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RESEARCH PAPER

Expression analysis of key curding and flowering genes in diverse thermosensory groups of cauliflower

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Abstract

Cauliflower is a highly thermosensitive crop with complex developmental transitions that are intricately regulated by genotype-specific, environment-responsive genetic mechanisms. Understanding the genetic behavior of key genes is crucial for mitigating temperature factors in cauliflower production. Substantial differences in expression patterns were observed among the cauliflower genotypes. During curd initiation, Pusa Sharad displayed notable upregulation of *BoREM1*, *CCE1*, *BoFT*, and *BoVRN2* genes; Pusa Shukti showed increased expression of *BoREM1* and *BoFUL-d*; and in Pusa Snowball Kt-25, *BoREM1* and *CCE1* genes were remarkably upregulated. The expression of *BoLFY*, *BoCAL*, and *BoTFL1* genes was downregulated in all three genotypes at curd initiation. Interestingly, the expression of all genes was relatively lower in the temperate genotype Pusa Snowball Kt-25 compared to Indian types. Increased expression of *BoREM1*, *BoLFY*, and *BoFUL-d* genes at curd loosening in Pusa Sharad indicated their probable association with curd to bolting stage transition. The study holds the potential for further understanding the genetic regulation of curding and flowering, to expand the thermosensitive plasticity of cauliflower genotypes.

Keywords: *Brassica oleracea* var. *botrytis*, Meristem identity genes, MIGs, Curding, Flowering pathways, Gene expression.

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Introduction

The vegetative-to-reproductive growth transition is a critical phase in the life cycle of flowering plants. In the model plant *Arabidopsis*, extensive studies have revealed six major pathways deciding flowering time: photoperiod, vernalization, gibberellins, thermosensory (ambient temperature), age, and autonomous (Cheng *et al*., 2017). The aging pathway is to ensure flowering and seed production before plant death, even in the absence of floral inductive signals. It is largely regulated by two microRNAs (miRNAs), miR156 and miR172, and *SQUAMOUS PROMOTER BINDING PROTEIN-LIKE* (*SPL*) transcription factors (Ridge, 2012). Genes of the autonomous pathway promote flowering independently of the environment through repression of the central flowering repressor and vernalization regulatory gene *FLOWERING LOCUS C (FLC)*; they are, *LUMINIDEPENDENS* (*LD*), *FLOWERING LOCUS D* (*FLD*), *FLOWERING LOCUS PA (FPA), FLOWERING LOCUS CA (FCA), FLOWERING LOCUS Y (FY), and FLOWERING LOCUS VE* (*FVE)* (Simpson and Dean, 2002). The hormone pathway is predominantly governed by gibberellins (GAs), which promote flowering by upregulating the expression of the floral integrators, such as *LEAFY* (*LFY*) and *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*), via a *DELLA*-dependent mechanism (Moon *et al*., 2003; Achard *et al*., 2004). The photoperiod pathway is responsible for sensing day length in plants, which is achieved by

the association between components of light signal transduction pathways and circadian clock function. Major genes in this pathway are *CRYPTOCHROME2/FHA* (*CRY2*), *GIGANTEA* (*GI*), *FLOWERING LOCUS T* (*FT*), *CONSTANS* (*CO*), and *FLOWERING WAGENINGEN* (*FWA*) (Khan *et al*., 2014). Global warming has highlighted the importance of understanding the thermosensitivity or ambient temperature pathway critically regulated by *FLOWERING LOCUS M* (*FLM*) (Lutz *et al*., 2015). The vernalization pathway is under the control of two major dominant flowering repressor genes, *FRIGIDA* (*FRI*) and *FLC*. *FRI* delays flowering in winter-annual plants by promoting the expression of *FLC*, whereas the genomic deletion in the *FRI* allele fails to upregulate *FLC*, resulting in an early flowering phenotype in summer-annual plants (Kim, 2020).

Cauliflower (*Brassica oleracea* var. *botrytis* L., 2n = 2x = 18) is one of the crops with highly complex developmental transitions governed by a complex network of internal and external cues. In cauliflower, the transition from the vegetative to the reproductive stage is separated by the intermediate 'curd' stage (arrested inflorescence meristems), which defines flowering in two phases: first induction of flowering followed by subsequent 'bolting' of the curd to produce normal flowers (Ridge, 2012; Singh, *et al*. 2018; Singh *et al*., 2021). In India, based on temperature requirements for curd initiation and development, cauliflower is classified as early (20–27°C), mid-early (16–20°C), mid-late (12–16°C), and late group (10–16°C) (Sharma and Singh, 2003). The reproductive transition physiologies in these maturity groups of cauliflower require investigation to develop varieties with better curding plasticity and flowering. In *Brassica oleracea*, researchers have identified and studied homologs of *Arabidopsis* genes associated with curding and flowering time regulation (Duclos and Björkman, 2008; Matschegewski *et al*., 2015; Akter *et al*., 2021). Though *B. oleracea* orthologues of *Arabidopsis* flowering time genes play similar roles, research is still required to identify the behavior of these gene paralogues.

In cauliflower, allelic variation in *BoFLC2* and an insertion or deletion in the second intron of *BoFLC1* in cabbage has shown variations for flowering time (Ridge *et al*., 2015; Abuyusuf *et al*., 2019). In *Arabidopsis*, an increase in *LFY* expression and resultant suppression of *TERMINAL FLOWER 1* (*TFL1*) initiate flowering as a consequence of the upregulation of *APETALA 1* (*AP1*) and *CAULIFLOWER* (*CAL*), and homologs of these genes behaved similarly in *B. oleracea* as well (Duclos and Björkman, 2008). The prodigious role of these key flowering pathway genes necessitates the investigation of these genes to widen the curding and flowering plasticity of cauliflower. Mangal and Singh (2023) investigated 13 genes at two sowing time points and showed variable expression of genes in Indian and Snowball materials. However, the complex genetic regulation demands further supportive studies to establish the changes in genes governing developmental transitions. Hence, the present study was undertaken with representative varieties from all four maturity groups to understand these changes.

Materials and Methods

The expression pattern of key curding and flowering genes was studied in four representative varieties: Pusa Ashwini (early, curd initiation at 20–27°C), Pusa Sharad (mid-early, 16–20°C), Pusa Shukti (mid-late, 12–16°C), and PSB Kt-25 (late, 10–16°C). Genotypes were sown on October 30, 2022,

Table 1: Details of the genes selected for the study and their primer sequences

Gene	*Full form*	*Forward Primer (5'→3')*	*Reverse Primer (3'→5')*	*Reference*
BoREM1	*Brassica oleracea REPRODUCTIVE MERISTEM-1*	CCACGTTAAGTTTCCTTTTCAGTATTT	TGAGCCATGGAACCGAACA	Duclos and Björkman (2008)
CCE1	*CAULIFLOWER CURD EXPRESSION 1*	TCGTTCCACCACCTTCCAAA	ACGAGCCTGAAATGGTCTAAT	Duclos and Björkman (2008)
BoLFY	*Brassica oleracea LEAFY*	AGCGACTTTGGTTGGTGGTATT	AACCACAGCAACTCATGAACTAATTAA	Duclos and Björkman (2015)
BoCAL	*Brassica oleracea CAULIFLOWER*	AAACCGCAGCCACCATGTA	AAGGAGATGATCCATTAAGGA	Duclos and Björkman (2008)
BoTFL1	*Brassica oleracea TERMINAL FLOWER1*	CGTGAATTTGCGATCGAGAAT	TTTCTCTCTGAGCGTTGAAGAAGA	Duclos and Björkman (2008)
BoFT	*Brassica oleracea FLOWERING LOCUS T*	GCCAAAGAGAGGTGACAAATGG	CCAACCAATGGAGATATTCTCGT	Ridge *et al*. (2015)
BoVRN2	*Brassica oleracea VERNALIZATION 2*	TCGTCACAAGAGGAGGAGGT	AAAGGGAGCGAATGCGAAGA	Matschegewski *et al*. (2015)
BoFUL-d	*Brassica oleracea FRUITFUL-d*	TCGTCGTTGATTGAACCAAACT	AGTCACCAAAAAAGCTGATACATTATGA	Duclos and Björkman (2008)

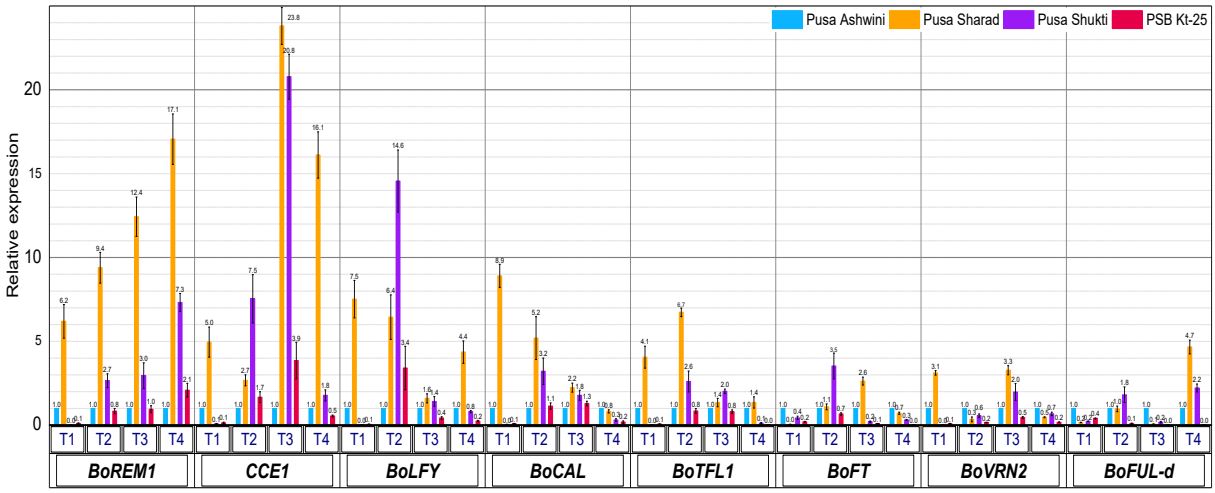

Figure 1: Relative expression of key curding and flowering genes at 35 DAP (T1), 55 DAP (T2), 75 DAP (T3), and 95 DAP (T4) in four varieties of cauliflower. Pusa Ashwini as calibrator

and the crop was raised as per recommended cultivation practices (Sharma and Singh, 2003). A total four time points, i.e., 35, 55, 75, and 95 days after planting (DAP), were chosen for the study. The samples were collected in liquid nitrogen and kept at -80°C until further processing. The study was conducted with three biological and three technical replications. RNA isolation was performed using Tri-Xtract (G Biosciences, Geno Technology, Inc., St. Louis, USA) following the manufacturer's guidelines. Qualitative estimation of RNA was done by gel electrophoresis using agarose gel (1.2%) in tris-acetate-EDTA (TAE) buffer and quantitative estimation using a NanoDrop spectrophotometer (MicroDigital, USA). Two micrograms of RNA from each sample were used for the cDNA synthesis by a Verso cDNA synthesis kit (Thermo Scientific, USA) as per the manufacturer's instructions. For substantiation of cDNA, PCR was performed to amplify the housekeeping gene *BoACTIN*, and cDNA was validated with gel electrophoresis of the amplified product performed using 3% agarose gel. From the publicly available gene sequences, eight specific genes were used for expression analysis (Table 1). The specific cDNA targets were amplified and detected through RT-qPCR (Bio-Rad, USA) using SYBR™ Green PCR Master Mix (Applied Biosystems™) as the fluorescent chemistry. 200 ng of cDNA was used as a template for 10 µL qPCR reactions, each containing 5 µL of SYBR green, 1-µL of the chosen gene primer, and 3 µL of nuclease-free water. The RT-qPCR program comprised enzyme activation at 95°C for 3 minutes, 40 cycles of denaturation at 95°C for 10 seconds; primer annealing and extension at 55°C for 30 seconds, and a final hold at 4°C. The assessment of relative gene expression was accomplished utilizing the ΔΔCt method. The normalization of gene ΔCt values was carried out using the internal control *BoGAPDH* (Glyceraldehyde 3-Phosphate Dehydrogenase), with Pusa Ashwini serving as the calibrator for relative gene expression. Also, the 35 DAP was taken as a calibrator for observing the relative change in respective varieties. The presented data represent the means ± standard deviation (SD) derived from three independent replicates for each variety. Data analysis was conducted using MS Excel, and graphical presentations were created using OriginPro2023b.

Results and Discussion

Understanding the genetic influence on a plant's shift from vegetative-curd-reproductive helps us understand the plant development process, and by altering prominent genetic factors, we can expand the cultivation horizon of *Brassica* crops. The expression pattern of eight genes was studied in Pusa Ashwini, Pusa Sharad, Pusa Shukti, and PSB Kt-25 at 35 DAP (T1), 55 DAP (T2), 75 DAP (T3), and 95 DAP (T4) considering Pusa Ashwini as a calibrator. Regarding developmental transitions, at T1 all varieties were at the young stage, at T2, Pusa Ashwini was at the full curd stage while others were at the adult stage (Table 2). At T3 Pusa Ashwini was at floral initiation, Pusa Sharad at curd initiation, while Pusa Shukti and PSB Kt-25 were still at the vegetative stage. At T4 Pusa Ashwini was at floral termination, Pusa Sharad had curd loosening, while Pusa Shukti and PSB Kt-25 were at curd initiation.

At the young stage (T1), all genes were downregulated in the mid-late genotype Pusa Shukti and late genotype PSB Kt-25 (Figure 1, Table 3). In contrast, except for *BoFT* and *BoFUL-d*, all genes were upregulated in the mid-early genotype Pusa Sharad. During the adult stage (T2), *BoTFL1*, *BoFT*, and *BoFUL-d* were upregulated in Pusa Shukti and Pusa Sharad, while *CCE1*, *BoLFY*, and *BoCAL* were upregulated in Pusa Shukti and PSB Kt-25 but downregulated in Pusa Sharad. At curd initiation, Pusa Sharad exhibited upregulation of *BoREM1*, *CCE1*, *BoFT*, and *BoVRN2* genes; *BoREM1* and *BoFUL-d* showed increased expression in Pusa

Table 2: Developmental stages of genotypes at corresponding time points

Variety	*35 DAP*	*55 DAP*	*75 DAP*	*95 DAP*
Pusa Ashwini	YS	FC	FI	FT
Pusa Sharad	YS	AS	CI	Curd loosening
Pusa Shukti	YS	AS	AS	CI
PSB Kt-25	YS	AS	AS	CI

DAT- Days After Transplanting, YS- Young Stage, AS- Adult Stage, CI- Curd Initiation, FC- Full Curd,
FI- Flower Initiation, FT- Flower Termination

Shukti; and *BoREM1* and *CCE1* genes were upregulated in Pusa Snowball Kt-25. Meanwhile, the expression of *BoLFY*, *BoCAL*, and *BoTFL1* genes was downregulated in all three genotypes during curd initiation. In Pusa Sharad, at the curd loosening stage (T4), *BoREM1*, *BoLFY*, and *BoFUL-d* genes were upregulated, while *BoFT* and *BoVRN2* were downregulated. The specific gene-wise expression across time points and its interpretation are presented under headings corresponding to each gene.

Brassica oleracea REPRODUCTIVE MERISTEM-1 (BoREM-1)

In Pusa Sharad, the *BoREM-1* gene expression was 6.2-fold at T1, and it continued to increase at T2, T3, and reached its highest at T4, with values of 9.4, 12.4, and 17.1 folds, respectively (Figure 1). In Pusa Shukti, there was no expression at T1, but it increased in T2 (2.7), had a slight change at T3 (3.0), and reached its highest at T4 (7.3). In PSB Kt-25, there was no expression at T1, followed by a slight expression lower than Pusa Ashwini at T2 (0.84). At T3, the expression values were almost similar to those of Pusa Ashwini, and they doubled at T4 (2.1). The *CCE1* and *BoREM1* genes are specifically associated with cauliflower curd. *BoREM1* is highly expressed during cauliflower curd development, promoting the proliferation of meristematic cells and increasing curd size (Franco-Zorrilla *et al*., 1999). In all the varieties, *BoREM1* increased with time points, reaching its maximum at curd initiation (T4) in Pusa Shukti, PSB Kt-25, and full curd stage (T4) in Pusa Sharad, indicating its strong relationship with curd growth and development.

CAULIFLOWER CURD EXPRESSION 1 (CCE1)

In Pusa Sharad, *CCE1* gene expression nearly halved from 5.0 folds at T1 to 2.7 folds at T2 (Figure 1). It then rose to about 24 folds at T3 and decreased to 16.1 folds at T4. In Pusa Shukti, expression remained below Pusa Ashwini (1) at T1, upregulated to 7.5 folds at T2, peaked at T3 (20.8 folds), and notably downregulated to 1.8 folds at T4. For PSB Kt-25, expression was below 1 at T1 and T4, increasing more than double from T2 (1.7) to T3 (3.9). The *CCE1* gene was previously reported to control meristem arrest in cauliflower (Palmer *et al*., 2001). However, its expression in both the inflorescence and floral primordium of cauliflower revealed that it was not the cause of meristem arrest (Duclos and Björkman, 2008). The expression of the *CCE1* gene during flower initiation in Pusa Ashwini (T3), curd initiation in Pusa Sharad (T3), and the adult stage in Pusa Shukti and PSB Kt-25 (T3) supports the findings of Duclos and Björkman (2008), suggesting that it is not the responsible factor for the arrest of meristem and indicating its association in both vegetative and reproductive phases.

Table 3: Gene expression changes across time points in respective varieties

Variety	*35 DAP (T1)*		*55 DAP (T2)*		*75 DAP (T3)*		*95 DAP (T4)*	
	Up	*Down*	*Up*	*Down*	*Up*	*Down*	*Up*	*Down*
Pusa Sharad	*BoREM1 CCE1 BoLFY BoCAL BoTFL1 BoVRN2*	*BoFT BoFUL-d*	*BoREM1 BoTFL1 BoFT BoFUL-d*	*CCE1 BoLFY BoCAL BoVRN2*	*BoREM1, CCE1 BoFT, BoVRN2*	*BoLFY BoCAL BoTFL1 BoFUL-d*	*BoREM1 BoLFY BoFUL-d*	*CCE1 BoCAL BoFT BoVRN2*
Pusa Shukti	-	*All*	*BoREM1 CCE1 BoLFY BoCAL BoTFL1 BoFT BoFUL-d*	*BoVRN2*	*BoREM1 CCE1 BoVRN2*	*BoLFY BoCAL BoTFL1 BoFT BoFUL-d*	*BoREM1 BoFUL-d*	*CCE1 BoLFY BoCAL BoTFL1 BoFT BoVRN2*
PSB Kt-25	-	All	CCE1 BoLFY BoCAL	BoREM1 BoTFL1 BoFT BoVRN2 BoFUL-d	BoREM1 CCE1 BoCAL	BoLFY BoTFL1 BoFT BoVRN2 BoFUL-d	BoREM1 CCE1	CCE1 BoLFY BoCAL BoTFL1 BoFT BoVRN2 BoFUL-d

Up- Upregulation, Down- Downregulation, Bold- Downregulation but expression above calibrator, DAP- Days After Planting

Brassica oleracea LEAFY (BoLFY)

In Pusa Sharad, *BoLFY* expression peaked at T1 (7.5), decreased at T2 (6.4), hit a minimum at T4 (1.6), and rose again at T4 (4.4) (Figure 1). Pusa Shukti had minimal expression at T1 and T4. The highest expression for Pusa Shukti was at T3 (14.6), decreasing sharply at T3 (1.4). PSB Kt-25 had consistently lower expression than Pusa Ashwini at T1, T3, and T4, with higher expression at T2 (3.4-fold). In *Arabidopsis*, *LFY* functions as a master regulator, activating numerous floral meristem genes to specify the identity of the floral meristem; similarly, in *B. oleracea* (*BoLFY*), it is reported to be essential for inflorescence meristem initiation (Duclos and Björkman, 2008). In Pusa Sharad, the BoLFY gene was downregulated at curd initiation, but it exhibited upregulation during the transition from a full curd to bolting in T4, aligning with observations reported by Duclos and Björkman (2008) and Mangal and Singh (2023). In contrast, in Pusa Shukti and PSB Kt-25, expression levels remained lower at the adult stage (T3) and curd initiation stages (T4). However, in all tested genotypes, there was an unusual upregulation during the juvenile stage at T2, suggesting its association with the pre-reproductive stage.

Brassica oleracea CAULIFLOWER (BoCAL)

In Pusa Sharad, *BoCAL* expression peaked at T1, then decreased to a minimum at T4. In Pusa Shukti, minimal expression occurred at T1 and T4, with a 3.2-fold increase at T3, reduced to 1.8-fold at T3. PSB Kt-25 had low expression at T1 and T4, slightly higher than Pusa Ashwini at T2 (1.1) and T3 (1.3) stages (Figure 1). In *Arabidopsis*, *CAL* acts as a redundant partner with *AP1* in specifying floral meristem identity, and a mutation in the allele causes a cauliflower phenotype in *Arabidopsis* (Duclos and Björkman, 2008). In *B. oleracea*, *BoCAL* and *BoAP1* are reported to be under independent regulatory control, and the expression of *BoCAL* was not consistent with up-regulation to initiate floral primordium (Duclos and Björkman, 2008). The expression of *BoCAL* decreased in all genotypes over time, reaching a minimum at T4. At curd initiation, expression was significantly downregulated in all tested genotypes, further reduced during curd loosening in Pusa Sharad. Mangal Singh (2023) also reported a reduction in expression from curd initiation to full curd transition in Pusa Sharad.

Brassica oleracea TERMINAL FLOWER1 (BoTFL1)

In Pusa Sharad, *BoTFL* expression increased from T1 (4.1) to T2 (6.7), decreased to 1.4 folds at T3, and stayed almost the same at T4 (Figure 1). In Pusa Shukti, it was nearly absent at T1, increased to 2.6 folds at T2, remained similar at T3 (2.0) with a slight decrease, and reduced to almost zero at T4. Similarly, in PSB Kt-25, expression was minimal at T1 and T4, remaining lower than Pusa Ashwini at T2 and T3. *TFL1* is reported as a flowering repressor and maintainer of the indeterminate shoot apical meristem (Conti and Bradley, 2007). However, in *B. oleracea* and *B. napus*, its role as a floral repressor has been inconsistent, instead, it is reported to be associated with the enlargement of the curd (Duclos and Björkman, 2008). Correspondingly, the expression of *BoTFL1* was upregulated before curd initiation in Pusa Sharad. Additionally, its expression was upregulated during the adult stage, but it was subsequently reduced during the later stages of development.

Brassica oleracea FLOWERING LOCUS T (BoFT)

In Pusa Sharad, *BoFT* expression was nearly absent at T1 (Figure 1). It increased to the level of Pusa Ashwini at T2, upregulated to 2.6 folds at T3, but later decreased below that of Pusa Ashwini. For Pusa Shukti, except at T2, the expression remained lower than in Pusa Ashwini, reaching its maximum at 3.5 folds at T2. In PSB Kt-25, the expression stayed lower than in Pusa Ashwini across all time points. The *BoFT* acts as a flowering activator, stimulating the process of flowering (Shalit *et al*., 2009). In the present study, the expression increased from T1 after sowing to T2 in Pusa Shukti and PSB Kt-25. The expression level of *BoFT* was increased from T1 to T3 in Pusa Sharad, however, it was reduced thereafter. This expression pattern indicates for onset of flowering activities in Pusa Sharad which were maintained up to T3 days.

Brassica oleracea Vernalization 2 (BoVRN2)

In Pusa Sharad, the expression of the *BoVRN2* gene was higher in T1 and T3, with a value of up to 3 folds (Figure 1). However, it was downregulated at T2 (0.3) and T4 (0.5), falling below that of Pusa Ashwini. In Pusa Shukti, there was no expression at T1. The highest expression was observed at T3, nearly up to 2-fold, while it remained below 1 at T2 and T4. In PSB Kt-25, the expression consistently stayed lower than that of Pusa Ashwini across all time intervals. The *VRN2* gene plays a role in vernalization and codes for a nuclear-localized zinc finger protein that shares similarities with Polycomb group (PcG) proteins found in both plants and animals (Gendall *et al*., 2001). *VRN2* functions to sustain the repression of *FLC* after exposure to cold, serving as a mechanism for the cellular memory of vernalization (Gendall *et al*., 2001). Non-vernalization or high-temperature conditions likely led to lower expression of *BoVRN2* in the genotypes; however, the relatively higher expression observed in genotypes until T3 suggests the potential associations of the gene with the vegetative stage in plants until conducive floral signals are perceived.

Brassica oleracea FRUITFUL-d (BoFUL-d)

In Pusa Sharad, the expression of *BoFUL-d* was almost absent in T1 and T3 (Figure 1). It increased to the level of Pusa Ashwini in T2 and reached its maximum at T4 with a value of 4.7. In Pusa Shukti, the expression was much lower than Pusa Ashwini in T1 (0.2) and T3 (0.18), and higher in T2 (1.8), and T4 (2.2). In PSB Kt-25, there was minimal expression in T1 (0.4) and almost no expression in T2, T3, and T4. The

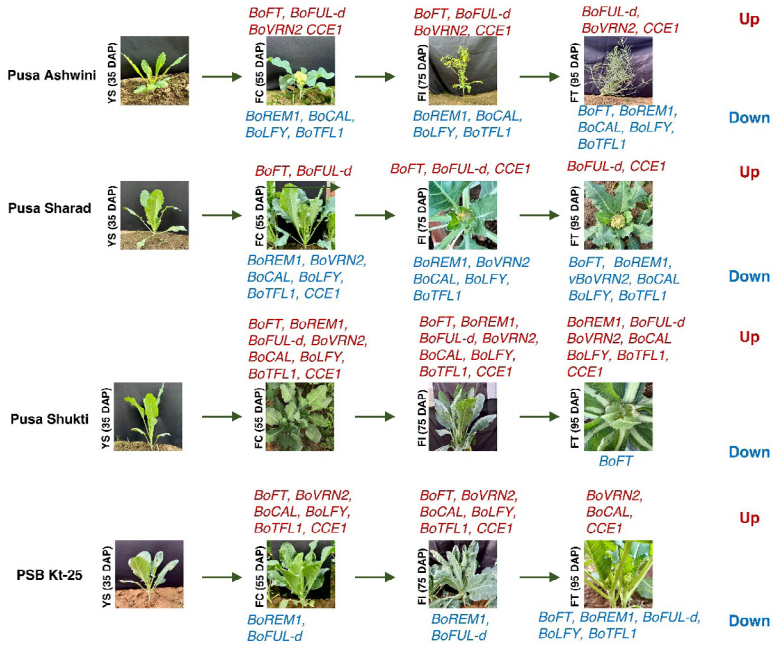

Figure 2: Proposed model for developmental transitions in different maturity groups of Indian cauliflower (35 DAP as calibrator, Up- Upregulation Down- Downregulation**,** Details of the genes in Table 1).

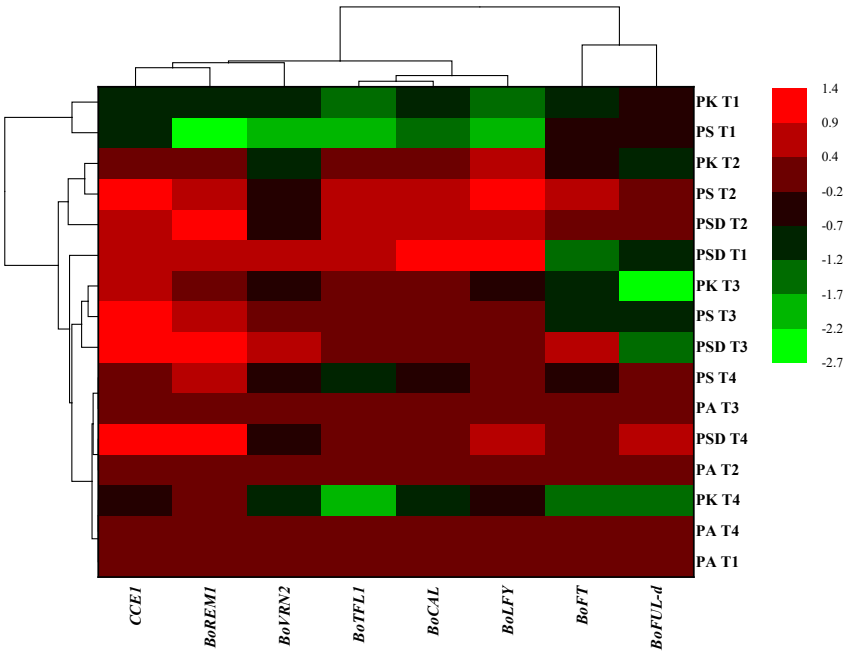

Figure 3: Heat map of the curding and flowering-related genes in Pusa Ashwini (PA), Pusa Sharad (PSD), Pusa Shukti (PS), and Pusa Snowball Kt-25 (KT) at four-time intervals: 65 DAS (T1), 85 DAS (T2), 105 DAS (T3), and 125 DAS (T4)

BoFUL genes are crucial regulators of floral meristem identity in *Brassica oleracea*. Among the four *BoFUL* paralogues, *BoFUL-b*, *BoFUL-c*, and *BoFUL-d* exhibited identical expression patterns, while *BoFUL-a* displayed a slightly different pattern. Specifically, *BoFUL-b*, *BoFUL-c*, and *BoFUL-d* are reported to have maximum expression at the inflorescence meristem stage, with *BoFUL-c* and *BoFUL-d* being the most abundant transcripts (Duclos and Björkman, 2008). In the study, the expression of *BoFUL-d* was reported to be maximum at the curd loosening (T4) in Pusa Sharad, followed by Pusa Shukti at curd initiation (T4), supporting the findings of Duclos and Björkman (2008), and Mangal and Singh (2023).

A model using eight candidate genes for the regulation of developmental transitions was proposed for all four maturity groups of Indian cauliflower (Figure 2). Notably, the relative expression pattern (35 DAP as calibrator for respective variety) revealed that the Pusa Ashwini (early group) and Pusa Sharad (mid-early group) followed same pattern while Pusa Snowball Kt-25 behaved differently. The intermediate pattern was reflected by Pusa Shukti, however, the upregulation of all the genes except *BoFT* at FT (95 DAP) needs further investigation. The similar pattern on Pusa Ashwini and Pusa Sharad could be attributed to their typical Indian/tropical nature while the different pattern of Pusa Snowball Kt-25 was due to its different lineage than the Indian type (Singh and Kalia, 2020; Mangal and Singh, 2023).

Correlation analysis of genes led to the formation of two primary clusters (Figure 3). The first cluster included *BoFUL-d*, and *BOFT* genes, while the rest of the genes comprised the second cluster. Similarly, when examining the expression of genotypes at four time intervals, two clusters resulted. Pusa Shukti and Pusa Snowball Kt-25 at 65 DAS (T1) exhibited similar expression patterns forming one cluster, while the remaining expression study of genotypes formed the second cluster.

Conclusion

In conclusion, this study sheds light on the significant role of candidate genes in the curd induction and bolting processes, revealing distinct expression patterns across four thermosensory cauliflower groups. The complexity of flowering pathways underscores the need for further investigations involving other genes of the studied pathway and genes of other pathways influencing developmental transitions for their application in future breeding programs.

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सारांश

फूलगोभी जटिल विकासात्मक परिवर्तनों वाली तापमान के प्रति असहिष्णु फसल है जो जीनोटाइप-विशिष्ट, पर्यावरण-उत्तरदायी आनुवंशिक तंत्र द्वारा जटिल रूप से नियंत्रित होती है। फूलगोभी उत्पादन में तापमान कारकों को कम करने के लिए प्रमुख जीनों के आनुवंशिक व्यवहार को समझना महत्वपूर्ण है। फूलगोभी जीनोटाइप के बीच जीन की अभिव्यक्ति पैटर्न में पर्याप्त अंतर देखा गया। फूल के आने के प्रारंभिक (कर्ड इनिशीएशन) चरण में, पूसा शरद ने *BoREM1*, *CCE1*, *BoFT*, और *BoVRN2* जीन का उल्लेखनीय ऊपर विनियमन प्रदर्शित किया; पूसा शुक्ति ने *BoREM1* और *BoFUL-d* की बढ़ी हुई अभिव्यक्ति दिखाई; और पूसा स्नोबॉल Kt-25 में, *BoREM1* और *CCE1* जीन का उल्लेखनीय रूप से ऊपर विनियमन पाया गया । फूल के आने के प्रारंभिक (कर्ड इनिशीएशन) चरण में तीनों जीनोटाइप में *BoLFY*, *BoCAL* और *BoTFL1* जीन की अभिव्यक्ति का अधोनियमन पाया गया। दिलचस्प बात यह है कि शीतोष्ण जीनोटाइप पूसा स्नोबॉल Kt-25 में सभी जीनों की अभिव्यक्ति भारतीय (फूलगोभी) प्रकारों की तुलना में अपेक्षाकृत कम थी। पूसा शरद में बोल्टिंग पर *BoREM1*, *BoLFY*, और *BoFUL-d* जीन की बढ़ी हुई अभिव्यक्ति ने फूल से बोल्टिंग चरण संक्रमण के साथ उनके संभावित संबंध का संकेत दिया। इस अध्ययन में फूलगोभी की तापसुग्राही सुघट्यता का विस्तार करने के लिए, फूल आने और पुष्पन के आनुवंशिक नियमन की समझ को आगे बढ़ाना की क्षमता है।