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Cloning and expression studies of a *LFY* cDNA from *Brassica juncea*

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ABSTRACT

A *LEAFY* cDNA was cloned from the short-day (SD) plant *Brassica juncea* cv Varuna. (*Bj*LFY) consists of 1261 bp encoding for a protein of 420 amino acids and an estimated isoelectric point of 6.8. The deduced amino acid showed 99% and 86% identity to *Arabidopsis thaliana* and *Brassica oleracea LFY* cDNA respectively. The *LFY* transcript was detected throughout the vegetative and reproductive phase but an increase in transcript level was observed during transition. The earlier induction of *Bj*LFY was observed in early flowering variety of *B. juncea* as compared to late flowering varieties further proving the critical role LEAFY plays in floral transition.

***Keywords*:** *Brassica juncea*; *LFY*; Flowering; Expression

1. INTRODUCTION

Flowering has been extensively studied in the model plant, *Arabidopsis*; where it is regulated by at least four parallel pathways, *i.e.*, long day, vernalization, GA dependent, and the autonomous pathway. These pathways converge at a single point and switch on the expression of the floral meristem identity genes such as *LEAFY* (*LFY*), *AP1* (*APETALA*1) and *CAULIFLOWER* (*CAL*) as described by [1-4]. Among the meristem identity genes, *LFY* is the first gene to be expressed preceding flower formation and the mutated form has the most drastic effects on the floral transition [5]. After turning on the meristem identity switch, the *LFY* gene has a second role as a transcription factor in the activation of the floral homeotic genes that specify the identity of organs in the flower [6,7]. Despite its pivotal role in the development of *Arabidopsis thaliana*, the molecular events downstream of LFY activation (e.g. signaling and regulatory steps, the identity of the genes regulated by LFY) are poorly understood. Among the few direct target genes of LFY that are currently known are *APETALA1* (*AP*1), *AGAMOUS* (*AG*), and *APETALA3* (*AP*3) [2,8-10].

The expression of *LFY* gene was reported to increase with the age of the plant till it reached a threshold level which is required for transition from vegetative to the reproductive phase [11]. Overexpression of the *LFY* gene has been found to accelerate flowering in a variety of plants like, aspen and tobacco [12], rice [13], poplar [14] and *Citrus* [15]. This ability to accelerate flowering has generated considerable interest in its role in transition from vegetative to the reproductive phase and its potential for modulating flowering in agriculturally important crops.

The *LFY* gene has been cloned and its function has been studied from a few species viz. *Antirrhinum* [16], tobacco [17], *Eucalyptus* [18], *Pinus* [19], rice [20], tomato [21], poplar [14], violet cress [22], apple [23], grapevine [24], papaya [25], rubber [26] and cedar [27].

In the present investigation *Brassica juncea* was selected as the experimental interest as it is an important oilseed crop of India. It is an amphidiploid (2n = 36, genome AABB) species raised by the hybridization of *B. rapa* (2n = 20, genome AA) and *B. nigra* (2n = 16, genome BB). *B. juncea* along with *B. carinata* provides 12% of the world wide edible vegetable oil supplies. When compared to other edible oils, the rapeseed/mustard oil has the lowest amount of harmful saturated fatty acids. It also contains adequate amounts of the two essential fatty acids, linoleic and linolenic acid, which are not present in many of the other edible oils. Our earlier studies showed accelerated flowering in transgenic *B. juncea* by overexpressing the *LFY* cDNA from *A. thaliana* [28]. In the present study with the future aim of expressing homologous *LFY* in *B. juncea*, we have isolated *LFY* from *B. juncea* and studied its temporal and spatial expression in *B. juncea* in different cultivars with differences in flowering time.

2. MATERIALS AND METHODS

2.1. Plant Material

Brassica juncea cv. Varuna, RNBL, TN-1, PNMB seeds were obtained from I.A.R.I, Pusa, New Delhi, India. The TN-1 is an early flowering cultivar while PNMB and

RNBL are late flowering cultivars.

2.2. Cloning of *LFY* cDNA

Approximately 1 μg of total RNA isolated from floral buds of *Brassica juncea* was denatured at 65˚C for 10 min and reverse transcribed using MuMLV reverse transcriptase at 42˚C for 1 hr. An aliquot of the reverse transcribed cDNA formed was used for PCR with *LEAFY* cDNA specific primers. A mixture of Pfu and Taq Polymerase in the ratio of 1:3 was used for the amplification. A band of ~1.3 kb was obtained. The amplicon obtained was cloned in pGEMT-Easy vector, transformed in *E. coli* and the positive colonies identified by colony PCR. The positive clone was sequenced using automatic sequencer at DNA sequencing facility, Department of Biochemistry, University of Delhi, South Campus, New Delhi, India.

2.3. Analysis of *LFY* Expression Profile

RNA was isolated from freshly harvested tissues (floral buds, leaves, roots, stem, silique and shoot apices) of *B. juncea* according to a modified protocol of Chomozynski and Sacchi [29]. Total RNA was fractionated on 1.5% formaldehyde denaturing agarose gel according to Sambrook *et al.* [30] and transferred to nitrocellulose membrane in the presence of 20X SSC (3.0 M NaCl, 0.30 M sodium citrate, pH 7.0) by capillary transfer for 14 - 18 h. The blot was crosslinked by uv irradiation at 254 nm for 2 min. Hybridization with the radiolabeled *BjLFY* cDNA was carried out at 58 C in buffer containing 0.5 M sodium phosphate buffer, pH 7.2, 1 mM EDTA and 7% SDS for 16018 h. The membrane was washed twice in 1X SSC containing 0.1% SDS for 15 min at 58 C and kept in phosphoimager plates. The signals were detected by phosphoimager (FLA-500, Japan). Equal loading and proper transfer of RNA was monitored by methylene blue staining of the nylon membrane.

3. RESULTS AND DISCUSSION

3.1. Cloning and in Situ Characterization of *LFY* cDNA from *B. Juncea* cv. Varuna

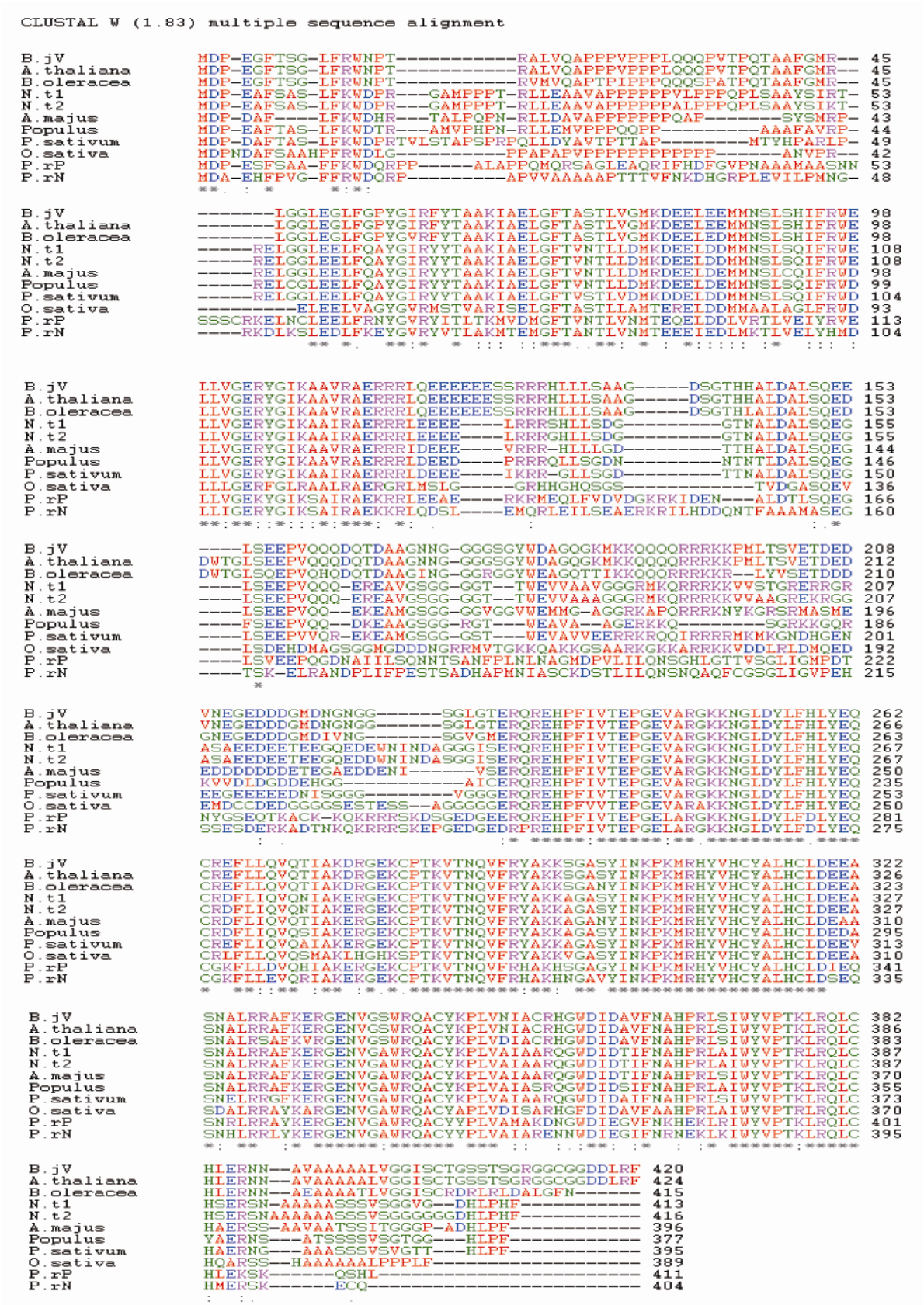
A cDNA (DQ471932) encoding for LEAFY protein was cloned from *B. juncea* cv. Varuna. The deduced polypeptide comprised of 420 amino acids with predicted molecular weight 47 kDa and pI 6.48. The putative protein encoded by *Bj*LFY in this study, shares a number of sequence motifs with a *LFY/FLO* like proline rich region near the amino terminus and a highly acidic region in the centre of the protein which are thought to be characteristic of plant transcription factors. These were present in the variable regions (low similarity) of the *FLO/LFY* proteins. The *B. juncea* LFY protein was evolutionary more closely related to the *LFY* proteins from *A. thaliana* and *B. oleracea* as was expected.

An alignment of the predicted amino acid sequence with the reported sequences revealed that *Bj*LEAFY-V shares 99% identity with *Arabidopsis thaliana* LFY protein (*At*LFY) and 86% identity with *Brassica oleracea* LFY protein (BOFH) at the amino acid level. Between *Bj*LFY-P and *Bj*LFY-V there was 88% identity. *Bj*LFY-V also had the typical FLO/LFY regions- the proline-rich region near the amino terminus and an acidic central region present within the encoded amino acid sequence. Sequence comparison of FLO/LFY-like proteins (accession numbers in parentheses) PrFLL from *Pinus radiata* (U92008); NLY from *Pinus radiata* (U76757); BOFH from *Brassica oleracea* (Z18362); LEAFY from *Arabidopsis thaliana* (M91208); NFL1 and NFL2 from *Nicotiana tabacum* (U16172 and U16174, respectively); PEAFLO from *Pisum sativum* (AF010190); FLO from *Antirrhinum majus* (M55525); PtFL from *Populus balsamifera* (U93196); and RFL from *Oryza sativa* (AB005620) was done with the *Brassica juncea* LFY amino acid sequence using ClustalW 1.83 (**Figure 1(a)**).

The amino acid sequence from 115 - 132 of *Bj*LEAFY-V correspond to the bipartite nuclear targeting sequences. The tools at Expasy could also identify four sites, 126 - 128; 229 - 331; 339 - 341and 408 - 410 in *Bj*LFY-V. Besides these two caesin kinase II phosphorylation sites at 201 - 204 and 205 - 208, three sites at 150 - 153, 201 - 204 and 205 - 208 in *Bj*LEAFY-V are also predicted to be present. SUMOplot™ predicted that the region 79 - 82 (MKDE) has a high probability to be engaged in SUMO attachment. Myristoylation sites were also predicted to be present in the proteins.

Dendrogram drawn by comparing the amino acid sequences of *LFY* proteins from *Brassica oleracea*, *Arabidopsis thaliana*, *Nicotiana tabacum*, *Pisum sativum*, *Antirrhinum majus*, *Oryza sativa* and *Pinus radiata* using the software Genebee (**Figure 1(b)**). As expected this analysis showed a close relationship between *LFY* proteins from *B. juncea*, *B. oleracea* and *A. thaliana.*

Southern blot analysis of *B. juncea* genomic DNA using the *BjLFY*-V cDNA as a probe gave multiple bands (**Figure 1(c)**). *B. juncea* is an amphidiploid species derived from *B. rapa* and *B. nigra*. The multiple signals obtained in *B. juncea* may probably be due to the presence of multiple copies of *LFY* in *B. juncea* genome inherited from each progenitor species or through the presence of these sites within the introns of *LFY* gene. In most plants for which *LFY* homologues have been determined, these *LFY*-homologous genes had a single copy in each genome (*A. thaliana*, *A. majus*, pea, tomato, petunia and poplar) [6,14,16,21,31,32]. In apple genome there are two homologous copies of *LFY* genes (AFL1



(a)

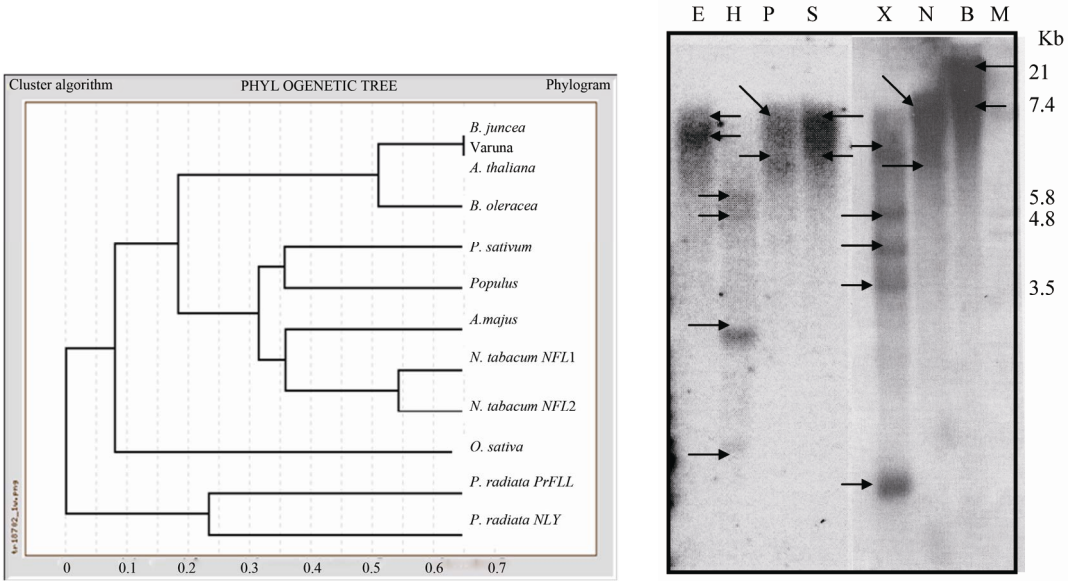


Figure 1. (a) Multiple sequence alignment of *Bj*LFY-Varuna. ClustalW was used for the multiple sequence alignment. "*" means that the residues or nucleotides in that column are identical in all sequences in the alignment, ":" means that conserved substitutions have been observed and "." means that semi-conserved substitutions are observed; (b) Dendrogram of *Bj*LFY-Varuna protein obtained using Genebee software; (c) Southern hybridization analysis of *Brassica juncea* cv. Varuna genome. Genomic DNA (20 µg) from *Brassica juncea* was digested with Bam HI (B), Eco RI (E), Hind III (H), Nco I (N), Pst I (P) or Sal I (S) at 37˚C overnight. The digested DNA was separated on 1% agarose gel, blotted on nylon membrane and hybridized with a probe prepared from a *Bj*LFY cDNA. M indicates λ DNA EcoR I/digest. The numbers at right indicate molecular size (kilo basepairs).

and AFL2) [23] and so also in tobacco (NFL1 and NFL2) [17]. *Eucalyptus* has three homologous genes in the genome, but two of them were found not to be expressed and had stop codons in their coding region [18]. *P. radiata* has two homologous genes, *NEEDLY* and *PRFLL* which are expressed during vegetative development and in male cones during reproductive development respectively [19,33].

3.2. Spatial and Temporal Expression of *Bj*LFY during *B. juncea* Development

The expression of *LFY* gene was studied in different organs, viz. adult leaves, root, stem, silique and bracts of *B. juncea*. Northern blot analysis revealed a very high expression of the *LFY* gene in mature leaves, followed by stems and bracts. There was very faint expression in the silique and no expression in the roots of the plants (**Figure 2**). The *LFY* gene expression was detected in all the four floral organs although there were differences in the level of expression. This is similar to the expression pattern observed in *A. thaliana* and *A. majus* [6,16]. The expression of the *LFY* gene in the floral organs of *B. juncea* reflects their role in establishing floral organ identity like the other *FLO/LFY* genes. Unlike *Bj*LFY its homologue from other plants (*BOFH* gene from *B. oleracea*, *ELF* gene from *Eucalyptus*, *AFL1* gene from apple and *NFL* genes from tobacco) have not been reported to express in leaf and stem [17,18,23,34].

As we observed considerable expression in leaves of *B. juncea* we decided to study the changes in *LFY* expression in leaves at different developmental stages. The *LFY* transcript could be detected very early in the cotyledonary leaves within one week after germination, after which it decreased during vegetative growth in the next two weeks. When the plant was ca. 26 - 34 days old

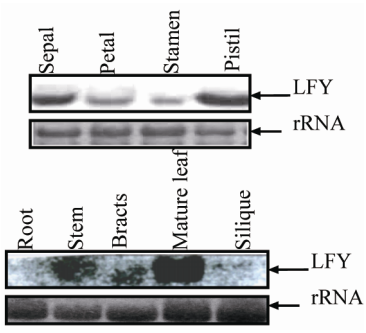
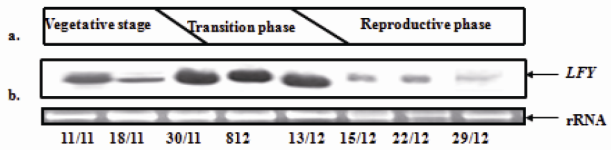


Figure 2. Expression of the *LFY* gene in different organs in wild type plants of *B. juncea* cv. Varuna.

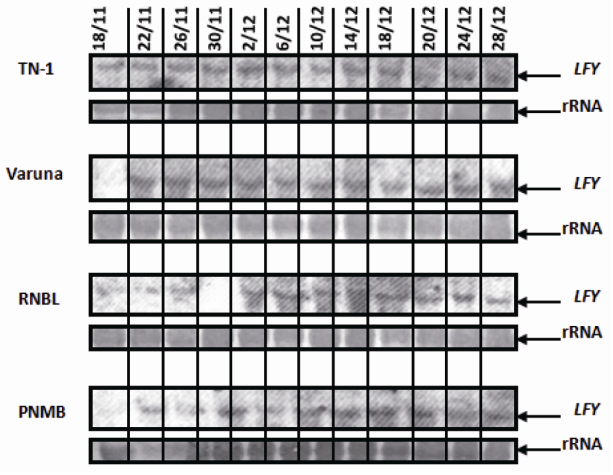
with no visual signs of flowering, there was a sudden increase of *LFY* transcript, which was sustained till the appearance of the floral buds. The *LFY* transcript level decreased on initiation of flower development. Therefore, the kinetics of the *LFY* gene expression in *B. juncea* cv. Varuna suggested that there is an increase in level of *LFY* transcript during the transition from vegetative to the reproductive phase (**Figure 3(a)**). Most species for which expression of *LFY* genes has been investigated have simple leaves and where *LFY* expression occurs in leaves, it is low and restricted to early phases of leaf development (*A. thaliana*; [35], *N. tabacum*; [17], *Impatiens* species; [36]). An interesting observation by Lee *et al*. (1997) who reported the formation of lobed leaves in transgenic *Arabidopsis* plants overexpressing *UNUSUAL FLORAL ORGANS* (*UFO*) gene and this phenotype required the presence of functional *LFY* gene [37]. Therefore, *LFY* could indirectly be involved in controlling the morphology of simple leaves. In the present investigation, the *LFY* transcript in the leaves of *B. juncea* was abundant during the initial one week of growth and decreased during the vegetative growth. It showed an increase during the transition phase (~26-34-day-old) from the vegetative to the reproductive phase and decreased slightly when re-productive development started. The modulation of *LFY* gene expression during the vegetative to reproductive development in *B. juncea* suggests that it has an important role to play. An in-depth study is needed to support this.

The *LFY* expression was also studied in shoot apices of different cultivars at different time period starting from 10-day-old seedlings to 50-day-old adult plants. The *LFY* gene expression was detected very early in the shoot apices of *B. juncea* during the vegetative phase. The level of the transcript was more abundant in the early flowering cultivar, TN-1 followed by Varuna. The expression in PNMB and RNBL cultivars which are late flowering was very less in the early stages of growth (10 - 24 days after germination) but subsequently increased with age (**Figure 3(b)**). The appearance of the *LFY* transcript much earlier and at higher levels in early flowering cultivates indicate that the threshold level required for floral transition is attained faster in these as compared to the late flowering ones. Though the *LFY* and *FLO* genes are expressed in the floral apices, the *NFL* transcript could be detected in the vegetative apices also [17].

Although, the expression studies of *LFY* gene of *B. juncea* showed that it is expressed throughout the life cycle of the plant, there is a variation in the amount of transcript at different developmental stages and in different organs. Despite the differences in expression pattern, these expression studies indicate that *Bj*LFY could be involved in regulating floral meristem identity and



(a)



(b)

Figure 3. (a) Northern blot of total RNA isolated from leaves of *B. juncea* cv.Varuna at different developmental stages; (b) Northern blot of total RNA isolated from shoot apices at different stages of development in various cultivars of *B. juncea* varying in flowering time.

flowering time as in *LFY/FLO* homologs from other systems. Detailed *LFY* expression through *in situ* hybridizations in the leaf and floral primordia would give an idea about the specific regions of its activity and thus help in elucidating its functions. The expression pattern of *LFY* in *B. juncea* was different from its closely related species *A. thaliana* and *B. oleracea*, though there was high level of sequence similarity amongst them. Isolation and characterization of the promoter and cis regulatory elements of the *LFY* gene from *B. juncea* may provide explanation for the variation in transcript profiles. The higher level of transcripts could be due to the presence of multiple copies of *LFY* gene in *B. juncea.* The analysis of the *LFY* promoter from *B. juncea* will also help in deciphering whether the transcriptional control of these regulators is direct or indirect and which proteins might act as mediators between these and the *LFY* gene.

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REFERENCES

[1] Bowman, J.L., Alvarez, J., Weigel, D., Meyerowitz, E.M. and Smyth, D.R. (1993) Control of flower development in Arabidopsis thaliana by APETALA1 and interacting genes. *Development*, **119**, 721-743.

[2] Busch, M.A., Bomblies, K. and Weigel, D. (1999) Activation of a floral homeotic gene in Arabidopsis. *Science*, **285**,585-587. doi:10.1126/science.285.5427.58

[3] Gustafson-Brown, C., Savidge, B. and Yanofsky, M.F. (1994) Regulation of the Arabidopsis floral homeotic gene APETALA1. *Cell*, **76**,131-143. doi:10.1016/0092-8674(94)90178-3

[4] Komeda, Y. (2004) Genetic regulation of time to flower in Arabidopsis thaliana. *Annual Review Plant Biology*, **55**, 521-535. doi:10.1146/annurev.arplant.55.031903.141644

[5] Schultz, E.A. and Haughn, G.W. (1991) LEAFY, a homeotic gene that regulates inflorescence development in Arabidopsis. *Plant Cell*, **3**, 771-781.

[6] Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F. and Meyerowitz, E.M. (1992) LEAFY controls floral meristem identity in Arabidopsis. *Cell*, **69**, 843-859. doi:10.1016/0092-8674(92)90295-N

[7] Huala, E. and Sussex, I.M. (1992) LEAFY interacts with floral homeotic genes to regulate Arabidopsis floral development. *Plant Cell*, **4**, 901-913.

[8] Lamb, R.S., Hill, T.A., Tan, Q.K. and Irish, V.F. (2002) Regulation of APETALA3 floral homeotic gene expression by meristem identity genes. *Development*, **129**, 2079-2086.

[9] Parcy, F., Nilsson, O., Busch, M.A., Lee, I. and Weigel, D. (1998) A genetic framework for floral patterning. *Nature*, **395**, 591-596. doi:10.1038/26956

[10] Wagner, D., Sablowski, R.W.M. and Meyerowitz, E.M. (1999) Transcriptional activation of APETALA1 by LEAFY. *Science*, **285**, 582-584. doi:10.1126/science.285.5427.582

[11] Blazquez, M.A., Green, R., Nilsson, O., Sussman, M.R. and Weigel, D. (1998) Gibberellins promote flowering of Arabidopsis by accelerating the LEAFY promoter. *Plant Cell*, **10**, 791-800.

[12] Weigel, D. and Nilsson, O. (1995) A developmental switch sufficient for flower initiation in diverse plants. *Nature*, **377**, 495-500. doi:10.1038/377495a0

[13] He, Z., Zhu, Q., Dabi, T., Li, D., Weigel, D. and Lamb, C. (2000) Transformation of rice with the Arabidopsis floral regulator LEAFY causes early heading. *Transgenic Research*, **9**, 223-227. doi:10.1023/A:1008992719010

[14] Rottmann, H.W., Meilen, R., Sheppard, L.A., Brunner, M.A., Skinner, J.S., Caiping, Ma, Cheng, S., Jouanin, L., Pilate, G. and Strauss, S.H. (2000) Diverse effects of overexpression of LEAFY and PTLF, a poplar (Populus) homolog of LEAFY/FLORICAUA, in transgenic poplar and Arabidopsis. *Plant Journal*, **22**, 235-245. doi:10.1046/j.1365-313x.2000.00734.x

[15] Pena, L., Trillo, J.M.M.J., Pina, A.J., Navarro, L. and Martinez-Zapater. M.J. (2001) Constitutive expression of Arabidopsis LEAFY or APETALA1 genes in Citrus reduces their generation time. *Nature Biotechnology*, **19**, 263-267. doi:10.1038/85719

[16] Coen, E.S., Romero, J.M., Doyle, S., Elliot, R., Murphy, G. and Carpenter, R. (1990) Floricaula: A homeotic gene required for flower development in Antirrhinum majus. *Cell*, **63**, 1311-1322. doi:10.1016/0092-8674(90)90426-F

[17] Kelly, A.J., Bonnlander, M.B. and Meeks-Wagner, R.D. (1995) *NFL*, the tobacco homolog of *FLORICAULA* and *LEAFY*, is transcriptionally expressed in both vegetative and floral meristems. *Plant Cell*, **7**, 225-234.

[18] Southerton, S.G., Strauss, S.H., Olive, M.R., Harcourt, R.L., Decroocq, V., Zhu, X., Llewellyn, D.J., Peacock, W.J. and Dennis, E. (1998) *Eucalyptus* has a functional equivalent of the *Arabidopsis* floral meristem identity gene *LEAFY*. *Plant Molecular Biology*, **37**, 897-910. doi:10.1023/A:1006056014079

[19] Mouradov, A., Glassick, T., Hamdorf, B., Murphy, L., Fowler, B., Marla, S. and Teasdale, R.D. (1998) *NEEDLY*, a *Pinus radiata* ortholog of *FLORICAULA/LEAFY* genes, expressed in both reproductive and vegetative meristems. *Proceedings of the National Academy of Sciences*, **95**, 6537-6542. doi:10.1073/pnas.95.11.6537

[20] Kyozuka, J., Konishi, S., Nemoto, K., Izawa, T. and Shimamoto, K. (1998) Down regulation of RFL, the FLO/LFY homolog of rice, accompanied with panicle branch initiation. *Proceedings of the National Academy of Sciences*, **95**, 1979-1982. doi:10.1073/pnas.95.5.1979

[21] Molinero-Rosales, N., Jamilena, M., Zurita, S., Gomez, P., Capel, J. and Lozano, R. (1999) *FALSIFLORA*, the tomato orthologue of *FLORICAULA* and *LEAFY*, controls flowering time and floral meristem identity. *Plant Jounal*, **20**, 685-693. doi:10.1046/j.1365-313X.1999.00641.x

[22] Shu, G., Amaral, W., Hileman, L.C. and Baum, D.A. (2000) LEAFY and the evolution of rosette flowering in violet cress (Jonopsidium acaule, Brassicaceae). *American Journal of Botany*, **87**, 634-641. doi:10.2307/2656849

[23] Wada, M., Cao, Q.F., Kotoda, N., Soejima, J.I. and Masuda, T. (2002) Apple has two orthologues of FLORICAULA/LEAFY involved in flowering. *Plant Molecular Biology*, **49**, 567-577. doi:10.1023/A:1015544207121

[24] Carmona, M.J., Cubas, P. and Martinez-Zapater, J.M. (2002) VFL, the grapevine FLORICAULA/LEAFY ortholog, is expressed in meristematic regions independently of their fate. *Plant Physiology*, **130**, 68-77. doi:10.1104/pp.002428

[25] Yu, Q., Moore, P.H., Albert, H.H., Roader, H.K. and Ming, R. (2005) Cloning and characterization of a FLORICAULA/LEAFY ortholog, PFL, in polygamous papaya. *Cell Research*, **15**, 576-584. doi:10.1038/sj.cr.7290327

[26] Dornelas, M.C. and Rodriguez, A.P. (2005) The rubber tree (Hevea brasiliensis Muell. Ag.) homologue of the LEAFY/FLORICAULA gene is preferentially expressed in both male and female meristems. *Journal of Experimental Botany*, **56**, 1965-1974. doi:10.1093/jxb/eri194

[27] Dornelas, M.C. and Rodriguez, A.P. (2006) The tropical cedar tree (Cedrela fissilis Vell. Meliaceae) homolog of the Arabidopsis LEAFY genes is expressed in reproductive tissues and can complement Arabidopsis leafy mu-

tants. *Planta*, **223**, 306-314. doi:10.1007/s00425-005-0086-y

[28] Deb R.S., Saxena, M. and Sarin-Bhalla, N. (2009) Overexpression of AtLEAFY accelerates flowering in Brassica juncea. *Crop Science*, **49**, 930-936.

[29] Chomczynski, P. and Sacchi. N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochemistry*, **162**, 156-159. doi:10.1016/0003-2697(87)90021-2

[30] Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) Molecular cloning. 2nd Edition, Cold Spring Harbour Laboratory, Cold Spring Harbour, New York.

[31] Hofer, J., Turner, L., Hellens, R., Ambrose, M., Matthews, P., Michael, A. and Ellis, N. (1997) UNIFOLIATA regulates leaf and flower morphogenesis in pea. *Current Biology*, **7**, 581-587.

[32] Souer, E., Krol van der, A., Kloos, D., Spelt, C., Bliek, M., Mol, J. and Koes, R. (1998) Genetic control of branching pattern and floral identity during *Petunia* inflorescence development. *Development*, **125**, 733-742.

[33] Mellerowicz, E.J., Horgan, K., Walden, A., Coker, A. and Walter, C. (1998) PRFLL-a Pinus radiata homologue of FLORICAULA and LEAFY is expressed in buds containing vegetative shoot and undifferentiated male cone primordial. *Planta*, **206**, 619-629. doi:10.1007/s004250050440

[34] Anthony, R.G., James, P.E. and Jordan, B.R. (1993) Cloning and sequence analysis of a *FLO/LFY* homologue isolated from cauliflower (Brassica oleracea L. var. botrytis). *Plant Molecular Biology*, **22**, 1163-1166. doi:10.1007/BF00028986

[35] Blazquez, M.A., Soowal, L.N., Lee, I. and Weigel, D. (1997) LEAFY expression and flower initiation in Arabidopsis. *Development*, **24**, 3835-3844.

[36] Pouteau, S., Nicholls, D., Tooke, F., Coen, E. and Battey, N. (1997) The induction and maintenance of flowering in *Impatiens*. *Development*, **124**, 3343-3351.

[37] Lee, I., Wolfe, D.S., Nilsson, O. and Weigel, D. (1997) A *LEAFY* co-regulator encoded by *UNUSUAL FLORAL ORGANS*. *Current Biology*, **7**, 95-104. doi:10.1016/S0960-9822(06)00053-4

