Towards a physiological analysis of *CONSTANS* role at floral transition in *Sinapis alba*

Maria D'ALOIA and Claire PERILLEUX
Laboratory of Plant Physiology, University of Liège, B22, Sart tilman, 4000 Liège, Belgium
Corresponding author: maria.daloia@ulg.ac.be

Flowering in *Sinapis alba* and *Arabidopsis thaliana* - two Brassicaceae – is accelerated by long days (LD) and can be experimentally induced by a single LD. At the physiological level, this photoperiodic control has been shown to involve production and export from the leaves of a multifactorial floral stimulus, translocated in phloem towards the shoot apical meristem. Although – *in Sinapis* - nutritional and hormonal components have been identified (**1**), nature of the floral stimulus remains unsolved. On the other hand, genetic studies in *Arabidopsis* have revealed the central role of *CONSTANS* (*CO*) and its target *FLOWERING LOCUS T* (*FT*) in the photoperiodic control of flowering. Both genes could be involved in production and/or translocation of the floral stimulus since they are expressed in companion cells of the phloem (**2,3**). In order to integrate these genetical and physiological data, we are interested in cloning and analysing *CO* function in *Sinapis*.

PCR primers were designed based on sequences of *AtCO* and *BniCOa* from *Brassica nigra* (Figure 1) (Primer_For: 5'-GTTCACT CTGCCAATCGCGTTGCTTCC-3' and Primer_rev: 5'-ATCTAGTATTCTTTATTTTGGCC-3'). A partial *CO*-like sequence of 1037 bp – hereafter called *SaCO* - was amplified from cDNA prepared from leaves of *Sinapis* plants induced to flower by a single LD (Figure 2a). The predicted amino acid sequence of SaCO showed 88% identity with BniCOa and 69% identity with AtCO. Based on phylogenetic analysis, SaCO was much closer to BniCOa and AtCO than the other CO-like genes (Figure 2b). Thus *SaCO* is a putative orthologue of the flowering time gene *AtCO*.

Figure 1. Summary of structure of the *AtCO* cDNA showing positions of primers used to clone *S. alba* homolog.

(a)

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BniCOa  MLKQESNYNISNRENNRGARACDTCRSTICTVYCHADSAYLCNSCDAEVHSANRVASRHK   60
SaCO    ------------------------------------------------------VASRHK     6
AtCO    MLKQESNDIGS  ENNR ARPCDTCRSNACTVYCHADSAYLCMSCDAQVHSANRVASRHK   58
C       MLKQESN---S--ENNR-AR-CDTCRS--CTVYCHADSAYLC-SCDA-VHSANRVASRHK

BniCOa  RVPVCESCERAPAAFMCEADDVSLCTACDSEVHSANPLARRHQRVPVVPITGNSCSSLAT  120
SaCO    GFRVCESCERALAAFICEADDVSXCTACDSEVHSANPLARRHQRVPVVPITGNSCSSLAT   66
AtCO    RVRVCESCERAPAAFLCEADDASLCTACDSEVHSANPLARRHQRVPILPISGNSFSSMTT  118
C       rvrVCESCERApAAF-CEADDvSlCTACDSEVHSANPLARRHQRVPvvPItGNScSSlaT

BniCOa  THHTAVTEPE----KRAVLVQDDEEGKEDAKETASWMFPYSDKGSPNHNNNNNNNQNNE  176
SaCO    -HHTTVTEPE----KRAVLVQDDQEGKEDAKETASWMFPYSDK-S-NHN----TSNQNNE  115
AtCO    THHQSEKTMTDPEKRLVVDQEEGEEGDKDAKEVASWLFPNSDK---------NNNNQNNG  169
C       tHHt-vtepe----kraVlvqddeEGkeDAKEtASWmFPySDK-s-nhn-n-NnnNQNNe

BniCOa  LLFSDDYLDLADYNSSMDYKFTGQYNQPQHKQDCTVPQTNYGGDRVVPLQLEETRGNVRH  236
SaCO    LLFSDGYLDLADYNSSMDYKFTGQYNQHQNKQDCTVPQTNYGGDRVVPLQLEETKGNLRH  175
AtCO    LLFSDEYLNLVDYNSSMDYKFTGEYSQHQQN--CSVPQTSYGGDRVVPLKLEESRGHQCH  227
C       LLFSD-YLdLaDYNSSMDYKFTGqYnQhQ-kqdCtVPQTnYGGDRVVPLqLEEtrGn-rH

BniCOa  KKE----KITYGSSGSQYNYNDSINHNAYNPSMETDFVPEPTARETTVSHQKTPK--IHQ  290
SaCO    KEQ----NITYGSSGSQYNYNGSINHNAYNPSVETDFVPEPTARDTTVSHQKTPKGAIHN  231
AtCO    NQQNFQFNIKYGSSGTHYNDNGSINHNAYISSMETGVVPESTACVTTASHPRTPKGTVEQ  287
C       k-q----nItYGSSGsqYNyNgSINHNAYnpSmETdfVPEpTAr-TTvSHqkTPKg-ihq

BniCOa  LPEPLVQILSP-----MDREARVLRYREKKKRNKFEKTIRYASRKAYAERRPRINGRFAK  345
SaCO    XPEPLIQILSP-----MDREARVLRYREKKKRNKFEKTIRYASRKAYAERRPRINGRFAK  286
AtCO    QPDPASQMITVTQLSPMDREARVLRYREKRTRKFEKTIRYASRKAYAEIRPRVNGRFAK  347
C       -PeP--Qilsp-----MDREARVLRYREKkKrRKFEKTIRYASRKAYAErRPRiNGRFAK

BniCOa  MSETEVEDQEYNTMLMYYDTGYGIVPSFYGQNKEY                          380
SaCO    MSETEAEDQDFNSMLMYYDTGYGIVPSFYGQNKEY                          321
AtCO    R-EIEAEEQGFNTMLMYN-TGYYGIVPSF------                          373
C       msEtEaEdO-fNtMLMYvdTGYGIVPSFvgqnkev
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(b)

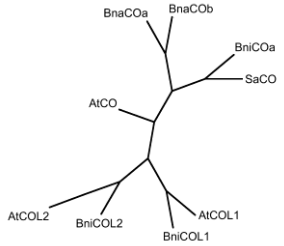

Figure 2. (a) Amino acid alignement of BniCOa, SaCO and AtCO sequences. (b) Phylogenetic relationships of Brassica CO-like proteins using maximum-parsimony. Aligned amino acid sequences of CO homologs from *Brassica napus (Bna), B. nigra (Bni), A. thaliana (At) and S. alba (Sa)* were used for phylogenetic analyses.

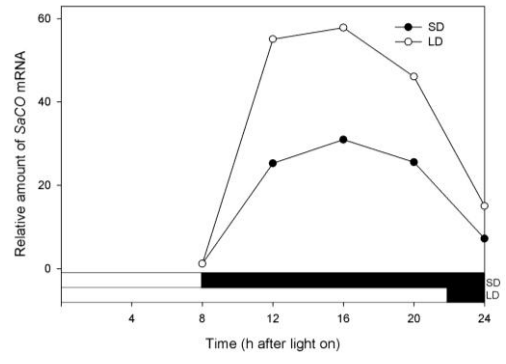

Figure 3. *SaCO* expression pattern in leaves of *Sinapis* plants kept in control SD or exposed to an inductive 22h-LD. White and black bars show light and dark periods respectively. The expression level of *SaTUBULINE* was used for data normalization.

Time course analyses of *SaCO* expression were performed by real-time RT-PCR on total RNA extracted from leaves harvested during the single inductive LD, or in control short day (SD) (Figure 3). The expression pattern found was quite similar to the kinetics described in *Arabidopsis* : a peak of *SaCO* mRNA was observed 16h after light on, i.e. during the night in SD and during the light period in LD. Further experiments are on the way to analyse the physiological function of SaCO.

References
(**1**) Bernier G., Corbesier L. and Périlleux C. (2002) The flowering process: on the track of controlling factors in *Sinapis alba*. *Russ. J. Plant. Physiol.*, 49, 445-450.
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