validated in sugar beet. In our study, we confirmed that the highest expression level of *BvRAV1-like* was observed in the vernalised samples via an experimental test. The expression levels were higher at Dv-1W and

flowering than in the nonvernalised samples. The expression levels were almost unchanged throughout all periods in the nonvernalised samples (Fig. 3), which further supported the transcriptome-scale analysis (Mutasa-Göttgens *et al.* 2012).

Taken together, the expression levels of *BvRAV1-like*, *AGL15X1* and *AGL15X2* reached a peak at time point V-16W then decreased. *BvFT1* reached the highest level at time point bolting (Fig. 5a). By contrast, the expression level of *BvFT2* increased consistently (Fig. S4) and *BvRAV1-like* upregulated slightly at time point flowering in the vernalised sugar beet (Fig. S3) (Fig. 5a). We speculated that *BvRAV1-like* and *BvFT2* coregulated *BvFT1* after bolting under LDs in the vernalised samples. *AGL15X1* and *AGL15X2* might be sensitive to photoperiod (Fig. 5a). *AGL15X1* reached to its greatest expression level and then decreased gradually in the nonvernalised samples under LDs, which might be indicated the influence by LDs. The highest peak of *BvFT1* expression was observed at time point 19 weeks of nonvernalised samples, and then decreased dramatically with a slight upregulation of *BvRAV1-like* and *BvFT2* under LDs, which was consistent with the vernalisation data (Fig. 5B). Investigations confirmed that *BvRAV1-like* and *BvFT2* initiated the repression mechanism of *BvFT1* both in the vernalised and nonvernalised samples under LDs (Fig. 6). Vernalisation might be modified *BvFT1* expression through *AGL15X2*, *BvRAV1-like* and *BvFT2*, resulting in the postvernalisation response under LDs. However, few lncRNAs have been explored in *B. vulgaris*. Future studies are essential to better understand the role of lncRNAs in *B. vulgaris*.

Conclusion

AGL15X, *AGL15X2* and *CAULIFLOWER A* were discovered by the BLAST search against the public database of *B. vulgaris*. *AGL15X2* was identified as a candidate lncRNA gene that was sensitive to LDs in *B. vulgaris*. *BvRAV1-like* expression level was indeed upregulated in response to vernalisation. A new complementary flowering model of sugar beet was proposed. New technologies will be essential for the exploration of lncRNAs because only a few lncRNAs have been explored in *B. vulgaris* and thus further studies will be necessary to elucidate the molecular mechanism(s) of vernalisation in sugar beet.

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