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Spatio-temporal Analysis of Florigenic Signals in *Arabidopsis thaliana, Sinapis alba* and *Brassica napus*

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List of Abbreviations

°C	Degrees Celsius
µ	Micro
aa	Amino acid
APS	Ammonium persulphate
CC	Companion Cell
DTT	Dithiothreitol
EDTA	Ethylene diamine tetraacetic acid
ESI	Electrospray ionisation
FW	Fresh weight
g	Gram
GC-MS	Gas Chromatography-Mass Spectrometry
GC-TOF-MS	Gas Chromatography-Time of Flight-Mass Spectrometry
IgG, IgM	Immunoglobulin g, immunoglobulin m
kDa	Kilodalton
l	Litre
LDs	Long Days
LDP	Long Day Plant
m	Milli
M	Molar concentration (mol/litre)
m/z	Mass to charge ratio
min	Minutes
MS	Mass spectrometry
MSTFA	N-methyl-N-(trimethylsilyl)trifluoroacetamide
N	Nano
OD	Optical density

PAGE	Polyacrylamide gel electrophoresis
PD	Plasmodesmata
pH	negative decaic logarithm of the proton concentration in mol per litre
Q-TOF	Quadrupole time of flight
RT	Room temperature
s	Seconds
SAM	Shoot Apical Meristem
SDs	Short Days
SDP	Short Day Plant
SDS	Sodium dodecyl sulphate
SE	Sieve Element
TEMED	N,N,N,N-tetramethyl ethylene diamine
TFs	transcription factors
Tris	2-amino 2-hydroxymethylpropane-1,3-diol
UV	Ultraviolet
v/v	Volume per volume
VIS	Visible light
w/v	Weight per volume
w/w	Weight per weight
WT	Wild type
x g	Times gravity speed

List of Genes

ADS2	*delta 9 desaturase*
AFO	*abnormal floral organs protein*
AGL20	*AGAMOUS LIKE 20*
AGP17	*Cell wall arabinogalactan-protein*
AIP1	*ABI3-INTERACTING PROTEIN 1*
AMP1	*glutamate carboxypeptidase*
AP1	*APETALA1*
AP2	*APETALA 2*
ART1	*AERIAL ROSETTE*
ATA1	*alcohol dehydrogenase*
ATHIM	*DNA (cytosine-5-)-methyltransferase*
BFT	*BROTHER OF FT*
bHLH	*basic helix-loop-helix*
CAL	*CAULIFLOWER*
CCA1	*CIRCADIAN CLOCK ASSOCIATED*
CER 1	*aldehyde decarbonylase*
CLV	*CLAVATA*
CO	*CONSTANS*
COL1	*CONSTANS-LIKE 1*
COL2	*CONSTANS-LIKE 2*
CRY2	*CRYPTOCHROME 2*
DDF 1	*DWARF AND DELAYED FLOWERING 1*
DDF1	*DREB subfamily A-1 of ERF/ AP2 transcription factor family*
ELF4	*EARLY FLOWERING 4*
ELF8	*EARLY FLOWERING 8*

EMF1	*EMBRYONIC FLOWER 1*
EMF2	*EMBRYONIC FLOWER 2*
ESD4	*EARLY IN SHORT DAYS 4*
EXO	*DEFENCE/ STRESS PHOSPHATE-RESPONSIVE PROTEIN, PUTATIVE*
FIE	*FERTILIZATION INDEPENDENT ENDOSPERM*
FIL	*FILAMENTOUS FLOWER PROTEIN*
FKF1	*FLAVIN BINDING KELCH REPEAT, F-BOX*
FLC	*FLOWERING LOCUS C*
FLK	*KH domain-containing protein*
FLM	*FLOWERING LOCUS M*
FPF1	*FLOWERING PROMOTING FACTOR 1*
FRI	*FRIGIDA*
FRL	*FRIGIDA LIKE*
FT	*FLOWERING LOCUS T*
FUL	*FRUITFULL*
GCR1	*cytokinin*
GI	*GIGANTEA*
GSH1	*glutamate-cysteine ligase / gamma-glutamylcysteine synthetase*
HB-7	*homeobox-leucine zipper protein 7*
LD	*LUMINIDEPENDENS*
LFY	*LEAFY*
LHY	*LATE ELONGATED HYPOCOTYL*
LPK1	*LOV kelch protein 1*
LTI65	*low-temperature-responsive 65 kD protein*
LTP	*lipid transfer protein*
MAF1	*MADS AFFECTING FLOWRING 1*

MFT	*MOTHER OF FT*
NAM	*NO APICAL MERISTEM*
PEBP	*phosphatidylethanolamine-binding protein*
PR-1	*pathogenesis-related protein 1*
PRE	*PRECOCIOUS*
PSD	*PAUSED*
RD29B	*desiccation-responsive protein 29B*
SEP3	*SEPATALLA 3*
SNZ	*SCHNARCHZAPFEN*
SOC1	*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1*
SPL3	*squamosa promoter-binding protein-like 3*
SPT	*SPATULA*
SVP	*short vegetative phase protein*
SVP	*SHORT VEGETATIVE PHASE*
SYD	*SPLAYED*
TFL1	*TERMINAL FLOWER 1*
TOC1	*TIMING OF CAB EXPRESSION 1*
TSF	*TWIN SISTER OF FT*
YABBY1	*axial regulator YABBY1*
ZTL	*ZEITLUPE*

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INTRODUCTION

Although the body plan of plants is established during embryogenesis, most of plant development occurs postembryonically, through the reiterative production of organ primoridia at the shoot apical meristem (SAM; Levy and Dean, 1998). In most species, the SAM gives rise to vegetative organs such as leaves but when a plant undergoes the transition to reproductive growth, the SAM reprograms its development and produces flowers. The decision to flower has repercussions regarding the survival of the species based on whether or not seeds are set. Understandably, therefore, the transition from vegetative to floral growth is carefully controlled by factors of physiological and genetic nature.

Important environmental cues such as light, temperature and photoperiod influence housekeeping plant developmental processes and need to be gauged. Garner and Allard (1920) demonstrated that the photoperiod (i.e. the duration, rather than the quantity, of light in the daily cycle) regulated flowering by exposure to long days (LDs) or short days (SDs) depending on the plant species (Corbesier and Coupland 2005, reviewed in Thomas and Vince-Prue, 1997).

This change in the developmental fate of primordia initiated at the SAM is also controlled by factors intrinsic to plant development, such as the age of the plant. Effectively, the vegetative meristem is thought to transit from a 'juvenile' phase to an adult one (Levy and Dean, 1998). More specifically, the shoot apex needs to attain a state of responsiveness to allow the plant to respond to both these external and endogenous signals in order to become committed to flowering (King 1973, Zeevart 1976, Corbesier *et al*., 1996, Bradley *et al*., 1997).

The mechanism perceiving the exogenous cues and integrating them within the context of plant development is a circadian clock. Light, perceived by phytochromes and cryptochromes (input signals), entrains the clock (central oscillator) to keep a 24-hour rhythm. Briefly, this autoregulated mechanism functions by negative feedback loops which

are set at the dawn and dusk transitions, allowing plants to remain synchronised with the daily light and dark periods and has been referred to as the external coincidence model. Aspects of plant development that are under circadian control include leaf movement, the opening and closing of stomatal pores and the expression of genes that are involved in the photosynthetic process, cell elongation and flowering-time regulation (Yanovsky and Kay 2003). Output genes like *CONSTANS* (*CO*) link circadian regulation to physiological processes such as floral induction.

Genetics of Flowering Time

Valuable genetic knowledge about the regulation of flowering has been continuously growing, expanding the links and cross talk among the four pathways controlling flowering, as detailed in Figure 1 (Amasino 1996, Korneef *et al*., 1998, Simpson and Dean 2002, Mouradov *et al*., 2002, Yanovsky and Kay 2003, Coupland *et al*., 2005, Searle and Coupland 2004, Searle *et al*., 2006).

In *Arabidopsis*, two of these pathways mediate signals from the environment: the photoperiod promotion pathway is responsible for floral induction in response to inductive photoperiods and the vernalization pathway allows flowering to occur after experiencing an extended period of cold. The remaining gibberellin (GA) and autonomous promotion pathways act independently from these external signals and the latter appears to monitor the endogenous developmental and physiological status of a plant.

As seen in Figure 1, one of the four pathways controls the response to vernalization. In response to extended exposures to low temperature this pathway reduces the abundance of the mRNA encoding the MADS box transcription factor *FLOWERING LOCUS C* (*FLC*), which is a potent repressor of flowering, predominantly expressed in shoot and root apices and in vasculature (Michaels and Amasino 1999; Sheldon *et al*., 1999).

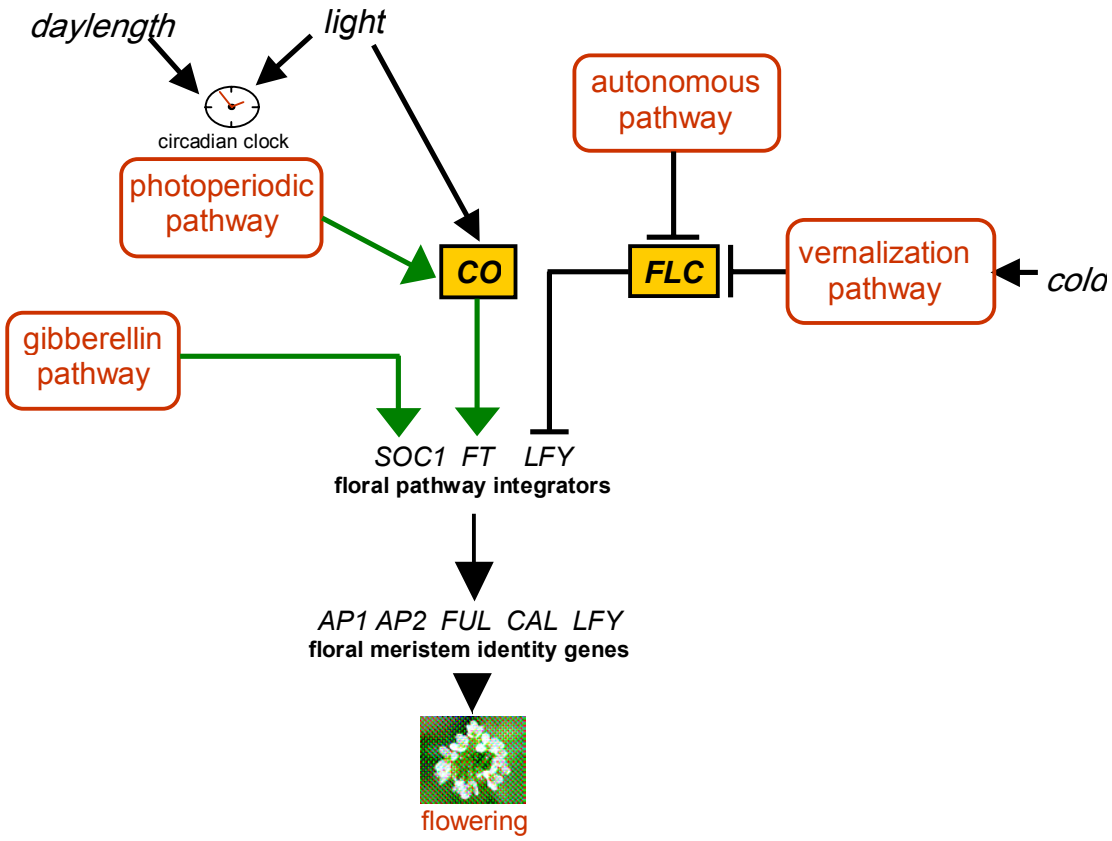


Figure 1: Pathways controlling flowering-time in *Arabidopsis*.

The flowering-time pathways control the expression of the floral pathway integrators *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*), *FT* and *LEAFY* (*LFY*). These genes encode proteins that activate the floral meristem identity genes *APETALA1* (*AP1*), *APETALA2* (*AP2*), *FRUITFULL* (*FUL*), *CAULIFLOWER* (*CAL*) and *LFY*, which convert the vegetative meristem to a floral fate. The photoperiodic and gibberellin pathways activate floral pathway integrators. The *CONSTANS* (*CO*) transcription factor functions in the photoperiod pathway; long-day photoperiods promote flowering by circadian clock-dependent and independent mechanisms, which control the activity of CO. Activation of flowering is antagonised by the floral repressors encoded by *FLOWERING LOCUS C* (*FLC*). *FLC* expression is controlled by the autonomous and vernalization pathways. Promoter and repressive activities are denoted by arrowheads and T-bars respectively. The picture was combined and modified from Henderson and Dean (2004) and from Quesada *et al*. (2005).

Therefore, vernalization accelerates flowering by reducing FLC expression. Mutations in the second pathway, the autonomous pathway, delay flowering under both LDs and SDs, and cause an increase in FLC mRNA levels (reviewed in Boss *et al*., 2004). This second genetic pathway also regulates *FLC* expression but independently of vernalization so that the high FLC mRNA levels observed in these mutants can be corrected by vernalization. Mutants

affected in this pathway also show an altered flowering time in response to ambient temperatures (Blázquez *et al*., 2003). The autonomous pathway appears to represent protein complexes involved in histone modification and RNA processing (He *et al*., 2003; Simpson *et al*., 2003; Ausin *et al*., 2004), and probably also has a more general role than the regulation of FLC expression. Thirdly, application of the growth regulator GA_3 promotes flowering of *Arabidopsis*, and mutations that affect genes required for GA biosynthesis delay flowering, particularly under SDs (Wilson *et al*., 1992). Finally, the photoperiodic pathway controls the response to daylength, and specifically promotes flowering in response to LDs (Yanovsky and Kay 2003; Hayama and Coupland 2003; Searle and Coupland 2004). Mutations in this pathway can either delay flowering under LDs or accelerate flowering under SDs. The last gene that is specifically involved in this pathway is *CONSTANS* (*CO*), which encodes a zinc finger protein that promotes transcription of downstream flowering-time genes (Putterill *et al*., 1995; Robson *et al*., 2001). This photoperiodic pathway probably also plays a role in the effect of light quality on flowering, because high ratios of far-red to red light promote flowering and stabilize the CO protein (Valverde *et al*., 2004), although the flowering response to light quality also involves a *CO*-independent pathway (Cerdan and Chory 2003).

These distinct genetic pathways finally converge to regulate the expression of a small group of downstream genes, sometimes described as floral integrators (Mouradov *et al*., 2002; Simpson and Dean 2002). This group includes two genes that promote flowering, *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*), and *LEAFY*, a gene encoding a transcription factor required to confer floral identity on developing floral primordia. *FT* encodes a protein with similarity to RAF kinase inhibitors of animals (Kardailsky *et al*., 1999; Kobayashi *et al*., 1999) whereas *SOC1* encodes a MADS box transcription factor (Borner *et al*., 2000; Lee *et al*., 2000; Samach *et al*., 2000). Mutations in each of these genes delay flowering, whereas their overexpression from the viral CaMV 35S promoter causes extreme early flowering. The expression of *SOC1* and *FT* is

increased by *CO* and reduced by *FLC*, indicating that they are downstream of the point of convergence of the vernalization and photoperiod pathways (Samach *et al*., 2000; Hepworth *et al*., 2002). Furthermore, the expression of *SOC1* is increased by treating plants with GA, suggesting that it acts downstream of all three pathways (Moon *et al*., 2003).

In addition, less dramatic changes in ambient conditions also strongly influence flowering time. For instance, exposure to lower temperatures of 16ºC instead of typical 20-24ºC as well as exposure to the high ratios of far-red and red light associated with shading conditions accelerates flowering (Blázquez *et al*., 2003, Cerdan and Chory, 2003).

Of noteworthy importance is the extent of genetic conservation amongst different plant species. Homologues of the key regulators, showing at least partial conservation, were isolated in evolutionarily distant dicots and monocots (rice), and in plants with differing photoinductive requirements (SDPs, and day neutral species) compared to *Arabidopsis* (Colasanti 2005).

Physiological Control of Flowering

Over the years, physiological studies have led to three models of control of flowering time (reviewed in Bernier *et al*. 1998; Thomas and Vince-Prue, 1997). The *florigen* concept was based on the transmissibility of substances or signals across grafts between 'donor' shoots and vegetative 'recipients' (Chailakhan 1936). A second general model, the *nutrient diversion hypothesis*, proposes that inductive treatments result in an increase in the amount of assimilates moving to the apical meristem, which in turn induces flowering (Sachs and Hackett, 1969). The latter view was superseded by *the multifactorial control model*, which proposed that a number of promoters and inhibitors, including phytohormones and assimilates, are involved in the controlling the developmental transition (Bernier *et al*. 1998). According to this model, flowering can only occur when the limiting factors are present at the apex in the appropriate concentration and at the right times, accounting for the diversity of

flowering responses by proposing that different factors could be limiting for flowering in different genetic backgrounds and/or under particular environmental conditions.

The Florigen Hypothesis

Evidence obtained from interspecies grafting experiments led to the hypothesis that a signal produced in the photoinduced leaves, is transported via the phloem before triggering the switch to flower formation at the shoot apex (Figure 2). Later extensions to this hypothesis include the existence of an anti-florigenic component as part of the complex mixture representing the updated 'florigen'.

The existence of a mobile floral stimulus was conceived at the time when relatively simple compounds, the phytohormones, were found to have key roles in orchestrating plant growth and plant development and in mediating the plant's response to the environment (Colasanti 2005). Further, these substances were found to have similar effects in a wide variety of plant species. Thus, by extension, the idea was put forward that the florigen might be a simple compound with a universal role in flowering. Properties of the florigen included: 1) the signal originates in the leaf and moves to the SAM, 2) the signal has a measurable rate of movement, 3) the signal is graft-transmissible and must pass through living tissue and 4) the signal is universal (reviewed in Zeevart 1976).

Young mature leaves have generally been assumed to be the most effective for photoperiod perception and generation of long-distance information substances (Bernier *et al*., 1981a, Perilleux and Bernier 2002). Soon after it is formed, the florigen moves out of the induced leaves from cell to cell in the leaf parenchyma (Chailakhan 1940). The translocation of this signal from the induced leaves to the shoot apex occurs in the phloem tissue of vascular bundles of the petioles and stem, as proven by mechanical or physiological disruption of phloem resulting in no florigen transport (Lang 1965, Thomas and Vince-Prue 1997). Additional details regarding the general nature and role of the phloem are

presented in a later section of this introduction. Indirect estimations involving measuring the time needed to move a certain distance were used and were therefore greatly variable, reporting velocities ranging between from 2.4 to 3.5 mm h^{-1} depending on the species (Chailakhan 1936).

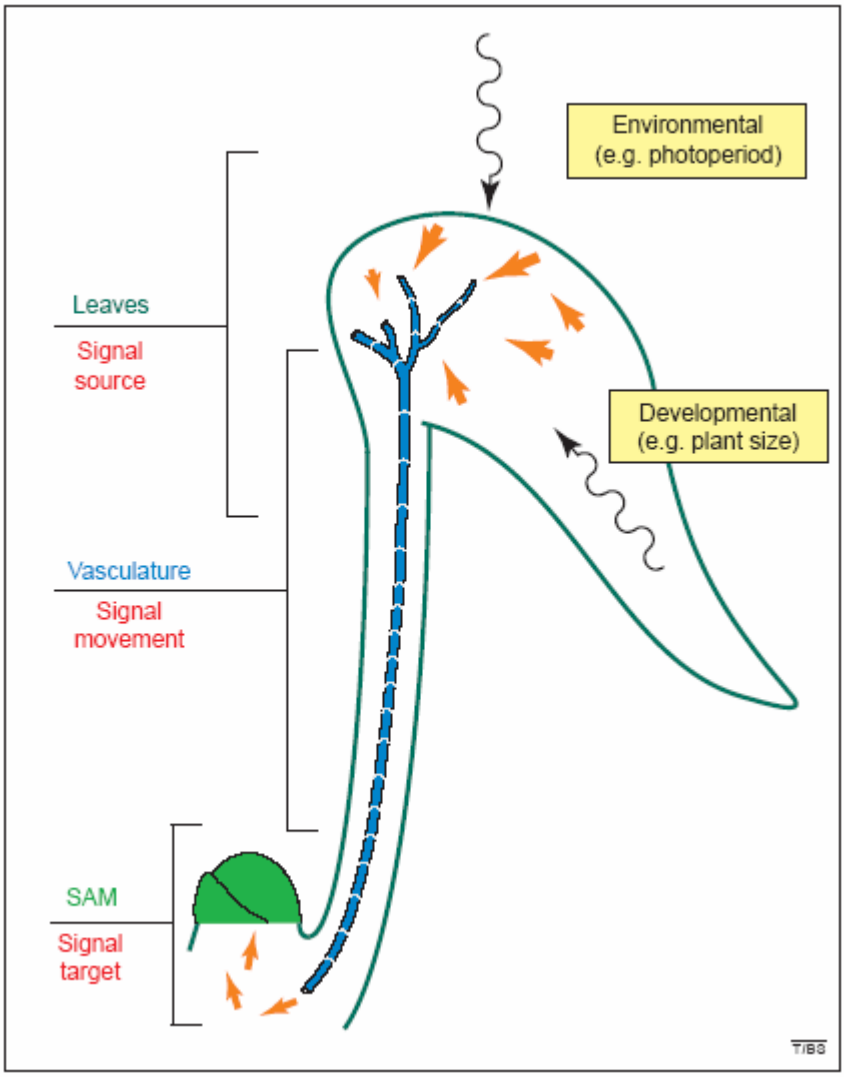


Figure 2: The Florigen Hypothesis. Plants integrating internal cues related to development (e.g. age) and external factors such as photoperiod, light or temperature can produce a signal in their leaves. Transport of this long-distance messenger occurs in the phloem and eventually reaches its target the shoot apical meristem (SAM) where it triggers the formation of floral structures instead of leaves. Picture from Colasanti and Sundaresan (2000).

Once in the upper part of the shoot, the florigen presumably moves from cell to cell beyond the ends of the protophloem strands towards the SAM (Bernier *et al*., 1981a). Events detected in SAMs of photoinduced plants before the start of flower initiation include increased respiratory activity, RNA and protein synthesis, growth rate and cell division, changes in protein complement, alterations in cell wall properties and synchronisation of cell division (Lyndon 1998, Bernier 2005). At the morphological level, the SAM is irreversibly enlarged to a taller inflorescence meristem and young axillary buds develop into inflorescences (Vaughan 1955).

Evidence from interspecific and intergeneric grafts proved the unsuccessful transmission of the florigen between donor and receptor plants, indicating that the universality of this floral stimulus is an exception rather than the rule (Bernier *et al*., 1981b, Bernier 2005).

The focus of numerous studies attempted to isolate this mysterious florigen. Some of the major compound classes that could be involved in floral signalling are discussed below.

Candidates for the Florigen

Gibberellins (GAs) are present in phloem and xylem sap (Perilleux and Bernier 2002). In the grass *Lolium temulentum*, GAs were proven to be a long day signal transmitted to the shoot apex where they induce flowering (Evans and King 1985, Evans *et al*., 1990, King *et al*., 1993, King and Evans 2003, King *et al*., 2003, King *et al*, 2006). When exogenously applied, GAs upregulate *LFY* expression in *Arabidopsis* and eventually activate flowering in *Sinapis alba* as well, but only in combination with other treatments (Blázquez *et al*., 1998, Lang 1965, Mouradov *et al*., 2002, Perilleux and Bernier 2002, Zeevart 1983).

In *Chenopodium rubrum* (SDP) and *Chenopodium murale* (LDP), a transient but significant increase in cytokinins accompanied floral induction in the shoot apex (Macháčková *et al*., 1993, Krekule and Macháčková 2005). Cytokinin levels rose in induced

Arabidopsis and *Sinapis alba* and correlated in time with an increased flux in the *S. alba* shoot apex and with the movement of the floral stimulus in *Arabidopsis*. However, exogenous cytokinin application at the SAM even stimulated cell division, induced the expression of genes associated with floral induction and increased plasmodesmal frequency, but ultimately did not promote flowering (Bonhomme *et al*., 2000, Ormenese *et al*., 2000, Ormenese *et al*., 2006).

Sucrose has also been extensively studied as a long-distance signal for flowering in *Arabidopsis* and *S. alba*, since increasing levels of sucrose coincide with the start of mobile signal transport (Bernier *et al*., 1993, Corbesier *et al*., 1998). However, given the role of sucrose in the phloem for rapid assimilate transport, clear conclusions were not reached.

The presence of proteins and peptides in phloem sap has been well documented (Fisher *et al*., 1992, Marentes and Grusak 1998, Haebel and Kehr 2001, Ruiz-Medrano *et al*., 2001, Hoffmann-Benning *et al*., 2002, Giavaliasco *et al*., 2006) as well as the graft transmissibility of some of them (Golecki *et al*., 1999, Xonocastle-Cázares *et al*., 1999). However, the only work linking floral induction to the role of peptides as long distance signalling molecules used samples that were harvested 3 weeks after the start of the inductive treatment (Hoffmann-Benning *et al*., 2002).

mRNA molecules transcribed in the companion cells of the mature phloem were trafficked to the vegetative plant shoot apex and accumulated in specific cell-types in the SAM and developing organs (Kim *et al*., 2001, Ruiz-Medrano *et al*., 1999). Some mRNAs isolated in the phloem of pumpkin (Ruiz-Medrano *et al*., 1999), cucumber (Ruiz-Medrano *et al*., 1999, Xonocastle-Cázares *et al*., 1999) and tomato (Kim *et al*., 2001), could move across a graft junction. This raised the possibility that mRNA could be part of the mobile signal for developmental events like flowering. Recent data supports the idea that either the *FT* mRNA or the FT protein, or both, is the florigen or at least one of its components (Huang *et al.,* 2005, An *et al*., 2004, Giavalisco *et al*., 2006).

Small RNAs were also detected in the phloem sap of various plants (Yoo *et al*., 2004a). Small RNAs regulate gene expression through several mechanisms and include microRNAs (miRNAs) and short interfering RNAs (siRNAs) (Nakahara and Carthew 2004). siRNAs have been suggested to participate in the spread of RNA silencing (Yoo *et al*., 2004a). Schmid *et al.* (2003) have demonstrated that miRNAs are involved in flowering. One of these miRNAs precursors showed a *CO*- and *FT*-dependent upregulation after floral induction. Auckerman and Sakai (2003) also demonstrated flowering time regulation by microRNAs.

The discovery of signalling functions associated with the following compounds implied that they could be the focus of studies aimed at determining their florigenic potential: steroids, sterols and lipid derivatives are now known to be active signalling components in plants (Bishop and Yogota 2001, Jang *et al*., 2000, Schrick *et al*., 2000, Ng *et al*., 2001), nitric oxide, shown to have a clear role in mediating a defence response against pathogens and small molecules (Beligni and Lamattina 2001).

Even after decades of investigation and scrutiny, the identity of the elusive 'florigen' remains unknown, confirming the complexity of signalling inputs that evolved to orchestrate floral induction under a broad range of environmental, physiological and developmental conditions (Lucas, 2005).

Integration of Genetic, Physiological and Spatio-Temporal Information in Flowering

Only recently, did the integration of the physiology with the genetic work, in the context of spatial resolution, provide additional insights into the regulation of the floral transition.

Promoter-driven tissue-specific expression studies revealed that the site of *CO* expression is the phloem, more specifically the companion cells of mature source leaves. *CO* was classified as part of the photoperiod pathway, encodes a zinc-finger binding transcription

factor and acts upstream of the florigen. Neither the CO protein nor its mRNA move in the phloem, but grafting studies confirmed that *CO* was necessary for the production of a phloem-borne substance that could influence flowering in a wild type scion grown under non-inductive conditions (An *et al*., 2004; Ayre and Turgeon, 2004). The *CO* homolog in maize, (indeterminate gene, *ID1*) was also shown to act in a non-cell autonomous manner and is thought to regulate the production or transport of a signal exported to the shoot apex to induce flowering. (Colasanti *et al*., 1998).

FT expression was also localised to the phloem but could be additionally detected in the meristem. These arguments together with the estimated small protein size (23KDa, Kardailasky *et al*., 1999, Kobayashi *et al*., 1999), and the high sequence homology to mammalian RAF-kinase-inhibitor proteins (RKIP) or phosphatidylethanolamine-binding proteins (PEBP) made FT a strong possible candidate as the mobile graft-transmissible signal or as one of the florigenic components (Kardailasky *et al*., 1999, Kobayashi *et al*., 1999, reviewed in Suarez-Lopez 2005, Bernier 2005). In fact, some studies focusing on either the FT mRNA (Huang *et al*., 2005) or its protein (An *et al*., 2004) suggested their role as part of this stimulus.

Although the expression of *FT* by *CO* could be justified at a spatial level, there still remained a missing link as to how *FT* would activate its downstream target *SOC1* (Yoo *et al*., 2005), whose expression is confined to the SAM. Recently, FD was demonstrated to be FT's interacting partner in the shoot apex. FD is a bZIP transcription factor present in SAM cell walls well before the floral transition but its capacity to trigger inflorescence meristem formation and organ development is dependent upon the presence of FT (Wigge *et al*., 2005). Yeast-two-hybrid experiments and pull down assays confirmed the interactions between the proteins and the formation a FT/FD complex that can then up-regulate the MADS-box and floral meristem identity gene *AP1* (Abe *et al*., 2005; Wigge *et al*., 2005). These findings

provided a link between *FT*'s activation, transport and exertion of its effect, thereby validating classical physiological results.

The closest homolog of *FT*, *TWIN SISTER OF FT* (*TSF*), shares modes of regulation by major genetic pathways and by *CO* in response to plant exposure to LDs (Yamaguchi *et al*., 2005). Recent studies showed that *TSF* promotes flowering in a manner which is largely, but not entirely, redundant with *FT* (Michaels *et al*., 2005, Yamaguchi *et al.,* 2005). Indeed, *TSF* is expressed in phloem tissues, like *FT*, but shows non-overlapping expression pattern in photoinduced WT seedlings with *FT* expressed in cotyledons and *TSF* in hypocotyls. These two genes apparently exert a similar function via the activation of common downstream factors, such as *SOC1*, to trigger flowering, but in complementary parts of the vascular system. *TSF* appears to promote flowering to a greater extent than *FT* at lower ambient temperatures. Together, *FT* and *TSF* may fine tune the timing of the floral transition and provide robustness for the integration of multiple floral signals. It was proposed that the transcripts and/or proteins of these two genes are co-operative florigen components (Yamaguchi *et al.,* 2005). Effectively, the FT and TSF proteins, which share 82% sequence similarity, were both identified in the phloem of *Brassica napus* (Giavalisco *et al.,* 2006).

TFL1 is another member of the RKIP family involved in the establishment, development and differentiation of the inflorescence meristem. In contrast to *FT* and *TSF*, *TFL1* delays flowering and maintains indeterminate growth of the SAM by repressing floral meristem identity genes. In fact, if the timing of flowering is dictated by the fine balance between inducing and inhibitory signals, *FT* and *TFL1* would be these respective players (Kobayashi *et al*., 1999, Ahn *et al*., 2006). The differences in functions of TFL1 and FT were predominantly attributed to their protein sequence rather than their expression pattern (Kardailasky *et al*., 1999, Kobayashi *et al*., 1999, Ratcliffe *et al*., 1998). Similarly, in pea (*Pisum sativum*), genes which produce a floral stimulus in leaves (*gigas*) and others that

might produce floral inhibitors were also identified (Murfet and Reid 1987, Beveridge and Murfet 1996, Weller *et al*., 1997b).

The integration of signals from the above regulatory genes and/or proteins, at the transcriptional level, occurs in the phloem (Yamaguchi *et al*., 2005). This tissue is endowed with such functional capacity given its specialised structure which is discussed below.

The Phloem

Together with the xylem, the phloem constitutes the plant vascular system, a feature developed during the course of evolution which greatly contributed to the reproductive success of flowering plants as terrestrial organisms. This long-distance transport network permits the distribution of water and minerals and also efficiently transports signals to all developing plant parts. More specifically, the xylem achieves the transport of water and minerals from the roots to the aerial parts of the plant via the transpiration stream, while the phloem translocates small substances like inorganic ions, amino- and organic acids and minerals through the plant.

In angiosperms, the phloem is comprised of two main cell types, sieve elements (SEs) and companion cells (CCs). At maturity, the SEs are enucleate and become highly modified to create a low-resistance pathway for the translocation of assimilates. The CCs function in the maintenance of the associated SEs (van Bel 2003). Branched plasmodesmata (PD) connect these two cell types and form a CC-SE symplasmic complex. This specialised structure conducts photoassimilates from source to sink organs by mass flow and allows solute exchange between the phloem and the surrounding plant tissues (Thompson 2006). This combination of the enucleate sieve tube system and the symplasmic domains established by PD allowed angiosperms to develop a non-cell autonomous protein (NCAP)-based signalling network to integrate environmental cues at the whole-plant level (Lough and Lucas, 2006). Regulation of these local and long-distance macromolecular trafficking

networks is likely essential for the coordinated exchange of information between the distantly located plant organs, in order to orchestrate physiological and developmental events at the whole-plant level (Haywood *et al*., 2005).

The phloem is believed to coordinate events in defence and responses linked to stress signalling, with signals ranging from small communication molecules, such as phytohormones (Baker 2000) or certain metabolites, to macromolecules (Pearce *et al*., 2001) as well as messenger RNAs and small RNAs (Jorgensen *et al*., 1998, Lucas *et al*., 2001, Jorgensen 2002, Yoo *et al*., 2004a, Haywood *et al*., 2005) the latter having potential roles in long-distance gene silencing processes. Peptides and proteins (Hayashi *et al*., 2000, Walz *et al*., 2004) present in the phloem could act as generators or amplifiers of messages but can be themselves be the transported signals within the phloem (van Bel and Gaupels 2004, Kehr, 2006).

Therefore, in order to investigate the complement of signals as well as their roles in plant regulatory functions, finding effective ways of accessing the phloem content of plants is essential.

Phloem Sampling Techniques

Most plants do not exude large amounts of phloem sap because of the sealing mechanisms responsible for closing wounds in the phloem. Plant defence responses to wounding involve the formation of callose plugs, which act as a mechanical barrier against further pathogen or herbivorous invasion, while keeping the cell contents from being lost via leakage. The enzyme callose synthase catalyses this reaction and requires calcium ions as cofactors. Chelating agents, such as EDTA, bind divalent ions such as Ca^{2+}. Therefore, treating the cut plant surfaces with EDTA increases the exudation rates (King and Zeevart 1974, Tully and Hanson 1979).

In plant species such as *Ricinus*, cucumber (*Cucumis sativus*), lupine (*Lupinus*), yucca and oil seed rape (*Brassica napus*) (Sakuth *et al*., 1993, Marentes and Grusak 1998, Kehr *et al*., 1999, Giavalisco *et al*., 2006), relatively pure samples can be obtained by phloem sap exudation from wounds that sever sieve elements. Small incisions made with sterile needles, followed by subsequent collection by pipette, allow phloem sampling from members of the *Cucurbitaceae* family and from oil seed rape. The driving force for exudation is the high pressure in the sieve elements, but a major disadvantage of this wounding method is that the fluid collected may not represent the true composition of the translocated material. Contaminants can originate from leakage of damaged neighbouring parenchyma cells or even from the sieve elements themselves (Hanson and Cohen 1985, Ziegler 1975). The sap may also be diluted by water influx from the xylem or as a consequence of lowered turgor pressure in the sieve elements from surrounding cells.

Alternative aphid-based sampling techniques, which are probably more representative of phloem sap composition, include collection either by severing the stylet with a laser or using honeydew (Fisher and Frame 1984). Aphid stylectomy was successfully applied to both monocots (Fisher and Frame 1984) and dicots (Lohaus *et al*., 2000, Lohaus and Moellers 2000). However, inherent difficulties, such as placing the insects at the desired location and severing stylets without disrupting them, make this an altogether very tedious and time-consuming method. Other limiting factors are the compatibility of plant-insect combinations and the minute amounts of sample obtained (Hayashi and Chino 1986).

Collection of phloem sap by glass microcapillaries of fluorescently-labelled SEs has also been reported (Raps *et al*., 2001). In some plant species, final samples are in the microliter range and therefore allow further analysis.

Aims and Objectives of the Present Work

Although it was suggested that flowering can be triggered by pathways independent of *FT*, undeniably *CO* remains central in controlling the production of the floral signal. This florigen, regardless of its nature, can be assumed to be produced in the leaves (transiently in certain species and continually in others) and must be rapidly transported to the shoot apex. A large focus of this work therefore relied on the use of transgenic plants with inactive *CO* or overexpressing lines, with emphasis on the analysis of tissue-specific samples subjected to inductive conditions. The overall aims of this work are presented below:

1. Investigating transcript changes associated with the early events underlying the floral transition in *Arabidopsis thaliana* in leaves

(a) by specifying time windows using the expression of known marker genes associated with the floral transition via the comparison of two independent induction methods, a light extension and an artificial dexamethasone-based system.

(b) by identifying candidate genes showing consistent gene expression patterns between the two systems.

2. Characterization of significant metabolite changes potentially involved in the floral transition using GC-MS

(a) in *Arabidopsis* using the information obtained in 1(a).

(b) by comparing metabolites relevant to floral induction in *Sinapis alba*.

3. To obtain insights into the role of FT and floral induction

- by evaluating the reactivity and specificity of a peptide antibody generated against the FT protein present in the phloem of *Brassica napus*, and in phloem exudate samples of *Sinapis alba* and *Arabidopsis* collected in EDTA.

MATERIALS AND METHODS

Enzymes and Chemicals

All enzymes used were at least grade II and chemicals were at least of analytical purity. Biochemical enzymes and substrates were purchased from Roche (Mannheim) and Sigma (Munich). The chemicals were obtained from Roche (Mannheim), Merck (Darmstadt) or Sigma (Munich). All the reagents for SDS-PAGE were purchased from BioRad (Munich).

Plant Systems Employed

Arabidopsis thaliana

	Analysis				
	Transcripts		Metabolites		Proteins
Induction Experiment	ESD	DEX	ESD	DEX	
Arabidopsis Lines:					
L*er*	x		x		x
co-2	x	x	x	x	x
ft-7					x
ft-7/soc1-1					x
35S::CO:GR, *co-2*		x		x	
SUC2:FT					x

Table 1: Summary of the *Arabidopsis* wild type, flowering time mutants and overexpressing plants used for transcript, metabolite and protein analysis. Lines used for specific experiments are denoted by an 'x'. For transcripts and metabolites, plants were selected based on the induction system used. Experiments using plants subjected to additional light (an extended short day, ESD) utilised *CO* mutant (*co-2*) and the wild type *Landsberg erecta* (L*er*) lines. Floral induction triggered by the dexamethasone (DEX) system employed plants constitutively expressing the *CO* gene as a fusion to the rat glucocorticoid receptor (GR) generated in a mutant *CO* background (35S::CO:GR, *co-2*: see main text for details) and the *co-2* line. For protein analysis, the wild type, independent *CO* and *FT* (*ft-7*) knock out lines, the *FT* and *SOC1* double mutant and an *FT* overexpressor under the control of the phloem-specific sucrose promoter (SUC2:FT) were used.

All wild type and transgenic lines were created in the rapid-cycling progenitor *Landsberg erecta* (L*er*) ecotype background and were kindly provided by Prof. George Coupland (MPIZ, Cologne, Germany). Combinations of the lines used for transcript, metabolite and protein analysis are given in Table 1 above. The different flower induction systems used are described later.

Batches of plants used for induction were grown in soil under a short day light regime (8 h light, 16 h dark), under controlled conditions (at 20°C and 55% relative humidity) and at an irradiance of 150 μmol m^{-2} s^{-1} (PAR), with light provided by cool fluorescent tubes (Obeta, Potsdam, Germany) for three weeks.

Otherwise, seeds were sown in soil and plants were grown in LDs (16 h light, 8 h dark) under controlled conditions (20°C and 55% relative humidity) and at an irradiance of 150 μmol m^{-2} s^{-1} (PAR), with light provided by cool fluorescent tubes (Obeta, Potsdam, Germany).

Sinapis alba

Sinapis alba Grown in Belgium

Growth conditions for *S. alba* were as described elsewhere (Lejeune *et al*., 1988). Briefly, *Sinapis alba* L. seeds were sown and plants grown on a mixture of perlite and vermiculite (1:1) in 8 cm pots and were watered every 2 days with demineralised water and with a complete Hoagland solution once a week. Plants were grown for 7 weeks under 8 h short day conditions (under which they remained strictly vegetative) in controlled cabinets at a temperature of 20ºC and at a relative humidity of about 70%. Light was provided by Very High Output Sylvania fluorescent tubes (Sylvania, Zaventem, Belgium) at an irradiance of 150 μmol m^{-2} s^{-1} (PAR).

Sinapis alba Grown in Golm

Seeds of *Sinapis alba* L. (*Brassica hirta*) were sown in soil (Einheitserde Typ Topferde mit Quarzsand) and the plants were grown in short day conditions (8 h light, 20°C and 16 h dark, 18°C) in controlled cabinets (20°C, day and 18°C, night) at relative humidity of about 70% at light intensities of 150 μmol m^{-2} s^{-1} for 5 days. Plants were then transferred to regimes of light provided by fluorescent tubes (Obeta, Potsdam, Germany) at an irradiance of 300 μmol m^{-2} s^{-1} (PAR) for an additional 14 days. Plants were watered with fertiliser (Hakaphos in a concentration of 3g per litre) twice a week when they were 41 days old.

Brassica napus

Brassica napus plants cv. "Drakkar" (Serasem GIE, la Chapelle d'Armentiers, France) were grown in sterilised soil (Einheitserde® Typ T) in long days (16 h light, 8 h dark) under controlled conditions (25°C day, 20°C night, 55% relative humidity) and were automatically watered three times a day with tap water containing fertilizer (Hakaphos® spezial). Batches of 30 plants were divided into 3 subgroups and used for phloem collection on a rotational basis for protein analyses.

Flower Induction Systems

Light Extension System (Arabidopsis thaliana)

Three-week old batches of wild-type *Ler* plants grown under short day conditions were subjected to extended light periods of 10, 12, 14, 16 and 20 hours to test for discrepancies between the critical daylength of reported Columbia accessions (Corbesier *et al*., 1996). Repeated experiments demonstrated that exposure to 14 additional hours of light, provided by incandescent bulbs was sufficient to irreversibly induce at least 90% of plants to flower. Four independent repetitions of the inductive cycles were performed on individual batches of approximately 100 plants and consistently yielded a satisfactory

phenotype. These conditions were therefore used for large-scale experiments as outlined in Figure 3. Also depicted are the conditions under which the control plants were kept as well as the corresponding sampling points.

Dexamethasone-Inducible System (Arabidopsis thaliana)

A number of pilot inductions were performed by spraying soil-grown 35S:*CO*::*GR* and *co-2* plants with dexamethasone (DEX) solution. The working concentration of 10 μM (in water) was diluted from a 10 mM stock solution prepared in ethanol (w/v). The application of DEX, a strong synthetic glucocorticoid (Kang *et al*., 1999), activates proteins containing the GR domain by allowing the fusion protein to be imported to the nucleus and rapidly promotes transcription (Samach *et al*., 2000). In this case, after *CO* is transported to the nucleus and once transcribed, it can trigger downstream signalling cascades associated with the initiation of floral development. Control treatment was applied as a 1% ethanol solution, matching the dilution factor of the DEX solution. The final design of these experiments in terms of the induction times is shown in Figure 4. Sampling points were based on previously reported florigen movement information and on experiment size (Corbesier *et al*., 1996). The efficiency of induction was assessed when shoot apices were dissected 2 weeks later - at least 90% of all plants were flowering. Replicate experiments were performed on 6 separate occasions using 75 plants per treatment and per genotype. A consistent and satisfactory flowering phenotype was obtained on all occasions.

Photoextension System (Sinapis alba)

65 day-old plants grown in Belgium and 21 day-old plants grown in Golm were subjected to 14 additional hours of light. Control plants were kept under short day conditions and sampled in parallel. Details of exact sampling times and exudation intervals are provided in Figure 5 for metabolites and in Figure 6 for proteins. The photoperiodic extension was

given with the same light source and at the same irradiance as during the standard short day regime. Dissection of shoot apices 2 weeks later showed that all induced plants had initiated flowers while all control plants kept continuously under short day conditions remained vegetative.

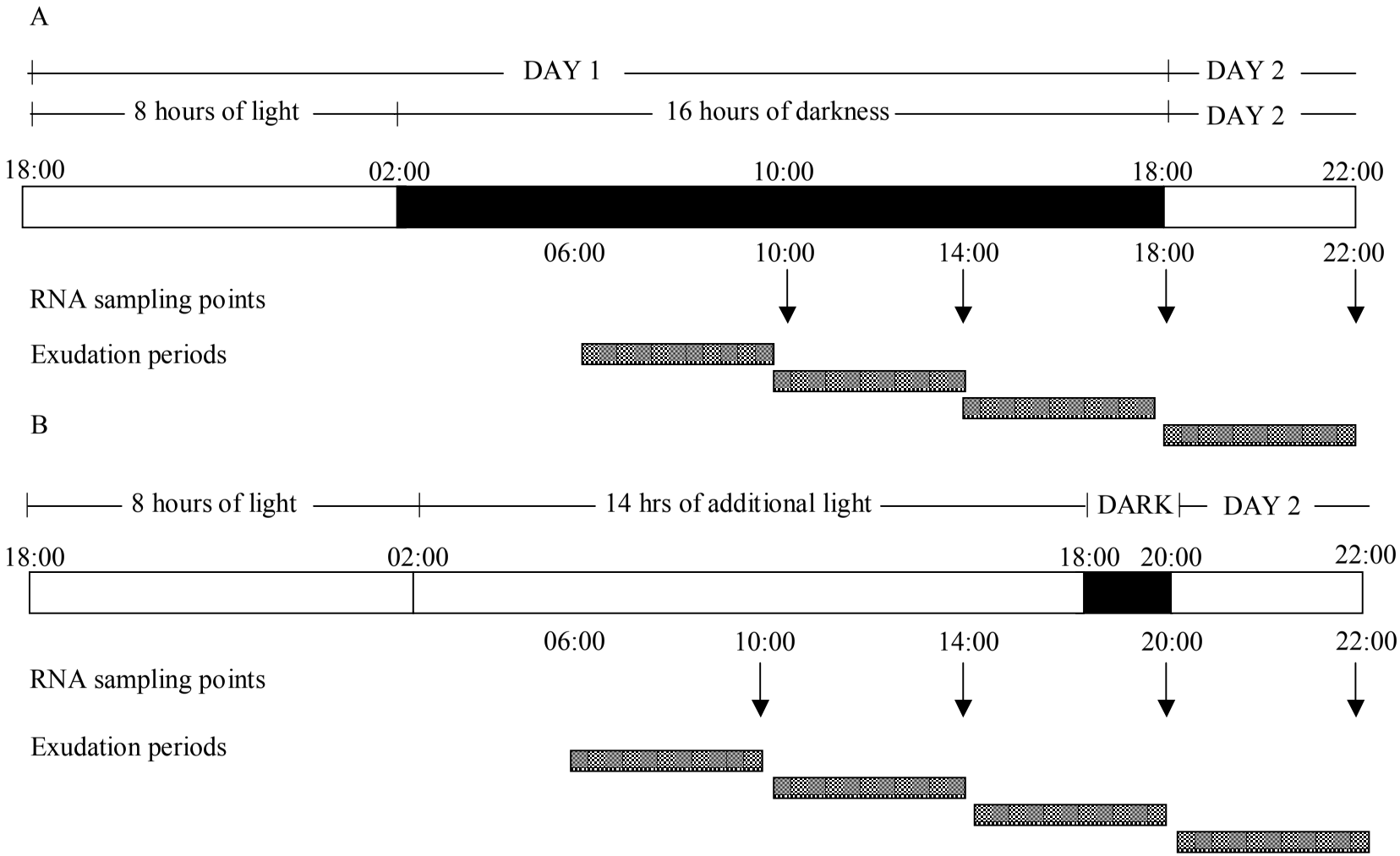


Figure 3: Growth, induction conditions and sampling details details of *Arabidopsis thaliana* for the light extension inductive system. (A): All *Arabidopsis* plants were raised under short day conditions (8 hours of light). (B): Batches of plants subjected to induction were exposed to 14 additional hours of incandescent light and RNA samples were taken at the time points indicated with arrows (at time 0 and at 4, 8 and 12 hours post-induction) to coincide with the ends of the exudation times for metabolite sampling. Control batches of plants were kept in short day conditions and were sampled in parallel.

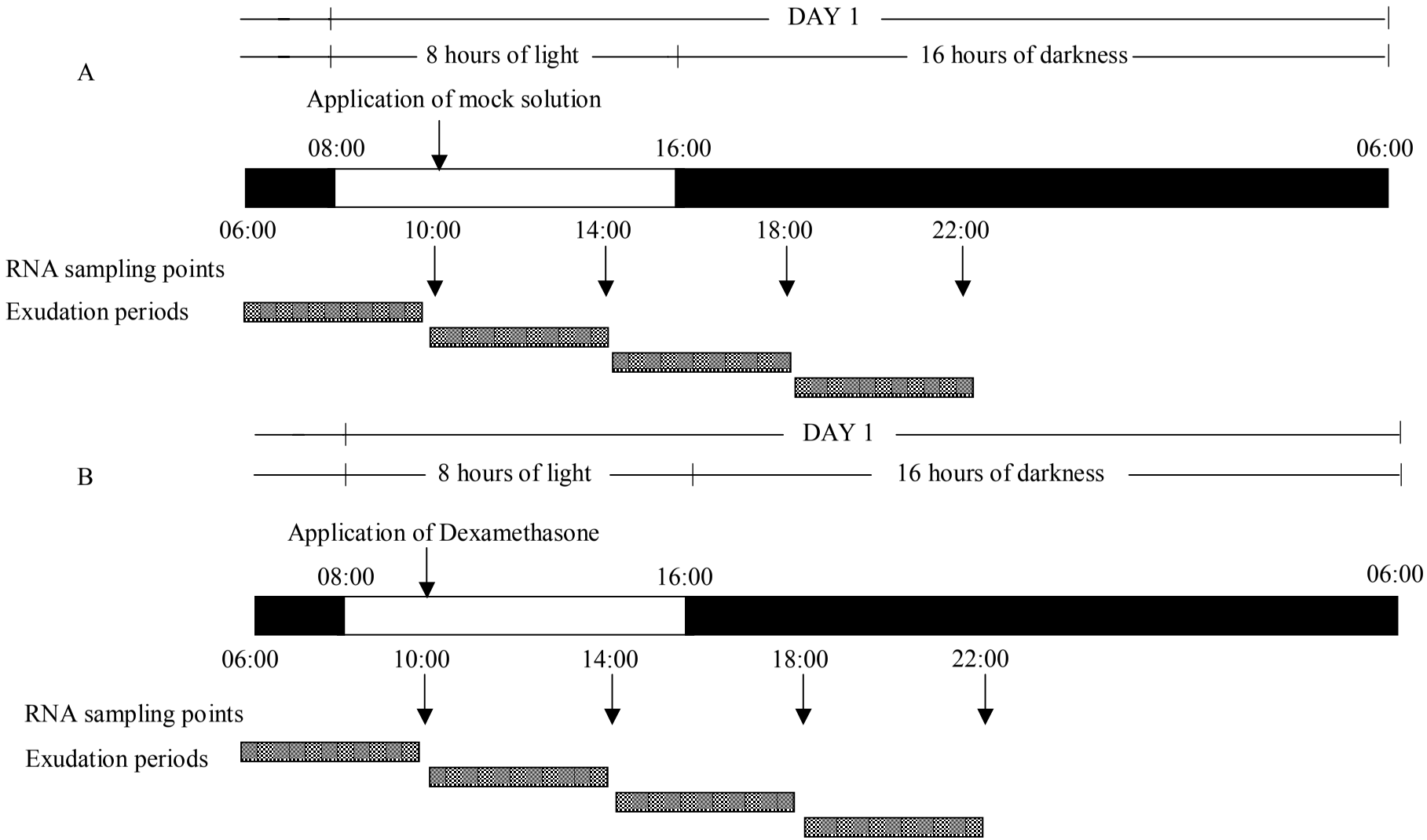


Figure 4: Growth, induction conditions and sampling details of *Arabidopsis thaliana* for the dexamethasone (DEX) system. (A): All *Arabidopsis* plants were raised under short day conditions (8 hours of light). (B): DEX was sprayed as indicated (at 10:00) and RNA samples were taken at the time points indicated with arrows (at time 0 and at 4, 8 and 12 hours post-induction) to coincide with the ends of the exudation times for metabolite sampling. Control batches of plants were sprayed with a mock solution of 1% ethanol and were sampled in parallel.

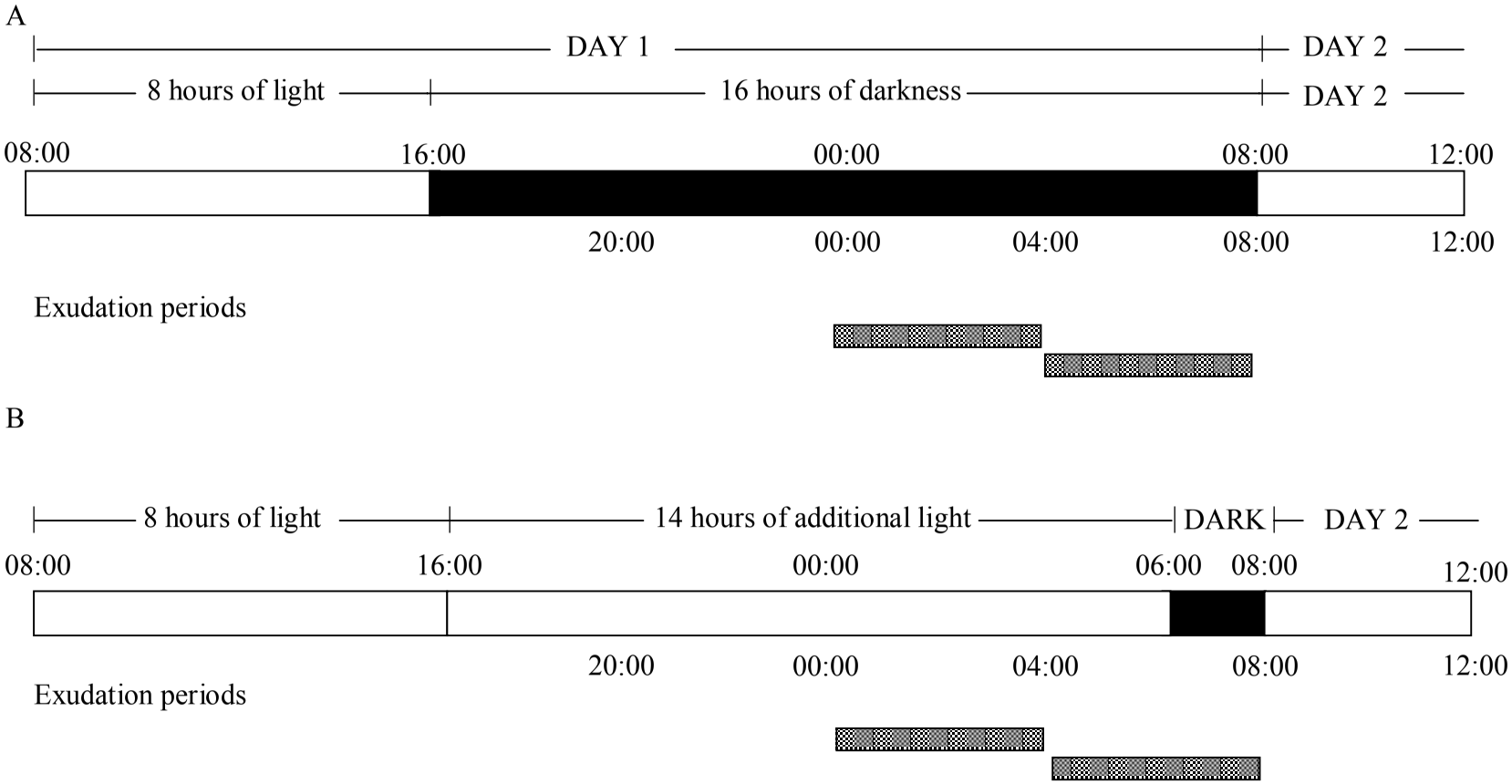


Figure 5: Growth, induction conditions and sampling details for *Sinapis alba* grown Liège, Belgium. (A): All plants were raised under short day conditions (8 hours of light). (B): Batches of plants subjected to induction were exposed to 14 additional hours of light. Phloem exudation in EDTA was performed for leaf and shoot apex samples as indicated by the shaded rectangles. Each exudation lasted 4 hours. Control batches of plants were kept in short day conditions and were sampled in parallel.

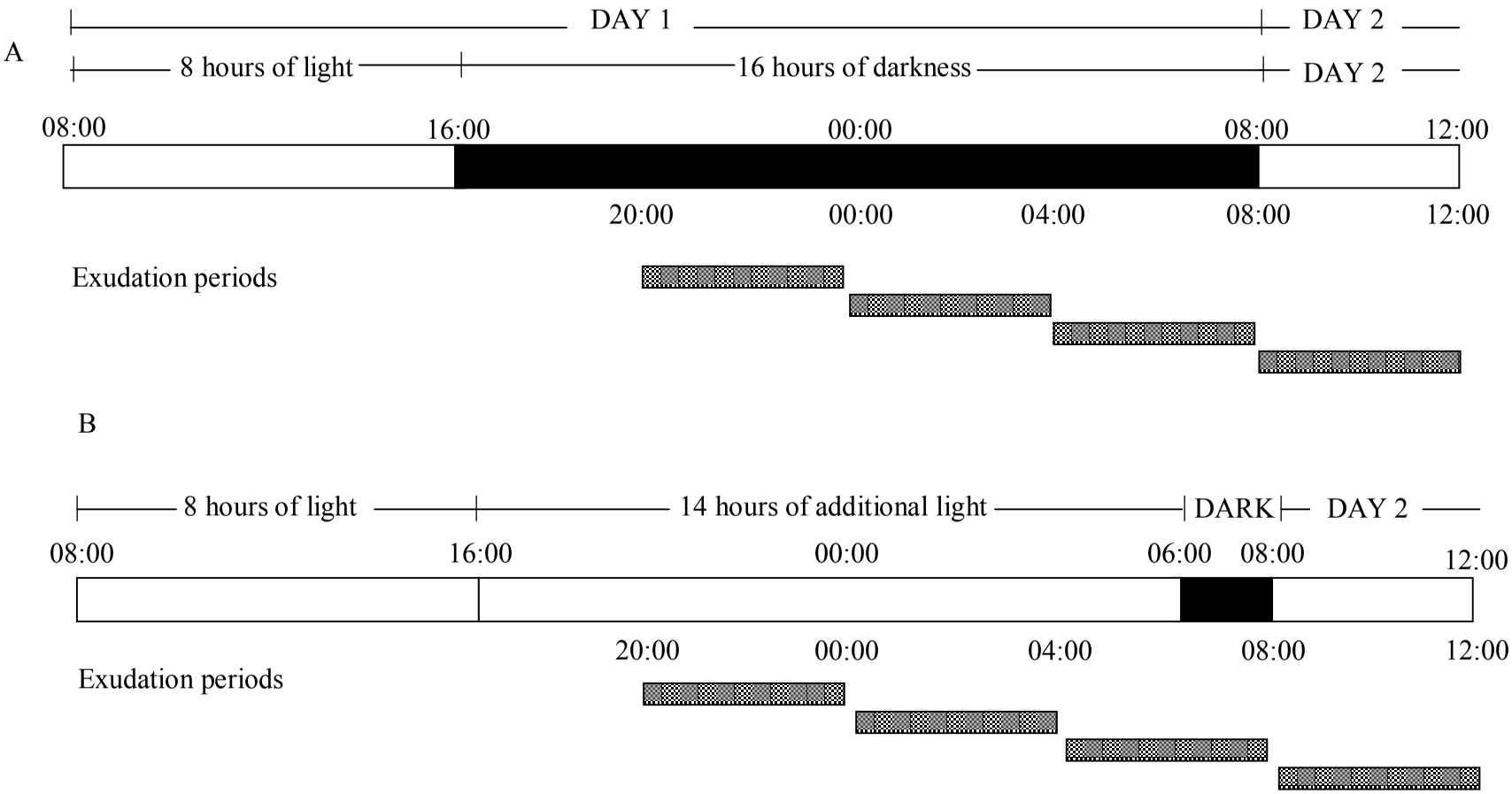


Figure 6: Growth, induction conditions and sampling details for *Sinapis alba* grown in Golm. (A): All plants were raised under short day conditions (8 hours of light, 16 hours of darkness). (B): Batches of three week-old plants subjected to induction were exposed to one cycle of 14 hours of additional light. Exudation times for protein samples lasted four hours each are indicated by the hatched boxes. Control batches of plants were kept in short day conditions and were sampled in parallel. Sub-batches of plants that were not sampled were returned to short day conditions and the flowering phenotype was later checked to confirm the induction efficiency.

RNA Analyses

Sampling and RNA Isolation for Transcript Profiling

15 complete *Arabidopsis* plants were collected (without roots) at the times indicated in Figures 3 (photoextension inductive system) and 4 (dexamethasone-inducible system), pooled, snap frozen in liquid nitrogen, and stored at -80 ºC until further processing. All plant tissue was homogenised using cooled pestles and mortars. Total RNA was extracted with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Briefly, 100 mg of homogenised plant tissue was used and extractions were carried out in triplicates for every sample. The RNA quality was checked by gel analysis according to the Affymetrix technical manual.

RNA Labelling and Hybridisation

Quality-checked RNA was sent to the German Resource Center for Genomic Research (RZPD, Berlin, Germany) for probe preparation and Arabidopsis GeneChip hybridisation (Affymetrix, Santa Clara, CA). Twenty µg of total RNA was used for double-strand cDNA synthesis (SuperScript Choice system, Gibco/BRL). Biotin-labelled cRNA was synthesized using the BioArray High Yield RNA Transcript Labeling Kit (Enzo Life Sciences, Farmingdale, NY). Affymetrix GeneChip experiments including washing and scanning procedures were performed as described in the Affymetrix technical manual.

Data Processing and Normalisation

Data were analysed using the bioconductor software project (Gentleman *et al*., 2004). The starting point for all analyses were the .CEL files generated by the Affymetrix GCOS software. These contain signal intensities for 11 probesets per gene for ~22k genes. Each probeset consists of a perfect match (PM) complementary to the target sequence and a mis-

match (MM) probe which has a base switched at the middle position and so should give information on non-specific hybridisation

Data quality was assessed using functions in the Affy (Gautier *et al*., 2004) and AffyPLM packages to visualise the uniformity of raw intensity distributions, RNA degradation/labelling efficiency and probe intensities between arrays. The robust multichip average (RMA) algorithm was used to obtain expression estimates (Irizarry *et al*., 2003). RMA uses quantile normalization and median polish of background corrected PM probe intensities to generate robust expression estimates. The median polish algorithm fits probe- and array-specific effects to give more precise expression estimates.

The Affymetrix GCOS software (www.affymetrix.com) uses both PM and MM probes. After background correction MM (or, when MM>PM, a computed MM) values are subtracted from PM values and a one-step Tukey's biweight estimate is used to give a robust mean across the 11 probesets as an expression estimate. Expression estimates are then scaled by setting the trimmed mean across all probesets to a target intensity of 500 to allow comparison between arrays. These expression estimates are more accurate particularly at low signal intensities. Therefore, they are used in combination with a detection call. The detection call assigns a 'present' or 'absent' to each probeset using a one-sided Wilcoxan's signed rank test to determine if the 11 PM probes are significantly higher than the MM probes. To reduce the number of false-positives, a gene must show a signal change and in addition be called 'present' before or after a treatment to respectively be considered as decreased or increased.

Sampling and Preparation

Phloem Sampling

Collection of Arabidopsis thaliana Leaf Phloem Exudate

The EDTA exudation method (King and Zeevart 1974) was used. Briefly, plants which do not readily exude phloem sap were blocked in their wounding response. The formation of callose was prevented by using EDTA to chelate the calcium ions required to catalyse the formation of callose. Hence treating plants with EDTA resulted in phloem exudation. This method was previously adapted to *Arabidopsis thaliana* (Corbesier *et al*., 1998) and was modified as follows. At the times indicated in Figures 3 and 4, the 5 youngest, fully-expanded leaves of individual plants were cut at the base of the petiole and placed together in a glass vial (Chromacol, Herts, UK) with the base of their petioles immersed in 200 µl of 20 mM EDTA solution adjusted to pH 8.5. All glass vials were inserted in Eppendorf tubes which were placed in airtight containers. Inside the containers, the atmosphere was water-saturated to prevent uptake of the EDTA solution by the leaves. Rinsing glass vials and Eppendorfs tubes with methanol ensured that contaminants such as dust were eliminated. Individual exudations were carried out for 4 hours but sampling periods during an entire inductive cycle lasted 16 hours. All exudates were immediately frozen after collection and stored at -20°C until further analysis.

Modified Collection of Arabidopsis thaliana Leaf Phloem Exudate

Leaf phloem exudates were collected as above with the following modifications. Five leaves, cut from 50 plants, were pooled and allowed to exude in 20 ml of 20 mM EDTA containing 1.5% (v/v) of protease inhibitor cocktail for plant cell and tissue extracts (PIC, Sigma, Steinheim, Germany) to prevent protein degradation. Inflorescence stems were used in the case of the SUC2:FT line. Once the 4-hour exudation periods were over, DTT was added in a final concentration of 4 mM to prevent the formation of protein complexes and samples were stored at -20°C until further analysis.

Collection of Sinapis alba Leaf Phloem Exudate

The EDTA exudation method (King and Zeevart 1974) adapted to *Sinapis alba* L. (Corbesier *et al*., 1998) was modified as follows. The uppermost 5 leaves, below the half-expanded one, of 15 independent plants were cut. Petioles recut in distilled water (to ensure the removal of any air bubbles that may block phloem from exudating from the stem) were placed together in a 25 ml beaker with their bases immersed in 1.5 ml of 20 mM EDTA adjusted to pH 7.5. The beakers were placed in airtight containers in which the atmosphere was water-saturated to prevent uptake of the EDTA solution by the leaves. Samples were collected over 2 exudation periods each lasting 4 hours at the times indicated in Figure 5. All exudates were immediately frozen after collection and stored at -20°C until further analysis.

For protein analyses, leaf phloem exudates were collected as described for 'Modified Collection of Arabidopsis thaliana Leaf Phloem Exudate' above. Leaves from 20 individual plants were used for exudations.

Collection of Sinapis alba Apical Phloem Exudate

Apical exudates were prepared after Lejeune *et al*. (1993) with the following modifications. 15 plants per batch were detopped below the third leaf longer than 1 cm. A 0.5 ml microcentrifuge tube containing 1% agarose (Electrophoresis Grade, Pharmacia, Uppsala, Sweden) prepared in 20 mM EDTA (pH 7.5) was immediately placed on the cut stump. 15 independent plants were used for each exudation in every treatment. Exudation periods were complementary to petiole exudate collection, were started in parallel at the times indicated in Figure 5 and also lasted 4 hours. The tubes were then removed and the agar blocks immediately transferred to Vivaclear centrifugal filters (Vivascience, Hannover, Germany). After centrifugation at 10000 rpm for 20 minutes at 4°C, the flow-through was collected and stored at -20°C until further analysis.

Modified Collection of Sinapis alba Apical Exudate

Apical phloem exudates were collected as described above with the following changes. Preparation of 0.5 ml microcentrifuge tubes containing 1% agarose (Electrophoresis Grade, Pharmacia, Uppsala, Sweden) in 20 mM EDTA (pH 7.5) were mixed with 1.5% (v/v) of protease inhibitor cocktail for plant cell and tissue (PIC, Sigma, Steinheim, Germany). 30 plants were used to collect shoot apex exudate samples.

Metabolite Analyses

Setting up a Metabolite Profiling Procedure for Reliable Peak Quantification

The main goal was to analyse and detect the maximum number of metabolites while simultaneously quantifying highly abundant as well as the lower abundant, noisier peaks.

Removing/Eliminating Contaminants

Precautions to ensure that the influence of any contaminating compounds was minimised were discussed above. Rinsing of all the vials improved the 'purity' of the chromatograms as published by Schad *et al.* (2005). A further precaution was the parallel use of blanks interspersed among the biological samples during measurements. This ensured that artifactual peaks could later be identified and discarded.

Extraction and Derivatisation

Given that the EDTA exudates were already in solution, no extraction was necessary and samples were aliquoted before they were dried down in pre-washed glass vials. Initial volumes had to be adjusted to ensure that highly abundant carbohydrates were not oversaturated.

The dried aliquots were dissolved in 5 µL of methoxamine hydrochloride (20 mg/ml pyridine) and incubated at 30°C for 90 min with continuous shaking. Then 45 µL of N-methyl-

N-trimethylsilyltrifluoroacetamide (MSTFA) were added to derivatise polar functional groups at 37°C for 30 min. These reduced volumes relative to the standard protocol ensured that the peaks of lower abundance would not become too diluted and would still be detectable, thereby remaining quantifiable, even in the presence of more abundant peaks eluting close to or at the same elution times. The derivatised samples were stored at room temperature for 120 min before injection. The use of internal standards was also omitted since ion suppression would result and very likely eliminate candidate molecules of interest. Alternative ways of correcting for the retention time shifts were looked into and employed.

Metabolite Profiling by GC-MS Analysis

GC-TOF MS (Leco Pegasus II GC-TOF mass spectrometer; Leco, St. Joseph, MI, USA) analyses were performed on an HP 5890 gas chromatograph with tapered, deactivated split/splitless liners containing glasswool (Agilent, Böblingen, Germany) and 1.5 µL splitless injection at 230°C injector temperature. Before each injection, the liner was rinsed with a pure MSTFA injection (1 µl). Sample injection was carried out without sample wash steps due to the limited amount of total sample volume. The GC was operated at constant flow of 2 ml/min helium and a 30 m 0.32 mm id 0.25 µm MDN35 column (Macherey-Nagel, Düren, Germany). The temperature gradient started at 80°C, was held isocratic for 2 min, and subsequently ramped at 15°/min to a final temperature of 330°C which was held for 6 min. Twenty spectra per second were recorded between m/z 85–500. Peak identification and quantification were performed using the Pegasus software package ChromaTOF 1.61 (Leco, St Joseph, MI, USA). Retention time shifts were corrected by linear interpolation using known metabolites as reference markers. All files were subsequently processed against a reference which was generated using a signal/noise threshold of 10 with automated peak identification based on mass spectral

comparison to a standard NIST 98 library and available in house customized mass spectral libraries.

Protein Analyses

Purification and Analysis of Proteins from Sinapis alba and Arabidopsis thaliana Exudates

Pooled petiole and apical exudates were loaded on Amicon Ultra-4 centrifugal filter devices (Millipore, Eschborn, Germany) with a nominal molecular weight size exclusion limit of 10 KDa and centrifuged for 30 minutes at 4000 rpm at 4°C. The separate protein fractions were then further analysed independently. The protein fraction above 10 KDa was run on 1-DE.

Total Leaf Protein Extraction – Arabidopsis thaliana

Leaves of *Arabidopsis thaliana* (inflorescence stems in the case of the early flowering SUC2:FT overexpressor) were ground in liquid nitrogen, incubated in extraction buffer [50 mM Hepes 7.5, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM benzamidine, 1 mM e-amino caproic acid, 20 % glycerol, 1 mM PMSF (hydroxy ethyl piperazine sulfonic acid), 20 µM leupeptin, 1 % Triton X-100, 5 mM DTT] for 15 minutes on ice and then subjected to centrifugation at 14000 rpm for 15 minutes at 4°C. The protein concentration of the supernatants (total protein extracts) was determined using the BioRad protein assay kit (BioRad, Munich, Germany), following instructions of the supplier and as described below.

Brassica napus

Phloem Sampling and Protein Extraction

All initial tests for antibody specificity were carried out on phloem sap collected from young flowering stems of *Brassica napus* L., as described in Giavalisco *et al.* (2006). Briefly, small punctures were made a sterile hypodermic needle on inflorescence stems of 8-10 week-old

plants. Phloem exudation was restricted to this location. To minimise contamination by destroyed cells, the first exuding droplets were discarded and only the subsequent exudate was collected. Sample volumes from one wounding site varied between 10-200 µl. 300 µl of phloem sap were collected in 5 µl of PIC and concentrated using Microcon columns (Millipore, Eschborn, Germany) with nominal molecular weight size exclusion limit of 10 KDa by centrifugation for 2 hours at 12000 rpm at 4°C. The flow-through was discarded and 100-200 µl of 20 mM sodium phosphate buffer was added to the sample on top of the column and centrifuged for a further 1-2 hours until about 50 µl of the samples was still above the membrane. Concentrated and purified samples were stored at -20°C until further use.

Spectrophotometric Estimation of Protein Concentration

Protein content of the samples was determined using the BioRad protein assay kit (BioRad, Munich, Germany). This method is based on unspecific binding of the staining agent Coomassie brilliant blue to the cationic and non-polar, hydrophobic sites of proteins. Following this reaction the absorption maximum rises from 465 to 595 nm. Absorbance was measured using a UV-VIS Biophotometer (BioRad) calibrated with BSA.

Production of an Antibody against the FT Protein

The FT peptide sequence obtained from rape phloem (as shown in the alignment in Figure 11) was synthesised and conjugated with hemocyanine from *Limulus polyphemus* for the immunisation of rabbits by subcutaneous injection (BioGenes GmbH, Berlin, Germany). Four weekly injections of the same amount of peptide were given before bleeding. Crude sera were used for initial reactivity tests. Antisera immunopurified from the fourth bleeding were used for all Western blot assays.

Gel Electrophoresis

SDS-PAGE

Phloem, exudate and total protein extract samples were separated on 1-DE on pseudo-native gels. Sample volumes corresponding to 50 µg of protein were mixed with an equal volume of non-denaturing sample buffer [50 mM Tris, adjusted to pH 8.0 with HCl, 1 mM EDTA, 10% SDS, 15% glycerol and 0.05% bromophenol blue]. Proteins were heat denatured at 75°C for 5 minutes. Separation was performed in 12% separation gels with 4% stacking gels in a Mini-PROTEAN® III system (BioRad) at 66 V (stacking gel) and 100 V (separating gel). Proteins were visualised by colloidal Coomassie staining overnight (Novex ™ Colloidal Blue Staining Kit, Invitrogen, Karlsruhe, Germany).

Tris-Tricine SDS-PAGE

This system is suitable for the separation of proteins in the range of 1 to 100 KDa. Separation was performed in 16.5% separating gels, with 10% spacing gels and 4% stacking gels, using gel buffer composed of 3M Tris adjusted to pH 8.45 with HCl and 0.3% SDS. Electrophoresis was carried out using anode (0.2M Tris-HCl, pH 8.9) and cathode (0.1M Tris, 0.1M Tricine, 0.1% SDS) buffers. Samples were incubated with equal volumes of sample buffer (4% SDS (w/v), 12% glycerol (w/v), 5 mM Tris, pH 6.8, 2 % β-mercaptoethanol (v/v), 0.01 % bromophenol blue)

Immunoblotting

A second gel containing the same samples was immunoblotted as described in Buhtz *et al.* (2004). After gel electrophoresis, gels were incubated for 10 minutes in standard transfer buffer containing 20% methanol. Subsequently, the gel-separated proteins were transferred onto nitrocellulose membranes (0.2 µm Porablot, Schleicher-Schuell, Germany) using a mini

transblot cell system (BioRad) for 1 hour at 80 V. Blots were stained for total protein using Ponceau (0.1% (w/v) in 5% acetic acid) and destained in water. After overnight blocking with a 3% solution of bovine serum albumin (BSA), blots were incubated with the produced peptide antibody directed against the FLOWERING LOCUS T (FT) protein in *Brassica napus* L. phloem sap (BioGenes GmBH, Berlin, Germany) for 3 hrs in a working dilution of 1:1000. Three steps of washing in TBS-T solution (20 mM Tris-base, 150 mM NaCl adjusted to pH 7.4, 0.05% Tween 20) were applied after the incubation with antibodies. For detection of antibody-bound proteins, secondary IgM rabbit antibodies coupled to alkaline phosphatase (Sigma-Aldrich, Steinheim, Germany) were used in a 1:5000 dilution and complexes were visualised by adding NBT/BCIP solutions (Roche Diagnostics GmbH, Mannheim, Germany). A 1µl aliquot of the peptide used to generate the antibody against FT was dotted on the membrane and used as a positive control on all Western blots.

In-gel Tryptic Digestion

All stained bands corresponding to signals obtained in immunoblots or showing differential intensity and/or presence/absence in either control/induced or petiole/apical sample combinations were excised from gels, transferred to 0.5 ml siliconised, nuclease-free reaction tubes (Ambion, Huntingdon, UK), and destained for 2 hours in a solution containing 40% (v/v) acetonitrile and 60% (v/v) NH_4HCO_3. Destained spots were dehydrated in 100% acetonitrile (ACN) for 5 minutes and air dried for 5 minutes. 20 µl of modified trypsin solution (0.001 µg/µl in 50 mM NH_4HCO_3) were added to each sample and after complete absorption of the solution by the gel piece (about 30 minutes), 30 µl of 50 mM NH_4HCO_3 solution were added. The digestion reactions were incubated overnight at 37˚C. For the elution of the proteolytic peptides, gel pieces were incubated first with 50 µl of 5% (v/v) trifluoroacetic acid (TFA) for 20 minutes then three times with 50 ul of 5% TFA in 50% ACN for 20 min. Digestion supernatants and

eluates of each gel piece were collected together in a new reaction tube. Collected samples were dried by vacuum centrifugation.

Partial Sequence Analysis by Tandem Mass Spectrometry

After extraction, the tryptic peptides were dried and pre-treated with C_{18} ZipTips (Millipore, Eschborn, Germany) to concentrate and desalt the peptide mixtures before MS analysis. Digests were individually ionised by nanoelectrospray and analysed with a quadrupole time-of-flight hybrid mass spectrometer (Q-TOF, Micromass, Altrincham, UK). Conditions for the measurements, instrument settings and description of the procedure are specified in Walz *et al.* (2002). Partial sequences were determined from fragmentation spectra in a software-assisted (MaxEnt3 and PepSeq, MassLynx, Micromass) procedure. Theoretical molecular weights of the identified proteins were calculated using the MassLynx software.

Database Searches

The database searches using partial sequences from tryptic peptides determined by Q-TOF-tandem-MS were performed using the BLAST resources at NCBI (http://www.ncbi.nlm.nih.gov/blast/), first using the short sequence Blast algorithm limited to green plants. If no similar protein could be found with this algorithm, further searches in the EST database were performed with the tblastn algorithm (database EST, limited to Viriplantae, expect value 20000). Amino acid sequence alignments were performed using the freely accessible program ClustalW (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_clustalw.html).

RESULTS - TRANSCRIPTS

Two independent systems, a photoextension (extended short day, ESD) and a dexamethasone-based (DEX) one were used to induce flowering in *Arabidopsis*. All significant changes were classified in functional classes, within the individual systems and are summarised in Table 2 for the ESD and Table 3 for DEX. Additional details associated with the altered genes are given in Appendices 1-6 for the ESD system and in Appendices 7-14 for the DEX samples.

	Hours post induction					
	8		12		16	
	Up	Down	Up	Down	Up	Down
Total number of genes with significantly changed expression	(71)	(5)	(85)	(219)	(33)	(18)
Functional Classes						
Transcription Factors	13	1	18	48	2	4
Signalling	18	-	5	22	2	3
Hormone Signalling	-	1	7	5	-	-
Defence	11	-	3	26	2	-
Stress	-	-	4	7	2	-
Light-mediated Responses	-	-	3	-	-	-
Circadian Clock and Flowering	-	-	-	4	-	-
Cell Wall	1	-	2	3	1	-
Carbohydrate Metabolism	4	-	7	5	3	1
Lipid Metabolism	-	-	3	1	-	1
Amino Acid Metabolism	-	-	-	1	-	1
Sulphur Metabolism	-	-	-	1	-	-
Secondary Metabolism	-	-	-	4	-	-
Photosynthesis/Respiration	-	1	2	7	3	1
Protein Regulation/Degradation	-	-	1	4	-	-
Transport	-	-	1	-	-	-
DNA	-	-	-	-	1	-

Table 2: Summary of all transcript changes listed as functional classes over the eight-hour inductive period of the photoextension system. Significance was assessed using a threshold of 2 between the treatments for both the responsive (L*er*) and non-responsive (*co-2*) line. Transcripts showing expression changes below or above the threshold in both pair-wise comparisons are listed. Induced samples were compared to corresponding control ones for each time point. Up- or downregulated genes are distinguished. Seventeen categories are listed. The miscellaneous and unidentified classes were omitted.

	Hours post induction							
	0		4		8		12	
	Up	Down	Up	Down	Up	Down	Up	Down
Total number of genes with significantly changed expression	(7)	(107)	(122)	(149)	(76)	(378)	(60)	(9)
Functional Classes								
Transcription Factors	2	20	15	14	5	68	10	1
Signalling	-	18	13	8	2	35	2	-
Hormone Signalling	-	-	2	2	2	6	4	1
Defence	1	17	2	3	-	20	2	-
Stress	-	2	2	6	4	10	1	-
Light-mediated Responses	-	-	-	-	-	-	-	-
Circadian Clock and Flowering	-	-	-	-	2	-	2	-
Cell Wall	-	2	4	8	-	11	1	3
Carbohydrate Metabolism	1	2	8	6	7	1	4	-
Lipid Metabolism	-	3	2	-	3	-	1	-
Amino Acid Metabolism	-	-	1	4	4	-	1	-
Sulphur Metabolism	-	-	1	1	-	-	-	-
Secondary Metabolism	-	-	2	-	4	-	-	-
Photosynthesis/Respiration	-	2	2	3	-	8	2	1
Protein Regulation/Degradation	-	4	7	10	4	1	2	-
Transport	-	-	-	-	-	5	2	-

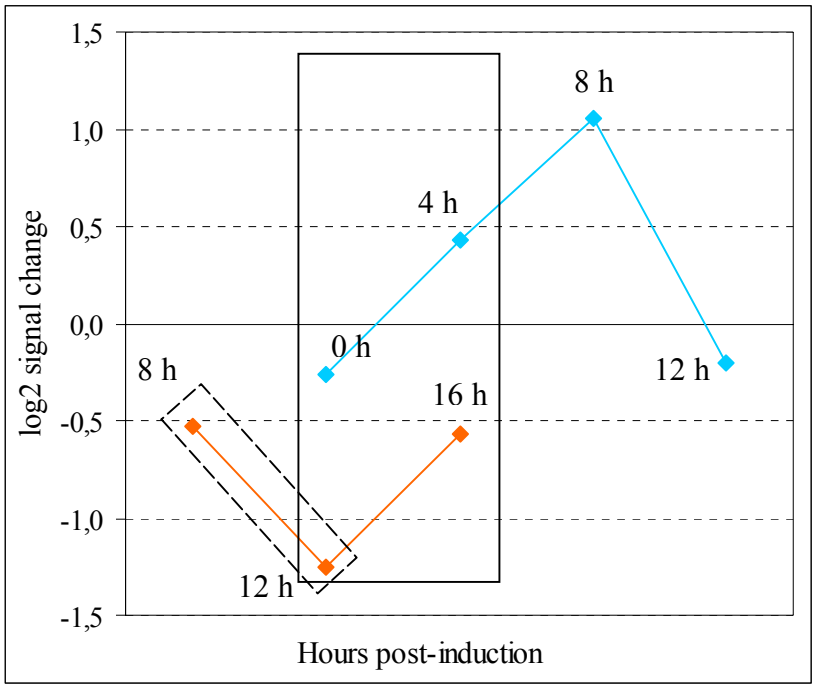
Table 3: Summary of all transcript changes listed as functional classes over the twelve-hour inductive period using the dexamethasone-inducible system. Significance was assessed using a threshold of 2 between the treatments for both the responsive (35S::CO:GR) and non-responsive (*co-2*) line. Transcripts showing expression changes below or above the threshold in both pair-wise comparisons are listed. Induced samples were compared to corresponding control ones for each time point. Up- or downregulated genes are distinguished. Seventeen categories are listed. The miscellaneous and unidentified classes were omitted.

In order to gain insights into whether the expression of known genes showed similar behaviour between the systems, two genes central to regulating the floral transition, *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*), were used. Figure 7 shows that in both cases, the second and third sampling times in the photoextension experiment matched the first and second points of the DEX system. The trends of both genes were also strikingly similar both within and between the systems.

These consistent expression patterns therefore became the basis for identifiying signals preceding and following the activation of *CO*. More specifically, the comparison between the second sampling time to the first in the light extension system would identify signals preceding the activation of *CO*. Similarly, the comparison between the third and second sampling times in the light extension system would focus on signals produced after the activation of *CO*. Given the conserved expression pattern of the *CO* and *FT* genes in both induction systems, the comparison between the second and first sampling times in the dexamethasone system would also achieve the analogous result. Therefore, the latter two comparisons would identify candidates common to both inductive treatments between the two systems. For all comparisons, the difference between the induced samples was calculated at the specified time points. All values were normalised and log2-transformed.

As far as signals preceding *CO*, approximately 8 000 genes were obtained when using a significance threshold of 2 when comparing the second time point of the light extension system to the first. In order to understand the behaviour of flowering specific genes within these overlapping transcripts, an initial list of approximately 80 loci, generated by literature and database searches, was used.

A



B

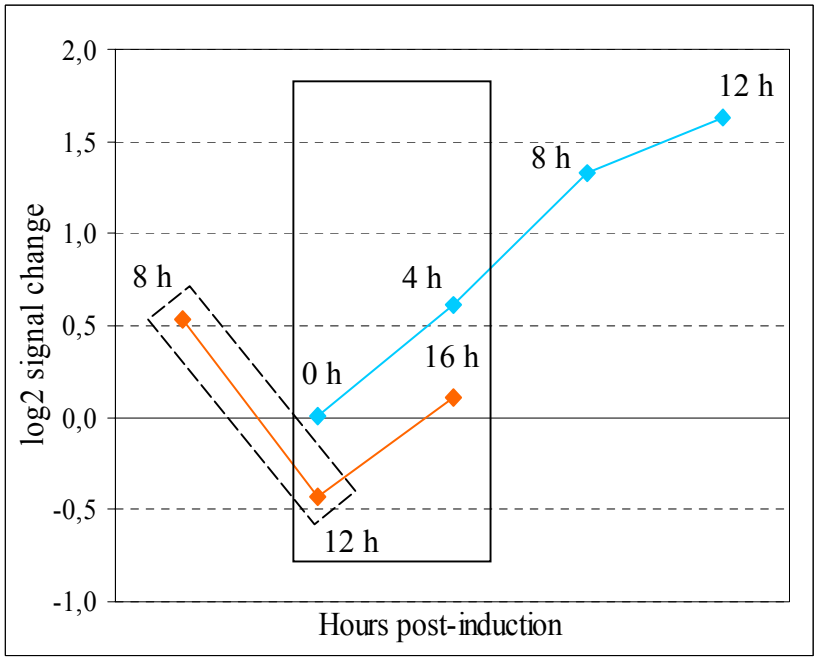


Figure 7: Expression levels of flowering time genes central to regulating the floral transition in *Arabidopsis*. Transcripts levels of (A) *CONSTANS* (*CO*) and (B): *FLOWERING LOCUS T* (*FT*). The sampling points were shifted in order to match the corresponding trend between the experiments. Sampling times refer to 8, 12 and 16 hours post-induction for the light extension (shown in orange) and to 0, 4, 8 and 12 hours after the start of the inductive treatment using dexamethasone (in blue). Transcript levels are plotted as the log2 of the change in signal intensity. Comparing transcripts included in the boxes with dotted lines in the *CO* (A) and *FT* plots (B) refers to events occurring prior to the activation of *CO*. The time points enclosed within the larger solid rectangles, represent common events occurring after the activation of *CO* between the two systems (in both A and B).

Table 4 lists all the flowering-specific genes which were upregulated, positive and negative regulators are distinguished. *CONSTANS* and two of its holomogs *CONSTANS-LIKE 1* (*COL 1*) and *CONSTANS-LIKE 2* (*COL2*) were observed as significant using a threshold of 0.5. All three transcription factors possess zinc finger regions and CCT motifs but unlike *CO*, the *COL* genes do not participate in the regulation of flowering (Ledger *et al*., 2001). Interestingly, while the expression of *CO* was negatively regulated, both *COL1* and *COL2* appeared upregulated. Next seen was *FT*, whose activation is partly *CO*-dependent, as was demonstrated by both sets of expression values. One of *FT*'s close members, *MOTHER OF FT* (*MFT*; Yoo *et al*., 2004b) also appeared downregulated. A paralog of *FD*, a probable *FD* and *FT* interactor, was the next item on the list (Abe *et al*., 2005), followed by *CAULIFLOWER* (*CAL*), a floral meristem identity gene whose expression was suppressed (Ferrandiz *et al*., 2000). Then came *ABI3-INTERACTING PROTEIN 1* (*AIP1 or TIMING OF CAB EXPRESSION 1*, *TOC1*; Mas *et al*., 2003) and *CIRCADIAN CLOCK ASSOCIATED* (*CCA1*), a myb-related transcription factor (Alabadi *et al*., 2002), both of which are involved in regulation of the circadian clock. A transcription factor of the YABBY family, involved in abaxial cell type specification in leaves, was listed as the next entry. A squamosa promoter-binding protein-like 3 (*SPL3*), another positive floral regulator (Cardon *et al*., 1997) which could interact with AP1 was next. The last entries were a delta desaturase, involved in fatty acid chain desaturation and a transcription factor of the *NO APICAL MERISTEM* (*NAM*) family (Riechmann *et al*., 2000).

Negative floral regulators included *TERMINAL FLOWER 1* (*TFL1*), which showed upregulated transcription; *SCHNARCHZAPFEN* (*SNZ*), a transcription factor (TF, Schmid *et al*., 2003); *DDF1*, a drought responsive element binding TF (Magome *et al*., 2004); *AMP1*, encoding a glutamate carboxypetidase (Helliwell *et al*., 2001) and a FAD-binding domain-containing protein. Since the changes in flowering genes were not dramatic, the analysis was expanded to the most drastically-changed transcripts, as indicated in Table 5.

AGI Code	Description	ESD T2-T1	Floral Regulator
At5g15840	zinc finger protein CONSTANS (CO)	-0,72	+
At5g15850	zinc finger protein CONSTANS-LIKE 1 (COL1)	1,31	+
At3g02380	zinc finger protein CONSTANS-LIKE 2 (COL2)	4,56	+
At1g65480	flowering locus T protein (FT)	-0,97	+
At1g18100	mother of FT and TFL1 protein (MFT)	-0,50	+
At2g17770	FD paralog,	-0,56	+
At1g26310	ABA-responsive element binding protein, putative, CAULIFLOWER, CAL, AP1 homolog, MADS-box protein, putative	-0,64	+
At5g61380	ABI3-interacting protein 1 (AIP1), timing of CAB expression 1 protein (TOC1)	-1,55	+
At2g46830	myb-related transcription factor (CCA1)	4,33	+
At2g45190	axial regulator YABBY1 (YABBY1) / abnormal floral organs protein (AFO) / filamentous flower protein (FIL)	1,15	+
At2g33810	squamosa promoter-binding protein-like 3 (SPL3)	0,59	+
At2g31360	delta 9 desaturase (ADS2)	-0,65	+
At1g69490	no apical meristem (NAM) family protein	-1,95	+
At5g03840	terminal flower 1 protein (TFL1)	0,65	-
At2g39250	SNARCHZAPFEN, SNZ,	-1,55	-
At1g12610	AP2 domain-containing transcription factor, DDF1, encodes a member of the DREB subfamily A-1 of ERF/ AP2 transcription factor family	-1,77	-
At3g54720	glutamate carboxypeptidase, putative (AMP1)	0,61	-
At4g20830	FAD-binding domain-containing protein	-0,86	-

Table 4: Changes in expression levels of flowering-specific genes preceding the activation of *CO* in the photoextension induction system. This subset of listed genes was extracted from a list of 8000 where the double comparison of the treatments for both the responsive (L*er*) and non-responsive (*co-2*) line satisfied a threshold of 2. The second and first time points of the light extension system were compared by calculating differences between induced samples. The data were normalised and log2 transformed beforehand. All listed genes showed altered expression at a threshold greater than 0.5 or less than -0.5. Floral regulators are indicated by a '+' and '-'denotes negative ones.

The flowering genes category included five members, of which *LATE ELONGATED HYPOCOTYL* (*LHY*), *CONSTANS-LIKE 2* (*COL2*), *CIRCADIAN CLOCK ASSOCIATED* (*CCA1*) were upregulated. *GIGANTEA* (*GI*) was downregulated. Calmodulin-binding, WRKY and NAM transcription factors (TFs) were part of TFs/signalling class. The two cell wall entries were upregulated while four defence- or stress-related transcripts were found. Five hits completed the miscellaneous group. Two genes were unidentified.

AGI Code	Description	ESD T2-T1
	Flowering Genes	
At1g01060	LATE ELONGATED HYPOCOTYL (LHY), myb family transcription factor	4,72
At3g02380	zinc finger protein CONSTANS-LIKE 2 (COL2)	4,56
At1g22770	gigantea protein (GI)	-5,91
At2g46830	myb-related transcription factor (CCA1)	4,33
At5g17300	myb family transcription factor, similar to CCA1	4,22
	Transcription Factors /Signalling	
At1g73805	calmodulin-binding protein	-4,08
At3g01830	calmodulin-related protein, putative	-6,16
At2g46400	WRKY family transcription factor	-4,52
At5g22570	WRKY family transcription factor	-5,90
At3g44350	no apical meristem (NAM) family protein	-4,16
At1g52890	no apical meristem (NAM) family protein	-6,08
At5g22380	no apical meristem (NAM) family protein	-6,63
At4g34410	encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF / AP2 transcription factor family	-4,70
At4g23150	protein kinase family protein	-4,20
At1g07430	protein phosphatase 2C, putative	-5,47
	Cell Wall	
At2g23130	arabinogalactan-protein (AGP17)	4,59
At1g10550	xyloglucan:xyloglucosyl transferase, putative	4,35
	Defence/ Stress	
At4g08950	phosphate-responsive protein, putative (EXO)	4,04
At5g18470	curculin-like (mannose-binding) lectin family protein	-4,23
At2g14610	pathogenesis-related protein 1 (PR-1)	-4,43
At1g28480	glutaredoxin family protein	-5,16
	Miscellaneous	
At4g23610 At4g23620	expressed protein, 50S ribosomal protein-related	-4,00
At3g50930	AAA-type ATPase family protein	-4,24
At3g56710	sigA-binding protein	-4,25
At4g10500	oxidoreductase, 2OG-Fe(II) oxygenase family protein	-4,60
At5g52760	heavy-metal-associated domain-containing protein	-4,85
	Unidentified	
At2g14560	expressed protein	-4,78
At1g13470	expressed protein	-5,66

Table 5: Changes in expression levels of transcripts preceding the activation of *CO* in the photoextension induction system. The transcripts shown are a subset extracted from a list 8000 where the double comparison of the treatments for both the responsive (L*er*) and non-responsive (*co-2*) line satisfied a threshold of 2. The second and first time points of the light extension system were compared by calculating differences between induced samples. The data were normalised and log2 transformed beforehand. All listed genes showed altered expression at a threshold greater than 4 or less than -4.

In case of signals produced following the activation of *CO*, approximately 13 000 transcripts were found using a significance threshold of two for the ESD system when comparing the third and second sampling times. In the DEX treatment, however, about 10 000 genes fulfilled the same significance criteria, when looking at the second and first sampling times. When considering overlaps between the systems, about 3000 common genes were obtained. The behaviour of flowering-specific genes, within these overlapping transcripts, was also investigated using the previously mentioned list of approximately 80 loci. Table 6 lists the transcripts with genes upregulated in both systems; positive and negative regulators are distinguished.

The fourteen positive floral regulators present in both inductive treatments included *CO* which showed very consistent ratios in both systems. Two members of the phosphatidylethanolamine-binding protein (PEBP) family, *FT* and its homolog *BROTHER OF FT* (*BFT*) were also listed. *AGAMOUS LIKE 20* (*AGL20*, or *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1*, *SOC1*), a downstream target of FT, showed expression patterns matching the latter's trend. *LEAFY* (*LFY*), a floral meristem identity gene which is partly downstream of *SOC1*, was also detected as significantly increased. Also associated with the regulation of floral development, was *CLAVATA2* (*CLV2*), a receptor-like protein containing leucine-rich repeats (Jeong *et al*., 1999). The next entry was *LUMINIDEPENDENS* (*LD*), which encodes a homeodomain-containing transcription factor and functions in the autonomous pathway (Auckerman *et al*., 1999). Another member of the autonomous pathway, *FY*, encoding an mRNA processing factor was also listed (Simpson *et al*., 2003). The LOV kelch protein 1 (*LPK1*) or clock-associated protein *ZETILUPE* (*ZTL*), which influences flowering via circadian rhythm was also part of this list (Somers *et al*., 2004). One of the two transcription factors listed, *SPATULA* (*SPT*), encodes the first enzyme of glutathione (GSH) biosynthesis, gamma-glutamylcysteine synthetase and is required for cell proliferation at the root tip (Heisler *et al*., 2001). *NO APICAL MERISTEM* (*NAM*), the

second transcription factor, was reported to have a role in multidimensional cell growth (Riechmann *et al*., 2000). *PAUSED* (*PSD*) a karyopherin, involved in nucleotide and nucleic acid transport (such as tRNAs) with likely involvement in shoot apical meristem initiation and development, was also found (Hunter *et al*., 2003). Then came *EARLY IN SHORT DAYS 4* (*ESD4*), a gene encoding a SUMO protease that is predominantly located at the periphery of the nucleus (Murtas *et al*., 2003). The last upregulated positive floral regulator was *FLOWERING PROMOTING FACTOR 1* (*FPF1*), whose gene product encodes a small protein of 12.6 kDa that is involved in gibberellin signalling pathway (Melzer *et al*., 1999). It is expressed in apical meristems immediately after the photoperiodic induction of flowering.

Of the negative regulators of flowering listed, *DWARF AND DELAYED FLOWERING 1* (*DDF1*), which encodes a drought responsive element-binding transcription factor probably involved in gibberellic acid biosynthesis, was found (Magome *et al*., 2004). *EMBRYONIC FLOWER 1* (*EMF1*) was reported to possess transcription regulator activity (Aubert *et al*., 2001). Two transcription factors, *VERNALIZATION1* (*VRN1*) and *VERNALIZATION2* (*VRN2*), encoding a transcriptional factor B3 family protein and a nuclear-localised zinc finger protein (Levy *et al*., 2002), both regulate levels of the floral repressor *FLOWERING LOCUS C* (*FLC*, Michaels and Amasino, 1999). *VERNALIZATION INDEPENDENCE 4* (*VIP4*), however, encodes a highly hydrophilic protein involved in positively regulating FLC expression (Zhang *et al*., 2002). Next was *SHORT VEGETATIVE PHASE* (*SVP*, or *AGAMOUS LIKE 22*, *AGL22*), a MADS box transcription factor involved in the regulation of flower development (Levy *et al*., 2002). *AERIAL ROSETTE* (*ART1*, Poduska *et al*., 2003), a TRAF-type zinc factor, activates *FLC*. *PRECOCIOUS* (*PRE*) was the next item and encodes a nucleoporin (Zhang and Li, 2005). The last member, *EARLY FLOWERING 8* (*ELF8*), is involved in the methylation of *FLC* and *FLOWERING LOCUS M* (*FLM*, or *AGAMOUS LIKE 27*, *AGL27* or *MADS AFFECTING FLOWERING 1*, *MAF1*; He *et al*., 2004).

AGI code	Description	ESD T3-T2	DEX T2-T1	Floral Regulator
At5g15840	zinc finger protein CONSTANS (CO)	0.68	0.69	+
At1g65480	flowering locus T protein (FT)	0.54	0.61	+
At5g62040	brother of FT and TFL1 protein (BFT)	0.24	0.54	+
At2g45660	MADS-box protein (AGL20)	0.41	0.30	+
At5g61850	floral meristem identity control protein LEAFY (LFY)	0.07	0.25	+
At1g65380	receptor-like protein CLAVATA2 (CLV2)	0.21	0.16	+
At4g02560	homeobox protein LUMINIDEPENDENS (LD)	0.18	0.11	+
At5g13480	similar to WD-40 repeat family protein,	0.19	0.06	+
	similar to putative FY protein (Oryza sativa)			
At5g57360	F-box family protein / LOV kelch protein 1 (LKP1),	0.01	0.27	+
	identical to clock-associated protein ZTL, ZEITLUPE			
At4g36930	basic helix-loop-helix (bHLH) protein SPATULA (SPT)	0.32	0.33	+
At1g69490	no apical meristem (NAM) family protein	2.74	0.37	+
At1g72560	tRNA export mediator exportin-t, putative PAUSED, (PSD)	0.41	0.53	+
At4g15880	EARLY IN SHORT DAYS 4 (EDS4), Ulp1 protease family protein	0.31	0.10	+
At5g24860	FLOWERING PROMOTING FACTOR 1 (FPF1)	0.14	0.32	+
At1g12610	encodes a member of the DREB subfamily A-1 of ERF/ AP2 transcription factor family (DDF1).	0.22	1.58	-
At5g11530	embryonic flower 1 (EMF1)	0.20	0.13	-
At3g18990	vernalization 1 protein (VRN1)	0.92	0.27	-
At4g16845	vernalization 2 protein (VRN2)	0.21	0.03	-
At5g61150	VERNALIZATION INDEPENDENCE 4 (VIP4), leo1-like family protein	0.46	0.10	-
At2g22540	short vegetative phase protein (SVP)	0.25	0.25	-
At1g09920	AERIAL ROSETTE 1 (ART1) TRAF-type zinc finger-related	0.29	0.12	-
At1g80680	PRECOCIOUS (PRE), nucleoporin family protein	0.08	0.13	-
At2g06210	EARLY FLOWERING 8 (ELF8), phosphoprotein-related	0.28	0.29	-

Table 6: Upregulated expression levels of flowering-specific genes following the activation of *CO* in two independent induction systems. The subsets of listed genes were extracted from a list of 3000 overlapping genes that satisfied a threshold of 2 in the double comparison of the treatments for both the responsive (L*er* for ESD and 35S::CO:GR for DEX) and non-responsive (*co-2*) lines. The third and second time points of the light extension system were compared to the third and second times of the DEX systems. Differences were calculated between the induced samples. The data were normalised and log2 transformed beforehand. All listed genes showed altered expression at a threshold of zero. Floral regulators are indicated by a '+' and '-'denote negative ones.

In Table 7, two floral homeotic genes, *APETALA 1* (*AP1*) and 2 (*AP2*) are the first downregulated members detected in the two induction systems (Ferrandiz *et al*., 2000). *SPLAYED* (*SYD*), the next transcript, was demonstrated to act with LFY to regulate shoot apical meristem identity (Wagner *et al*., 2002). A transcription factor of the YABBY family, involved in abaxial cell type specification in leaves, was listed as the next entry. Then, glutamate-cysteine ligase / gamma-glutamylcysteine synthetase (*GSH1*), the first enzyme of glutathione biosynthesis was seen (Wachter *et al*., 2005). An alcohol dehydrogenase (*ATA1*) with oxidoreductase activity, a putative RNA-binding protein (*FLK*; Mockler *et al*., 2004) and a G protein coupled receptor associated with sensitivity to cytokinin (*GCR1*; Colucci *et al*., 2002) were the last positive floral regulators.

Five negative floral regulators were downregulated, of which *FRIGIDA* (*FRI*) was the first example. The protein encoded by this gene positively regulates the floral repressor FLC and is part of the autonomous pathway (Clarke and Dean, 1994). The second entry was a *FRIGIDA LIKE 2* (*FRL2*) gene. *EMBRYONIC FLOWER 2* (*EMF2*) encodes a Polycomb group protein with zinc finger domain (Yoshida *et al*., 2001). *FERTILIZATION INDEPENDENT ENDOSPERM* (*FIE*) also encodes a protein similar to the transcriptional regulator of the animal Polycomb group which is involved in regulation of establishment of anterior-posterior polar axis in the endosperm and repression of flowering during vegetative phase. The last member was a DNA cytosine methyltransferase.

Since the changes specific to the flowering genes were not dramatic, as for the signals before *CO*, the analysis was expanded to the most drastically-changed transcripts with annotated functions. Table 8 shows the two main categories, transcription factors (TFs) and defence- or stress-related. Of the six transcription factors entries, the first two were potentially associated with mediating drought responses. In fact, the homeobox-leucine zipper protein 7 was transcriptionally regulated in an ABA-dependent manner. In addition to the myb, NAM and bHLH classes of TFs, the AZF2 encodes a zinc finger protein.

AGI code	Description	ESD T3-T2	DEX T2-T1	Floral Regulator
At1g69120	floral homeotic protein APETALA1 (AP1) /	-0.68	-0.31	+
	agamous-like MADS box protein (AGL7)			
At4g36920	floral homeotic protein APETALA2 (AP2)	-0.09	-0.49	+
At2g28290	chromatin remodeling protein, putative SPLAYED (SYD)	-0.04	-0.38	+
At2g45190	axial regulator YABBY1 (YABBY1) /	-1.42	-0.10	+
	abnormal floral organs protein (AFO) /			
	filamentous flower protein (FIL)			
At4g23100	glutamate-cysteine ligase /	-0.10	-0.50	+
	gamma-glutamylcysteine synthetase (GSH1)			
At3g42960	alcohol dehydrogenase (ATA1)	-0.28	-0.02	+
At3g04610	KH domain-containing protein (FLK)	-0.22	-0.48	+
At1g48270	G protein coupled receptor-related	-0.13	-0.09	+
At4g00650	FRIGIDA protein	-0.02	-0.17	-
At1g31814	FRIGIDA LIKE 2 (FRL2), expressed protein	-0.13	-0.49	-
At5g51230	embryonic flower 2 (EMF2)	-0.16	-0.08	-
At3g20740	fertilization-independent endosperm protein (FIE)	-0.05	-0.26	-
At5g49160	DNA (cytosine-5-)-methyltransferase (ATHIM)	-0.48	-0.06	-

Table 7: Downregulated expression levels of flowering-specific genes following the activation of *CO* in both systems. The subsets of listed genes were extracted from a list of 3000 overlapping genes that satisfied a threshold of 2 in the double comparison of the treatments for both the responsive (L*er* for ESD and 35S::CO:GR for DEX) and non-responsive (*co-2*) lines. The third and second time points of the light extension system were compared to the third and second times of the DEX systems. Differences were calculated between the induced samples. The data were normalised and log2 transformed beforehand. All listed genes showed altered expression at a threshold of zero. Floral regulators are indicated by a '+' and '-'denote negative ones.

AZF2 mRNA levels appeared upregulated in response to ABA, high salt, and mild dessication potentially associated with responding to an abscisic acid stimulus (Sakamoto *et al*., 2004). In the next defence/stress category, were found a thioltransferase, a late embryogenesis abundant protein and a cyteine proteinase. The last member, a low-temperature-responsive 65 kD protein (*LTI65*) or a desiccation-responsive protein 29B (*RD29B*) has been shown to be produced in response to water deprivation, salt stress and responded to abscisic acid stimulus (Parvez *et al.*, 2001). Two lipid transfer proteins (LTP3 and 4) were listed in the miscellaneous class, both of which are lipid-binding and respond to

ABA (Arondel *et al*., 2000). Three other entries for the miscellaneous class were a phosphodiesterase, a thioesterase and a remorin family protein. Last was the CER1 protein, associated with production of stem epicuticular wax and pollen fertility. The CER 1 protein is an aldehyde decarbonylase, converting stem wax C30 aldehydes to C29 alkanes (Aarts *et al*., 1995).

CONSTANS encodes a transcription factor containing conserved DNA binding motifs, which allow it to regulate genes (Samach *et al*., 2000). Although the link of CO to flowering is known, it is likely that additional target genes are under its control. Since the expression of *CO* could be followed in two parallel systems, once induced, genes whose expression pattern matched that of *CO* and those showing reciprocal trends were investigated. The candidates present in both induction systems are displayed in Table 9. Two transcription factors are seen, one of which is induced by auxin, and the other belongs to the basic helix-loop-helix family. Two additional enzymes involved in the synthesis of the distinct complex carbohydrates, starch and cellulose, were also found. The last candidate was an unidentified protein.

AGI Code	Functional Class	**ESD T3-T2**	**DEX T2-T1**	ESD T2-T1
	Transcription Factors			
At4g34410	encodes a member of the ERF	1.71	3.13	-4.70
	(ethylene response factor) subfamily B-3 of ERF/			
	AP2 transcription factor family			
At2g46680	homeobox-leucine zipper protein 7 (HB-7) /	2.68	1.62	-3.05
	HD-ZIP transcription factor 7			
At1g75250	myb family transcription factor	-1.74	-1.59	1.48
At5g22380	no apical meristem (NAM) family protein	2.91	2.88	-6.63
At1g10585	similar to basic helix-loop-helix (bHLH) family protein	-1.70	-2.44	3.40
At3g19580	zinc finger (C2H2 type) protein 2 (AZF2)	1.81	1.72	-1.86
	Defence /Stress			
At1g28480	glutaredoxin family protein (thioltransferase)	4.07	1.54	-5.16
At1g52690	late embryogenesis abundant protein, putative	1.99	3.30	-1.85
At5g52300	low-temperature-responsive 65 kD protein (LTI65) /	2.51	1.61	-2.51
	desiccation-responsive protein 29B (RD29B)			
At4g11320	cysteine proteinase, putative	-2.18	-1.65	2.47
At4g11310				
	Miscellaneous			
At5g59320	lipid transfer protein 3 (LTP3)	2.39	3.93	-3.19
At5g59310	lipid transfer protein 4 (LTP4)	2.03	3.95	-1.70
At3g02040	glycerophosphoryl diester phosphodiesterase family protein	1.60	1.57	0.13
At4g17470	palmitoyl protein thioesterase family protein	-2.66	-2.88	2.58
At3g57540	remorin family protein	1.90	1.52	-1.89
At1g02205	CER1 protein,	1.60	1.50	-1.59
	identical to maize gl1 homolog (glossy1 locus)			
	Unidentified			
At5g45630	expressed protein	1.72	3.03	-3.13
At3g17800	expressed protein	2.63	1.87	-2.50
At3g17790				
At5g03210	expressed protein	2.19	1.95	-3.22
At2g25625	expressed protein	2.06	1.70	-2.16
At3g51750	expressed protein	1.86	1.67	-2.94
At2g34600	expressed protein	1.50	1.54	-1.44

Table 8: Changes in expression levels of common transcripts following the activation of *CO* in the photoextension and dexamethasone induction systems. The transcripts shown were compared by calculating the difference between the third and second time points of the ESD system to that of the third and second times of the DEX system. Therefore, only the values in the columns with bold headers were used for comparisons. The data were normalised and log2 transformed beforehand. The genes listed gave the highest changes and showed similar tendencies in both experiments. Altered expression was set at a threshold of 1.5.

Affymetrix Identifier	Functional Class	DEX 0h	DEX 4h	DEX 8h	DEX 12h	ESD1 8h	ESD2 12h	ESD3 16h	AGI Code
246525_at	CONSTANS	0.83	1.35	2.07	0.87	0.69	0.42	0.67	At5g15840
	Transcription factors								
263664_at	indoleacetic acid-induced protein 17	0.16	0.51	1.59	1.07	-0.05	2.10	-0.28	At1g04250
255694_at	basic helix-loop-helix (bHLH)	0.15	1.16	1.82	1.04	-0.19	1.74	-0.68	At4g00050
	Carbohydrate Metabolism								
261191_at	starch synthase	-0.18	1.69	1.65	2.38	0.12	2.60	-1.51	At1g32900
260592_at	cellulose synthase family protein	-0.51	-1.29	-1.48	-1.23	-0.16	-0.86	0.07	At1g55850
	Unknown Proteins								
248028_at	expressed protein	-0.19	1.70	2.57	1.70	0.30	2.14	-0.74	At5g55620

Table 9: Classes of potential targets of *CONSTANS* (*CO*) found in the two independent induction systems. Genes whose expression pattern matched, or was entirely inverted to, that of CO and which overlapped in both systems (DEX and ESD) are shown. The expression pattern of CONSTANS is provided as a reference. Individual sampling times with hours post induction are indicated. All values used were the calculated differences between the induced responsive lines and the *co-2* line. The Affymetrix identifier and AGI codes of the candidate transcripts are shown.

RESULTS - METABOLITE PROFILING

General Considerations

Leaf and shoot apex phloem exudates (the latter applies to *Sinapis* only) were collected in EDTA, aliquoted and profiled for metabolites using GC-MS. The data obtained was processed as summarised in the materials and methods section and is shown in Figure 8. Batches of samples usually required chromatographic runs lasting several days or sometimes several weeks and were always randomised to avoid effects of machine drift. Once the chromatograms were acquired, the baseline corrected and the peaks deconvoluted. In order to correct for retention time shifts occurring between measurement days, comparisons and/or corrections are generally performed against internal standards. As mentioned before, internal standard markers were omitted in sample processing to avoid ion suppression due to the low abundance of some of the analytes in the phloem exudates. Therefore, 16 compounds consistently present in all EDTA phloem samples were chosen. These analytes were also selected such that their elution times spanned the 20-minute run and are listed in Table 10. These internal marker peaks were used to correct for the error in the 15 spanning intervals by linear interpolation, making day-to-day variation minimal. Hence, comparison of replicate samples of a defined genotype, subjected to an induction treatment and harvested at a specific time but measured on separate days were realigned and were ready for peak searches and metabolite assignment, for instance.

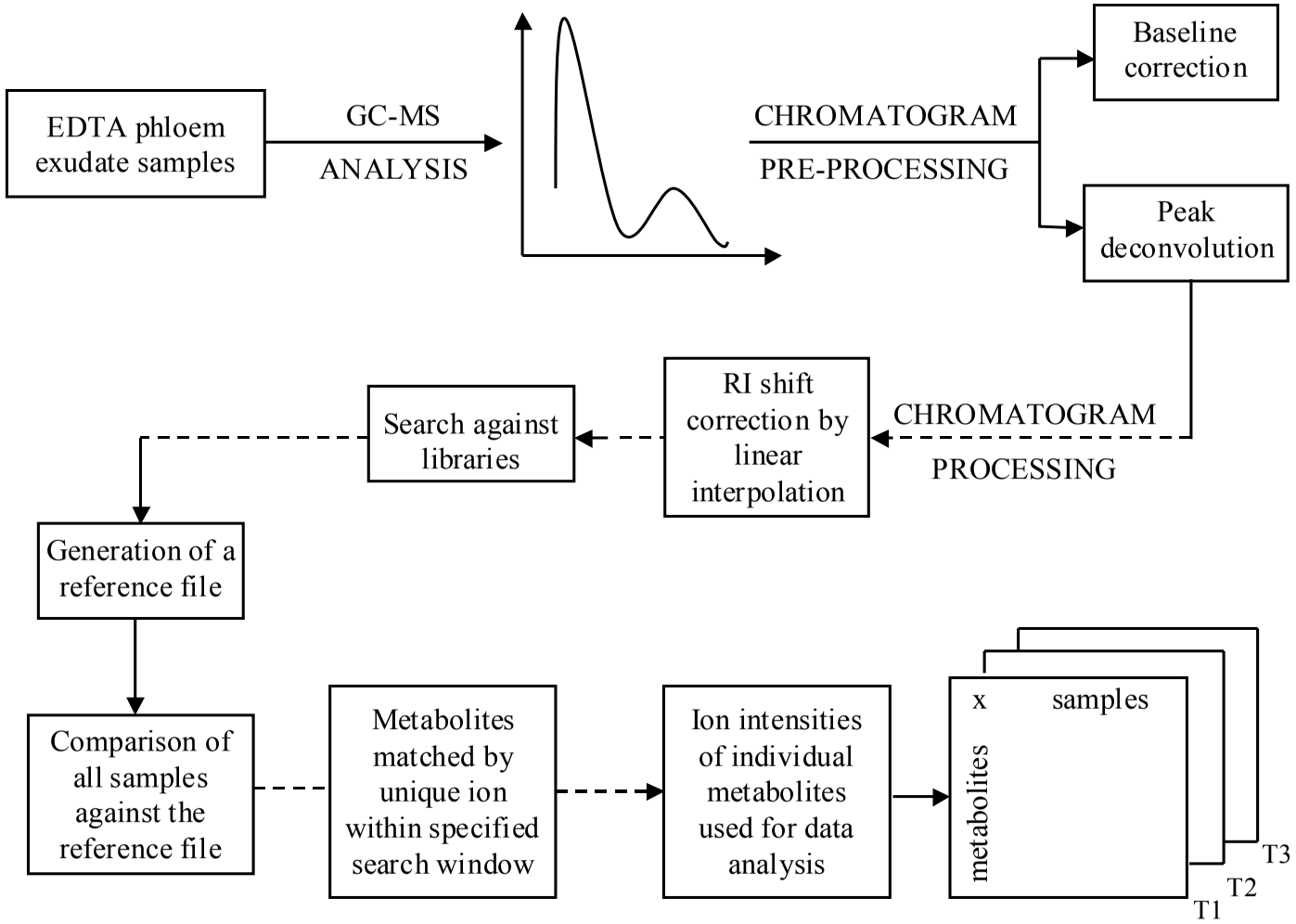


Figure 8: Flowchart representing steps in sample processing after chromatographic measurements. Once EDTA samples were run, pre-processing steps included baseline correction and peak deconvolution. Actual processing then involved correction of the shift in retention index (RI) by linear interpolation and searches against libraries. Next, a reference file was generated specifying peak assignments and manually selecting ions unique to analytes for their quantification. Artifacts were also removed in the process. All samples were compared against this reference; matches relied on a specified time window search. Ion intensities were then used to find statistical differences between treatments during the sampled times.

	Metabolite Name	Retention Index (arbitrary units)
1	Alanine	208300
2	Valine	272150
3	Glycerol	292660
4	Glycine	325060
5	Serine	358010
6	Aspartic Acid	458350
7	Methionine	475170
8	2-Ketoglutaric Acid	525160
9	Citric Acid	592510
10	Dehydroascorbic Acid	638650
11	Myo-inositol	654460
12	Palmitic Acid	696730
13	Stearic Acid	771390
14	Maltose (minor)	880640
15	Galactinol dihydrate	940280
16	Galactinol dihydrate minor-like	1137900

Table 10: Analytes used as internal standards in phloem exudate samples collected in EDTA. Sixteen compounds in *Arabidopsis* and *Sinapis* phloem exudate samples found to be consistently present at high abundances were selected based on their elution times and high mass spectral purity. All except one (retention index marker 16) are known compounds whose identity can be easily confirmed by library comparisons. Together, all 16 analytes spanned the 20-minute chromatographic run thereby allowing retention shift corrections to be performed within the retention marker intervals.

Once all samples from one batch were RI-corrected, a reference chromatogram processed over a signal/noise threshold of 10 was used for automated peak identification based on mass spectral comparison to a standard NIST 98 library and available in house customised mass spectral libraries. Within the frame of this study, samples subjected to induction regimes were assumed more complex and were chosen as the group of interest. From the harvesting times post-induction (4 or 8 or 12 hours), the crucial interval was decided based on the gene expression pattern of marker genes as detailed in the transcript profiling section of the results (Figure 7). Now focusing on this group of interest, a sample containing a number of deconvoluted peaks averaging the means of the remaining samples was usually selected. Care was taken to verify that the intensities of highly abundant compound classes were not saturated - sugars were one example. Also, the quality of the spectra was checked throughout the run for abundant amino acids, for instance. In this reference file, peaks were then identified and/or annotated, time windows specified for each metabolite as well as unique ions for which intensities (peak heights) would be extracted. Automated assignments of unique fragment ions for each individual metabolite were taken as default as quantifiers, and manually corrected where necessary. All comparisons were performed in R using custom scripts (Lisec *et al*., 2006). In parallel, all artifactual peaks resulting from the derivatisation procedure, column bleeding and contaminant peaks present in blank samples were removed and thus not considered for later analysis. Remaining metabolite data were normalised to the variable median of all detected metabolites and log-transformed. Statistical analyses were performed by Matlab version 6.5 (The MathWorks, MA, USA).

Further steps were then taken to ensure that the metabolites analysed met certain requirements and cleared a number of checkpoints. Figure 9 shows that the first important selection criterion was the presence of the analyte in at least half of the replicate samples run. All experiments were designed and performed such that fifteen replicates of each genotype undergoing a specific treatment at a defined time were available, thereby enabling robust

comparisons and later contributing to relevant statistical conclusions. Once normalised, ion intensity values were corrected with respect to the control samples. Metabolites chosen further were required to fulfill the following threshold, a two fold change between the treatments for both the responsive and non-responsive line. The ratios of the induced and control samples were then calculated for all time points for the separate experiments.

Analytes showing changed either two-fold up or down were then checked for correct peak assignment. The presence of any of the metabolites of interest in blank samples was an additional selection parameter. All final candidates were then assigned to compound classes and will be further discussed here. The physiological relevance of these compounds was not achieved in this study but remains the long-term goal of this work.

The identification of metabolite signals was performed as for the transcripts and was based on the upregulation of *CO* and *FT* expression as depicted in Figure 7. As described previously, the comparison of the first two time points of the light extension system would identify signals preceding the activation of *CO*. In contrast, identifying significant differences between times 2 and 3 in the same system would represent signals induced after the activation of *CO*. Comparing the latter to the first two sampling points in the dexamethasone-inducible would allow the identification of common metabolic candidates between the inductive systems.

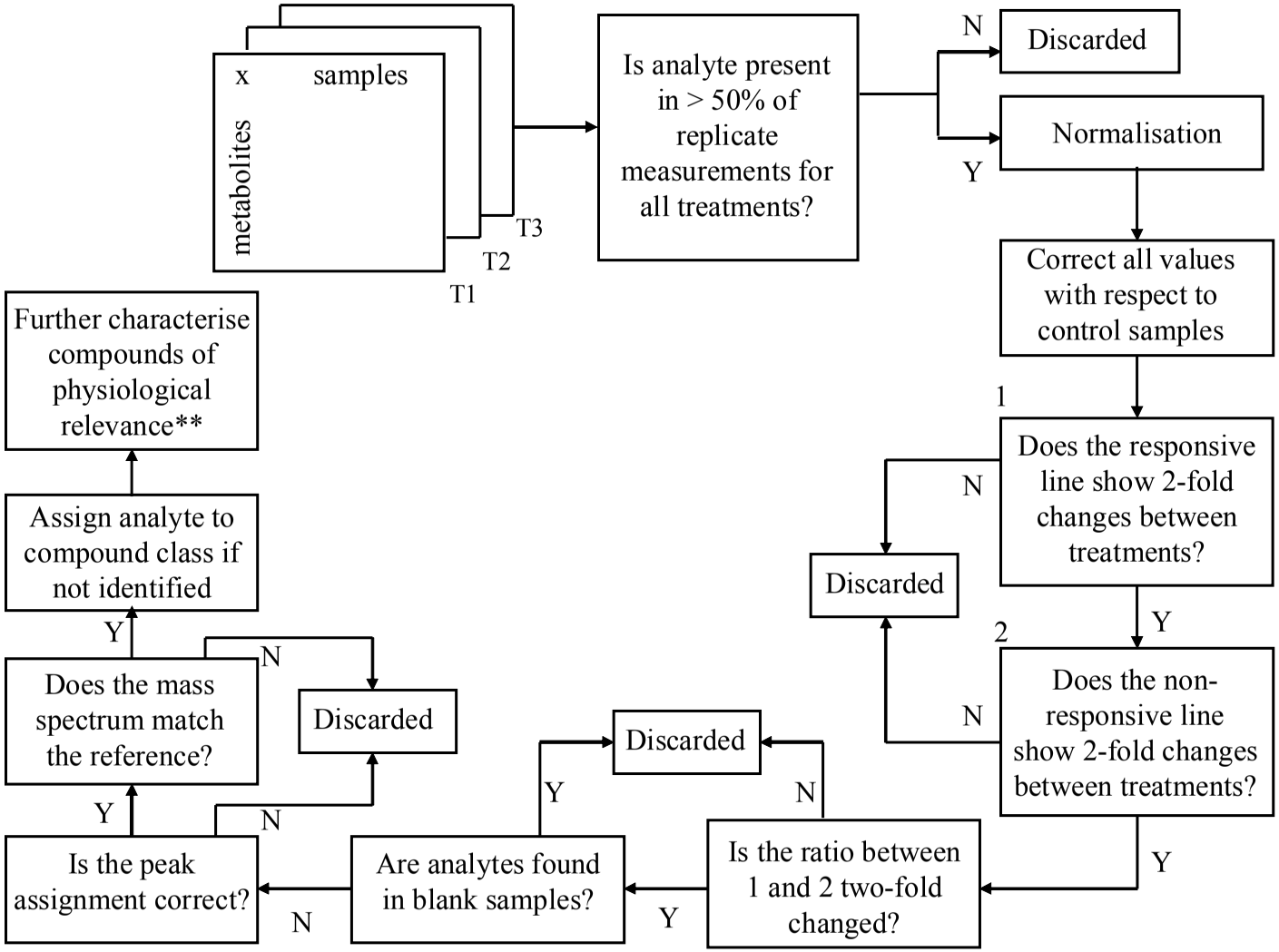


Figure 9: Flowchart representing steps in data matrix reduction for statistical analysis to identify and further characterise metabolites showing significant differences between groups specific to photoperiodically-induced samples.

Metabolite Changes Related to Flower Induction

Metabolites Changes Preceding CO

Significantly Changed Metabolites in Photoextension Time 2 (12-16 hours pi) Compared to Photoextension Time 1 (8-12 hours pi)

Table 11 lists the thirteen final compounds selected from the twenty-six identified analytes which were a subset of the initial seventy-eight significantly changed metabolites. The ratios between corrected ion intensity values of induced and control wild-type plants are shown. Amino acids appeared as mostly changed. A few sugars, sugar alcohols and one intermediate of glycolysis or the TCA cycle category could also be quantified. All miscellaneous entries were acids, ranging from lactic acid to aminohydroxybutyric acid, dehydroascorbic acid to galacturonic acid. The unknowns with consistent spectra are listed in Appendix 15.

Metabolites Changes after CO

Significantly Changed Metabolites in Photoextension Time 3 (16-20 hours pi) Compared to Photoextension Time 2 (12-16 hours pi)

Table 12 shows the final fifteen analytes resulting from the comparison of the second and third time points of the light extension induction. As before, amino acids were mostly changed and included eight members of which asparagine was seen before. Sugars included maltose; myo-inositol was the only sugar alcohol that was also previously detected. 2-ketoglutaric acid was the only glycolysis/TCA cycle intermediate. The miscellaneous category mainly consisted of acids, namely glyceric acid, iminodiacetic acid and phosphoric acid. Adenine was the last member of this group. Unidentified metabolites that were present in more than half of the replicate samples and with reasonably consistent spectra are listed in Appendix 16.

Amino Acids	**RT (s)**	**RI**	**Ratio T2/T1**
Alanine	202.755	208300	11.94
Asparagine	539.205	NA	3.59
Glutamic Acid	497.605	508700	6.74
Proline	330.655	338690	2.05
Sugars			
Glucose	578.255	NA	10.55
Sugar alcohols			
Myo-inositol	641.655	654460	2.60
Galactinol dihydrate	925.105	933070	2.81
Glycolysis / TCA Cycle			
Glucose-6-phosphate	751.905	774990	12.78
Miscellaneous			
Lactic acid	184.155	192920	30.79
4-amino, 2-hydroxy butyric acid	221.755	423840	14.93
Dehydroascorbic acid	614.655	638650	7.46
Galacturonic acid	612.005	624630	19.54

Table 11: Changes in metabolites, classified by compound class, preceding the activation of CO in the light extension system. The metabolites listed fulfilled the double requirement of a 2-fold change when comparing both the induced and control treatments of the responsive line and the equivalent comparison for the non-responsive line. All values for induced samples were corrected for the extended short day treatment and for the mutation by calculating corresponding ratios. The corrected numbers for induced samples of the responsive line were compared against the control treatment of the same line. The latter ratios were then used to generate the ratios listed as fold changes between the second and first time exudation periods. Retention times and retention indices of most metabolites are provided. Retention times were missing ones if commercial libraries were used for identification of the analytes.

Amino Acids	**RT (s)**	**RI**	**Ratio T3/T2**
Asparagine	469.305	NA	3.22
Cysteine	469.055	479510	54.48
Histidine	665.455	679450	2.12
Methionine	464.505	475170	36.85
Phenylalanine	520.405	531850	2.94
Valine	264.755	271250	4.89
Gamma-Amino-n-Butyric Acid	442.705	453270	17.22
Ornithine	480.605	NA	27.84
Sugars			
Maltose	866.005	880640	14.61
Sugar alcohols			
Myo-inositol	641.655	654560	2.59
Glycolysis / TCA Cycle			
2-Ketoglutaric Acid	513.255	525160	17.76
Miscellaneous			
Adenine	666.605	679770	10.99
Glyceric Acid	336.555	345840	3.74
Iminodiacetic Acid	453.205	463230	14.27
Phosphoric Acid	325.205	333190	51.99

Table 12: Changes in metabolites, classified by compound class, following the activation of CO in the light extension system. The metabolites listed fulfilled the double requirement of a 2-fold change when comparing both the induced and control treatments of the responsive line and the equivalent comparison for the non-responsive line. All values for induced samples were corrected for the extended short day treatment and for the mutation by calculating corresponding ratios. The corrected numbers for induced samples of the responsive line were compared against the control treatment of the same line. The latter ratios were then used to generate the ratios listed as fold changes between the third and second time exudation periods. Retention times and retention indices of most metabolites are provided. Retention times were missing ones if commercial libraries were used for identification of the analytes.

Significantly Changed Metabolites in Dexamethasone Time 2 (4-8 Hours pi) Compared to Dexamethasone Time 1 (0-4 hours pi)

Table 13 shows the twelve candidates changed between times one and two of the dexamethasone induction. Following the trend seen in the photoextension system, its five members made amino acids the class with the most members. Talose and xylose represented the sugars, galactinol dihydrate was the only sugar alcohol and citric acid made up the glycolysis/TCA cycle category. Unidentified metabolites that were present in more than half of the replicate samples and with reasonably consistent spectra are listed in Appendix 17.

Amino Acids	**RT (s)**	**RI**	**Ratio T2/T1**
Asparagine	463.194	NA	47.24
Methionine	457.844	475170	12.01
Serine	342.644	358010	12.35
Tryptophan	769.744	791850	10.90
Tyrosine	639.144	659260	8.75
Sugars			
Talose	581.494	593440	12.49
Xylose	484.594	488240	16.95
Sugar alcohols			
Galactinol dihydrate	919.144	933070	5.76
Glycolysis/ TCA cycle			
Citric Acid	575.494	592510	2.71

Table 13: Changes in metabolites, classified by compound class, following the activation of CO in dexamethasone system. The metabolites listed fulfilled the double requirement of a 2-fold change when comparing both the induced and control treatments of the responsive line and the equivalent comparison for the non-responsive line. All values for induced samples were corrected for the extended short day treatment and for the mutation by calculating corresponding ratios. The corrected numbers for induced samples of the responsive line were compared against the control treatment of the same line. The latter ratios were then used to generate the ratios listed as fold changes between the second and first exudation periods. Retention times and retention indices of most metabolites are provided. Retention times were missing ones if commercial libraries were used for identification of the analytes.

Comparisons of all unknowns between the systems did not provide clear candidates whose behaviour appeared consistent in *Arabidopsis*.

Amino Acids	RI	ESD Ratio T3/T2	DEX Ratio T2/T1
Asparagine	NA	3,22	47,24
Methionine	475170	36,85	12,01

Table 14: Common metabolites present after the activation of *CO* in the light extension and dexamethasone-dependent systems. The third exudation period of the light extension (ESD) induction method was compared to the second exudation period and the second exudation period of the dexamethasone (DEX) induction method was compared to the first exudation period.

Common Metabolites between the Light Extension and the Dexamethasone Inductive Systems after CO induction

Table 14 lists the common known metabolites obtained when comparing times two and three of the light extension system to times one and two of the DEX system. Two amino acids, asparagine and methionine showed overlaps between the inductive regimes. Since asparagine was also detected as a potential signal before *CO* in the extended short day (ESD) experiment, it may or may not be involved with events linked to floral induction.

Changes in Metabolites during Floral Induction in Sinapis alba

Timing of the sampling was based on previously published data regarding this established induction system (Bernier et *al*., 1998). All plants were sampled in Belgium after they were grown under established, standard conditions. Sampling times and additional information is provided in Figure 5. Chromatograms were processed as described earlier. Further data analysis also followed the steps described in Figure 9. Leaf and shoot apex samples were treated separately. Only two time points were selected for comparison and significance ratios were calculated for those. Table 15 lists metabolites of interest for leaves and Table 16 for apex samples.

Amino Acids	**RT (s)**	**RI**	**T2/T1 ratio**
Glutamine	516,983	NA	14,53
Glycine	314,633	325060	10,06
Methionine	461,483	475170	11,53
O-acetyl Serine	478,033	490740	2,75
Sugars			
Glucose	576,683	590380	2,08
Miscellaneous			
Dehydroascorbic Acid	623,583	635300	6,29
Glyceric Acid	334,083	344750	8,57
Glycolic Acid	237,633	NA	14,43

Table 15: Significantly changed metabolites in leaf phloem exudates of *Sinapis alba*. The eight final candidates classified in three main categories are listed. The retention times (RT), and retention indices (RI) are provided for most metabolites. Retention indices are missing if commercial libraries were used for the identification of the analytes. The indicated ratios are comparisons of induced samples taken 16 to 20 hours after induction with respect to induced samples taken 12 to 16 hours post-induction. All induced samples were corrected against control ones beforehand.

Amino Acids	**RT (s)**	**RI**	**T2/T1 Ratio**
Proline	402.390	338690	3.04
Valine	350.990	271250	10.85
Gamma-Amino-n-Butyric Acid	439.890	453270	2.02
Sugars			
Cellobiose	858.790	872230	3.50
Maltose	856.290	871000	2.60
Glycolysis/TCA Cycle			
Fumaric Acid	359.640	371410	2.89
Miscellaneous			
Benzyl alcohol	268.590	278830	3.17
4-amino, 2-Hydroxy Butyric Acid	220.590	423840	22.93
Levodopa	519.190	612380	2.38
Oxamic Acid	337.390	348370	6.30
Malonic Acid	256.440	300730	4.60

Table 16: Significantly changed metabolites in shoot apex phloem exudates of *Sinapis alba*. The eleven final candidates are listed. The retention times (RT), and retention indices (RI) are provided for most metabolites. Retention indices are missing if commercial libraries were used for the identification of the analytes. The indicated ratios are comparisons of induced samples taken 16 to 20 hours after induction with respect to induced samples taken 12 to 16 hours post-induction. All induced samples were corrected against control ones beforehand.

Sinapis Exudate Samples

Significantly Changed Metabolites in Leaf Phloem Exudate Samples

Table 1 lists metabolites obtained when comparing leaf phloem exudate samples collected 12-16 hours post-induction to those sampled 8-12 hours after induction. The amino acid class contained glutamine, glycine, methionine and O-acetylserine. Glucose was the only sugar; three acids, dehydroascorbic acid, glyceric acid and glycolic acid were placed in the miscellaneous group. Unidentified metabolites which were present in more than half of the replicate samples and with reasonably consistent spectra are listed in Appendix 18.

Significantly Changed Metabolites in Shoot Apex Phloem Samples

Proline, valine and gamma-amino-butyric acid were the changed amino acids. Cellobiose and maltose constituted the sugars category while fumaric acid was the sole member of the glycolysis/TCA cycle group. The miscellaneous class contained five entries of which three were acids (4-amino, 2-hydroxy-butyric acid, oxamic acid and malonic acid); benzyl alcohol and levodopa were the other two members. Unidentified metabolites which were present in more than half of the replicate samples and with reasonably consistent spectra are listed in Appendix 19.

Overall, there were no metabolites which appeared in both leaf and shoot apex samples of *Sinapis*. Although some of the categories overlapped, the individual members were all different. For instance, the main sugars seen leaf exudates was glucose while cellobiose and maltose were detected in the shoot apex instead. The same holds true for the miscellaneous class where different acids were mainly seen.

Amino Acids	**RI**	**ESD Ratio T3/T2**	**DEX Ratio T2/T1**	**Sinapis Ratio T2/T1**
Methionine	475170	36,85	12,01	11,53

Table 17: Common metabolites in leaf phloem exudates samples of *Arabidopsis thaliana* induced by two systems and of *Sinapis alba*. Fold changes are indicated.

One amino acid, methionine was found in *Arabidopsis* leaf samples induced either by light extension, or by dexamethasone application and in induced *Sinapis* leaf samples

RESULTS – PROTEINS

The direct regulation of *FT* by *CO*, its small protein size (23KDa, Kardailasky *et al*., 1999, Kobayashi *et al*., 1999), and the high sequence homology to mammalian RAF-kinase-inhibitor proteins (RKIP) made FT a strong possible candidate as the mobile graft-transmissible signal or as one of the florigenic components (Kardailasky *et al*., 1999, Kobayashi *et al*., 1999, reviewed in Suarez-Lopez 2005, Bernier 2005). Therefore, it was followed with an antibody. The identification of FT and TSF in the phloem sap of *Brassica napus* is depicted in Figure 10 below (Giavalisco *et al*., 2006). Partial sequences obtained using tandem mass spectrometry allowed the design of a peptide antibody against these proteins.

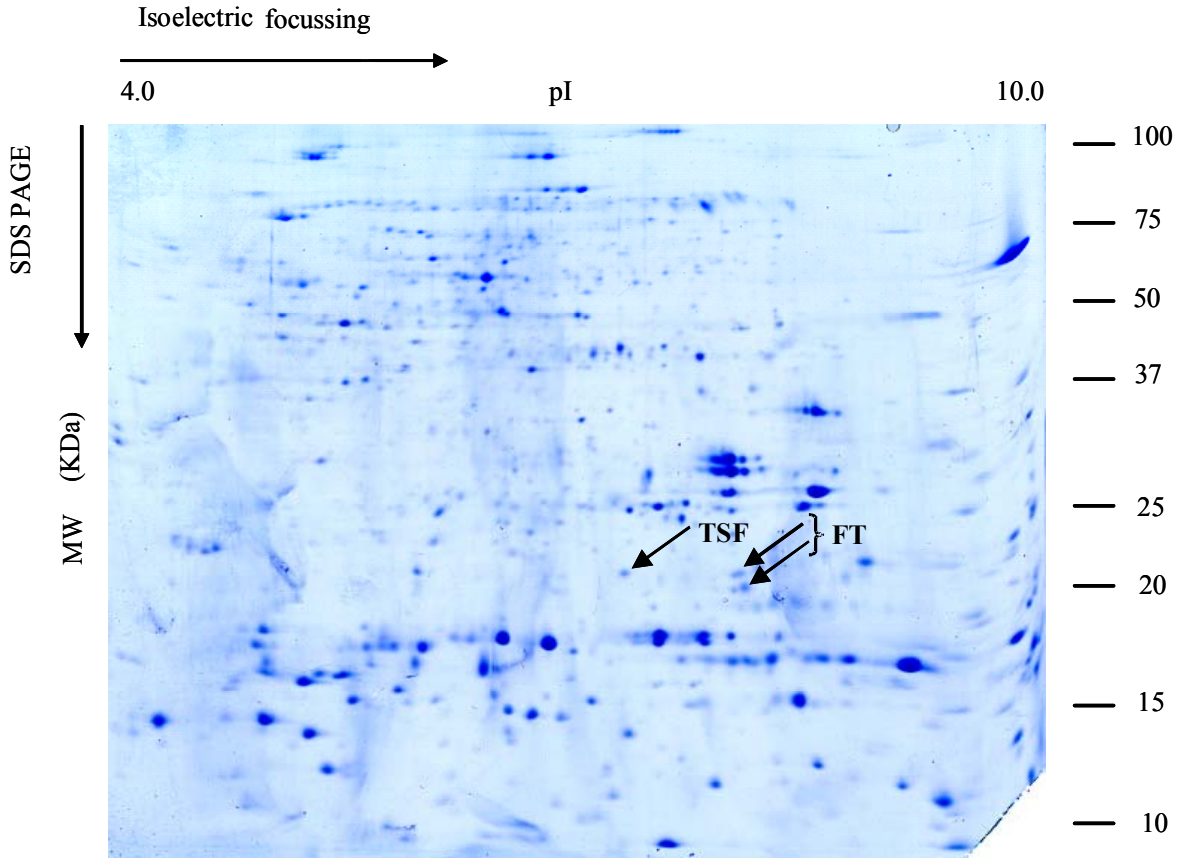


Figure 10: Identification of FT and TSF after 2D SDS-PAGE separation of proteins from *Brassica napus* phloem samples (Giavalisco *et al.,* 2006). Proteins were stained with colloidal Coomassie after electrophoresis and identified by tandem MS. The arrows indicate that two isoforms of FT were identified and one protein spot corresponded to TSF.

Antibody Generation

The partial sequences obtained from the phloem of rape were aligned against all other available FT sequences available in NCBI (http://www.ncbi.nlm.nih.gov/) to select conserved and potentially immunogenic regions. Sequences with the highest homology to the conserved regions of all FT and FT-like proteins lied within the first 100 residues (Figure 11). The stretch of residues between 38 and 77 was tested for hydrophobicity and the peptide spanning amino acids 68 to 81 was selected for synthesis (the sequence of which was confirmed by Q-TOF measurements and is detailed in Figure 12). An antibody was generated against this peptide.

Antibody Reactivity Tests

Initial Tests

Phloem sap was collected from 7-week-and-5-day-old *Brassica napus* plants as described in the materials and methods section. Incisions were made on stems of young inflorescence stems (Figure 13A) and after discarding the initial exudating drops, sap was collected using a pipette (Giavalisco *et al*., 2006). 300 µl of phloem sap were then washed and reconcentrated. This sample was then run on 1-DE, immunoblotted, incubated with crude antisera of the peptide antibody and the signals visualised are shown in Figure 13B. Four bands, two of which were intense and are marked with solid arrows, with respective sizes of 23 and 18 KDa were clearly visible. Two additional fainter bands of estimated sizes 17 and 16 KDa are indicated with dotted arrows. Comparison of the total protein pattern with the Ponceau-stained membrane confirmed that the signals were specific and did not represent any major phloem proteins (data not shown). Successive tests using plants of different ages revealed that the signal sizes appeared to vary and seemed dependent on the plant developmental stage. The next step was to investigate whether the variation observed in the pattern of antigen-specific signals to the antibody was in fact dependent on plant age.

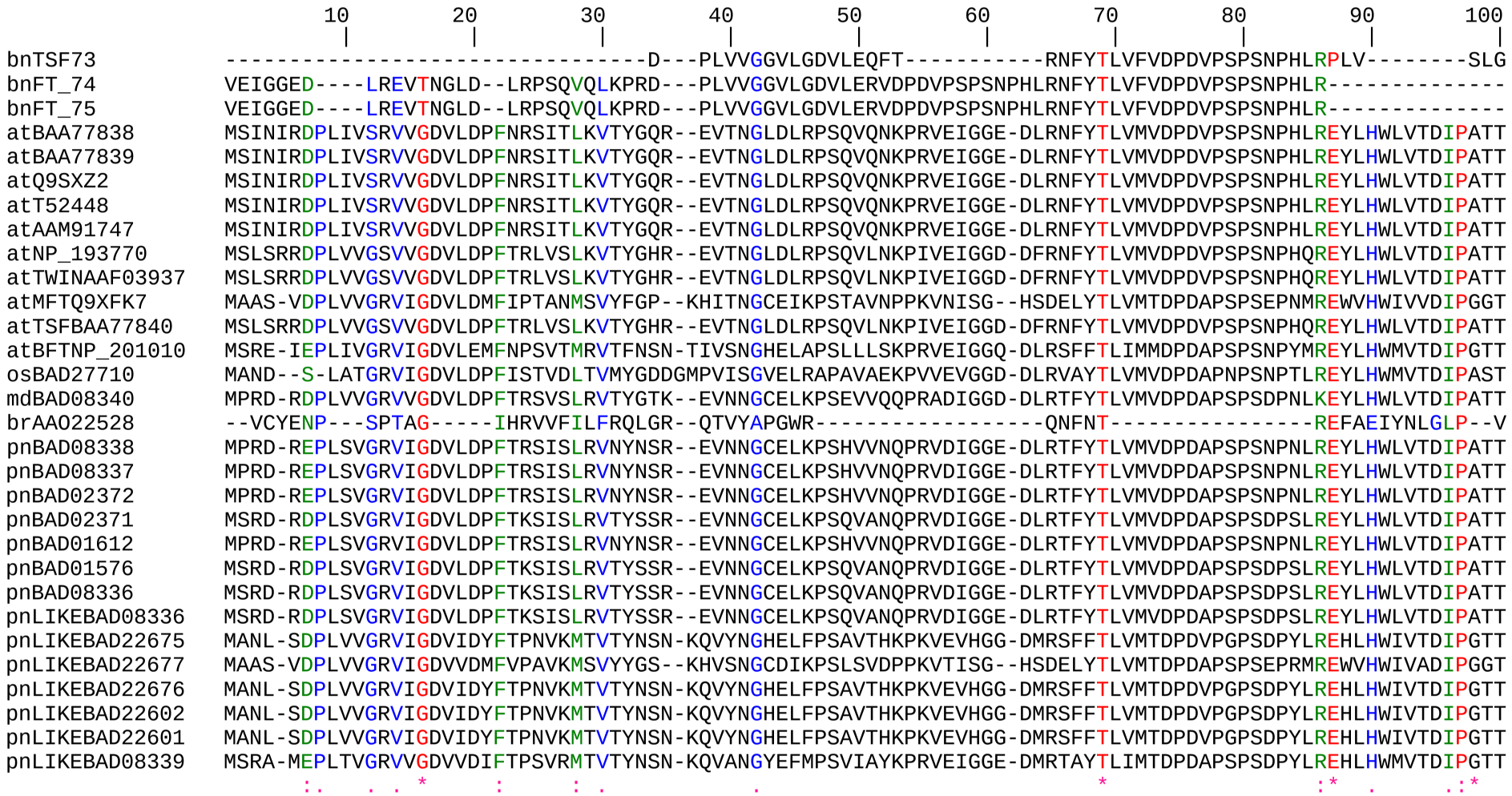


Figure 11: FT sequence comparisons for the generation of a peptide antibody. Protein FT sequences obtained from NCBI (http://www.ncbi.nlm.nih.gov/) were compared to partial sequences of FT and TSF identified in rape phloem using the alignment software ClustalW (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_clustalw.html). Only the first 100 amino acids are shown. The highest matches lied within the conserved domains, as indicated by the asterisks (no discrepancy), full stops (1 amino acid change) and semicolons (2 amino acid changes). The region between amino acid residues 38 and 77 was tested for hydrophobicity, the final sequence used for antibody production lied between residues 69 to 82.

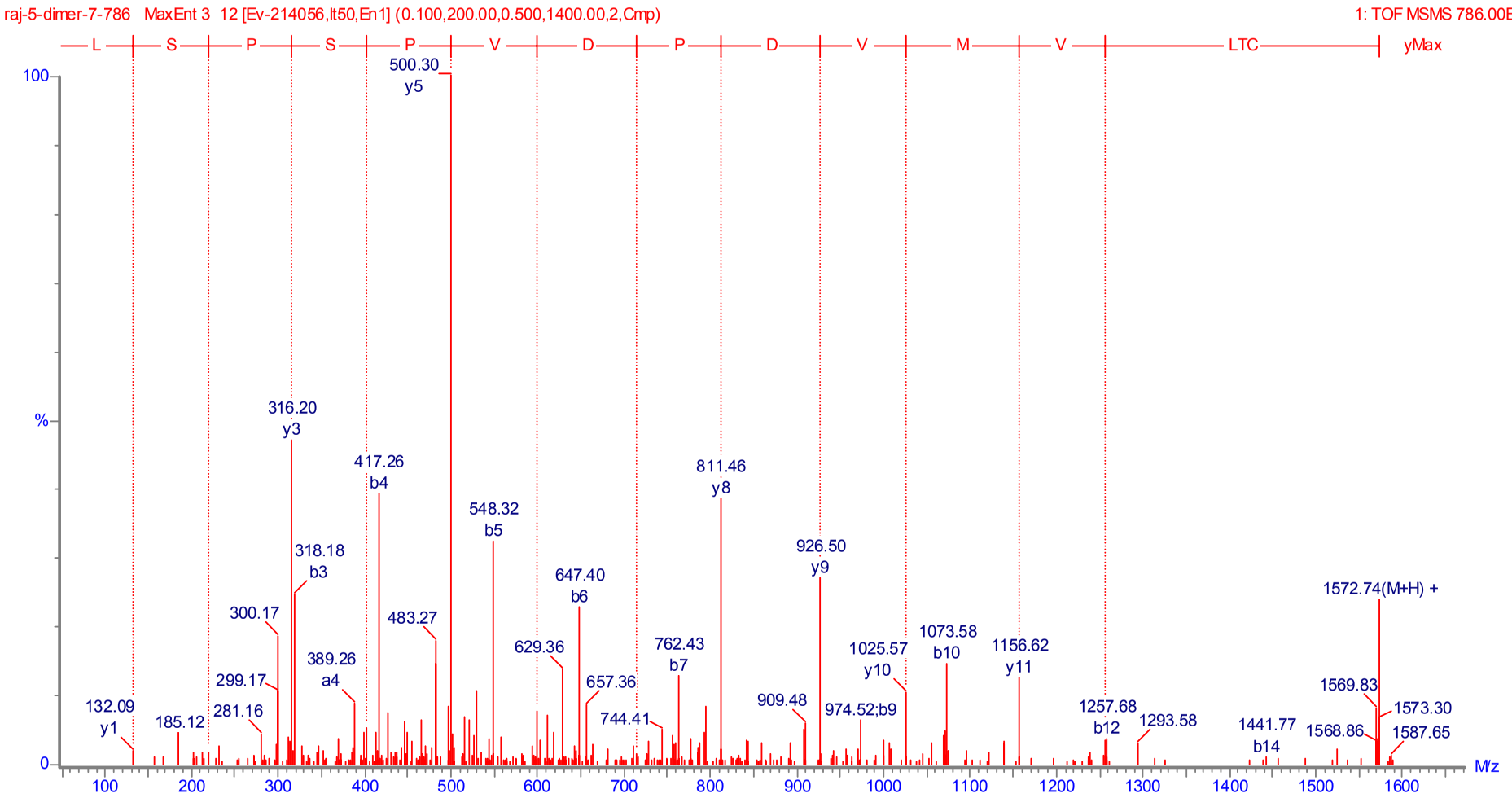


Figure 12: Q-TOF analysis of the final sequence chosen for peptide synthesis and antibody production. Depicted is the fragmentation pattern from a tandem MS experiment using the peptide chosen to generate the peptide antibody against FT. The amino acid sequence is deduced from the spectrum and amino acids are indicated in the upper part of the figure.

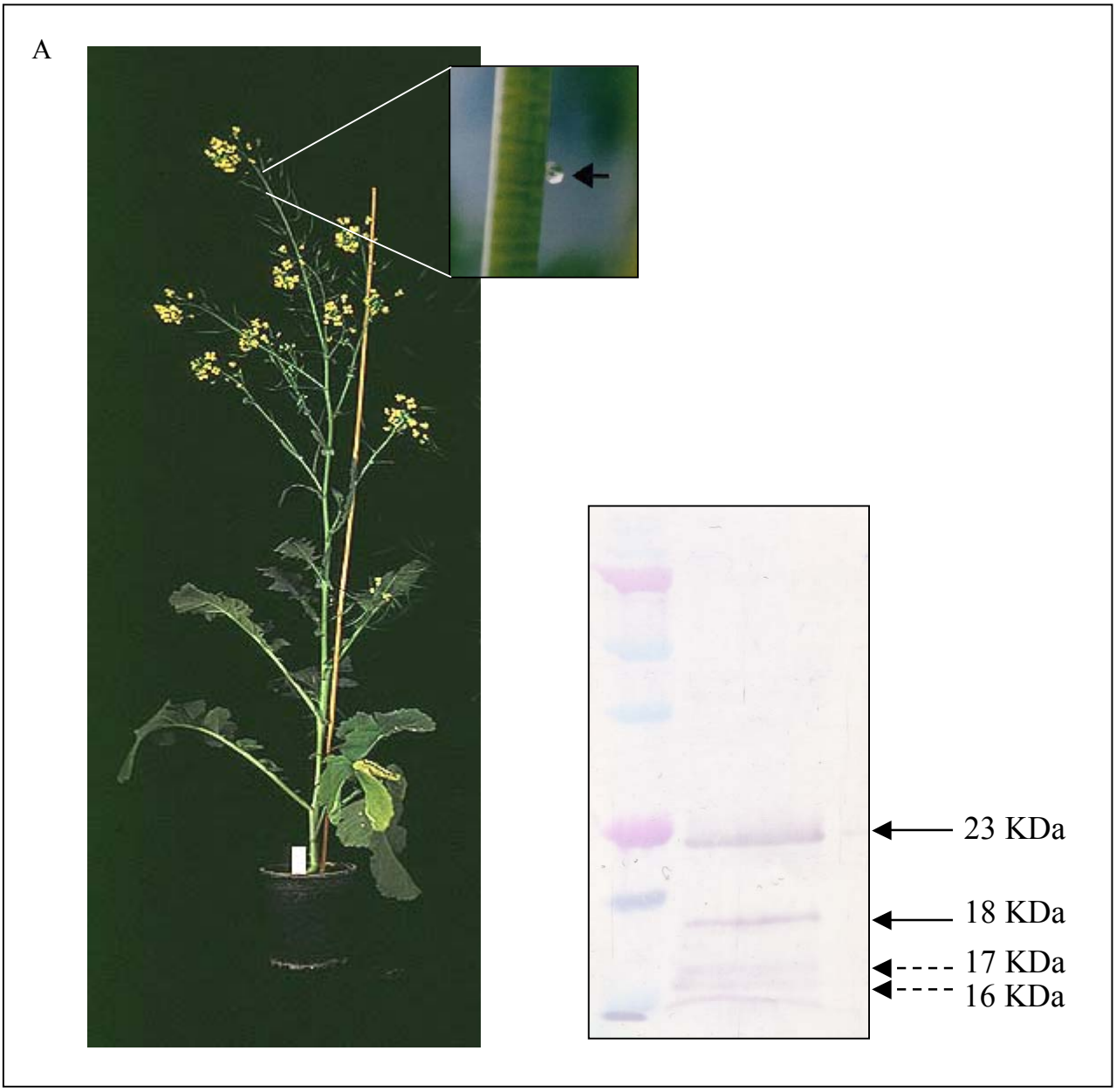


Figure 13: Phloem sap collection and immunodetection of FT in rape phloem sap by Western blotting. A: Phloem sap sampling from *Brassica napus* plants. A small incision was made with a sterile needle into the inflorescence stem and exuding phloem sap (indicated by an arrow) was collected with a pipette. B: About 50 ug of phloem proteins from seven weeks and five day-old *Brassica napus* plants were separated by 1D PAGE and blotted to a nitrocellulose membrane. A peptide anti-FT antibody and AP-conjugated anti-rabbit IgG secondary antibodies were used for detection. Four phloem antigens reacted with the FT antibody and are indicated by arrows. Two distinct bands are seen at sizes 23 and 18 KDa (solid arrows) and two fainter bands can be observed at sizes 17 and 16 KDa (dashed arrows). The Precision Plus Dual Colour marker (BioRad) was used for the estimation of protein molecular masses.

Daily Phloem Sampling – Time Course

Phloem sampling in rape is specific to plant developmental stages and is feasible from young inflorescence stems but not from vegetative plants. Therefore, rape plants with an emerging flowering stem were used daily for phloem collection at the same time of the day. Sub-batches of plants were rotated and re-used on a two- to three-day basis to allow the wounds resulting from sampling to seal. Daily sampling was continued for 14 consecutive days and subsequently spaced out to two one-week intervals, covering a sampling period totalling four weeks. The phloem sap was washed, concentrated, run on 1-DE and immunoblotted on the day that it was collected. All membranes were stained using Ponceau before overnight blocking; incubation with antibodies and visualisation of signals was performed on the next day.

Seven weeks and five day-old plants were used for the first day of sampling. Figure 14A shows that the major signals corresponded to proteins of 70 and 23 KDa (as depicted by solid arrows). The additional fainter signals were estimated to be 250, 100, 40, 35, 30, 20, 18, 17 and 16 KDa (shown in dashed arrows). When comparing this pattern of positive signals to plants used five days later (Figure 14B), the 70 KDa band remained a major signal however, the band of 23 KDa is much fainter in comparison, while the intensity of the signal in the 18 KDa range was considerably higher. The fainter bands followed the pattern described above with the addition of two signals appearing at 75 and 22 KDa respectively. Signals obtained from a phloem sample taken from nine weeks and two days old plants matched the previously reported band sizes (Figure 14C). The strength of the 18 KDa band was strongly intensified and in fact became the only major signal visible on the immunoblot. Figure 14D represents the signals obtained from the last phloem sampling, taken from eleven weeks and three day-old plants. The most obvious and most intense signal originated from a 30 KDa protein. The other fainter bands were the previously observed 250, 75, 70, 40, 23, 20 and 18 KDa proteins. For all the

sampling days, none of the signals observed corresponded to highly abundant proteins in the phloem samples (data not shown).

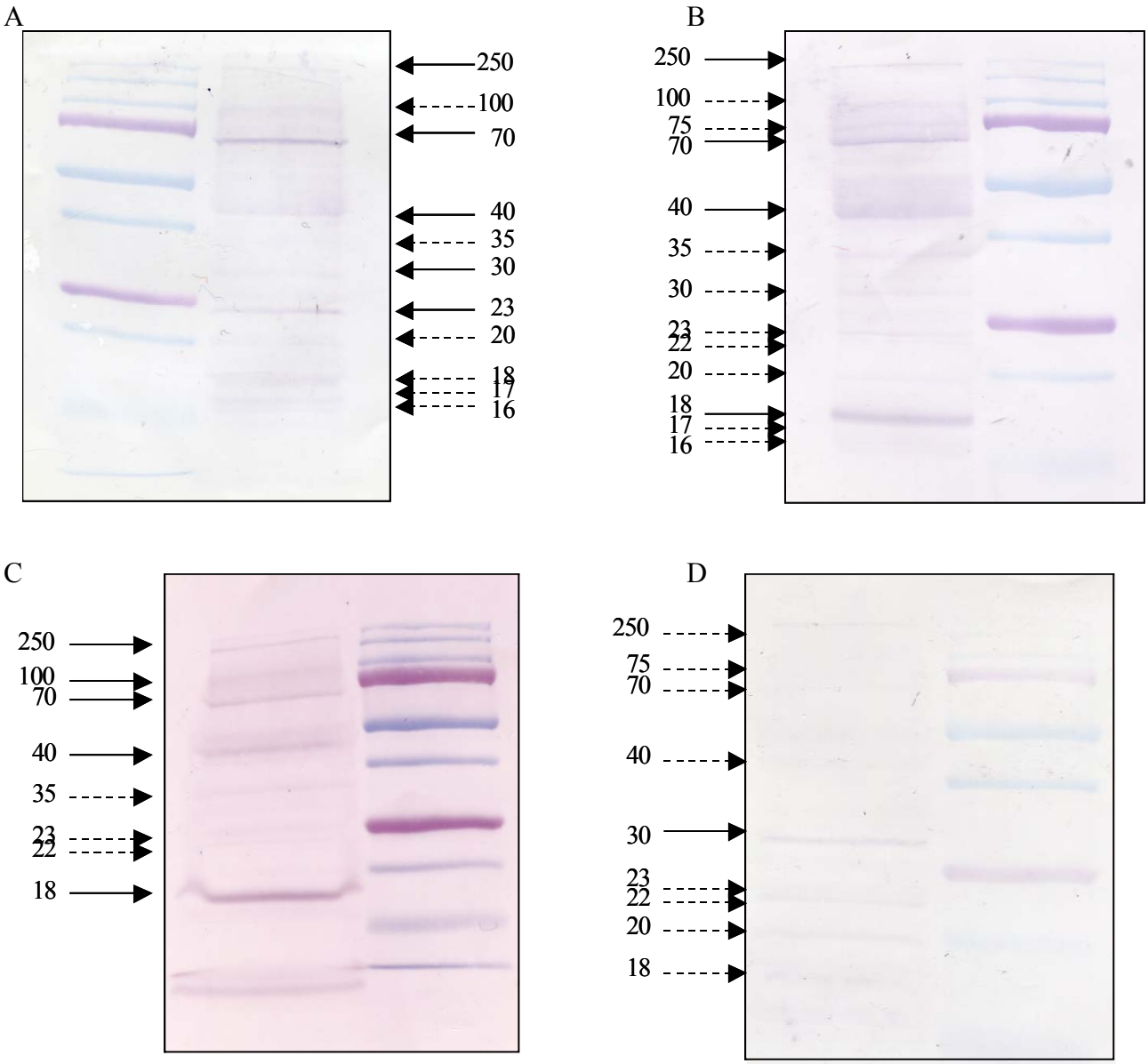


Figure 14: Immunoblotting and signal detection of phloem samples taken throughout rape development. Phloem collected daily from one batch of plants was washed, reconcentrated, blotted and used for detection using the peptide antibody produced against FT in rape phloem. (A): Phloem from plants with an emerging inflorescence stem (aged seven weeks and five days) gave 2 strong signals indicated by the solid arrows and additional fainter ones (dotted arrows). (B): Eight weeks and three day-old plants showed a similar pattern of antigens reacting to the antibody although the relative intensities were changed as seen in the case of the 18 KDa band for instance. Addition of signals also appeared in the ranges of 75, 35 and 22 KDa. (C): No major changes in the relative intensities or in the sizes of the signals could be seen in 9 week and three day-old plants. (D): Plants which were starting to set seed (eleven weeks and three days old) showed the presence of bands whose reported sizes were consistent but whose intensities were relatively lower and different, with the 30 KDa band becoming a clearly visible signal.

Phloem was collected in double amounts and 2 gels were run in parallel to allow excision of the band(s) corresponding to the signals for later tryptic digestion and sequencing. However, since the signals obtained originated from faint bands, the presence from proteins of higher abundance did not allow generation of further sequence information to confirm the specificity of the antibody. To obtain more information regarding the nature of the phloem antigens giving the observed signals, immunoprecipitation was performed.

Phloem Immunoprecipitation

Immunoprecipitation of the phloem antigens was attempted on many occasions, one of which is depicted in Figure 15. The bands from the two independently collected phloem samples are marked with arrows. Bands common to both samples were pooled (i.e. PHL^+_1 to PHL^+_10) while PHL12/11_1 and 2 were treated separately. The sizes of the antigens fished out from the phloem samples did not always match the signal sizes seen on the immunoblots: bands as previously reported were visible at 100 (PHL12/11_2), 75 (PHL^+_1), 70 (PHL^+_2), 40 (PHL^+_7) and 18 KDa (PHL^+_10) but others at 150 (PHL12/11_1), 60 (PHL^+_3), 55 (PHL^+_4), 52 (PHL^+_5), 50 (PHL^+_6), 34 (PHL^+_8) and 28 (PHL^+_9) KDa were also stained on the gel. All bands were excised from the gel, destained, tryptically-digested and sequenced. Database searches revealed that the identified proteins seemed to be isolated unspecifically, since previously identified proteins such as myrosinase, cyclophilins and heat shock proteins were found (Giavalisco *et al*., 2006).

The low success rate of the immunoprecipitations was initially difficult to understand. Therefore, experiments to check if phloem sap inhibits immunoprecipitation were performed. As a result, parallel immunoprecipitations of the peptide used to generate the antibody only worked in the absence of phloem samples (Figure 16). Apparently, therefore, some

component(s) of the phloem interfered with the procedure and hindered antigen-antibody complex formation and/or antigen elution.

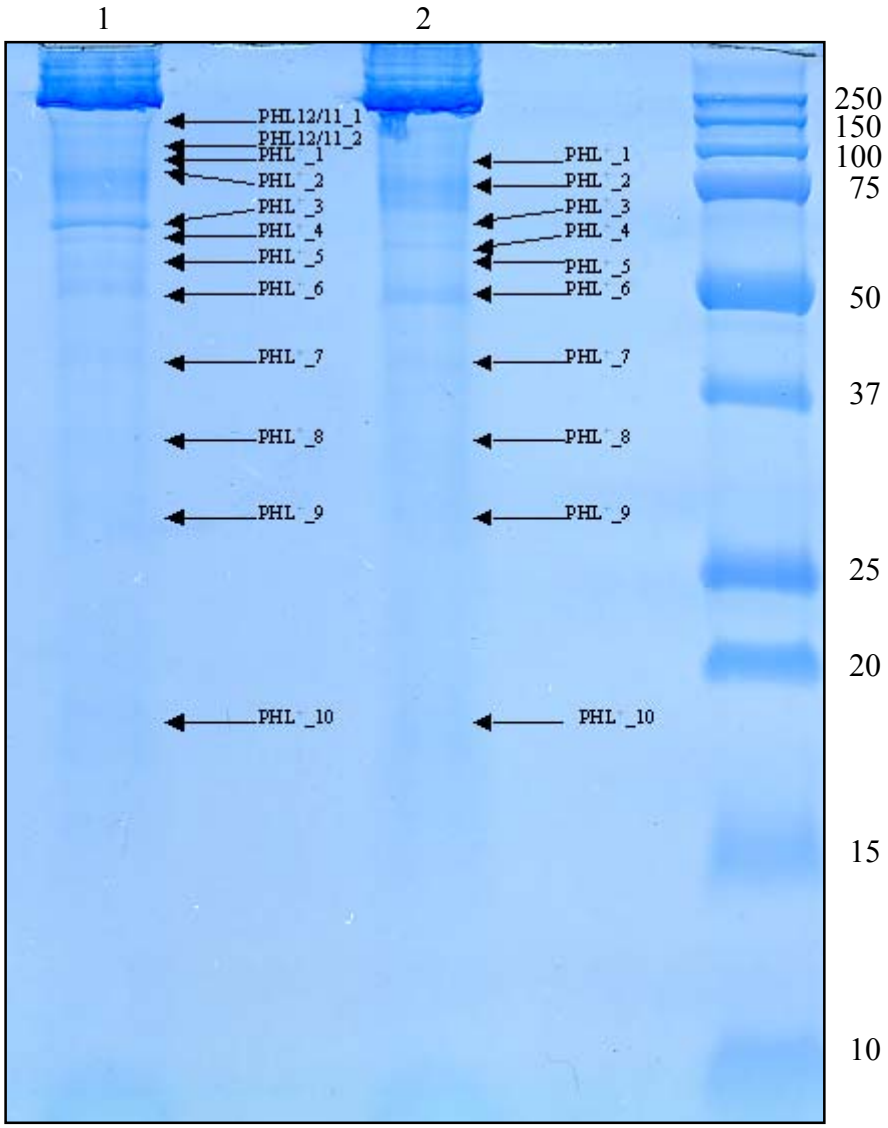


Figure 15: Immunopurification of antigens from phloem using protein A-linked Dynabeads coupled to the FT antibody. Ferrule beads coupled with protein A were incubated with the purified FT antibody and were allowed to form protein A/FT-IgG complexes that were bound to the beads. Two independently collected phloem samples taken from nine week and four day-old plants (lane 1) and from nine weeks and two day-old plants (lane 2) were used as antigens. This allowed the formation of protein A/FT-IgG/FT-antigen complexes, which were eluted and run on 1D SDS-PAGE. Precision Plus Dual Colour marker (BioRad) was used for estimation of protein molecular masses (in KDa). Antigens reacting with the FT antibody are shown by the arrows. Bands corresponding to the same protein were pooled during excision from the gel (PHL^+_1 – PHL^+_10), remaining bands were treated individually (PHL12/11_1 and PHL12/11_2). All gel pieces were tryptically-digested and partial sequences were generated using a Q-TOF.

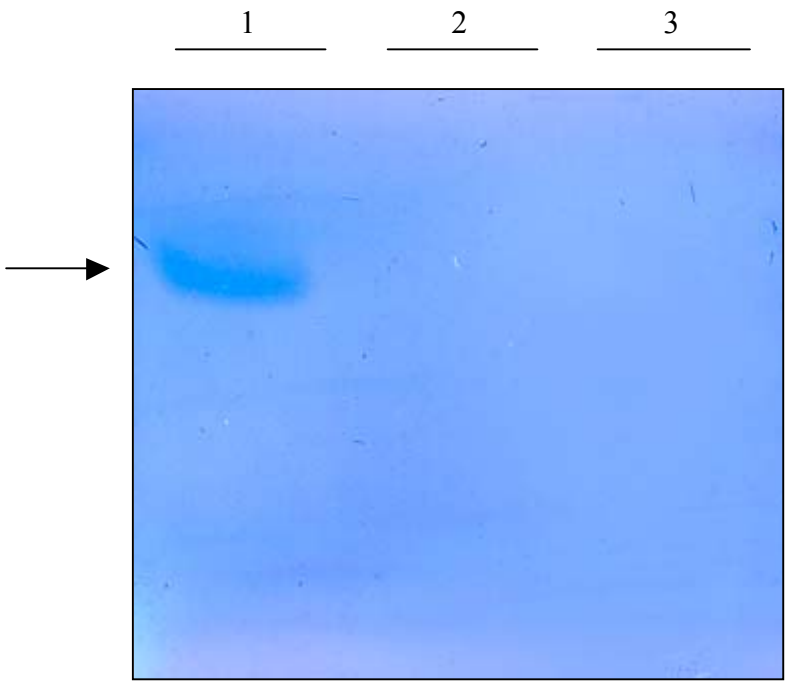


Figure 16: Interference of phloem samples during the immunopurification of antigens using protein A-coupled Dynabeads. The peptide used for antibody generation was incubated with Dynabeads coupled to FT-specific IgG (lane 1). A phloem sample was used as a separate antigen (lane 3) and a third sample contained the peptide mixed with phloem (lane 2). After elution, all samples were run on a Tris-Tricine gel. The low range Rainbow molecular weight marker (Amersham Biosciences) was used for protein size estimations. The peptide was recovered only in the absence of the phloem in the sample (indicated by an arrow).

Antibody Cross-reactivity Tests

Arabidopsis thaliana

Total protein extracts of *Arabidopsis* plants were used to evaluate the extent of cross-reactivity of the antibody raised against FT in the phloem. Single knock out lines in the flowering time genes *CO* and *FT,* a double mutant line with inactive *FT* and *SOC1* genes, as well as wild type *Landsberg erecta* and an *FT* overexpressor under the control of a phloem-specific promoter were used. Leaf tissue (inflorescence stems in the case of SUC2::FT) collected from two week-old plants was ground and proteins were extracted and quantified. Volumes of extracts corresponding to 50 µg of protein were run on SDS-PAGE, immunoblotted

and incubated with the peptide antibody. However, visualisation of signals was difficult given the presence of relatively highly abundant proteins such as RUBISCO originating from the leaf tissue.

In order to minimise this contamination, the abovementioned lines were subjected to EDTA exudation instead. Results after electrophoresis, immunoblotting and antibody incubation are shown in Figure 17. The signals of highest intensity were seen in the line overexpressing *FT* under the control of the companion cell-specific sucrose transporter promoter line (SUC2:FT). Clear bands were seen in the sizes 250, 60, and 14 KDa, with additional bands visible at 125, 85, 45, 35 and 30 KDa. In the flowering time gene mutants, *co-2*, *ft-7* and *ft-7*/*soc1-1*, proteins of sizes 26 and 24 KDa represented the main signals. Other bands in the range of 100, 70, 60, 35 and 18 KDa showed fainter reactions to the antibody. Phloem exudates collected in EDTA for the wild type *Landsberg erecta* line gave results similar to those seen in the flowering time mutants (not shown).

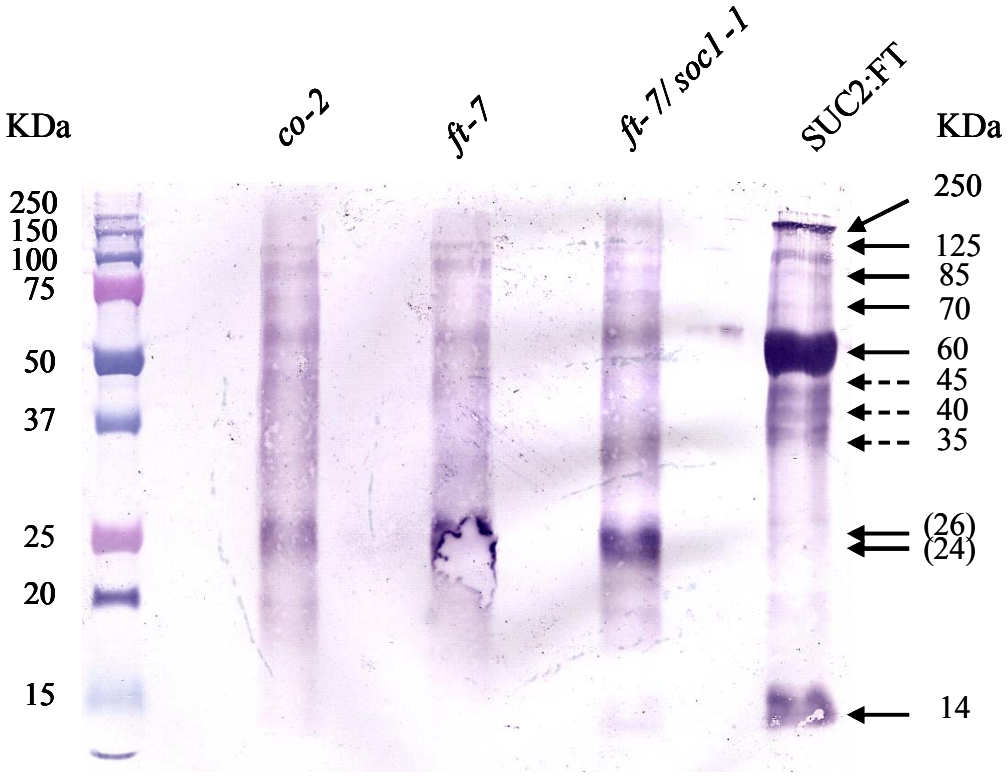


Figure 17: Cross-reactivity antibody test with *Arabidopsis* phloem exudate samples collected in EDTA. Leaves (from the flowering time gene mutants *co-2*, *ft-7* and *ft-7*/*soc1-1*) and stem tissue in the case of the FT overexpressor under the control of the companion cell-specific sucrose transporter promoter (SUC2:FT) were used for exudation in EDTA. All phloem exudates were reconcentrated using size exclusion columns, washed and quantified. 20 µg equivalents were run on 1-DE and blotted. After incubation with the FT antibody generated against FT in rape phloem, the main signals for the flowering gene mutants were of 26 and 24 KDa and are indicated in brackets. Other signals were fainter in the 100, 70, 60, 35 and 18 KDa range. The pattern of signals obtained from the FT overexpressor was entirely different. Clear bands were seen in the sizes 250, 60, and 15 KDa with additional bands visible at 125, 85, 45, 35 and 30 KDa. Precision Plus Dual Colour marker (BioRad) was used for estimation of protein molecular masses sizes are indicated on the left (in KDa).

Sinapis alba

Initial tests using phloem EDTA exudates from leaf and shoot apices were promising both in terms of protein amounts and quality obtained, since the observed pattern on 1D gels closely matched rape phloem sample protein sizes. Batches of three week-old plants grown in non-inductive conditions (SDs) were induced to flower by a single exposure to fourteen additional hours of light. The times of sampling are detailed in Figure 6 in the Materials and Methods section. Western blots comparing induced and control *Sinapis* also yielded signals

whose masses corresponded to rape phloem immunoblots (Figure 18). However, for the samples collected from the four independent induction experiments from plant batches representing 4, 8, 12 and 16 hours post-induction samples, protein amounts seemed insufficient. Since all exudates were frozen for a number of weeks before processing, protein degradation and/or denaturation could account for the above. Therefore, no clear conclusions can be drawn as far as FT, floral induction and *Sinapis*.

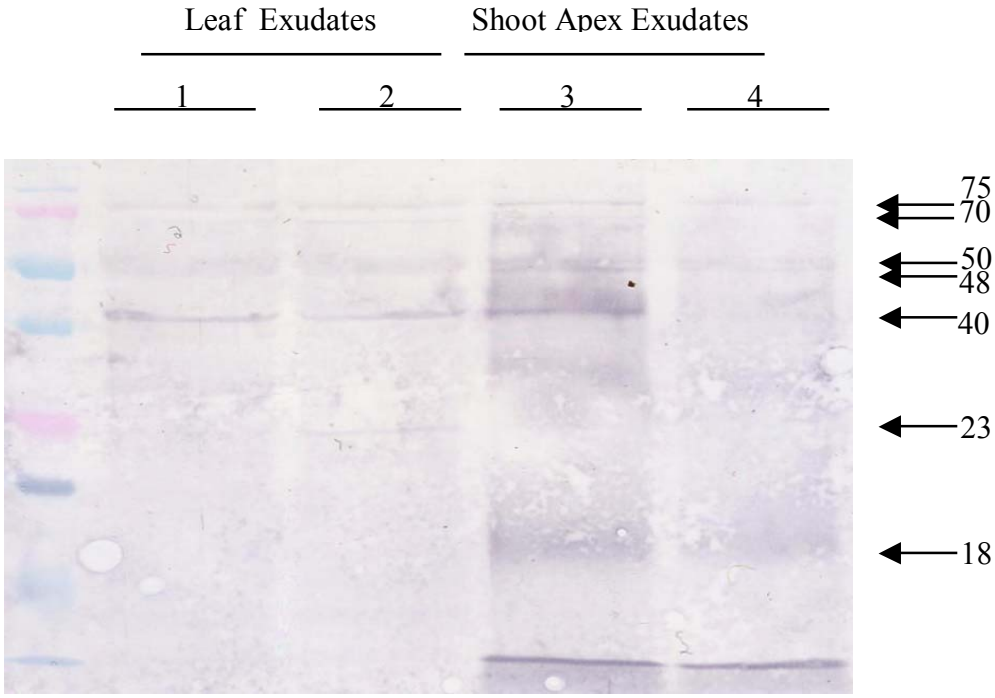


Figure 18: Cross-reactivity antibody test with *Sinapis alba* phloem exudate samples collected in EDTA. Leaf and shoot apex samples of *Sinapis* plants were analysed. Plants were induced to flower by exposure to 14 hours of additional light. Samples from control (1 and 3) and induced (2 and 4) plants were taken 8 hours after the start of the light extension period. All EDTA exudates were immediately centrifuged through size fractionation columns to eliminate the contaminating EDTA. The Precision Plus protein ladder was used to estimate protein sizes (KDa). After immunoblotting, antibody incubation and signal visualisation, bands at 75, 70, 50, 48, 40, 23 and 18 KDa were visible.

DISCUSSION

General Remarks

Growth Conditions

Oscillations in plant homeostasis (e.g. related to the local environment/developmental differences, sample location, plant position with regard to light/shading at the time of sampling, diurnal rhythm or tissue differences are commonplace (Hall 2006). Therefore, to minimise variation in plant cultivation, plantation using a large-volume growth chamber was preferred (Trethewey, 2004). Also, the positions of pots/trays in the chamber were periodically rotated to reduce growth differences associated with local fluctuations. All sources contributing to variation were kept as constant as possible. Given that biological variation can vary several-fold compared to acceptably low technical variations in GC-MS measurements (standard deviation usually ≤ 10%, Hall 2006), the minimum number of replicates measured was 15. Moreover, both the growth stage and the exact time of sampling were controlled, with great attention paid to post-harvest treatment.

In pilot experiments performed before planning full-scale metabolomic analyses, all samples for comparison were grown together and harvested under identical conditions so that maximum biological relevance could be linked to conclusions drawn from statistical analyses.

Tissue Sampling

The majority of metabolite or protein analyses present information obtained from pooled or non-sorted (non-fractionated) tissues such as ground leaf tissue used for the extraction of metabolites. Although interesting conclusions can be drawn from such surveys, they overlook a crucial aspect of plant evolution namely the differentiation of specialised cells whose individual metabolic activities constitute the spatially-separated, function-specific plant organs. In effect, any information obtained under such experimental design represents an average situation (snapshot) and very likely dilutes out the relative contributions of the individual cell types of various organs. More valuable information would be gained if tissue-specific sampling and subsequent analysis were

emphasised, as described by Schad *et al*. (2005) for instance. A large focus of this work was dedicated to achieving high sampling resolution in order to investigate signals produced during the early floral induction events.

As reported in Schmid *et al*. (2003), excised SAMs from Arabidopsis were used for transcript profiling studies. In effect, plants were dissected under a binocular microscope at room temperature and the shoot apex was immediately frozen in liquid nitrogen. Although dissections could be performed fairly rapidly, concerns still remained as to whether the metabolite composition would remain unaltered during the process. In addition, quite a large number of plants would need to be sampled for further processing. Given that time course experiments were planned with large numbers of sample replicates, genotypes and sampling points, this option was deemed too labour-intensive, would complicate experimental design and was therefore not pursued.

In the case of *Sinapis alba*, the shoot apical meristem is comparably larger and is therefore relatively more accessible for sampling. Initial tests using slightly modified metabolite extraction and derivatisation procedures gave good chromatograms but the problem of neighbouring plant cells/tissue contributed to 'contaminant' peaks in the samples. The same issue arose when deciding to opt for petiole sampling from *Arabidopsis* leaves – the tissue-specificity of the sampling remained unacceptable.

One alternative was the use of laser microdissection. Standard preparation procedures had been modified to allow the detection of metabolites within their localised tissue in microscope sections of reasonable morphological quality (Schad *et al*., 2005). In the case of shoot apex (both for *Arabidopsis* and *Sinapis*) and leaf (*Arabidopsis*) tissue, sections were always of poor quality because of the soft nature of the starting materials, which became brittle after freezing. Only in the case of stem tissue was the cell structure maintained. It was therefore only possible to further customise laser microdissection and cell capture in the case of *Sinapis* stem and petiole samples. Since comparison to a complementary leaf sample would not be possible and this approach was not considered worthwhile to continue.

Although it is theoretically possible to obtain phloem samples from *Arabidopsis* plants, phloem collection by aphid stylectomy yields samples in the nanoliter ranges and the concentration of compounds in the phloem is very dilute in comparison that of *Brassica napus* (rape), for instance. A method identical to that used in rape, employing sterile needles to make small incisions on plant stems was tested in *Sinapis* without success.

In the end, phloem exudate samples collected in EDTA were the closest alternative to pure phloem available and would also solve the previously observed problem of leaf or cell tissue contamination. In principle, plants respond to wounding, when sampling phloem for example, by the formation of callose plugs, which act as a mechanical barrier against further pathogen or herbivorous invasion while keeping the cell contents from being lost via leakage. Callose synthase requires calcium ions as cofactors to catalyse this reaction. Chelating agents, such as EDTA, bind divalent ions such as Ca^{2+}. Therefore, treating the cut plant surfaces with EDTA increases the exudation rates (King and Zeevart 1974, Tully and Hanson 1979). Although this technique is usually associated with distinct advantages such as unspecific contamination of cell debris and breakdown products, it would allow collection of both leaf and shoot apex samples in *Sinapis* and would permit parallel comparisons to be achieved so it became the method of choice. Furthermore, this same method would then be applicable to *Arabidopsis* enabling more relevant conclusions to be drawn when comparisons are made. Collection of phloem exudates in EDTA was carried out for leaf samples in *Arabidopsis* and for leaf and shoot apex samples in *Sinapis*.

Once the collection of phloem samples from both plant systems was finalised, conditions were optimised to create stable and reproducible induction regimes that would be later used in large-scale experiments.

Plant Induction Systems

Arabidopsis - Light Extension (Extended Short Day, ESD)

The samples subjected to the induction regimes would be essentially used for analysis by metabolite profiling using gas chromatography-time of flight-mass spectrometry (GC-TOF-MS). In this light, conditions were set up such that *Arabidopsis* plants had attained the competence to respond to floral signal(s). A compromise had to be achieved in terms of the age of the plants to be used, since the plants to be induced should not spontaneously start flowering as a result of the autonomous pathway activation. Given the high sensitivity of GC-MS detection, the amount of available material (size of the plant) was not a parameter which required consideration, unlike in Corbesier *et al*. (1996). Three-week old plants, grown under light regimes of 150 µmol m^{-2} sec^{-1} under short day conditions, provided sufficient leaves (5) for phloem exudation in EDTA and enough material to be analysed by GC-MS. All chromatograms obtained were of acceptable quality and included the major compound categories seen in leaf extracts for instance. In terms of testing the efficiency of three week-old plants to respond to prolonged periods of light and thereby trigger flowering, batches of one hundred plants were subjected to periods of ten, twelve, fourteen, sixteen and twenty additional hours of light. Experiments with wild type *Landsberg erecta* plants were performed on four separate occasions and satisfied the flowering phenotype threshold of 90% every time. Of all the extended light periods tested, it was observed that flowering resulted, two weeks later, if the light exposure was equal to or higher than fourteen hours. Hence, these conditions were applied to all subsequent experiments. These optimised inductive conditions, achieved by exposing plants to a single 14 hour-long light period, irreversibly triggered flowering, as demonstrated by the consistent flowering phenotypes and were therefore well-suited to the type of analysis planned.

Arabidopsis - Dexamethasone-Inducible System

Keeping the same considerations as for the ESD samples in mind, the developmental stage of the 35S::CO:GR plants was also restricted to three week-old plants. As discussed previously, plants of this age and size provided sufficient material for metabolite analysis. Although induction by spraying dexamethasone (DEX) would be more adequate using plants grown in sterile conditions, soil-grown plants were used since enough plant replicates could not always be obtained in tissue culture due to occasional contamination issues. Batches of seventy-five plants were grown under non-inductive short day conditions for three weeks, at light intensities equivalent to those used for the ESD system and were induced to flower by spraying a 10µM solution of DEX. Independent experiments performed on six occasions yielded the same, consistent results and fulfilled the 90% flowering phenotype requirement.

The experimental design of all induction experiments was further customised with respect to the minimum number of replicate samples needed for metabolite analyses. It was also ensured that enough plants would be available for the parallel sampling of whole plants for transcript analyses. In addition, at least 20 plants were kept to check the efficiency of induction after each experiment was carried out.

Sinapis alba in Belgium

All plants sampled in Belgium were grown and induced under conditions which have been optimised and reported (Lejeune *et al*., 1988). Plants subjected to a single exposure of 14 additional hours of light resulted in reliable flowering phenotypes. In addition, information provided by defoliation experiments have provided timings regarding the movement of the floral signal in *Sinapis alba*.

Sinapis alba in Golm

For the purpose of protein work, conditions were set up whereby *Sinapis alba* could be grown under short day conditions so that subsequent induction would be possible by exposure to an additional fourteen hours of light (Lejeune *et al*., 1998). Reported induction conditions for *Sinapis alba* used 65-day (9 week) old plants which were grown under 8-hour short days at an irradiance of 150 μmol m^{-2} sec^{-1}. Since multiple rounds of induction were planned, a system with a higher rate of plant rotation was sought. Therefore, plants were raised in vegetative conditions of 8-hour under light regimes of 300 μmol m^{-2} sec^{-1}. Initial tests regarding the age of the plants with respect to flowering times grown under these conditions revealed that plants should not be older than three-weeks at the time of induction. Inductive treatments were carried out at the same irradiance and flowering phenotypes, checked two weeks later, proved the reliability and consistency of this system. Initial tests were repeated five times independently of each other and satisfied the 90% flowering phenotype threshold, thereby providing evidence of a synchronous shift from vegetative to floral morphogenesis in at least 90% of all plants.

Changes Following the Induction to Flowering

Transcripts

Two entirely different systems were employed to study the events underlying the floral transition in *Arabidopsis* leaves at the transcript level. On the one hand, the photoextension system permitted investigating the events starting from the perception of the light signal, which is captured by the pigments constituting the clock's input pathways. In addition, the subsequent transduction resulting in downstream signalling events, which triggered the initiation of floral development. However, the dexamethasone-inducible system focused on events that were solely dependent on the activation of *CO* and its effects thereafter. Taken together, it was therefore possible to obtain insights into signalling events both prior to and after the upregulation of *CO* expression.

At first glance, as listed in Table 2, the number of changes in gene expression for the seventeen categories used, singled out the second time point of the extended short day system (representing samples taken 12 hours post-induction). By extension, an equivalent comparison would also apply for the second time point of the dexamethasone inductive treatment (Table 3). However, in order to find relevant overlaps between the systems, the expression trend of known marker genes was followed.

When investigating the behaviour of *CO* and *FT* throughout the inductive period, an overlap in the expression pattern was apparent between the systems for the individual genes, when the sampling times were shifted as shown in Figure 7A and B. Both genes also behaved in the same manner especially if the photoextension system is focused on. In other words, both these genes could be used as markers for the onset of floral induction, since they demonstrated some degree of commonality in the events occurring in those independent systems.

When comparing time point 2 of the ESD inductive treatment to time 1 of the same experiment to focus on signalling events occurring prior to the activation of *CO*, 8000 genes were obtained when using a significance threshold of 2. Briefly, it was required that the comparison between the induced and control treatments of non-responsive line (*co-2*) and that of the induced and control treatments of the responsive line (*Landsberg erecta*, L*er*) showed at least a two-fold change. In order to obtain insights into the behaviour of flowering genes, the 8000 genes fulfilling the latter criteria were compared against a list of approximately 80 flowering gene-specific loci. This list was generated by literature and database searches. Table 4 lists the floral regulators whose expression most severely increased or decreased. Included are *CO*, whose expression is downregulated and two of its homologues, *COL1* and *COL2*, showing contrasting transcript levels. Given the involvement of the *COL* genes in clock regulation and not in flowering time control per se, this is not entirely surprising (Ledger *et al*., 2001). Additionally, another *FT*-like gene *MFT*, showed a similar trend in expression. MFT is in fact a close homolog of mammalian RAF-kinase-inhibitor proteins (RKIP) and exhibited *FT*-like properties in terms of accelerating flowering (Yoo

et al., 2004b). It was suggested to act redundantly with *FT* or *TSF* to trigger flowering. Of notable interest was the detection of *TFL1*, a floral repressor. Together with the observed repression in *CO* and *FT* expression, this can be taken as proof that no events linked to floral induction had occurred at these times in this light extension system. Further downregulated genes supporting this fact were an *FD* paralog, which when associated to *FT* triggers flowering at the shoot apex (*Abe* et al., 2005, Wigge et al., 2005), and *CAL*, a meristem identity gene involved in floral development (Ferrandiz et al. 2000). The low levels of *TOC1*, a clock regulator, match the upregulation in *CCA1* seen given their antagonistic roles (Webb, 2003).

Using the same time comparison as Table 4 and considering all classes of genes and looking at the largest differences set to a threshold of 1.5 yielded Table 5. The changes in expression associated with transcripts preceding the activation of *CO* gave a small flowering genes class. *COL2* and *CCA1* were members that have been already discussed. *LHY* is also a clock regulator and is upregulated like *CCA1* and to the same extent. Together these two proteins create an autoregulation loop central to the oscillator of the circadian clock (Harmer and Kay, 2005). *GI* is another clock-associated gene that is proposed to function primarily in input to the clock (Park *et al*., 1999). The largest category contained ten entries and included several families of transcription factors. A few defence or stress related entries were listed, some of which may exert their functions under different stimuli. The same holds true for the members of the cell wall class and those listed in the miscellaneous category.

Signals following the activation of *CO*, common to both the extended light inductive conditions and the dexamethasone treatment showing upregulated gene expression are listed in Table 6. Comparing the previously mentioned time points representing times after the activation of *CO* gave 3000 overlapping genes when performing the double treatment comparison of the responsive and non-responsive line at threshold of 2. The 23 genes listed in Table 6 were obtained after comparing the 3000 genes to the abovementioned list of flowering genes. Differences between the equivalent time points between the induction experiments were calculated and those

listed were above or below zero. Known transcription factors such as *CONSTANS* (*CO*) and its immediate target FT appear to be increased. The consistency in their relative expression levels confirmed that the floral induction process had been initiated and could be tracked down to the specified windows mentioned above in both systems. The detection of the *FT* homolog *BFT* was interesting in the sense that other members of this small PEBP family of proteins have been suggested to be *FT*'s interacting florigenic partners. The closest FT homolog, *TWIN SISTER OF FT* (*TSF*), which promotes flowering like *FT*, was attributed such a role (Michaels *et al*., 2005; Yamaguchi *et al*., 2005) and was in fact identified in rape phloem alongside FT (Giavalisco *et al*., 2006). *TERMINAL FLOWER 1* (*TFL1*), a negative regulator of flowering, was suggested as being the antiflorigenic part of the floral signal assuming that FT would be the positive interactor (Kobayashi et al., 1999, Ahn et al., 2006). Interestingly, none of these previously reported genes were detected as increased or decreased in both systems here, suggesting that *BFT* may have taken over this function in *Arabidopsis*, possibly as a result of the evolution of species-specific features. Further proof that the induction cascade had in fact been activated was provided by the expression of *SOC1* (*AGL20*), a downstream target of *FT* and by *LFY*, which is itself a downstream target of *SOC1*. Moreover, the altered expression of genes regulating the floral repressor *FLC* such as upregulation of *LD* and *FY* (*FLC* repressors, the autonomous pathway), the downregulation of *FRI* and *FRL2* (positive regulators of *FLC*) together with the upregulation of the *VRN1* and *VRN2* (negative regulators of *FLC*, vernalization pathway) and upregulation of *VIP4*, *ART1* and *ELF 8* (negative regulators of flowering, *FLC* activators), imply that activation of the floral integrators by *FLC* is definitely repressed. Hence, the observed activation of the floral pathway integrators *FT*, *SOC1* and *LFY* is confirmed as being the result of events downstream of *CO* activation (via the photoperiod pathway in case of the light extension system and directly, as expected, in case of the DEX-dependent induction). Furthermore, these comparisons relate to early induction events corresponding to the regulation of integrator genes but not floral meristem identity genes as demonstrated by the downregulation of *AP1* and *AP2* (Table 7). Table 7 was generated in the same

manner as Table 6 but focused on downregulated flowering genes instead. Once again, differences between the equivalent time points between the induction experiments were calculated and genes listed were compared to a threshold of zero. Important genes seen as downregulated were *APETALA1*, *APETALA2*, *FRIGIDA* and *FRIGIDA-LIKE* as already discussed.

Table 8 was generated in the same manner as Tables 6 and 7 but was not specific to any class of genes. The differences used for the comparisons were at a threshold of 1.5 in this case. The transcripts listed were mostly related to stress or defence responses, even when examining transcription factor entries. A recurring theme was the production of certain of these proteins as a response to hormone signalling, especially ABA, or in response to biotic stresses. Interesting examples were the low temperature responsive protein and the lipid transfer protein of the LTP3 family (Arondel *et al*., 2000). Proteins of the LTP4 family, for instance, are generally small (~9 kD) and localise to the cell wall. Further investigating all these candidates with respect to the floral transition will prove interesting.

To further understand the role of *CONSTANS* as a transcription factor, outside the context of floral induction, genes whose expression levels followed that of CO in the two parallel floral induction systems were searched for. Table 9 showed AXR3, one of two transcription factors. This gene acts as a repressor of auxin-inducible gene expression (Davies, 2004). Auxin induced the relocalisation of the protein within the nucleus from a diffused nucleoplasmic pattern to a discrete particulated pattern named nuclear protein bodies in a process mediated by Rac1. This transcription factor co-localised with proteasome components. The second potential target, a basic helix-loop-helix transcription factor is not further well characterised as far as which regulatory pathways are influenced. Little can also be said about the two carbohydrate metabolism genes that appeared as possible targets of *CO*. Two further candidates, namely enzymes catalysing the synthesis of starch and cellulose would also need to be further investigated. The same applies for the unidentified protein, which may prove interesting to study once some preliminary indications regarding the pathway involvement are obtained.

Associating signalling and transport events occurring in the sampled leaves to the global genetic events surveyed here is not straightforward. The genes whose importance was revealed by significant overlaps between the independent inductive systems are likely to be part and parcel of this tightly regulated process. A number of unidentified candidates were seen and may be important as further study material in terms of elucidating their role and function in the context of early floral induction processes.

After obtaining more information regarding the timing of gene expression, it would be interesting to reduce the sampling time intervals to one or two hours in order to better understand the regulation of expression of genes involved in the switch to flowering. Analysing such information will be important in terms of gaining knowledge regarding the fine tuning of this developmental process. Moreover, the use of yet another but parallel system for triggering floral induction may yield more robust candidate transcripts. One option would be using a displaced short day (DSD), where the plants are exposed to a period of light equivalent to a normal short day (e.g. 8 hours), but at a time when they would usually experience darkness.

Metabolites

Metabolomics via GC-MS

The term metabolomics was coined by Oliver *et al.*, (1998) as being the metabolite complement of living tissues, to essentially provide an unbiased, comprehensive qualitative and quantitative overview of the complete set of small molecules, such as amino acids, lipids, carbohydrates, vitamins and hormones, present in an organism at a specific time point (Hall 2006). Therefore the metabolome, as the sum of the small molecules and metabolites of a living cell, represents the amplification and integration of signals from other functional genomic levels, such as the transcriptome and the proteome (Nielsen and Oliver 2005). Metabolic information represents the first level of responses to fluctuations in homeostatic systems and reflects the immediate changes associated with system regulation.

One of the most popular global metabolite analysis methods is gas chromatography-mass spectrometry (GC-MS) due to its robust separation and the electron impact ionisation technique (Hall 2006). There are several types of mass spectrometers and various ion sources available for the combination of GC and MS. The time-of-flight (TOF) mass spectrometer determines mass as a function of the time that it takes ions, with similar kinetic energy but different masses, to move through a flight tube to the detector. TOF analyzers have good mass accuracy, a high practical mass range and fast acquisition rates. They provide higher detection sensitivity than quadrupoles and have therefore been widely used for the analysis of small molecules.

Metabolite Profiling and Floral Induction

Using metabolite profiling in the context of floral induction in an attempt to find florigenic signals was one of the main goals of this work. Reasons behind the unsuccessful separation, identification and characterisation of the components of florigenic extracts were the non-tissue specific nature of the starting material and the use of techniques with low sensitivity. In this study, it was possible to adapt established metabolite platforms for the analysis of phloem exudates collected in EDTA.

Using the light-dependent extension system it was possible to investigate metabolite changes preceding the activation of *CO* in phloem exudates. Metabolites listed were required to fulfill the double requirement of a 2-fold change when comparing both the induced and control treatments of the responsive line and the equivalent comparison for the non-responsive line. All values for induced samples were corrected for the extended short day treatment and for the mutation by calculating corresponding ratios. The corrected numbers for induced samples of the responsive line were compared against the control treatment of the same line. The latter ratios were then used to generate the ratios listed as fold changes between the second and first time exudation periods and are listed in Table 11. Alanine, asparagine, glutamic acid and proline were the changed amino acids seen. Of these, glutamic acid matched the report of being one of the most abundant

amino acids sampled from single sieve element cells in *Arabidopsis* (Zhu *et al*., 2005). The three remaining amino acids were also detected albeit as less abundant ones in the same paper. The role of all other metabolite classes was not reported in the context of flowering or of phloem sampling. With respect to floral induction and phloem exudate sampling, glutamic acid and asparagine were previously reported as two of five most abundant amino acids in *Arabidopsis* and their increase in this study matched the sampling times published. Alanine was also detected but in lower amounts (Corbesier *et al*., 2001) while proline has also been associated with the floral transition in other plants species such as chicory, cabbage and tobacco (Shvedskaya and Kruzhilin, 1966, Vallee *et al*., 1968, Bouniols *et al*., 1973).

With regard to metabolite changes following the activation of *CO*, significant metabolites in the photoextension induction were primarily amino acids. The eight listed in Table 12 did not constitute any of the major or most abundant ones. However, asparagine, histidine, phenylalanine, methionine, valine and ornithine corresponded to amino acids previously detected in *Arabidopsis* phloem sap (Zhu *et al*., 2005); cysteine and gamma-amino-butyric acid were not. Given its role as a neurotransmitter, the role of GABA as a signalling molecule has been studied in animals and plants (Bouché and Fromm, 2004). Biotic and abiotic stresses appear to regulate the production of GABA in plants (Shelp *et al*., 1999; Snedden and Fromm, 1999; Kinnersley and Turano, 2000). A GABA gradient is required in the pistil to allow pollen tube guidance and growth (Wilhelmi and Preuss, 1996). Furthermore, a potential role for GABA as a long-distance signal in the regulation of nitrate uptake was recently reported in *Brassica napus* (Beuve *et al*., 2004). In the comparison of the matching time points in the DEX system (Table 13) asparagine, methionine, serine and tyrosine were previously reported in the composition of phloem sap of *Arabidopsis* (Zhu *et al*., 2005) while tryptophan was not. Therefore, common between these two induction systems were two amino acids asparagine and methionine (Table 14). Their relative amounts were previously measured during flower induction (Corbesier *et al*., 2001) and the data obtained in this study may therefore confirm a connection between changes in these metabolites and flowering. The

discrepancy between additional amino acids deemed significant and those reported could be explained by the difference in exudation times. When considering phloem exudate sampling in terms of floral induction events, amino acids seen in the ESD system are lacking glutamine, whose export from the leaves was shown to drastically increase between 16 and 20 hours post-induction in *Arabidopsis* (Corbesier *et al*., 2001). Interestingly, this amino acid was also not detected in the DEX system at this sampling time.

To investigate whether some metabolites are consistently related to flowering in other plants species, *Sinapis* samples were harvested in Belgium using conditions identical to those reported in Corbesier et *al*. (2001). The comparison of leaf exudate samples matched the increase in glutamine seen in the leaves 16-20 hours post-induction as described in Corbesier *et al*. (2001). However, in contrast to the 3-4 fold increase reported, a ratio of 14-fold was observed with respect to the control in this metabolite dataset. As expected, no changes in glutamate or asparagine levels were observed, but the reported decrease in aspartate and serine was not detected. Defoliation experiments in *Sinapis* provided estimates of florigen movement 16 hours post-induction (Bernier 1989). The detection of dehydroascorbic acid showing a six-fold difference in analyte levels was interesting given reports linking ascorbic acid to the floral transition (Ye *et al*., 2000; Barth et al., 2006). *Arabidopsis* plants that underwent bolting possessed a five-fold less peroxidase activity and plants showed visible senescence symptoms. Although no clear evidence supports this in other plants species, the long-distance transport of this small antioxidant molecule was shown to occur in potato (Tedone *et al*., 2004). The direct and reciprocal conversions of dehydroascorbate to ascorbate may account for either of these molecules as potential signals in this system and should be investigated further.

Analysis of the shoot apex samples did not match any of the reported changes for glutamine and glutamate. Valine was the most abundant amino detected in this study but was classified as part of the less abundant group by Corbesier *et al*. (2001). Proline was the next increased followed by GABA. None of the latter were discussed in terms of signals for floral induction, therefore

investigating these further as well as the diverse compound classes listed in the miscellaneous category may prove interesting.

On the whole, the main changes linking floral events to metabolite levels, within and between the floral induction systems in *Arabidopsis*, were compounds with broad functions in primary metabolism. For this reason, it is difficult to associate such analytes to specific plant developmental events. Interspecies comparisons also resulted in no clear, common metabolites related to flowering. Given also that the non-identified metabolites could not always be reliably found, drawing further conclusions is not possible. Since metabolites are small molecules which are rapidly influenced by various reactions and fluctuations, it not unlikely that their rate of turnover is too fast to be captured using the methods used here. Other issues such as the relatively long exudation periods in EDTA may be another factor explaining why no dramatic changes are seen in this metabolite survey. In the end, it may also be that metabolites have no involvement in flower induction. Changes at the gene, transcript and protein levels probably in different combinations and with tightly regulated time and space expression patterns may be the more conceivable answer to the floral signal mystery.

The above points also apply to *Sinapis*. However, it seemed somewhat surprising that no changes were seen even when using such a well-defined system in terms of florigen movement and reliable induction. The sampling window was narrowed down to the two exudation periods of 4 hours based on the latter facts. Since not all the published findings could be reproduced GC-MS, a more sensitive technique than HPLC, it may be possible that the timing of the signal movement needs to be reviewed. It was expected that changes occurring in the leaf between 8 and 12 hours post-induction would be captured again during the next sampling point but in the shoot apex instead. No such overlap was found.

Proteins

The direct regulation of *FT* by *CO*, its small protein size (23KDa, Kardailasky *et al*., 1999, Kobayashi *et al*., 1999), and the high sequence homology to mammalian RAF-kinase-inhibitor proteins (RKIP) made FT a strong possible candidate as the mobile graft-transmissible signal or as one of the florigenic components (Kardailasky *et al*., 1999, Kobayashi *et al*., 1999, reviewed in Suarez-Lopez 2005, Bernier 2005). Therefore an analysis focused on the level of FT was performed in this work by Western Blot analyses. To achieve this, a FT peptide antibody was produced. The high homology of the FT rape sequences and the hydrophobicity tests confirmed that the peptide chosen for synthesis and antibody generation was highly immunogenic (Figures 11 and 12). Database searches ascertained that the selected sequence was homologous to only FT and TSF.

Initial antibody reactivity tests using rape phloem proved that the antibody reacted with the protein it was raised against and was not solely specific for the peptide sequence (Figure 13). Using crude antisera, a signal corresponding to the previously estimated 23 KDa FT protein in *Arabidopsis* was observed in samples of rape phloem (Kardailsky *et al*., 1999; Kobayashi *et al*., 1999). Database entries report an additional size of FT around 20 KDa (19.809 KDa) in *Arabidopsis*, matching the sizes of the FT protein spots seen in 2D gels (Giavalisco *et al*., 2006), Figure 10.

FT shows sequence similarity to the precursor of an eleven amino acid peptide, hippocampal cholinergic neurostimulating peptide (HCNP), which is generated by proteolytic cleavage at the amino end of the 21 KDa precursor (Tohdoh *et al*., 1995). This inherent signalling capacity and the fact that the size of the FT protein is below the size exclusion limit of plasmodesmata supported FT as a strong candidate for the mobile floral signal (Lucas 2005). Interestingly, additional bands of 18 (strong signal), 17 and 16 KDa (both fainter) were also seen. Given the similarity between FT, TSF and HCNP, it is probable that proteolytic processing of FT and TSF gives rise to peptides of smaller molecular weights, representing the corresponding

cleavage products. An alternative to the processing of the FT and TSF proteins is alternative splicing of the DNA sequence. Database searches report a splice variant of FT in *Arabidopsis* with a final length of 122 aa whose estimated size is approximately 14 KDa (13.741 KDa). This information matches the signal size observed in *Arabidopsis* phloem exudates in the case of the companion cell-specific FT overexpressing line. Therefore, such a mechanism might also explain the presence of the intense 18 KDa band in rape.

Throughout the phloem sampling course in rape, bands of consistent sizes showed differential intensities related to the plant developmental stage (Figure 14). As an extension to the abovementioned point, given the high sequence homology among all members of the RKIP family, the number of bands observed at one time point could result from differential post-translational modifications of these respective proteins (Kardailasky et al., 1999, Kobayashi et al., 1999), a total of 7 members: FT (175 aa, 19.809 KDa), twin sister of FT (177 aa, 19.688 KDa), brother of FT (BFT, 177 aa, 20.008 KDa), mother of FT (MFT, 173 aa, 19.134 KDa), terminal flower 1 (TFL1, 177 aa, 20.008 KDa), terminal flower 2 (TFL2, 177 aa, 20.008 KDa) and the *Antirrhinum majus* CENTRORADIALIS homolog in *Arabidopsis* (ATC, 175 aa, 19.893 KDa). However, since the amino acid sequence used to generate the antibody showed highest similarity to FT and TSF, the signals can be assumed to be specific to the latter proteins. Thus, posttranslational modifications of the precursor and processed peptides would account for the range of size variants seen at one time.

Given the difficulties of obtaining phloem sap during non-flowering stages of *Brassica napus*, associating FT to florigenic transport was not possible. It is interesting to note that not only one but two FT protein spots were identified in 2-DE analysis of phloem, both of which are also phosphorylated (Julia Kehr, personal communication). One candidate suggested to be FT's florigenic partner is TFL1. This member of the PBEP family acts in a manner antagonistic to FT and delays flowering in *Arabidopsis*. Although these proteins show only 60% of sequence homology, their functional specificity could be assigned to one amino acid residue corresponding to the ligand-binding pocket (Hanzawa *et al*., 2005). Swapping the His88 to Tyr85 converts TFL1 to

FT and vice versa. Further investigations revealed that the key structural differences additionally lie in segment B of the fourth exon, contributing to an external loop which would be easily accessible to interactors (Ahn *et al*., 2006). Given that none of the proteins showed homologies to TFL1 (or its close homolog TFL2) in the phloem of flowering rape plants, the conversion of TFL1 to FT could trigger flowering. Further modifications would then activate the downstream signalling cascades leading to floral development.

Another suggested florigenic partner for FT is TSF. Like *FT*, *TSF* was shown to promote flowering via the activation of common downstream targets involved in the flowering transition (e.g. *SOC1*). The presence of TSF in rape phloem (Giavalisco *et al*., 2006) would then relate to its role of fine tuning the floral transition, one redundant with that of FT as reported by Yamaguchi *et al.* (2005). Predictions based on sequence and domain analysis in fact support the notion that TSF may form complexes with phosphorylated ligands by interfering with kinases and their effectors (www.expasy.org). Hence, it is possible that flowering is triggered once TSF is synthesised, in parallel with the two phosphorylated forms of FT previously discussed.

Cross reactivity of the antibody to evolutionarily-related species of the *Brassicaceae* family was not surprising. The degree of conservation of the signal sizes supports genetic evidence of a main conserved pathway module within the three species. In *Arabidopsis*, the 14 KDa signal appears analogous to the 18 KDa FT form in rape. Although less clear, an identical situation was observed in *Sinapis alba* with protein sizes corresponding to signals remaining consistent. Small variations were also observed but were likely due to species-specific divergence of the floral response. Since this protein is clearly absent in all flowering time mutants, the varying sizes of the protein (either the smaller peptides or the larger multimers) may in fact be underlying the regulation of triggering and/or maintaining the floral state.

Ultimately, proof that the antibody is in fact specific among the three plant species surveyed can only be obtained through sequence information of purified antigens. Identifying the

component(s) of phloem interfering with the immunoprecipitations will facilitate the isolation and identification of the FT interacting partners.

A remaining option as the graft-transmissible floral signal is the mRNA of *FT*. Transgenic plants harbouring the *FT* under the control of a heat shock promoter were used to time movement of the transcript (Huang et *al*., 2005). An increase in the transgenic *FT* mRNA was detected 6 hours post induction while the endogenous *FT* transcript was seen to increase in both leaves and in the shoot apex 6 to 12 hours post induction. Although no direct evidence supports the movement of native *FT* mRNA during floral induction in wild type plants, it could provide an answer to bridging the information between how the signal is generated in the leaves, then transduced and eventually transported to the shoot apex. It may therefore be worthwhile to also assess the RNA status of *FT* using the *Sinapis* system to gain information about potential links between floral induction and the *FT* mRNA and/or the FT protein.

Concluding Remarks

The major aim of this study was to identify components associated to the induction of flowering. Therefore, different plant systems were used and transcripts and metabolites were profiled. In addition, the abundance of the FT protein was analysed.

Transcript analyses of *Arabidopsis* leaves resulted in a list of known flowering-related genes but in addition revealed several new candidates that could be interesting for future studies.

Metabolite profiling of phloem exudates did not lead to any common substances that could be clearly related to flowering.

Western Blot analyses of FT in phloem samples showed no obvious relation of protein abundance to the stage of flower development. However, interesting size changes were observed that will be further investigated.

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Appendix 1: Classification of significantly upregulated transcripts in the Photoextension system 8 hours after induction

Affy Identifier	Functional Class	Fold change	AGI code
	Transcription Factors (10)		
245247_at	Scarecrow-like TF 13 (SCL13)	2.85	At4g17230
249890_at	WRKY family transcription factor	2.58	At5g22570
254231_at	WRKY family transcription factor,	3.08	At4g23810
	high mobility group (HMG1/2) family protein		At4g23800
261892_at	WRKY family transcription factor	6.18	At1g80840
263783_at	WRKY family transcription factor	4.83	At2g46400
249940_at	no apical meristem (NAM) family protein	11.07	At5g22380
252681_at	no apical meristem (NAM) family protein	5.70	At3g44350
260203_at	no apical meristem (NAM) family protein	2.82	At1g52890
263584_at	no apical meristem (NAM) family protein	2.28	At2g17040
246777_at	zinc finger family protein (C3HC4-type RING finger)	2.31	At5g27420
253259_at	encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/ AP2 transcription factor family	5.64	At4g34410
266821_at	encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/ AP2 transcription factor family	4.13	At2g44840
261470_at	encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/ AP2 transcription factor family	4.61	At1g28370
	Signalling		
249197_at	calmodulin-related protein, putative	6.52	At5g42380
258947_at	calmodulin-related protein, putative	10.50	At3g01830
253414_at	calmodulin-binding family protein	2.84	At4g33050
260046_at	calmodulin-binding protein	2.98	At1g73800
260068_at	calmodulin-binding protein	3.52	At1g73805
246821_at	similar to calmodulin-binding protein	3.21	At5g26920
262671_at	calcium-dependent protein kinase, putative	2.45	At1g76040
249417_at	calcium-binding EF hand family protein	3.63	At5g39670
			At5g39680
247723_at	protein phosphatase 2C, putative / PP2C,	2.79	At5g59220
259231_at	protein phosphatase 2C, putative / PP2C,	2.00	At3g11410
261077_at	protein phosphatase 2C, putative / PP2C,	2.21	At1g07430
259428_at	mitogen-activated protein kinase, putative	4.02	At1g01560
254265_s_at	receptor-like protein kinase 5 (RLK5)	2.39	At4g23140
			At4g23160
254271_at	protein kinase family protein	3.59	At4g23150
254869_at	protein kinase family protein	2.09	At4g11890
266037_at	protein kinase, putative	2.36	At2g05940
256366_at	serine/threonine protein kinase family protein	2.39	At1g66880

	Defence		
249264_s_at	disease resistance protein (TIR-NBS-LRR class)	4.35	At5g41750 At5g41740
256431_s_at	disease resistance family protein / LRR family protein	2.73	At3g11010 At5g27060
267546_at	disease resistance family protein, contains leucine rich-repeat (LRR) domains	3.30	At2g32680
252373_at	disease resistance protein (EDS1)	2.47	At3g48090
257083_s_at	non-race specific disease resistance protein (NDR1)	2.66	At3g20600 At3g20590
245034_at	serpin, putative / serine protease inhibitor, putative	2.55	At2g26390
249983_at	curculin-like (mannose-binding) lectin family protein	4.10	At5g18470
247602_at	lectin protein kinase family protein	2.23	At5g60900
255406_at	ankyrin repeat family protein	2.16	At4g03450
264434_at	ankyrin repeat family protein	2.39	At1g10340
261443_at	glutaredoxin family protein	3.66	At1g28480
	Plant Receptor		
266780_at	glutamate receptor family protein, plant glutamate receptor family	3.53	At2g29110
	Cell Wall		
266070_at	expansin family protein (EXPR3)	2.55	At2g18660
	Carbohydrate Metabolism		
254321_at	trehalose-6-phosphate phosphatase, putative	2.33	At4g22590
259550_at	arabinogalactan-protein (AGP5)	2.03	At1g35230
252179_at	similar to glycosyl transferase family 8 protein	2.87	At3g50760
245627_at	galactinol synthase, putative	2.08	At1g56600
	Miscellaneous		
248322_at	heavy-metal-associated domain-containing protein	3.99	At5g52760
248327_at	heavy-metal-associated domain-containing protein	2.37	At5g52750
246293_at	sigA-binding protein	2.41	At3g56710
246988_at	armadillo/beta-catenin repeat family protein / U-box domain-containing protein	2.14	At5g67340
252131_at	AAA-type ATPase family protein	5.56	At3g50930
252346_at	pseudogene, At14a-related protein	4.09	At3g48650
265993_at	pseudogene, leucine rich repeat protein family	2.71	At2g24160
260804_at	VQ motif-containing protein	2.50	At1g78410
254975_at	oxidoreductase, 2OG-Fe(II) oxygenase family protein	2.87	At4g10500
257763_s_at	leucine-rich repeat family protein	2.09	At3g23120 At3g23110
	Unidentified		
249918_at	expressed protein	2.34	At5g19240
250796_at	expressed protein	3.06	At5g05300
250956_at	expressed protein	2.02	At5g03210
251633_at	expressed protein	2.16	At3g57460
252345_at	expressed protein	2.25	At3g48640
252908_at	expressed protein	3.97	At4g39670

252977_at	expressed protein	2.95	At4g38560
253859_at	expressed protein	3.47	At4g27657
254229_at	expressed protein,	3.16	At4g23610
	50S ribosomal protein-related		At4g23620
258203_at	expressed protein	2.78	At3g13950
259385_at	expressed protein	2.68	At1g13470
259479_at	expressed protein	4.07	At1g19020
264635_at	expressed protein	2.23	At1g65500

Appendix 2: Classification of significantly downregulated transcripts in the Photoextension system 8 hours after induction

Affymetrix Identifier	Functional Class	Fold change	AGI code
	Transcription Factors		
265877_at	bZIP transcription factor family protein	0.49	At2g42380
	Hormone Signalling		
252965_at	Auxin-responsive protein, putative	0.41	At4g38860
	Photosynthesis/Respiration		
244966_at	Cytochrome b6-f complex, subunit 5	0.47	AtCg00600
	Miscellaneous		
253627_at	Hydrophobic protein, putative/		
	low temperature and salt responsive protein	0.41	At4g38860
264323_at	flavin-containing monooxygenase family protein	0.39	At1g04180

Appendix 3: Classification of significantly upregulated transcripts in the Photoextension system 12 hours after induction

Affymetrix Identifier	Functional Class	Fold change	AGI code
	Transcription Factors		
245362_at	homeobox-leucine zipper protein 1 (HAT1) / HD-ZIP protein 1	3.11	At4g17460
248564_at	DNA-binding protein-related	3.79	At5g49700
251575_at	bZIP transcription factor family protein	3.29	At3g58120
258349_at	bZIP transcription factor family protein / HY5-like protein (HYH)	3.24	At3g17609
252917_at	zinc finger (B-box type) family protein	6.36	At4g38960
263739_at	zinc finger (B-box type) family protein	4.29	At2g21320

263252_at	zinc finger (B-box type) family protein / salt tolerance-like protein (STH)	9.48	At2g31380
258497_at	zinc finger protein CONSTANS-LIKE 2 (COL2)	16.03	At3g02380
255694_at	basic helix-loop-helix (bHLH) family protein	6.40	At4g00050
263210_at	similar to basic helix-loop-helix (bHLH) family protein	17.20	At1g10585
258723_at	myb family transcription factor	4.48	At3g09600
261569_at	myb family transcription factor	4.61	At1g01060
258724_at	similar to myb family transcription factor	5.35	At3g09600
266719_at	myb-related transcription factor (CCA1)	10.21	At2g46830
250099_at	myb family transcription factor, similar to CCA1	4.22	At5g17300
260856_at	encodes a member of the DREB subfamily A-5 of ERF/ AP2 transcription factor family	2.94	At1g21910
264415_at	encodes a member of the ERF (ethylene response factor) subfamily B-4 of ERF/AP2 transcription factor family (RAP2.6)	4.74	At1g43160
	Signalling		
251017_at	protein phosphatase 2C family protein / PP2C family protein, similar to Ser/Thr protein phosphatase 2C (PP2C6)	2.38	At5g02760
255959_at	1-phosphatidylinositol-4-phosphate 5-kinase, putative	3.33	At1g21980
256751_at	chloride channel protein (CLC-b)	6.90	At3g27170
254250_at	protein kinase family protein	2.79	At4g23290
248179_at	protein kinase family protein	4.40	At5g54380
	Hormone Signalling		
255403_at	auxin-responsive GH3 family protein	3.91	At4g03400
257506_at	auxin-responsive family protein	5.05	At1g29440
260427_at	auxin-responsive protein-related	3.79	At1g72430
263664_at	auxin-responsive protein / indoleacetic acid-induced protein 17 (IAA17)	8.62	At1g04250
261768_at	gibberellin 3-beta-dioxygenase / gibberellin 3 beta-hydroxylase (GA4)	4.16	At1g15550
264195_at	gibberellin-responsive protein, putative	3.03	At1g22690
	Defence		
267411_at	disease resistance family protein, contains leucine rich-repeat domains	20.31	At2g34930
254770_at	leucine-rich repeat family protein / extensin family protein	5.11	At4g13340
260831_at	glutaredoxin family protein	2.78	At1g06830
	Stress		
245558_at	early-responsive to dehydration protein-related	11.80	At4g15430
257315_at	proline oxidase, mitochondrial / osmotic stress-responsive proline dehydrogenase (POX) (PRO1) (ERD5)	6.90	At3g30775
255064_at	phosphate-responsive protein, putative (EXO), similar to phi-1 (phosphate-induced gene) (Nicotiana tabacum)	3.61	At4g08950
263421_at	phosphate-responsive 1 family protein, similar to phi-1 (phosphate-induced gene) (Nicotiana tabacum)	6.31	At2g17230

	Light-mediated reponses		
251793_at	regulator of chromosome condensation (RCC1) family protein, UVB-resistance protein UVR8	2.98	At3g55580
261480_at	phytochrome kinase, putative	6.08	At1g14280
267614_at	encodes a member of the cytochrome p450 family. involved in brassinolide metabolism	4.07	At2g26710
	Cell Wall		
263207_at	xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative	3.06	At1g10550
264960_at	proline-rich extensin-like family protein	5.08	At1g76930
	Carbohydrate metabolism		
245275_at	beta-amylase (BMY1) / 1,4-alpha-D-glucan maltohydrolase	13.21	At4g15210
250007_at	beta-amylase, putative (BMY3) / 1,4-alpha-D-glucan maltohydrolase, putative	7.82	At5g18670
261191_at	starch synthase, putative	3.03	At1g32900
258528_at	glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein	41.21	At3g06770
249037_at	fasciclin-like arabinogalactan-protein	3.74	At5g44130
267260_at	arabinogalactan-protein (AGP17)	4.05	At2g23130
254773_at	glycosyl transferase family 2 protein	8.16	At4g13410
	Lipid Metabolism		
245422_at	palmitoyl protein thioesterase family protein	5.08	At4g17470
251013_at	short-chain dehydrogenase/ reductase (SDR) family protein	8.74	At5g02540
260957_at	delta 9 desaturase (ADS1)	8.86	At1g06080
261570_at	fatty acid elongase 3-ketoacyl-CoA synthase 1 (KCS1)	34.15	At1g01120
	Photosynthesis/Respiration		
262908_at	pyruvate dehydrogenase E1 component alpha subunit, mitochondrial (PDHE1-A)	3.32	At1g59900
245152_at	mitochondrial substrate carrier family protein		At2g47490
	Protein Degradation		
245262_at	aspartyl protease family protein	2.67	At4g16563
	Transport		
249071_at	MATE efflux family protein, similar to ripening regulated protein DDTFR18 (Lycopersicon esculentum)	2.93	At5g44050
	Miscellaneous		
246195_at	ubiquitin-conjugating enzyme 17 (UBC17)	13.59	At4g36410
248676_at	male sterility MS5 family protein	4.16	At5g48850
248683_at	protease inhibitor/seed storage/ lipid transfer protein (LTP) family protein	4.10	At5g48490
252989_at	multi-copper oxidase type I family protein,	3.73	At4g38420

	similar to pollen-specific BP10 protein (Brassica napus)		
251770_at	oxidoreductase, 2OG-Fe(II) oxygenase family protein, similar to leucoanthocyanidin dioxygenase (Malus domestica)	5.01	At3g55970
255302_at	methionine sulfoxide reductase domain-containing protein / SeIR domain-containing protein, low similarity to pilin-like transcription factor (Homo sapiens)	20.54	At4g04830
267147_at	oxidoreductase, 2OG-Fe(II) oxygenase family protein, similar to flavonol synthase (Citrus unshiu)	21.54	At2g38240
254122_at	eceriferum protein (CER2)	3.36	At4g24510
260438_at	bifunctional nuclease, putative	9.18	At1g68290
263796_at	kelch repeat-containing F-box family protein	3.04	At2g24540
264745_at	5'-adenylylsulfate reductase 2, chloroplast (APR2) (APSR)	6.06	At1g62180
264770_at	armadillo/beta-catenin repeat family protein / U-box domain-containing protein	4.49	At1g23030
265724_at	ovate protein-related, uncharacterized plant-specific domain	2.18	At2g32100
266363_at	haloacid dehalogenase-like hydrolase family protein	7.51	At2g41250
	Unidentified		
246200_at	expressed protein	3.09	At4g37240
247474_at	expressed protein	6.77	At5g62280
248028_at	expressed protein	3.22	At5g55620
249118_at	expressed protein	5.78	At5g43870
249752_at	expressed protein	3.29	At5g24660
251869_at	expressed protein	4.50	At3g54500
253811_at	expressed protein	4.37	At4g28190
253943_at	expressed protein	2.92	At4g27030 At4g27040
256096_at	expressed protein	3.69	At1g13650
256743_at	expressed protein	2.52	At3g29370
257057_at	expressed protein	8.98	At3g15310
257076_at	expressed protein	6.18	At3g19680
259275_at	expressed protein	6.49	At3g01060
265999_at	expressed protein	6.60	At2g24100
266364_at	expressed protein	11.20	At2g41230
266693_at	expressed protein	13.91	At2g19800

Appendix 4: Classification of significantly downregulated transcripts in the Photoextension system 12 hours after induction

Affymetrix Identifier	Functional Class	Fold change	AGI code
	Transcription Factors		
245247_at	scarecrow-like transcription factor 13 (SCL13)	0.36	At4g17230
245329_at	zinc finger family protein / (C3HC4-type RING finger)	0.40	At4g17030

	ankyrin repeat family protein		
246777_at	zinc finger family protein (C3HC4-type RING finger)	0.36	At5g26920
246933_at	zinc finger (C2H2 type) family protein (ZFP3)	0.49	At5g64510
247351_at	zinc finger family protein (C3HC4-type RING finger)	0.23	At5g63130
260753_at	zinc finger family protein (C3HC4-type RING finger)	0.28	At1g52690
259977_at	zinc-binding family protein, similar to zinc-binding protein (Pisum sativum)	0.23	At1g49230
251245_at	basic helix-loop-helix (bHLH) protein, putative	0.19	At3g51750
261713_at	basic helix-loop-helix (bHLH) protein (RAP-1)	0.30	At2g27920
249467_at	no apical meristem (NAM) protein; similar to cup-shaped cotyledon CUC2	0.24	At5g22570
249940_at	no apical meristem (NAM) protein	0.11	At5g10760
252681_at	no apical meristem (NAM) family protein	0.32	At4g32340
256300_at	no apical meristem (NAM) family protein	0.20	At3g14440
258809_at	no apical meristem (NAM) family protein	0.22	At1g73800
260203_at	no apical meristem (NAM) family protein	0.04	At1g19960
261564_at	no apical meristem (NAM) family protein	0.33	At2g42760
263584_at	no apical meristem (NAM) family protein	0.20	At2g43010
258395_at	no apical meristem (NAM) family protein (NAC3)	0.46	At1g20510
253872_at	no apical meristem (NAM) family protein (RD26)	0.17	At4g23810
			At4g23800
249890_at	WRKY family transcription factor	0.04	At5g13320
251705_at	WRKY family transcription factor	0.18	At3g48020
253485_at	WRKY family transcription factor	0.28	At4g25490
254231_at	WRKY family transcription factor	0.38	At4g11650
257382_at	WRKY family transcription factor	0.15	At3g15500
263783_at	WRKY family transcription factor	0.21	At2g37130
267028_at	WRKY family transcription factor	0.44	
250810_at	myb family transcription factor	0.34	At5g01600
252475_s_at	myb family transcription factor	0.13	At4g33050
265359_at	myb family transcription factor	0.34	At2g47130
255250_at	myb family transcription factor (MYB74)	0.30	At1g56300
255753_at	myb family transcription factor (MYB51)	0.35	At3g24520
260581_at	myb family transcription factor (MYB2)	0.31	At1g56140
			At1g56130
			At1g56120
261431_at	myb family transcription factor (MYB47)	0.32	At2g17040
257985_at	transcription factor jumonji (jmjC) domain-containing protein	0.46	At3g01830
258139_at	heat shock transcription factor family protein	0.32	At3g11410
259992_at	heat shock factor protein, putative (HSF5)	0.26	At1g78410
266327_at	homeobox-leucine zipper protein 7 (HB-7) / HD-ZIP transcription factor 7	0.20	
266555_at	G-box binding factor 3 (GBF3)	0.40	
245252_at	encodes a member of the ERF (ethylene response factor)	0.38	At4g17500

	subfamily B-1 of ERF/ AP2 transcription factor family		
248794_at	encodes a member of the ERF (ethylene response factor) subfamily B-1 of ERF/ AP2 transcription factor family	0.28	At5g39670 At5g39680
257053_at	encodes a member of the ERF (ethylene response factor) subfamily B-1 of ERF/ AP2 transcription factor family	0.35	At3g24520
261315_at	encodes a member of the ERF (ethylene response factor) subfamily B-1 of ERF/ AP2 transcription factor family	0.29	At1g05575
253259_at	encodes a member of the ERF (ethylene response factor) subfamily B-1 of ERF/ AP2 transcription factor family	0.22	At4g27657
261470_at	encodes a member of the ERF (ethylene response factor) subfamily B-1 of ERF/ AP2 transcription factor family	0.30	At2g46400
266821_at	encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/ AP2 transcription factor family	0.28	
250781_at	encodes a member of the DREB subfamily A-2 of ERF/ AP2 transcription factor family (DREB2A)	0.40	At5g02810
254074_at	encodes a member of the DREB subfamily A-1 of ERF/ AP2 transcription factor family (CBF1)	0.26	At4g22780
262211_at	encodes a member of the DREB subfamily A-5 of ERF/ AP2 transcription factor family	0.31	At1g10340
250286_at	auxin-responsive GH3 family protein	0.23	At5g05090
251342_at	auxin-responsive family protein, similar to auxin-induced protein SAUR-AC1	0.30	At3g50930
	Signalling		
245731_at	mitogen-activated protein kinase kinase (MAPKK), putative (MKK9)	0.36	At1g73480
259428_at	mitogen-activated protein kinase, putative MAPK, putative (MPK11)	0.27	At1g69260
246821_at	similar to calmodulin-binding protein	0.21	At5g26340
253414_at	calmodulin-binding family protein	0.27	At4g26080
267381_at	calmodulin-binding family protein	0.43	
258947_at	calmodulin-related protein, putative similar to regulator of gene silencing calmodulin-related protein from Nicotiana tabacum	0.15	At1g73805
260046_at	calmodulin-binding protein	0.21	At1g02470

260068_at	calmodulin-binding protein	0.21	At1g17380
247406_at	two-component responsive regulator / response regulator 6 (ARR6)	0.29	At5g62520
252374_at	two-component responsive regulator / response regulator 5 (ARR5) / response reactor 2 (RR2)	0.33	At4g34950
259466_at	two-component responsive regulator / response regulator 7 (ARR7)	0.26	At1g72520
247668_at	pseudo-response regulator 3 (APRR3)	0.30	At5g59320
250971_at	pseudo-response regulator 7 (APRR7)	0.23	At3g60420
247723_at	protein phosphatase 2C, putative	0.22	At5g54960
249417_at	calcium-binding EF hand family protein	0.28	At5g24530
259231_at	protein phosphatase 2C, putative	0.26	At1g73260
261077_at	protein phosphatase 2C, putative	0.05	At1g14870 At1g14880
250408_at	CBL-interacting protein kinase 5 (CIPK5)	0.20	At5g03350
254265_s_at	receptor-like protein kinase 5 (RLK5)	0.31	At4g05100
254271_at	protein kinase family protein	0.20	At1g18570
254416_at	S-locus protein kinase, putative (ARK3)	0.25	At1g33970
254660_at	receptor serine/threonine kinase, putative	0.35	At1g19250
	Hormone Signalling		
253994_at	protein phosphatase 2C ABI1 / abscisic acid-insensitive 1 (ABI1)	0.23	At4g23140 At4g23160
258498_at	ABA-responsive protein-related	0.24	At1g56510
266761_at	short-chain dehydrogenase/ reductase (SDR) family protein	0.34	
	Defence		
248169_at	ankyrin repeat family protein	0.13	At5g52300
264434_at	ankyrin repeat family protein	0.20	At2g18680
249264_s_at	disease resistance protein (TIR-NBS-LRR class), putative	0.47	At5g39610
259629_at	disease resistance protein (TIR-NBS-LRR class), putative	0.44	At2g43570
253997_at	disease resistance protein RPS2 (CC-NBS-LRR class), putative	0.38	At4g23150
256431_s_at	disease resistance family protein / LRR family protein	0.25	At2g40750
267546_at	disease resistance family protein, contains leucine rich-repeat (LRR) domain	0.26	
265723_at	similar to disease resistance protein (TIR class), putative	0.36	At2g40080
265597_at	Toll-Interleukin-Resistance (TIR) domain-containing protein	0.41	At2g41190
257083_s_at	non-race specific disease resistance protein (NDR1)	0.48	At3g17800 At3g17790
255980_at	avirulence-responsive protein, putative avirulence induced gene protein, putative	0.27	At3g11010 At5g27060
259925_at	pathogenesis-related protein 5 (PR-5)	0.29	At2g47190

266385_at	pathogenesis-related protein 1 (PR-1)	0.09	
247602_at	lectin protein kinase family protein, probable mannose binding	0.18	At5g60100
249983_at	curculin-like (mannose-binding) lectin family protein	0.22	At5g05410
250942_at	legume lectin family protein	0.13	At3g62550
260101_at	trypsin and protease inhibitor family protein / Kunitz family protein, similar to trypsin inhibitor propeptide (Brassica oleracea)	0.36	At1g07430
261443_at	glutaredoxin family protein (thioltransferase)	0.10	At2g46430 At2g46440
265067_at	glutaredoxin family protein (thioltransferase)	0.27	At2g38790
252421_at	chitinase, putative	0.35	At4g33980
260560_at	chitinase, putative	0.32	At1g01720
260568_at	chitinase, putative	0.31	At1g32640
256243_at	basic endochitinase	0.30	At3g20600 At3g20590
254889_at	osmotin-like protein (OSM34)	0.25	At1g19180
	Stress		
248352_at	low-temperature-responsive 65 kD protein (LTI65) / desiccation-responsive protein 29B (RD29B)	0.26	At5g47220
249850_at	DNAJ heat shock N-terminal domain-containing protein	0.16	At5g22380
256221_at	DNAJ heat shock N-terminal domain-containing protein	0.17	At3g15210
251221_at	universal stress protein (USP) family protein, similar to ER6 protein (Lycopersicon esculentum)	0.26	At3g56400
262128_at	late embryogenesis abundant protein, putative	0.30	At1g60190
257644_at	allene oxide cyclase, putative / early-responsive to dehydration protein, putative	0.50	At3g11840
265471_at	peroxidase 21 (PER21) (P21) (PRXR5)	0.43	At2g44840
	Circadian Clock Regulation and Flowering		
247525_at	ABI3-interacting protein 1 (AIP1), timing of CAB expression 1 protein (TOC1)	0.35	At5g60900
256060_at	CONSTANS-like protein-related	0.09	At3g28580
264211_at	gigantea protein (GI)	0.02	At2g27310
265248_at	phytochrome-interacting factor 4 (PIF4) / basic helix-loop-helix protein 9 (bHLH9) / short under red-light 2 (SRL2)	0.10	At2g46270
	Cell Wall		
245463_at	expansin-related	0.28	At1g56600
266070_at	expansin family protein (EXPR3)	0.19	At2g30770
254189_at	cellulose synthase family protein	0.28	At4g21380
	Carbohydrate Metabolism		
246831_at	hexose transporter, putative	0.26	At5g25210
260412_at	encodes a plastid localized alpha-amylase	0.20	At1g28480
245627_at	galactinol synthase	0.15	At1g73500

251673_at	similar to glycosyl hydrolase family 17 protein	0.16	At3g48100
256252_at	UDP-glucoronosyl/ UDP-glucosyl transferase family protein	0.14	At3g27210
	Lipid Metabolism		
262154_at	phospholipase/carboxylesterase family protein	0.32	At1g61800
	Amino Acid Metabolism		
267080_at	amino acid transporter family protein	0.34	
	Sulphur Metabolism		
246340_s_at	S-adenosyl-L-methionine:carboxyl methyltransferase family protein	0.34	At5g27420
	Secondary Metabolism		
249910_at	prephenate dehydratase family protein	0.30	At5g10930
259518_at	4-coumarate--CoA ligase family protein	0.41	At1g11080
248062_at	protease inhibitor/ seed storage/ lipid transfer protein (LTP) family protein	0.31	At5g52760
266098_at	protease inhibitor/ seed storage/ lipid transfer protein (LTP) family protein	0.29	
260399_at	lipoxygenase, putative	0.45	At1g18710
257280_at	9-cis-epoxycarotenoid dioxygenase, putative / neoxanthin cleavage enzyme, putative	0.21	At3g14280
	Photosynthesis/Respiration		
248138_at	pyruvate decarboxylase	0.23	At5g52750
248353_at	cytochrome P450, putative	0.39	At5g45630
257623_at	cytochrome P450 71B23, putative	0.31	At3g02480
267567_at	cytochrome P450 71A13, putative	0.47	
255941_at	mitochondrial import inner membrane translocase subunit Tim17	0.34	At1g69490
264400_at	glucose-6-phosphate/phosphate translocator	0.31	At2g24160
264783_at	phosphoenolpyruvate carboxylase kinase	0.18	At2g46680
	Protein Regulation/Degradation		
250445_at	aspartyl protease family protein	0.22	At5g03210
252131_at	AAA-type ATPase family protein	0.29	At5g59570 At3g46640
256989_at	AAA-type ATPase family protein	0.38	At3g20810
260475_at	serine carboxypeptidase S10 family protein	0.32	At1g28370
264071_at	serine carboxypeptidase S10 family protein	0.36	At2g20142 At2g20145
260005_at	Golgi transport complex protein-related	0.38	At1g02450
	Miscellaneous		
245734_at	hydrolase, alpha/beta fold family protein	0.38	At3g56880
246289_at	VQ motif-containing protein	0.31	At3g56710
260804_at	VQ motif-containing protein	0.34	At1g52700

246293_at	sigA-binding protein	0.13	At3g44860 At3g44870
247323_at	dentin sialophosphoprotein-related (Homo sapiens)	0.21	At5g63780 At5g63790
247393_at	octicosapeptide/Phox/ Bem1p (PB1) domain-containing protein	0.25	At5g62920
247431_at	Encodes a protein with similarity to RCD1 but without the WWE domain, role for the protein in ADP ribosylation	0.37	At5g61380
247717_at	lipid transfer protein 3 (LTP3)	0.15	At5g59310
247718_at	lipid transfer protein 4 (LTP4)	0.20	At5g59220
248322_at	heavy-metal-associated domain-containing protein	0.14	At5g52320
248327_at	heavy-metal-associated domain-containing protein	0.23	At5g50360
249754_at	oxidoreductase, 2OG-Fe(II) oxygenase family protein,	0.26	At5g22630
254975_at	oxidoreductase, 2OG-Fe(II) oxygenase family protein	0.12	At1g07050
256647_at	oxidoreductase, 2OG-Fe(II) oxygenase family protein	0.39	At3g26210
257763_s_at	leucine-rich repeat family protein	0.18	At3g04070
262082_s_at	leucine-rich repeat family protein / protein kinase family protein	0.17	At1g22770
265993_at	pseudogene, leucine rich repeat protein family	0.21	At2g32680
256012_at	flavin-containing monooxygenase family protein	0.34	At3g13610
251109_at	ferritin 1 (FER1)	0.36	At3g57540
251644_at	remorin family protein	0.32	At3g48650
252346_at	pseudogene, At14a-related protein	0.33	At4g39670
253215_at	nodulin family protein	0.23	At4g27654
254300_at	ACT domain-containing protein (ACR7)	0.36	At1g20350
256789_at	seven in absentia (SINA) family protein, developmental protein seven in Drosophila melanogaster	0.33	At3g23120 At3g23110
258787_at	U-box domain-containing protein	0.32	At1g67970
258792_at	glycine-rich protein, predicted proteins	0.40	At1g67930 At1g67920
260904_at	NPR1/NIM1-interacting protein 1 (NIMIN-1)	0.11	At1g74930
262448_at	transducin family protein / WD-40 repeat family protein	0.24	At1g09500
262930_at	harpin-induced protein-related	0.29	At1g03850
263776_s_at	cyclic nucleotide-regulated ion channel	0.28	At2g16720
267361_at	acid phosphatase class B family protein	0.13	
264217_at	armadillo/beta-catenin repeat family protein / U-box domain-containing protein	0.33	At2g32140
264514_at	similar to Eucalyptus gunnii alcohol dehydrogenase	0.27	At2g37870
264562_at	BTB/POZ domain-containing protein	0.25	At2g29350
265620_at	F-box family protein	0.43	At2g39920
266292_at	tropinone reductase, putative / tropine dehydrogenase, putative	0.28	
266462_at	benzodiazepine receptor-related	0.29	

	Unidentified		
245319_at	expressed protein	0.22	At4g14365
246929_at	expressed protein	0.38	At5g25160
247293_at	expressed protein	0.17	At5g64180
247295_at	expressed protein	0.34	At5g64170
248505_at	expressed protein	0.38	At5g41750
			At5g41740
248959_at	expressed protein	0.22	At5g39520
249454_at	expressed protein	0.16	At5g23240
			At5g23235
250956_at	expressed protein	0.22	At3g60690
251400_at	expressed protein	0.30	At3g48640
252073_at	expressed protein	0.21	At3g47540
252345_at	expressed protein	0.25	At3g44350
252400_at	expressed protein	0.29	At4g34410
252908_at	expressed protein	0.32	At4g31800
253322_at	expressed protein	0.26	At4g27410
253421_at	expressed protein	0.29	At4g26090
			At4g26100
253832_at	expressed protein	0.49	At4g24000
253859_at	expressed protein	0.28	At4g23610
			At4g23620
254229_at	expressed protein	0.20	At4g18250
256017_at	expressed protein	0.31	At3g13672
256766_at	expressed protein	0.17	At3g25780
257154_at	expressed protein	0.22	At3g13950
257670_at	expressed protein	0.50	At3g04640
258158_at	expressed protein	0.27	At1g01560
258203_at	expressed protein	0.30	At1g19050
258362_at	expressed protein	0.24	At1g15790
259385_at	expressed protein	0.05	At1g52890
259489_at	expressed protein	0.31	At1g69830
260357_at	expressed protein	0.36	At1g53170
260933_at	expressed protein, contains non-consensus splice sites;	0.28	At1g49450
261033_at	expressed protein	0.39	At1g11210
261221_at	expressed protein	0.12	At1g65690
262452_at	expressed protein, similar to cotton fiber expressed protein 1 (Gossypium hirsutum)	0.11	At1g55760
262832_s_at	expressed protein	0.24	At1g08650
263182_at	expressed protein	0.39	At1g23710
263972_at	expressed protein	0.28	At2g15890
265184_at	expressed protein	0.34	At2g47770
265478_at	expressed protein	0.33	At2g38470
265837_at	expressed protein	0.06	At2g26190
266071_at	expressed protein	0.48	
266396_at	expressed protein	0.33	
267364_at	expressed protein	0.14	

Appendix 5: Classification of significantly upregulated transcripts in the Photoextension system 12 hours after induction

Affymetrix Identifier	Functional Class Transcription factors	Fold change	AGI code
251245_at	Basic helix-loop-helix (bHLH) protein, putative	2.50	At3g62090
259977_at	Zinc-binding family protein	2.70	At1g76590
	Signalling		
246756_at	protein phosphatase 2C, putative / PP2C,	2.27	At5g27930
247867_at	CBL-interacting protein kinase	2.77	At5g57630
248910_at	CBL-interacting protein kinase 20 (CIPK20)	?? DOWN	
260728_at	serine/threonine protein kinase family protein	2.05	At1g48210
	Defence		
259925_at	Pathogenesis-related protein 5 (PR5)	3.69	At1g75040
266385_at	Pathogenesis-related protein 5 (PR1)	3.47	At2g14610
249983_at	curculin-like (mannose-binding) lectin family protein	3.23	At5g18470
260560_at	Chitinase, putative	2.51	At2g43590
	Stress		
263495_at	Cold-responsive protein/cold-regulated	3.58	At2g42530
258941_at	Monodehydroascorbate reductase, putative	6.67	At3g09940
	Cell Wall		
266070_at	expansin family protein (EXPR3)	3.12	At2g18660
	Carbohydrate Metabolism		
256252_at	UDP-glucoronosyl/ UDP-glucosyl transferase protein family	5.36	At3g11340
	Photosynthesis/Respiration		
244932_at	PsaC subunit of photosystem I	7.32	AtCg01060
256589_at	Cytochrome P450 family protein	2.97	At3g28740
259403_at	D-3-phosphoglycerate dehydrogenase (3-PGDH)	2.42	At1g17745
	DNA		
244999_at	Chloroplast DNA-dependent RNA polymerase B unit	3.30	AtCg00190
	Miscellaneous		
245749_at	heavy-metal-associated domain-containing protein	4.11	At1g51090
248062_at	protease inhibitor/seed storage/lipid transfer protein	2.89	At5g55450
254234_at	major latex protein-related	2.00	At4g23680
253268_s_at	CBS domain-containing protein	2.24	At4g34120 At4g34131 At4g34135
254975_at	Oxidoreductase, 2OG-Fe(II) oxygenase family protein	7.37	At4g10500
267425_at	FAD-binding domain-containing protein	3.31	At2g34810
244933_at	NADH dehydrogenase ND4L	4.00	AtCg01070

244934_at	NADH dehydrogenase ND6	6.52	AtCg01080
	Unidentified		
245319_at	expressed protein	2.40	At4g16146
256114_at	expressed protein	3.42	At1g16850
256766_at	expressed protein	2.86	At3g22231
257670_at	expressed protein	2.10	At3g20340
259385_at	expressed protein	4.46	At1g13470
260668_at	expressed protein	2.78	At1g19530
265837_at	expressed protein	3.76	At2g14560

Appendix 6: Classification of significantly downregulated transcripts in the Photoextension system 12 hours after induction

Affymetrix Identifier	Functional Class	Fold change	AGI code
	Transcription factors		
258947_at	Zinc finger protein CONSTANS-LIKE 2 (COL2)	0.28	At3g02380
258349_at	bZIP transcription factor family protein/ HY-like protein (HYL)	0.41	At3g17609
261569_at	Myb family transcription factor	0.45	At1g01060
263739_at	Zinc finger (B-box type) family protein	0.38	At2g21320
	Hormone Signalling		
252970_at	Auxin-responsive protein, small auxin up RNA (SAUR-AC1)	0.47	At4g38850
254746_at	Auxin-responsive protein, putative	0.43	At4g12980
261766_at	Auxin-responsive protein/ indole acetic acid induced protein	0.37	At1g15580
	Carbohydrate Metabolism		
261191_at	Starch synthase	0.35	At1g32900
	Lipid metabolism		
260957_at	Delta 9 desaturase (ADS 1)	0.43	At1g06080
	Amino Acid Metabolism		
246490_at	Adenosylmethionine decarboxylase family protein	0.36	At5g15950
	Photosynthesis/Respiration		
244966_at	Cytochrome b6-f complex, subunit 5	0.50	AtCg00600
	Miscellaneous		
248683_at	protease inhibitor/seed storage/lipid transfer protein	0.48	At5g48490
	Unidentified		
253643_at	expressed protein	0.41	At4g29780
253943_at	expressed protein	0.31	At4g27030 At4g27040
256096_at	expressed protein	0.39	At1g13650
256266_at	expressed protein	0.42	At3g12320

257070_at	expressed protein	0.33	At3g15310

Appendix 7: Classification of significantly upregulated transcripts in the dexamethasone system 0 hours after induction

Affymetrix identifier	Functional Class	Fold change	AGI code
	Transcription Factors		
246997_at	basic helix-loop-helix (bHLH) family protein	2,43	At5g67390
259432_at	myb family transcription factor	2,33	At1g01520
	Carbohydrate Metabolism		
261046_at	UDP-glucoronosyl/ UDP-glucosyl transferase family protein	2,16	At1g01390
	Defense		
265665_at	cysteine proteinase, putative	2,15	At2g27420
	Miscellaneous		
253764_s_at	casein kinase, putative	2,23	At4g28860
	Unidentified		
246997_at	expressed protein	2,76	At5g67390
255825_at	expressed protein	2,11	At2g40475

Appendix 8: Classification of significantly downregulated transcripts in the dexamethasone system 0 hours after induction.

Affymetrix identifier	Functional Class	Fold change	AGI code
	Transcription Factors		
245329_at	zinc finger (C3HC4-type RING finger) family protein/ ankyrin repeat family protein	0,49	At4g14365
245362_at	homeobox-leucine zipper protein 1 (HAT1) / HD-ZIP protein 1	0,49	At4g17460
246253_at	myb family transcription factor (MYB73)	0,49	At4g37260
	cadmium/zinc-transporting ATPase, putative (HMA1)		At4g37270
246777_at	zinc finger (C3HC4-type RING finger) family protein	0,37	At5g27420
246993_at	zinc finger (C2H2 type) protein 1 (AZF1)	0,36	At5g67450
261984_at	encodes a member of the DREB subfamily A-4 of ERF/ AP2 transcription factor family	0,20	At1g33760
259729_at	encodes a member of the DREB subfamily A-5 of ERF/ AP2 transcription factor family	0,35	At1g77640
260856_at	encodes a member of the DREB subfamily A-5 of ERF/ AP2 transcription factor family	0,39	At1g21910

248794_at	encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/ AP2 transcription factor family (ATERF-2)	0,28	At5g47220
247543_at	encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/ AP2 transcription factor family	0,35	At5g61600
248448_at	encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/ AP2 transcription factor family	0,35	At5g51190
259992_at	heat shock factor protein, putative (HSF5) / heat shock transcription factor	0,43	At1g67970
255753_at	myb family transcription factor (MYB51)	0,40	At1g18570
252193_at	myb family transcription factor	0,39	At3g50060
249940_at	no apical meristem (NAM) family protein	0,14	At5g22380
252278_at	no apical meristem (NAM) family protein	0,47	At3g49530
263783_at	WRKY family transcription factor	0,38	At2g46400
257382_at	WRKY family transcription factor	0,21	At2g40750
267028_at	WRKY family transcription factor	0,45	At2g38470
254231_at	WRKY family transcription factor	0,31	At4g23810
	high mobility group (HMG1/2) family protein		At4g23800
	Signaling		
246821_at	similar to calmodulin-binding protein	0,43	At5g26920
256100_at	calcineurin-like phosphoesterase family protein	0,36	At1g13750
252417_at	calcium-binding EF hand family protein	0,41	At3g47480
259879_at	calcium-binding EF hand family protein	0,31	At1g76650
260046_at	calmodulin-binding protein	0,28	At1g73800
260068_at	calmodulin-binding protein	0,33	At1g73805
249197_at	calmodulin-related protein	0,18	At5g42380
252136_at	calmodulin-related protein	0,47	At3g50770
258947_at	calmodulin-related protein	0,29	At3g01830
259428_at	mitogen-activated protein kinase, putative	0,37	At1g01560
254271_at	protein kinase family protein	0,35	At4g23150
260206_at	protein kinase family protein	0,49	At1g70740
258682_at	serine/threonine protein kinase (PK19)	0,49	At3g08720
251494_at	serine/threonine protein kinase, putative	0,46	At3g59350
258682_at	serine/threonine protein kinase (PK19)	0,49	At3g08720
251494_at	serine/threonine protein kinase, putative	0,46	At3g59350
259561_at	wall-associated kinase 1 (WAK1)	0,49	At1g21250
249417_at	calcium-binding EF hand family protein	0,33	At5g39670
	pentatricopeptide (PPR) repeat-containing protein		At5g39680
	Carbohydrate Metabolism		
259211_at	alpha 1,4-glycosyltransferase family protein	0,42	At3g09020
265841_at	glycogenin glucosyltransferase (glycogenin)-related	0,49	At2g35710

	Photosynthesis/Respiration		
248964_at	cytochrome P450 family protein	0,25	At5g45340
261986_s_at	cytochrome P450, putative	0,45	At1g33730
	cytochrome P450, putative		At1g33720
	Cell wall		
266070_at	expansin family protein (EXPR3)	0,31	At2g18660
253608_at	putative xyloglucan endotransglycosylase/hydrolase	0,25	At4g30290
	Stress		
255479_at	late embryogenesis abundant 3 family protein / LEA3 family protein	0,45	At4g02380
262128_at	late embryogenesis abundant protein, putative / LEA protein	0,10	At1g52690
	Lipid Metabolism		
260915_at	lipase class 3 family protein	0,39	At1g02660
256306_at	lipase class 3 family protein	0,47	At1g30370
245038_at	patatin, putative, similar to patatin-like latex allergen	0,48	At2g26560
	Defense		
248981_at	ankyrin repeat family protein	0,39	At5g45110
264434_at	ankyrin repeat family protein	0,35	At1g10340
263800_at	ankyrin repeat family protein	0,42	At2g24600
267546_at	disease resistance family protein	0,35	At2g32680
252373_at	disease resistance protein (EDS1)	0,47	At3g48090
262381_at	disease resistance protein (TIR-NBS class), putative	0,39	At1g72900
259629_at	disease resistance protein (TIR-NBS-LRR class), putative	0,47	At1g56510
260296_at	disease resistance protein (TIR-NBS-LRR class), putative	0,44	At1g63750
261443_at	glutaredoxin family protein	0,27	At1g28480
265067_at	glutaredoxin family protein	0,44	At1g03850
251625_at	glycosyl hydrolase family 17 protein	0,27	At3g57260
260568_at	chitinase, putative	0,39	At2g43570
249983_at	curculin-like (mannose-binding) lectin family protein	0,47	At5g18470
260904_at	NPR1/NIM1-interacting protein 1 (NIMIN-1)	0,46	At1g02450
266385_at	pathogenesis-related protein 1 (PR-1)	0,43	At2g14610
252060_at	phytoalexin-deficient 4 protein (PAD4)	0,39	At3g52430
253997_at	disease resistance protein RPS2 (CC-NBS-LRR class), putative	0,43	At4g26090
	casein kinase, putative		At4g26100
	Protein Regulation / Degradation		
252098_at	aspartyl protease family protein	0,50	At3g51330
250445_at	aspartyl protease family protein	0,40	At5g10760
251507_at	aspartyl protease family protein	0,46	At3g59080
264866_at	matrixin family protein, similar to matrix metalloproteinase	0,41	At1g24140

	Miscellaneous		
245757_at	phosphate-responsive protein, putative	0,24	At1g35140
245866_s_at	purine permease-related	0,47	At1g57990
	purine permease-related		At1g57980
246293_at	sigA-binding protein	0,40	At3g56710
247314_at	3'(2'),5'-bisphosphate nucleotidase, putative/ inositol polyphosphate 1-phosphatase	0,24	At5g64000
247717_at	lipid transfer protein 3 (LTP3)	0,11	At5g59320
247718_at	lipid transfer protein 4 (LTP4)	0,14	At5g59310
252131_at	AAA-type ATPase family protein	0,43	At3g50930
251336_at	BON1-associated protein 1 (BAP1)	0,27	At3g61190
255630_at	C2 domain-containing protein	0,42	At4g00700
248327_at	heavy-metal-associated domain-containing protein	0,42	At5g52750
248322_at	heavy-metal-associated domain-containing protein	0,27	At5g52760
263852_at	MutT/nudix family protein	0,35	At2g04450
254975_at	oxidoreductase, 2OG-Fe(II) oxygenase family protein	0,38	At4g10500
254573_at	pectinacetylesterase family protein	0,46	At4g19420
255064_at	phosphate-responsive protein, putative (EXO)	0,42	At4g08950
256933_at	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	0,37	At3g22600
252346_at	pseudogene, At14a-related protein	0,44	At3g48650
265993_at	pseudogene, leucine rich repeat	0,41	At2g24160
266292_at	tropinone reductase, putative	0,42	At2g29350
255895_at	12-oxophytodienoate reductase, putative	0,40	At1g18020
	12-oxophytodienoate reductase, putative		At1g17990
	Unidentified		
245119_at	expressed protein	0,30	At2g41640
245755_at	expressed protein	0,29	At1g35210
246200_at	expressed protein	0,39	At4g37240
247933_at	expressed protein	0,46	At5g56980
251400_at	expressed protein	0,38	At3g60420
251610_at	expressed protein	0,49	At3g57930
251684_at	expressed protein	0,48	At3g56410
252345_at	expressed protein	0,38	At3g48640
253044_at	expressed protein	0,24	At4g37290
256766_at	expressed protein	0,45	At3g22231
257076_at	expressed protein	0,44	At3g19680
259385_at	expressed protein	0,46	At1g13470
263182_at	expressed protein	0,28	At1g05575
266901_at	expressed protein	0,21	At2g34600
267230_at	expressed protein	0,43	At2g44080
267393_at	expressed protein	0,48	At2g44500
265837_at	expressed protein	0,37	At2g14560

262369_at	expressed protein	0,22	At1g73010
266800_at	expressed protein	0,42	At2g22870

Appendix 9: Classification of significantly upregulated transcripts in the dexamethasone system 4 hours after induction

Affymetrix identifier	Functional Class	Fold change	AGI code
	Transcription Factors		
254693_at	basic helix-loop-helix (bHLH) family protein	2,57	At4g17880
255694_at	basic helix-loop-helix (bHLH) family protein	2,23	At4g00050
258349_at	bZIP transcription factor family protein	5,05	At3g17609
264692_at	DNA-binding family protein	2,62	At1g70000
251272_at	homeobox-leucine zipper protein 12 (HB-12)	3,33	At3g61890
245758_at	myb family transcription factor (KAN2)	2,27	At1g32240
252534_at	myb family transcription factor (MYB48)	6,17	At3g46130
248246_at	myb family transcription factor (TRIPTYCHON)	4,18	At5g53200
258807_at	myb family transcription factor	2,05	At3g04030
260784_at	myb family transcription factor	3,57	At1g06180
260380_at	zinc finger (B-box type) family protein	3,25	At1g73870
263739_at	zinc finger (B-box type) family protein	3,01	At2g21320
257262_at	zinc finger (B-box type) family protein	3,22	At3g21890
248160_at	zinc finger (B-box type) family protein	2,18	At5g54470
251586_at	zinc finger (C2H2 type) family protein	2,96	At3g58070
	Signalling		
248191_at	calcium-binding EF hand family protein	2,08	At5g54130
252014_at	calmodulin-binding family protein	2,05	At3g52870
251060_at	CBL-interacting protein kinase 14 (CIPK14)	2,44	At5g01820
248910_at	CBL-interacting protein kinase 20 (CIPK20)	2,53	At5g45820
250408_at	CBL-interacting protein kinase 5 (CIPK5)	3,29	At5g10930
265939_at	DC1 domain-containing protein	2,55	At2g19650
263433_at	inositol-3-phosphate synthase isozyme 2 / myo-inositol-1-phosphate synthase 2 / MI-1-P synthase 2 / IPS 2	4,72	At2g22240
248888_at	inward rectifying potassium channel (KAT1)	2,01	At5g46240
266682_at	leucine-rich repeat family protein / extensin family protein	2,35	At2g19780
260146_at	phototropic-responsive NPH3 family protein	2,58	At1g52770
258677_at	serine/threonine protein kinase (PK1) (PK6)	2,11	At3g08730
249798_at	similar to transducin family protein / WD-40 repeat family protein	2,14	At5g23730
248607_at	sodium-inducible calcium-binding protein (ACP1)	3,26	At5g49480
	Hormone Signalling		
261150_at	S-adenosyl-L-methionine:jasmonic acid	3,17	At1g19640

	carboxyl methyltransferase (JMT)		
	Defence		
261914_at	disease resistance-responsive family protein	3,71	At1g65870
255622_at	the glycosyltransferase (UGT72B1)	2,03	At4g01070
	Stress		
262113_at	late embryogenesis abundant 3 family protein	14,63	At1g02820
251984_at	phenylalanine ammonia-lyase 2 (PAL2)	2,57	At3g53260
	Cell Wall		
258003_at	expansin, putative (EXP5)	2,07	At3g29030
264898_at	invertase/pectin methylesterase inhibitor family protein	6,07	At1g23205
245965_at	pectinesterase family protein	2,60	At5g19730
246403_at	similar to pectinacetylesterase, putative	2,72	At1g57590
	Carbohydrate Metabolism		
250007_at	beta-amylase, putative (BMY3)	2,16	At5g18670
261016_at	glycosyl hydrolase family 1 protein	2,19	At1g26560
264931_at	polygalacturonase, putative / pectinase, putative	3,04	At1g60590
261191_at	starch synthase, putative	3,22	At1g32900
255016_at	sucrose-phosphate synthase, putative	2,27	At4g10120
261046_at	UDP-glucoronosyl/ UDP-glucosyl transferase family protein	2,31	At1g01390
265197_at	UDP-glucoronosyl/ UDP-glucosyl transferase family protein	2,13	At2g36750
245277_at	UDP-glucose:indole-3-acetate beta-D-glucosyltransferase	3,95	At4g15550
	Lipid Metabolism		
252363_at	GDSL-motif lipase/hydrolase family protein	3,83	At3g48460
265646_at	lipase, putative	2,54	At2g27360
	Amino Acid Metabolism		
246597_at	L-aspartate oxidase family protein	3,41	At5g14760
	Sulphur Metabolism		
264745_at	5'-adenylylsulfate reductase 2	2,45	At1g62180
	Secondary Metabolism		
248311_at	beta-carotene hydroxylase, putative	2,47	At5g52570
266391_at	strictosidine synthase family protein	2,21	At2g41290
	Photosynthesis/Respiration		
257628_at	cytochrome P450 71B26, putative (CYP71B26)	3,44	At3g26290
264400_at	glucose-6-phosphate/phosphate translocator, putative	3,11	At1g61800

	Protein Regulation/Degradation		
264217_at	armadillo/beta-catenin repeat family protein	4,15	At1g60190
264770_at	armadillo/beta-catenin repeat family protein	2,16	At1g23030
248763_at	cysteine protease inhibitor, putative / cystatin, putative	3,84	At5g47550
265665_at	cysteine proteinase, putative	2,50	At2g27420
252606_at	serine carboxypeptidase III, putative	2,09	At3g45010
254791_at	serine carboxypeptidase S10 family protein	2,13	At4g12910
257748_at	U-box domain-containing protein	2,08	At3g18710
	Miscellaneous		
251826_at	ABC transporter family protein	2,21	At3g55110
256275_at	actin 11 (ACT11)	2,01	At3g12110
258299_at	alcohol oxidase-related	2,01	At3g23410
247074_at	allergen V5/Tpx-1-related family protein	3,76	At5g66590
267019_at	amino acid transporter family protein	2,39	At2g39130
255080_at	arabinogalactan-protein (AGP10)	2,67	At4g09030
266895_at	Bet v I allergen family protein	2,07	At2g26040
256751_at	chloride channel protein (CLC-b)	4,08	At3g27170
251005_at	chloroplast lumen common family protein	2,18	At5g02590
265066_at	fasciclin-like arabinogalactan-protein (FLA9)	2,83	At1g03870
264583_at	galactosyltransferase family protein	2,61	At1g05170
266363_at	haloacid dehalogenase-like hydrolase family protein	2,54	At2g41250
264729_at	heavy-metal-associated domain-containing protein	2,54	At1g22990
250335_at	hydrolase, alpha/beta fold family protein	2,99	At5g11650
262290_at	hydroxyproline-rich glycoprotein family protein	2,28	At1g70985
249071_at	MATE efflux family protein	2,94	At5g44050
248204_at	myosin heavy chain, putative	2,46	At5g54280
258181_at	nitrate transporter (NTP3)	2,37	At3g21670
248467_at	nodulin MtN3 family protein	3,61	At5g50800
254938_at	oligopeptide transporter OPT family protein	2,00	At4g10770
261407_at	phytochrome kinase substrate-related	2,02	At1g18810
252624_at	phytosulfokines-related	3,25	At3g44735
265111_at	protease inhibitor/seed storage/ lipid transfer protein (LTP) family protein	8,44	At1g62510
245427_at	transporter-related	2,25	At4g17550
259185_at	triose phosphate/phosphate translocator, putative	2,77	At3g01550
258757_at	zinc finger (C3HC4-type RING finger) family protein	2,48	At3g10910
260770_at	zinc finger (C3HC4-type RING finger) family protein	2,16	At1g49200
246439_at	zinc finger (C3HC4-type RING finger) family protein	2,30	At5g17600
248759_at	zinc finger (C3HC4-type RING finger) family protein	2,84	At5g47610
260727_at	expressed protein glycoside hydrolase family 28 protein	7,16	At1g48110; At1g48100
266720_s_at	pseudo-response regulator, putative pseudo-response regulator 9 (APRR9)	5,83	At2g46670; At2g46790

	Unidentified		
255723_at	expressed protein	2,71	At3g29575
256266_at	expressed protein	2,09	At3g12320
259856_at	expressed protein	2,90	At1g68440
252010_at	expressed protein	2,22	At3g52740
253814_at	expressed protein	2,87	At4g28290
254508_at	expressed protein	2,19	At4g20170
246125_at	expressed protein	3,06	At5g19875
261832_at	expressed protein	2,59	At1g10650
262875_at	expressed protein	2,27	At1g64970
256674_at	expressed protein	2,36	At3g52360
256926_at	expressed protein	2,57	At3g22540
264264_at	expressed protein	2,54	At1g09250
259373_at	expressed protein	2,24	At1g69160
249752_at	expressed protein	2,00	At5g24660
248819_at	expressed protein	2,90	At5g47050
249118_at	expressed protein	2,71	At5g43870
250158_at	expressed protein	3,24	At5g15190
249134_at	expressed protein	2,96	At5g43150
264102_at	expressed protein	2,61	At1g79270
253940_at	expressed protein	2,52	At4g26950
248959_at	expressed protein	3,26	At5g45630
254632_at	expressed protein	2,05	At4g18630
249011_at	expressed protein	2,77	At5g44670
263545_at	expressed protein	3,68	At2g21560
256603_at	expressed protein	2,55	At3g28270
245816_at	expressed protein	2,54	At1g26210
248028_at	expressed protein	3,26	At5g55620
260603_at	expressed protein	2,81	At1g55960
246716_s_at	hypothetical protein	2,82	At5g28960
	expressed protein		At5g28910
249191_at	expressed protein	2,63	At5g42765
	expressed protein		At5g42760

Appendix 10: Classification of significantly downregulated transcripts in the dexamethasone system 4 hours after induction

Affymetrix identifier	**Functional Class**	**Fold change**	**AGI code**
	Transcription Factors		
255742_at	AP2 domain-containing transcription factor, putative	0,38	At1g25560
260037_at	DNA-binding protein RAV2 (RAV2) / AP2 domain-containing protein RAP2.8	0,45	At1g68840
252214_at	encodes a member	0,41	At3g50260

	of the DREB subfamily A-5 of ERF/AP2 transcription factor family		
259793_at	encodes a member of the DREB subfamily A-6 of ERF/AP2 transcription factor family	0,48	At1g64380
253799_at	encodes a member of the DREB subfamily A-6 of ERF/AP2 transcription factor family	0,48	At4g28140
256255_at	myb family transcription factor	0,46	At3g11280
255794_at	no apical meristem (NAM) family protein	0,43	At2g33480
265248_at	phytochrome-interacting factor 4 (PIF4) / basic helix-loop-helix protein 9 (bHLH9) / short under red-light 2 (SRL2)	0,41	At2g43010
252367_at	speckle-type POZ protein-related	0,19	At3g48360
256332_at	trihelix DNA-binding protein / GT-2 factor (GT2)	0,48	At1g76890
267246_at	WRKY family transcription factor	0,48	At2g30250
257382_at	WRKY family transcription factor	0,40	At2g40750
259244_at	zinc finger (B-box type) family protein	0,36	At3g07650
254231_at	WRKY family transcription factor	0,46	At4g23810;
	high mobility group (HMG1/2) family protein		At4g23800
	Signaling		
246028_at	5'-AMP-activated protein kinase beta-2 subunit, putative	0,43	At5g21170
260046_at	calmodulin-binding protein	0,49	At1g73800
252136_at	calmodulin-related protein, putative	0,33	At3g50770
247867_at	CBL-interacting protein kinase 21, putative (CIPK21)	0,42	At5g57630
266873_at	cyclin family protein	0,34	At2g44740
253493_at	phototropic-responsive NPH3 family protein	0,48	At4g31820
266799_at	phytosulfokines 2 (PSK2)	0,47	At2g22860
252234_at	phytosulfokines 3 (PSK3)	0,42	At3g49780
	Hormone Signalling		
262092_at	auxin-responsive family protein	0,40	At1g56150
245076_at	encodes an IAA-amido synthase	0,33	At2g23170
	Defence		
266385_at	pathogenesis-related protein 1 (PR-1)	0,48	At2g14610
245196_at	pectate lyase family protein	0,49	At1g67750
258552_at	pectate lyase family protein	0,35	At3g07010
	Stress		
265471_at	peroxidase 21 (PER21) (P21) (PRXR5)	0,26	At2g37130
247327_at	peroxidase, putative	0,48	At5g64120
253161_at	senescence-associated protein (SEN1)	0,22	At4g35770
261144_s_at	wound-responsive family protein	0,44	At1g19660
	wound-responsive protein-related		At1g75380

253099_s_at	peroxidase, putative	0,36	At4g37530
	peroxidase 50 (PER50) (P50) (PRXR2)		At4g37520
255479_at	Late embryogenesis abundant 3 family protein / LEA3 family protein	0,45	At4g02380
	Cell Wall		
256787_at	Beta-fructosidase (BFRUCT1) / beta-fructofuranosidase / cell wall	0,49	At3g13790
260592_at	Cellulose synthase family protein	0,41	At1g55850
252437_at	Invertase/pectin methylesterase inhibitor family protein	0,45	At3g47380
254573_at	pectinacetylesterase family protein	0,33	At4g19420
245052_at	pectinesterase family protein	0,41	At2g26440
245148_at	pectinesterase family protein	0,49	At2g45220
259560_at	wall-associated kinase 2 (WAK2)	0,38	At1g21270
247925_at	xyloglucan:xyloglucosyl transferase / xyloglucan endotransglycosylase/ endo-xyloglucan transferase (TCH4)	0,46	At5g57560
	Carbohydrate Metabolism		
259211_at	Alpha 1,4-glycosyltransferase family protein / glycosyltransferase sugar-binding DXD motif-containing protein	0,41	At3g09020
247954_at	Beta-galactosidase, putative / lactase, putative	0,28	At5g56870
261211_at	Encodes a UDP-glucose epimerase	0,45	At1g12780
264339_at	trehalose-6-phosphate synthase, putative	0,49	At1g70290
253281_at	UDP-glucoronosyl/ UDP-glucosyl transferase family protein	0,49	At4g34138
260567_at	UDP-glucoronosyl/ UDP-glucosyl transferase family protein	0,32	At2g43820
	Amino Acid Metabolism		
257516_at	ACT domain containing protein (ACR4)	0,31	At1g69040
252415_at	Asparagine synthetase 1 (glutamine-hydrolyzing) / glutamine-dependent asparagine synthetase 1 (ASN1)	0,35	At3g47340
259403_at	D-3-phosphoglycerate dehydrogenase / 3-PGDH	0,44	At1g17745
250032_at	Glutamate dehydrogenase 1 (GDH1)	0,43	At5g18170
	Sulfur Metabolism		
247314_at	3'(2'),5'-bisphosphate nucleotidase, putative / Inositol polyphosphate 1-phosphatase, putative	0,38	At5g64000
	Photosynthesis/Respiration		
267567_at	Cytochrome P450 71A13, putative (CYP71A13)	0,46	At2g30770
257624_at	Cytochrome P450 family protein	0,43	At3g26220
258063_at	Cytochrome P450, putative	0,42	At3g14620

	Protein Regulation/Degradation		
259272_at	Band 7 family protein	0,48	At3g01290
261901_at	DNAJ heat shock N-terminal domain-containing protein	0,45	At1g80920
251356_at	F-box family protein / lectin-related	0,33	At3g61060
265620_at	F-box family protein	0,40	At2g27310
260303_at	protein kinase family protein	0,50	At1g70520
260362_at	protein kinase family protein	0,43	At1g70530
252991_at	protein kinase family protein	0,50	At4g38470
245637_at	purple acid phosphatase family protein	0,37	At1g25230
246195_at	ubiquitin-conjugating enzyme 17 (UBC17), E2	0,30	At4g36410
256337_at	DNAJ heat shock N-terminal domain-containing protein	0,48	At1g72070
	expressed protein		At1g72060
	Miscellaneous		
257216_at	Protein, putative	0,34	At3g14990
	AAA-type ATPase family protein		
252131_at	Alcohol dehydrogenase, putative	0,49	At3g50930
262870_at	Ankyrin repeat family protein	0,40	At1g64710
264434_at	C2 domain-containing protein	0,44	At1g10340
255630_at	CBS domain-containing protein	0,42	At4g00700
252323_at	Dormancy-associated protein, putative (DRM1)	0,42	At3g48530
245668_at	Dormancy/auxin associated family protein	0,22	At1g28330
267461_at	Embryo-abundant protein-related	0,37	At2g33830
251360_at	FAD-binding domain-containing protein	0,31	At3g61210
261021_at	Glutaredoxin family protein	0,43	At1g26380
251196_at	Glutaredoxin family protein	0,15	At3g62950
245504_at	Glutaredoxin family protein	0,37	At4g15660
265067_at	Glutaredoxin family protein	0,44	At1g03850
245392_at	Glycosyl hydrolase family 17 protein	0,48	At4g15680
251625_at	Glycosyl hydrolase family 3 protein	0,45	At3g57260
248622_at	Glycosyl transferase family 20 protein / trehalose-phosphatase Family protein	0,34	At5g49360
264246_at	Glycosyl transferase family 20 protein / trehalose-phosphatase	0,42	At1g60140
266072_at	Family protein	0,31	At2g18700
	Kelch repeat-containing F-box family protein		
259502_at	Kelch repeat-containing F-box family protein	0,35	At1g15670
260287_at	Latex-abundant family protein (AMC3) / caspase family protein	0,33	At1g80440
247282_at	Leucine-rich repeat family protein	0,30	At5g64240
259500_at	Leucine-rich repeat family protein / protein kinase family protein	0,41	At1g15740
260345_at	Leucine-rich repeat transmembrane protein kinase, putative	0,50	At1g69270
247383_at	MA3 domain-containing protein	0,45	At5g63410
247374_at	Mannitol transporter, putative	0,50	At5g63190

246238_at	Nodulin family protein	0,28	At4g36670
250217_at	Nodulin family protein	0,48	At5g14120
265414_at	Nodulin MtN3 family protein	0,45	At2g16660
249800_at	O-methyltransferase, putative	0,41	At5g23660
261459_at	Oligopeptide transporter OPT family protein	0,44	At1g21100
247284_at	Ovate protein-related	0,46	At5g64410
263953_at	Potassium channel tetramerisation domain-containing protein	0,40	At2g36050
265987_at	Proton-dependent oligopeptide transport (POT) family protein	0,49	At2g24240
262281_at	Proton-dependent oligopeptide transport (POT) family protein	0,40	At1g68570
248932_at	SEC14 cytosolic factor family protein / phosphoglyceride transfer	0,39	At5g46050
259803_at	Family protein	0,48	At1g72150
246114_at	similar to alkaline alpha galactosidase, putative similar to esterase, putative	0,29	At5g20250
252168_at	SOUL heme-binding family protein	0,34	At3g50440
263126_at	tolB protein-related	0,29	At1g78460
255543_at	transferase family protein	0,48	At4g01870
251144_at	transporter, putative	0,36	At5g01210
248276_at	zinc finger (C3HC4-type RING finger) family protein	0,46	At5g53550
253806_at	zinc finger (C3HC4-type RING finger) family protein	0,49	At4g28270
249862_at	extracellular dermal glycoprotein, putative / EDGP, putative	0,32	At5g22920
264365_s_at	extracellular dermal glycoprotein, putative / EDGP, putative	0,42	At1g03220
	AT1G18020, 12-oxophytodienoate reductase, putative		At1g03230
255895_at	12-oxophytodienoate reductase, putative	0,35	At1g18020
	FAD-binding domain-containing protein		At1g17990
263216_s_at	FAD-binding domain-containing protein	0,33	At1g30720
	purine permease-related		At1g30730
245866_s_at	purine permease-related	0,43	At1g57990
	S-adenosyl-L-methionine:carboxyl methyltransferase family protein		At1g57980
256376_s_at	S-adenosyl-L-methionine:carboxyl methyltransferase family protein	0,46	At1g66690
	curculin-like (mannose-binding) lectin family protein /		At1g66700
264279_s_at	PAN domain-containing protein	0,45	At1g78820
	curculin-like (mannose-binding) lectin family protein		
	kelch repeat-containing F-box family protein		At1g78830
267238_at	autophagy 4a (APG4a)	0,18	At2g44130
	methionine sulfoxide reductase domain-containing protein /		At2g44140
254385_s_at	SelR domain-containing protein	0,27	At4g21830
	methionine sulfoxide reductase domain-containing protein / SelR domain-containing protein		

	expressed protein		At4g21840
249923_at	GPI transamidase component family protein / Gaa1-like family protein	0,35	At5g19120
	expressed protein		At5g19130
247800_at	phosphatidate cytidylyltransferase family protein	0,31	At5g58570
			At5g58560
	Unidentified		
	expressed protein		
263182_at	expressed protein	0,46	At1g05575
264774_at	expressed protein	0,43	At1g22890
264580_at	expressed protein	0,43	At1g05340
253322_at	expressed protein	0,20	At4g33980
264445_at	expressed protein	0,44	At1g27290
247177_at	expressed protein	0,35	At5g65300
245353_at	expressed protein	0,35	At4g16000
259001_at	expressed protein	0,46	At3g01960
247754_at	expressed protein	0,35	At5g59080
259996_at	expressed protein	0,50	At1g67910
261075_at	expressed protein	0,45	At1g07280
258225_at	expressed protein	0,27	At3g15630
258939_at	expressed protein	0,47	At3g10020
265478_at	expressed protein	0,29	At2g15890
266259_at	expressed protein	0,38	At2g27830
266658_at	expressed protein	0,49	At2g25735
267209_at	expressed protein	0,36	At2g30930
260411_at	expressed protein	0,38	At1g69890
252040_at	expressed protein	0,43	At3g52060
265387_at	expressed protein	0,46	At2g20670
258472_at	expressed protein	0,41	At3g06080
252250_at	expressed protein	0,31	At3g49790
254193_at	expressed protein	0,45	At4g23870
253874_at	expressed protein	0,27	At4g27450
258402_at	expressed protein	0,46	At3g15450
259841_at	expressed protein	0,33	At1g52200
250028_at	expressed protein	0,37	At5g18130
249174_at	expressed protein	0,28	At5g42900
263799_at	expressed protein	0,46	At2g24550

Appendix 11: Classification of significantly downregulated transcripts in the dexamethasone system 8 hours after induction

Affymetrix Identifier	Functional Class	Fold Change	AGI code
	Transcription Factors		
263664_at	auxin-responsive protein / indoleacetic acid-induced protein 17 (IAA17)	3,00	At1g04250
252965_at	auxin-responsive protein, putative, auxin-induced protein 10A	2,54	At4g38860
255694_at	basic helix-loop-helix (bHLH) family protein	3,54	At4g00050
252534_at	myb family transcription factor (MYB48)	2,50	At3g46130
260770_at	zinc finger (C3HC4-type RING finger) family protein	3,39	At1g49200
	Stress		
253174_at	catalase 2	2,65	At4g35090
264436_at	glutathione S-transferase, putative (ERD9)	2,19	At1g10370
256245_at	heat shock protein 70, putative	2,30	At3g12580
262113_at	late embryogenesis abundant 3 family protein	6,76	At1g02820
	Protein Regulation/Degradation		
248763_at	cysteine protease inhibitor, putative / cystatin, putative	3,69	At5g47550
254791_at	serine carboxypeptidase S10 family protein	2,68	At4g12910
267256_s_at	serine carboxypeptidase S10 family protein	5,17	At2g23000 At2g23010
254915_s_at	cysteine proteinase, putative	2,70	At4g11320 At4g11310
	Amino Acid Metabolism		
246700_at	cysteine synthase	3,41	At5g28030
257173_at	adenosylhomocysteinase, putative / S-adenosyl-L-homocysteine hydrolase	2,38	At3g23810
246597_at	L-aspartate oxidase family protein	2,01	At5g14760
255298_at	methionine sulfoxide reductase domain-containing protein	4,41	At4g04840
	Signalling		
263433_at	inositol-3-phosphate synthase isozyme 2	4,38	At2g22240
249090_at	phosphotransferase-related	2,38	At5g43745
	Hormone Signalling		
261150_at	S-adenosyl-L-methionine: jasmonic acid carboxyl methyltransferase	3,16	At1g19640
245928_s_at	vegetative storage protein 2 (VSP2)	8,29	At5g24770 At5g24780
	Secondary Metabolism		

248311_at	beta-carotene hydroxylase, putative	2,02	At5g52570
250207_at	chalcone synthase / naringenin-chalcone synthase	4,38	At1g10370
250794_at	chalcone-flavanone isomerase family protein	3,15	At5g05270
252123_at	naringenin 3-dioxygenase / flavanone 3-hydroxylase (F3H)	3,83	At3g51240
	Circadian Clock and Flowering		
264638_at	flowering locus T protein (FT)	2,51	At1g65480
260146_at	phototropic-responsive NPH3 family protein	2,97	At1g52770
	Carbohydrate Metabolism		
245275_at	beta-amylase (BMY1) / 1,4-alpha-D-glucan maltohydrolase	4,56	At4g15210
252011_at	carbonic anhydrase family protein	4,19	At3g52720
254773_at	glycosyl transferase family 2 protein	2,53	At4g13410
264898_at	invertase/pectin methylesterase inhibitor family protein	8,74	At1g23205
264931_at	polygalacturonase, putative / pectinase, putative	5,92	At1g60590
261191_at	starch synthase, putative	3,14	At1g32900
255016_at	sucrose-phosphate synthase, putative	6,33	At4g10120
	Lipid Metabolism		
263809_at	GDSL-motif lipase/hydrolase family protein	2,51	At2g04570
252363_at	GDSL-motif lipase/hydrolase family protein	4,23	At3g48460
245422_at	palmitoyl protein thioesterase family protein	4,98	At4g17470
	Transport		
256751_at	chloride channel protein (CLC-b)	2,35	At3g27170
260676_at	integral membrane protein, putative / sugar transporter family protein	2,10	At1g19450
258181_at	nitrate transporter (NTP3)	3,56	At3g21670
263918_at	proline transporter, putative	2,03	At2g36590
259185_at	triose phosphate/phosphate translocator, putative	3,08	At3g01550
	Miscellaneous		
249970_at	extracellular dermal glycoprotein-related	2,02	At5g19100
260058_at	F-box family protein	2,42	At1g78100
264147_at	CER1 protein	2,37	At1g02205
258299_at	alcohol oxidase-related	3,63	At3g23410
247074_at	allergen V5/Tpx-1-related family protein	4,46	At5g66590
250860_at	amino acid permease family protein	2,47	At5g04770
264729_at	heavy-metal-associated domain-containing protein	2,06	At1g22990
253247_at	homeodomain-containing protein	3,01	At4g34610
252989_at	multi-copper oxidase type I family protein	2,60	At4g38420
259579_at	multidrug resistance P-glycoprotein, putative	2,37	At1g28010
265984_at	myrcene/ocimene synthase (TPS10)	2,30	At2g24210
248467_at	nodulin MtN3 family protein	3,38	At5g50800

259842_at	phosphoethanolamine N-methyltransferase 3, putative	3,03	At1g73600
265111_at	protease inhibitor/seed storage /lipid transfer protein (LTP) family protein	3,70	At1g62510
249798_at	similar to transducin family protein / WD-40 repeat family protein	2,09	At5g23730
249493_at	transferase family protein	2,98	At5g39080
255065_s_at	arginase, putative	2,12	At4g08870
	Unidentified		
248709_at	expressed protein	3,93	At5g48470
249118_at	expressed protein	3,18	At5g43870
249134_at	expressed protein	2,13	At5g43150
249752_at	expressed protein	6,15	At5g24660
249932_at	expressed protein	3,14	At5g22390
252073_at	expressed protein	2,64	At3g51750
252412_at	expressed protein	2,06	At3g47295
254208_at	expressed protein	3,34	At4g24175
255604_at	expressed protein	3,48	At4g01080
257207_at	expressed protein	5,26	At3g14900
263632_at	expressed protein	3,87	At2g04795
267034_at	expressed protein	2,89	At2g38310
256603_at	expressed protein	5,02	At3g28270
251058_at	expressed protein	2,57	At5g01790
248028_at	expressed protein	5,96	At5g55620
260727_at	expressed protein	3,69	At1g48110 At1g48100

Appendix 12: Classification of significantly downregulated transcripts in the dexamethasone system 8 hours after induction

Affymetrix identifier	Functional Class	Fold change	AGI code
	Transcription Factors		
255926_at	AP2 domain-containing transcription factor, putative	0,17	At1g22190
251282_at	AP2 domain-containing transcription factor, putative	0,26	At3g61630
259626_at	AtbZIP60 consists of a bZIP DNA binding domain followed by a putative transmembrane domain	0,31	At1g42990
265452_at	basic helix-loop-helix (bHLH) family protein	0,40	At2g46510
260037_at	DNA-binding protein RAV2 (RAV2) / AP2 domain-containing protein RAP2.8	0,21	At1g68840
256185_at	Dof-type zinc finger domain-containing protein (ADOF1)	0,43	At1g51700
254075_at	encodes a member of the DREB subfamily A-1 of ERF/ AP2 transcription factor	0,09	At4g25470
255937_at	encodes a member of the DREB subfamily A-1 of ERF/ AP2 transcription factor	0,09	At1g12610
250781_at	encodes a member of the DREB subfamily A-2 of ERF/	0,20	At5g05410

	AP2 transcription factor		
261984_at	encodes a member of the DREB subfamily A-4 of ERF/ AP2 transcription factor	0,31	At1g33760
262211_at	encodes a member of the DREB subfamily A-5 of ERF/ AP2 transcription factor	0,05	At1g74930
252214_at	encodes a member of the DREB subfamily A-5 of ERF/ AP2 transcription factor	0,18	At3g50260
262135_at	encodes a member of the DREB subfamily A-6 of ERF/ AP2 transcription factor	0,43	At1g78080
253799_at	encodes a member of the DREB subfamily A-6 of ERF/ AP2 transcription factor	0,29	At4g28140
259793_at	encodes a member of the DREB subfamily A-6 of ERF/ AP2 transcription factor	0,20	At1g64380
257053_at	encodes a member of the ERF (ethylene response factor) subfamily	0,12	At3g15210
261315_at	encodes a member of the ERF (ethylene response factor) subfamily	0,23	At1g53170
261470_at	encodes a member of the ERF (ethylene response factor) subfamily	0,07	At1g28370
258434_at	encodes a member of the ERF (ethylene response factor) subfamily	0,20	At3g16770
248799_at	encodes a member of the ERF (ethylene response factor) subfamily	0,32	At5g47230
245250_at	encodes a member of the ERF (ethylene response factor) subfamily	0,38	At4g17490
248448_at	encodes a member of the ERF (ethylene response factor) subfamily	0,10	At5g51190
253259_at	encodes a member of the ERF (ethylene response factor) subfamily	0,03	At4g34410
266821_at	encodes a member of the ERF (ethylene response factor) subfamily	0,12	At2g44840
249928_at	CCR4-NOT transcription complex protein, putative	0,09	At5g22250
252679_at	CCR4-NOT transcription complex protein, putative	0,08	At3g44260
246214_at	heat shock factor protein 4 (HSF4)	0,38	At4g36990
254592_at	heat shock transcription factor 21 (HSF21)	0,24	At4g18880
258139_at	heat shock transcription factor family protein	0,24	At3g24520
257919_at	myb family transcription factor (MYB15)	0,11	At3g23250
255753_at	myb family transcription factor (MYB51)	0,18	At1g18570
260237_at	myb family transcription factor (MYB95)	0,33	At1g74430
247455_at	myb family transcription factor (MYB96)	0,36	At5g62470
253872_at	no apical meristem (NAM) family protein (RD26)	0,18	At4g27410
249467_at	no apical meristem (NAM) family protein	0,18	At5g39610
249940_at	no apical meristem (NAM) family protein	0,09	At5g22380
259705_at	no apical meristem (NAM) family protein	0,23	At1g77450
261564_at	no apical meristem (NAM) family protein	0,29	At1g01720
252278_at	no apical meristem (NAM) family protein	0,23	At3g49530
260203_at	no apical meristem (NAM) family protein	0,07	At1g52890
263584_at	no apical meristem (NAM) family protein	0,25	At2g17040
256300_at	no apical meristem (NAM) family protein	0,08	At1g69490

252681_at	no apical meristem (NAM) family protein	0,49	At3g44350
247707_at	scarecrow-like transcription factor 11 (SCL11) transcription factor	0,32	At5g59450
260798_at	jumonji (jmjC) domain-containing protein	0,39	At1g78280
254074_at	Transcriptional activator that binds to the DRE/ CRT regulatory element	0,03	At4g25490
253485_at	WRKY family transcription factor	0,19	At4g31800
263783_at	WRKY family transcription factor	0,21	At2g46400
255568_at	WRKY family transcription factor	0,29	At4g01250
267028_at	WRKY family transcription factor	0,11	At2g38470
261892_at	WRKY family transcription factor	0,03	At1g80840
261648_at	zinc finger (C2H2 type) family protein (ZAT10)	0,06	At1g27730
247655_at	zinc finger (C2H2 type) family protein (ZAT12)	0,15	At5g59820
245711_at	zinc finger (C2H2 type) family protein	0,08	At5g04340
246993_at	zinc finger (C2H2 type) protein 1 (AZF1)	0,33	At5g67450
257022_at	zinc finger (C2H2 type) protein 2 (AZF2)	0,20	At3g19580
245329_at	zinc finger (C3HC4-type RING finger) family protein / ankyrin repeat family protein	0,24	At4g14365
245369_at	zinc finger (C3HC4-type RING finger) family protein	0,31	At4g15975
247708_at	zinc finger (C3HC4-type RING finger) family protein	0,22	At5g59550
252474_at	zinc finger (C3HC4-type RING finger) family protein	0,16	At3g46620
256093_at	zinc finger (C3HC4-type RING finger) family protein	0,36	At1g20823
265740_at	zinc finger (C3HC4-type RING finger) family protein	0,50	At2g01150
260327_at	zinc finger (C3HC4-type RING finger) family protein	0,31	At1g63840
246777_at	zinc finger (C3HC4-type RING finger) family protein	0,16	At5g27420
251745_at	zinc finger (CCCH-type) family protein	0,14	At3g55980
263379_at	zinc finger (CCCH-type) family protein	0,25	At2g40140
248524_s_at	squamosa promoter-binding protein, putative	0,37	At5g50670 At5g50570
254231_at	WRKY family transcription factor, AR411	0,09	At4g23810 At4g23800
	Signalling		
253284_at	C2 domain-containing protein, similar to calcium-dependent protein kinase	0,26	At4g34150
245251_at	calcineurin B-like protein 1 (CBL1), identical to calcineurin B-like protein 1	0,34	At4g17615
259137_at	calcium-binding EF hand family protein	0,38	At3g10300
253915_at	calcium-binding EF hand family protein	0,21	At4g27280
259879_at	calcium-binding EF hand family protein	0,03	At1g76650
248164_at	calcium-binding EF-hand protein, putative	0,20	At5g54490
247137_at	calcium-dependent protein kinase family protein / CDPK family protein	0,27	At5g66210
262671_at	calcium-dependent protein kinase, putative / CDPK, putative	0,31	At1g76040
251636_at	calcium-dependent protein kinase, putative / CDPK, putative	0,39	At3g57530
261650_at	calcium-transporting ATPase 1	0,36	At1g27770
247426_at	calmodulin-binding protein	0,34	At5g62570

266447_at	calmodulin-like protein (MSS3)	0,45	At2g43290
249197_at	calmodulin-related protein, putative	0,17	At5g42380
258947_at	calmodulin-related protein, putative	0,04	At3g01830
255872_at	CBL-interacting protein kinase 11 (CIPK11)	0,27	At2g30360
253550_at	CBL-interacting protein kinase 6 (CIPK6)	0,42	At4g30960
250556_at	diacylglycerol kinase 1 (DGK1)	0,26	At5g07920
247346_at	diacylglycerol kinase, putative	0,48	At5g63770
254271_at	protein kinase family protein	0,23	At4g23150
254241_at	protein kinase family protein	0,34	At4g23190
254996_at	protein kinase family protein	0,33	At4g10390
266037_at	protein kinase, putative	0,33	At2g05940
248821_at	protein kinase, putative	0,29	At5g47070
253323_at	protein phosphatase 2C family protein / PP2C family protein	0,33	At4g33920
247723_at	protein phosphatase 2C, putative / PP2C, putative	0,24	At5g59220
259231_at	protein phosphatase 2C, putative / PP2C, putative	0,30	At3g11410
253780_at	protein phosphatase 2C, putative / PP2C, putative	0,39	At4g28400
261077_at	protein phosphatase 2C, putative / PP2C, putative	0,17	At1g07430
258682_at	serine/threonine protein kinase (PK19)	0,25	At3g08720
266749_at	serine/threonine protein kinase, putative	0,25	At2g47060
245905_at	serine-rich protein-related	0,46	At5g11090
246821_at	similar to calmodulin-binding protein	0,17	At5g26920
257536_at	similar to tyrosine specific protein phosphatase family protein	0,36	At3g02800
249583_at	touch-responsive protein / calmodulin-related protein 2	0,24	At5g37770
249417_at	calcium-binding EF hand family protein	0,22	At5g39670
			At5g39680
	Hormone Signalling		
254926_at	1-aminocyclopropane-1-carboxylate synthase 6 / ACC synthase 6 (ACS6)	0,24	At4g11280
266139_at	auxin-responsive family protein	0,32	At2g28085
246861_at	auxin-responsive protein / indoleacetic acid-induced protein 28 (IAA28)	0,40	At5g25890
257644_at	allene oxide cyclase, putative	0,10	At3g25780
259445_at	gibberellin 2-oxidase, putative	0,21	At1g02400
266613_at	gibberellin-regulated family protein	0,30	At2g14900
	Carbohydrate Metabolism		
258507_at	beta-fructofuranosidase, putative / invertase, putative / saccharase, putative	0,31	At3g06500
259403_at	D-3-phosphoglycerate dehydrogenase	0,24	At1g17745
256633_at	galactinol synthase, putative	0,18	At3g28340
265841_at	glycogenin glucosyltransferase (glycogenin)-related	0,42	At2g35710
251804_at	glycosyl hydrolase family 17 protein / beta-1,3-glucanase	0,48	At3g55430
245393_at	glycosyl hydrolase family 17 protein	0,09	At4g16260

	Photosynthesis/Respiration		
258277_at	cytochrome P450 71B15, putative	0,07	At3g26830
253505_at	cytochrome P450 family protein	0,35	At4g31970
252368_at	cytochrome P450 family protein	0,09	At3g48520
248964_at	cytochrome P450 family protein	0,13	At5g45340
247949_at	cytochrome P450, putative	0,23	At5g57220
266246_at	cytochrome P450, putative	0,22	At2g27690
245020_at	encodes cytochrome f apoprotein	0,43	AtCg00540
245007_at	encodes psaA protein comprising the reaction center for photosystem I	0,48	AtCg00350
	Cell wall		
260592_at	cellulose synthase family protein	0,36	At1g55850
261226_at	expansin, putative (EXP11)	0,48	At1g20190
267158_at	expansin, putative (EXP3)	0,47	At2g37640
260015_at	caffeoyl-CoA 3-O-methyltransferase, putative	0,14	At1g67980
250149_at	cinnamoyl-CoA reductase-related	0,43	At5g14700
258552_at	pectate lyase family protein	0,32	At3g07010
259033_at	pectinacetylesterase family protein	0,21	At3g09410
245148_at	pectinesterase family protein	0,23	At2g45220
252740_at	pectinesterase family protein	0,46	At3g43270
247925_at	xyloglucan:xyloglucosyl transferase	0,20	At5g57560
245325_at	xyloglucan:xyloglucosyl transferase	0,28	At4g14130
	Stress		
264655_at	C2 domain-containing protein / src2-like protein, putative	0,21	At1g09070
255504_at	drought-responsive family protein	0,41	At4g02200
264389_at	early-responsive to dehydration protein-related / ERD protein-related	0,29	At1g11960
265530_at	12-oxophytodienoate reductase (OPR3) / delayed dehiscence1 (DDE1)	0,38	At2g06050
259516_at	dehydrin (ERD10)	0,32	At1g20450
247431_at	Encodes a protein with similarity to RCD1 but without the WWE domain	0,10	At5g62520
255479_at	late embryogenesis abundant 3 family protein / LEA3 family protein	0,22	At4g02380
262128_at	late embryogenesis abundant protein, putative / LEA protein, putative	0,24	At1g52690
256763_at	phytochelatin synthetase-related	0,32	At3g16860
259789_at	stress-responsive protein, putative	0,42	At1g29395
	Lipid Metabolism		
266977_at	esterase/lipase/thioesterase family protein	0,29	At2g39420

	Defense		
	ankyrin repeat family protein		
264434_at	ankyrin repeat family protein	0,17	At1g10340
263800_at	avirulence-responsive protein / avirulence induced gene (AIG1)	0,18	At2g24600
260116_at	basic endochitinase	0,22	At1g33960
256243_at	chitinase, putative	0,25	At3g12500
260560_at	curculin-like (mannose-binding) lectin family protein	0,06	At2g43590
249983_at	disease resistance protein (NBS-LRR class), putative	0,10	At5g18470
246406_at	disease resistance protein (TIR class), putative	0,23	At1g57650
264213_at	disease resistance protein (TIR-NBS class), putative	0,37	At1g65390
262381_at	disease resistance protein (TIR-NBS class), putative	0,26	At1g72900
262382_at	disease resistance protein (TIR-NBS class), putative	0,28	At1g72920
262383_at	disease resistance protein (TIR-NBS-LRR class), putative	0,38	At1g72940
259629_at	enhanced disease susceptibility 5 (EDS5) / salicylic acid induction deficient 1 (SID1)	0,20	At1g56510
252921_at	glutaredoxin family protein	0,36	At4g39030
261443_at	hevein-like protein	0,20	At1g28480
258791_at	legume lectin family protein	0,19	At3g04720
257206_at	pathogenesis-related thaumatin family protein	0,13	At3g16530
253104_at	plant defensin-fusion protein, putative	0,31	At4g36010
257365_x_at	similar to disease resistance protein (TIR class), putative	0,17	At2g26020
265723_at	disease resistance protein (TIR-NBS-LRR class), putative	0,30	At2g32140
249264_s_at		0,29	At5g41750 At5g41740
	Protein Regulation / Degradation		
256525_at	aspartyl protease family protein	0,35	At1g66180
	Miscellaneous		
	AAA-type ATPase family protein		
252131_at	AAA-type ATPase family protein	0,15	At3g50930
256989_at	ACT domain containing protein (ACR4)	0,45	At3g28580
257516_at	ACT domain-containing protein (ACR7)	0,39	At1g69040
254300_at	benzodiazepine receptor-related	0,29	At4g22780
266462_at	BON1-associated protein 1 (BAP1)	0,32	At2g47770
251336_at	2-oxoacid-dependent oxidase, putative	0,03	At3g61190
252265_at	4-coumarate--CoA ligase family protein / 4-coumaroyl-CoA synthase family protein	0,07	At3g49620
259518_at	4-coumarate--CoA ligase, putative / 4-coumaroyl-CoA synthase, putative	0,42	At1g20510
258037_at	4-methyl-5(b-hydroxyethyl)-thiazole monophosphate biosynthesis protein	0,34	At3g21230
257216_at	50S ribosomal protein-related	0,34	At3g14990
246495_at	9-cis-epoxycarotenoid dioxygenase, putative	0,23	At5g16200
257280_at	ABC transporter family involved in resistant to lead.	0,26	At3g14440

261763_at	arabinogalactan-protein (AGP1)	0,39	At1g15520
247279_at	arabinogalactan-protein (AGP20)	0,31	At5g64310
251281_at	cation/hydrogen exchanger, putative (CHX17)	0,35	At3g61640
254215_at	chloroplast gene encoding ribosomal protein s12	0,29	At4g23700
244939_at	choline kinase, putative	0,48	AtCg00065
261506_at	cyclic nucleotide-regulated ion channel (CNGC10)	0,41	At1g71697
261027_at	DNA-damage-repair/toleration protein, putative (DRT100)	0,31	At1g01340
256237_at	DSBA oxidoreductase family protein	0,43	At3g12610
249481_at	embryo-abundant protein-related	0,20	At5g38900
251360_at	embryo-abundant protein-related	0,45	At3g61210
251884_at	exocyst subunit EXO70 family protein	0,15	At3g54150
247693_at	exocyst subunit EXO70 family protein	0,34	At5g59730
256050_at	F-box family protein	0,24	At1g07000
264758_at	ferrochelatase I	0,18	At1g61340
246870_at	Fe-S metabolism associated domain-containing protein	0,38	At5g26030
245193_at	FAD-binding domain-containing protein	0,20	At1g67810
261020_at	FAD-binding domain-containing protein	0,37	At1g26390
261021_at	formin homology 2 domain-containing protein / FH2 domain-containing protein	0,26	At1g26380
262901_at	GCN5-related N-acetyltransferase (GNAT) family protein	0,41	At1g59910
265725_at	germin-like protein (GLP6)	0,20	At2g32030
249495_at	glutathione S-transferase, putative	0,39	At5g39100
260405_at	glutathione S-transferase, putative	0,39	At1g69930
260225_at	glycine-rich protein, predicted proteins	0,26	At1g74590
258792_at	GRAM domain-containing protein	0,09	At3g04640
250279_at	harpin-induced family protein (YLS9)	0,25	At5g13200
263948_at	heavy-metal-associated domain-containing protein	0,34	At2g35980
248327_at	heavy-metal-associated domain-containing protein	0,19	At5g52750
248322_at	histone H1-3 (HIS1-3)	0,12	At5g52760
265817_at	hypothetical protein	0,23	At2g18050
245008_at	hypothetical protein	0,42	AtCg00360
245019_at	hydroxyproline-rich glycoprotein family protein	0,39	AtCg00530
248592_at	L-ascorbate oxidase, putative	0,39	At5g49280
252862_at	lectin protein kinase family protein	0,15	At4g39830
255502_at	lectin protein kinase	0,29	At4g02410
251054_at	MATE efflux family protein	0,34	At5g01540
264289_at	MATE efflux family protein	0,32	At1g61890
258100_at	MATE efflux protein-related	0,24	At3g23550
248392_at		0,20	At5g52050
	Unidentified		
	expressed protein		
245041_at	expressed protein	0,20	At2g26530
245662_at	expressed protein	0,23	At1g28190
245677_at	expressed protein	0,16	At1g56660

245755_at	expressed protein	0,27	At1g35210
245840_at	expressed protein	0,11	At1g58420
246018_at	expressed protein	0,13	At5g10695
246270_at	expressed protein	0,31	At4g36500
246796_at	expressed protein	0,37	At5g26770
247177_at	expressed protein	0,05	At5g65300
247208_at	expressed protein	0,18	At5g64870
247215_at	expressed protein	0,29	At5g64905
248509_at	expressed protein	0,40	At5g50335
249378_at	expressed protein	0,25	At5g40450
250292_at	expressed protein	0,28	At5g13220
250956_at	expressed protein	0,32	At5g03210
251640_at	expressed protein	0,31	At3g57450
252133_at	expressed protein	0,41	At3g50900
252908_at	expressed protein	0,15	At4g39670
253044_at	expressed protein	0,40	At4g37290
253643_at	expressed protein	0,04	At4g29780
253830_at	expressed protein	0,05	At4g27652
253832_at	expressed protein	0,03	At4g27654
253859_at	expressed protein	0,04	At4g27657
254178_at	expressed protein	0,29	At4g23880
256017_at	expressed protein	0,17	At1g19180
256159_at	expressed protein	0,25	At1g30135
256442_at	expressed protein	0,04	At3g10930
257154_at	expressed protein	0,25	At3g27210
258203_at	expressed protein	0,26	At3g13950
258608_at	expressed protein	0,37	At3g03020
259479_at	expressed protein	0,09	At1g19020
259979_at	expressed protein	0,27	At1g76600
260227_at	expressed protein	0,17	At1g74450
260656_at	expressed protein	0,19	At1g19380
260744_at	expressed protein	0,03	At1g15010
261033_at	expressed protein	0,17	At1g17380
261193_at	expressed protein	0,21	At1g32920
261405_at	expressed protein	0,26	At1g18740
262801_at	expressed protein	0,36	At1g21010
263182_at	expressed protein	0,10	At1g05575
263931_at	expressed protein	0,13	At2g36220
263972_at	expressed protein	0,25	At2g42760
264342_at	expressed protein	0,38	At1g12080
264580_at	expressed protein	0,17	At1g05340
265184_at	expressed protein	0,21	At1g23710
266017_at	expressed protein	0,30	At2g18690
266101_at	expressed protein	0,37	At2g37940
266396_at	expressed protein	0,39	At2g38790

266545_at	expressed protein	0,37	At2g35290
266658_at	expressed protein	0,35	At2g25735
266901_at	expressed protein	0,24	At2g34600
267230_at	expressed protein	0,22	At2g44080
252938_at	expressed protein	0,39	At4g39190
253717_at	expressed protein	0,41	At4g29440
260411_at	expressed protein	0,29	At1g69890
245209_at	expressed protein	0,27	At5g12340
245119_at	expressed protein	0,26	At2g41640
247047_at	expressed protein	0,25	At5g66650
248959_at	expressed protein	0,17	At5g45630
265276_at	expressed protein	0,12	At2g28400
254158_at	expressed protein	0,36	At4g24380
262452_at	expressed protein	0,22	At1g11210
247933_at	expressed protein	0,35	At5g56980
249454_at	expressed protein	0,15	At5g39520
250216_at	expressed protein	0,39	At5g14090
249237_at	expressed protein	0,31	At5g42050
250796_at	expressed protein	0,19	At5g05300
246562_at	no_match	0,45	At5g15580
245613_at	no_match	0,23	no_match
245771_at	no_match	0,39	no_match
256046_at	similar to expressed protein	0,31	no_match
250676_at		0,27	At5g06310
	expressed protein		At5g06320
254229_at		0,18	At4g23610
	expressed protein		At4g23620
266800_at		0,09	At2g22870
			At2g22880

Appendix 13: Classification of significantly upregulated transcripts in the dexamethasone system 12 hours after induction

Affymetrix Identifier	Functional Class	Fold Change	AGI code
	Transcription Factors		
254693_at	basic helix-loop-helix (bHLH) family protein	2,72	At4g17880
255694_at	basic helix-loop-helix (bHLH) family protein	2,06	At4g00050
246962_s_at	bZIP transcription factor family protein	2,01	At5g24800
264692_at	DNA-binding family protein	2,23	At1g70000
249677_at	DNA-binding protein, putative	2,48	At5g35970
251272_at	homeobox-leucine zipper protein 12 (HB-12) / HD-ZIP transcription factor 12	2,30	At3g61890
252534_at	myb family transcription factor (MYB48)	3,50	At3g46130
248246_at	myb family transcription factor (TRIPTYCHON)	2,41	At5g53200

251586_at	zinc finger (C2H2 type) family protein	2,41	At3g58070
260770_at	zinc finger (C3HC4-type RING finger) family protein	4,05	At1g49200
	Signalling		
251060_at	CBL-interacting protein kinase 14 (CIPK14)	2,08	At5g01820
263433_at	inositol-3-phosphate synthase isozyme 2 / myo-inositol-1-phosphate synthase 2	4,35	At2g22240
	Hormone Signalling		
258498_at	ABA-responsive protein-related	3,05	At3g02480
263664_at	auxin-responsive protein / indoleacetic acid-induced protein 17 (IAA17)	2,09	At1g04250
261150_at	S-adenosyl-L-methionine: jasmonic acid carboxyl methyltransferase (JMT)	2,07	At1g19640
245928_s_at	vegetative storage protein 2 (VSP2)	3,96	At5g24770 At5g24780
	Defence		
265917_at	disease resistance family protein	2,08	At2g15080
262113_at	late embryogenesis abundant 3 family protein	8,03	At1g02820
	Stress		
253174_at	catalase 2	2,74	At4g35090
	Cell Wall		
264898_at	invertase/pectin methylesterase inhibitor family protein	3,92	At1g23205
	Circadian Clock and Flowering		
247511_at	brother of FT and TFL1 protein (BFT)	3,46	At5g62040
264638_at	flowering locus T protein (FT)	3,10	At1g65480
	Carbohydrate Metabolism		
261191_at	starch synthase, putative	5,20	At1g32900
255016_at	sucrose-phosphate synthase, putative	2,97	At4g10120
259185_at	triose phosphate/phosphate translocator, putative	5,12	At3g01550
252011_at	carbonic anhydrase family protein	5,17	At3g52720
	Lipid Metabolism		
252363_at	GDSL-motif lipase/hydrolase family protein	2,24	At3g48460
	Amino Acid Metabolism		
246597_at	L-aspartate oxidase family protein	2,40	At5g14760
	Photosynthesis/Respiration		
249125_at	2-oxoglutarate-dependent dioxygenase, putative	2,35	At5g43450
258239_at	chlorophyll A-B binding protein (LHCB2:4)	4,23	At3g27690

	Transport		
260676_at	integral membrane protein, putative / sugar transporter family protein	2,13	At1g19450
258181_at	nitrate transporter (NTP3)	2,10	At3g21670
	Protein Regulation/Degradation		
248763_at	cysteine protease inhibitor, putative	2,59	At5g47550
267256_s_at	serine carboxypeptidase S10 family protein	3,26	At2g23000 At2g23010
	Miscellaneous		
264217_at	armadillo/beta-catenin repeat family protein / U-box domain-containing protein	3,62	At1g60190
266363_at	haloacid dehalogenase-like hydrolase family protein	2,16	At2g41250
264729_at	heavy-metal-associated domain-containing protein	4,26	At1g22990
265939_at	DC1 domain-containing protein	2,45	At2g19650
254805_at	protease inhibitor/seed storage/ lipid transfer protein (LTP) family protein	2,02	At4g12480
250335_at	hydrolase, alpha/beta fold family protein	2,90	At5g11650
245734_at	hydrolase, alpha/beta fold family protein	2,31	At1g73480
259579_at	multidrug resistance P-glycoprotein, putative	2,05	At1g28010
248467_at	nodulin MtN3 family protein	6,97	At5g50800
259842_at	phosphoethanolamine N-methyltransferase 3, putative	2,41	At1g73600
249798_at	similar to transducin family protein / WD-40 repeat family protein	2,62	At5g23730
	Unidentified		
249134_at	expressed protein	2,16	At5g43150
249932_at	expressed protein	2,58	At5g22390
252073_at	expressed protein	3,45	At3g51750
252661_at	expressed protein	2,01	At3g44450
256096_at	expressed protein	2,61	At1g13650
262875_at	expressed protein	2,25	At1g64970
263632_at	expressed protein	2,11	At2g04795
264636_at	expressed protein	2,82	At1g65490
265698_at	expressed protein	2,20	At2g32160
264102_at	expressed protein	2,37	At1g79270
263545_at	expressed protein	2,54	At2g21560
256603_at	expressed protein	3,80	At3g28270
248028_at	expressed protein	3,24	At5g55620
260727_at	expressed protein	7,06	At1g48110 At1g48100

Appendix 14: Classification of significantly upregulated transcripts in the dexamethasone system 12 hours after induction

Affymetrix Identifier	Functional Class	Fold Change	AGI code
	Transcription Factors		
256503_at	myb family transcription factor	0,43	At1g75250
	Hormone Signalling		
252168_at	similar to esterase, putative similar to methyl jasmonate esterase [Solanum tuberosum]	0,38	At3g50440
	Cell Wall		
251791_at	expansin, putative (EXP16)	0,50	At3g55500
260592_at	cellulose synthase family protein	0,43	At1g55850
262978_at	tubulin beta-1 chain (TUB1)	0,31	At1g75780
	Carbohydrate Metabolism		
247189_at	arabinogalactan-protein (AGP7)	0,20	At5g65390
	Photosynthesis/Respiration		
244966_at	Cytochrome b6-f complex, subunit V	0,42	AtCg00600
	Miscellaneous		
266799_at	phytosulfokines 2 (PSK2)	0,30	At2g22860
244988_s_at	chloroplast ribosomal protein L23	0,41	AtCg00840 AtCg01300

Appendix 15: Classification of changes unidentified metabolites preceding the activation of *CO* on the light extension system in *Arabidopsis thaliana*. The metabolites listed fulfilled the double requirement of a 2-fold change when comparing both the induced and control treatments of the responsive line and the equivalent comparison for the non-responsive line. All values for induced samples were corrected for the extended short day treatment and for the mutation by calculating corresponding ratios. The corrected numbers for induced samples of the responsive line were compared against the control treatment of the same line. The latter ratios were then used to generate the ratios listed as fold changes between the second and first time exudation periods. Retention times of all metabolites are provided.

Unknowns	RT (s)	Ratio T2/T1
1	184.955	2.76
2	188.355	14.58
3	213.605	19.03
4	217.955	3.14
5	223.255	11.91
6	267.805	7.08
7	268.555	2.71
8	293.655	44.19
9	298.205	8.38
10	304.655	23.01
11	311.755	4.63
12	349.655	2.20
13	365.555	2.77
14	383.355	4.04
15	385.005	19.37
16	385.305	5.82
17	397.055	2.71
18	410.155	11.85
19	419.955	6.99
20	459.005	8.62
21	562.605	6.98
22	610.555	11.87
23	620.405	5.61
24	625.555	3.70
25	643.105	3.24
26	663.855	18.86
27	672.105	7.21
28	710.305	8.85
29	738.405	23.59
30	753.405	2.77
31	847.655	81.25
32	852.255	3.25
33	896.255	82.92

34	952.555	2.13

The metabolites listed fulfilled the double requirement of a 2-fold change when comparing both the induced and control treatments of the responsive line and the equivalent comparison for the non-responsive line. All values for induced samples were corrected for the extended short day treatment and for the mutation by calculating corresponding ratios. The corrected numbers for induced samples of the responsive line were compared against the control treatment of the same line. The latter ratios were then used to generate the ratios listed as fold changes between the second and first time exudation periods. Retention times of all metabolites are provided.

Appendix 16: Classification of changes unidentified metabolites following the activation of *CO* on the light extension system in *Arabidopsis thaliana*.

Unknowns	RT (s)	Ratio T3/T2
1	266.605	3.64
2	291.655	9.01
3	298.705	45.25
4	302.655	4.12
5	306.105	43.93
6	311.755	4.41
7	340.305	17.13
8	345.555	4.47
9	364.505	21.48
10	387.805	23.15
11	392.255	14.35
12	401.955	16.51
13	408.955	6.37
14	410.555	6.00
15	419.305	2.04
16	425.355	30.59
17	490.605	3.60
18	509.705	2.46
19	522.505	3.97
20	523.455	5.38
21	544.905	3.17
22	557.155	11.41
23	562.605	2.36
24	562.755	4.15
25	572.155	15.37
26	587.855	11.71
27	593.855	10.04
28	599.605	2.53
29	613.905	78.81
30	618.605	2.04
31	619.255	10.58

32	619.755	38.45
33	626.105	2.31
34	636.655	19.10
35	643.255	9.84
36	648.605	4.29
37	660.855	2.10
38	690.205	2.72
39	694.655	9.11
40	707.855	2.70
41	709.455	2.46
42	716.405	7.54
43	844.205	4.50

The metabolites listed fulfilled the double requirement of a 2-fold change when comparing both the induced and control treatments of the responsive line and the equivalent comparison for the non-responsive line. All values for induced samples were corrected for the extended short day treatment and for the mutation by calculating corresponding ratios. The corrected numbers for induced samples of the responsive line were compared against the control treatment of the same line. The latter ratios were then used to generate the ratios listed as fold changes between the second and first time exudation periods. Retention times of all metabolites are provided.

Appendix 17: Classification of changes unidentified metabolites following the activation of *CO* in the dexamethasone-inducible system in *Arabidopsis thaliana*.

Unknowns	RT (s)	Ratio T2/T1
1	187.894	8.49
2	188.544	3.32
3	212.844	39.65
4	220.294	39.59
5	220.444	4.33
6	242.244	15.01
7	272.644	4.71
8	284.244	13.80
9	302.494	6.47
10	336.844	3.66
11	355.344	68.95
12	357.894	2.55
13	376.094	7.25
14	437.394	18.01
15	449.094	4.58
16	452.794	8.27
17	497.144	2.71
18	499.794	35.77
19	503.044	4.58
20	511.944	14.71

21	523.994	36.58
22	527.694	8.88
23	555.144	6.53
24	555.894	7.79
25	565.294	3.96
26	576.244	14.55
27	601.544	2.43
28	624.144	3.48
29	651.794	7.58
30	654.344	18.60
31	666.144	2.10
32	674.094	6.23
33	687.294	11.51
34	687.494	2.42
35	698.094	3.52
36	733.894	5.53
37	737.094	7.38
38	776.194	9.49
39	785.444	46.47
40	858.844	26.71
41	604.544	8.77
42	622.694	5.64

The metabolites listed fulfilled the double requirement of a 2-fold change when comparing both the induced and control treatments of the responsive line and the equivalent comparison for the non-responsive line. All values for induced samples were corrected for the extended short day treatment and for the mutation by calculating corresponding ratios. The corrected numbers for induced samples of the responsive line were compared against the control treatment of the same line. The latter ratios were then used to generate the ratios listed as fold changes between the second and first time exudation periods. Retention times of all metabolites are provided.

Appendix 18: Classification of changes unidentified metabolites following in leaf phloem exudates of *Sinapis alba*.

1	177.583	0.47
2	189.883	0.89
3	198.383	1.00
4	203.033	0.13
5	205.183	0.50
6	208.983	0.73
7	226.033	0.30
8	230.083	0.88
9	247.933	0.11
10	265.333	0.83
11	270.083	0.10

12	271.683	3.44
13	276.933	0.54
14	289.783	0.07
15	331.283	0.09
16	336.933	1.17
17	342.633	0.05
18	344.733	0.09
19	347.333	1.16
20	361.583	1.33
21	374.933	0.06
22	376.233	0.72
23	386.883	1.02
24	394.283	0.12
25	396.383	1.09
26	397.333	0.31
27	406.533	1.01
28	411.383	0.37
29	411.933	2.22
30	419.583	0.38
31	455.583	1.23
32	470.433	0.09
33	474.883	0.09
34	507.933	1.34
35	508.083	1.25
36	511.483	0.09
37	523.583	1.21
38	525.683	0.10
39	526.033	0.00
40	527.633	0.10
41	529.233	0.77
42	533.733	1.16
43	536.383	0.06
44	536.983	1.05
45	539.383	0.64
46	541.383	0.09
47	547.133	0.15
48	556.383	1.22
49	556.933	1.24
50	561.233	1.09
51	590.733	0.94
52	594.583	0.05
53	597.283	1.75
54	606.083	0.99
55	620.133	0.14
56	631.683	1.76

57	642.733	0.08
58	674.783	0.10
59	679.383	1.01
60	686.433	0.00
61	704.233	0.09
62	715.533	0.04
63	730.683	0.92
64	738.183	0.09
65	804.033	1.40
66	827.783	0.10
67	843.083	2.03
68	886.683	1.03

Appendix 19: Classification of changes unidentified metabolites following in shoot apex phloem exudates of *Sinapis alba*.

Unknowns	RT (s)	Ratio T2/T1
1	202.09	2.07
2	209.14	7.24
3	204.24	5.29
4	219.09	2.55
5	224.04	3.18
6	224.19	4.02
7	226.29	5.00
8	239.19	9.17
9	230.29	3.17
10	198.69	82.56
11	207.69	4.03
12	218.59	12.45
13	264.19	3.91
14	284.34	90.21
15	287.44	6.33
16	329.14	2.66
17	341.09	2.75
18	306.49	5.51
19	300.59	2.25
20	357.14	3.68
21	353.24	2.52
22	311.79	63.15
23	335.64	15.20
24	376.34	3.74
25	406.19	8.41
26	357.69	5.20
27	361.69	8.27

28	416.24	3.58
29	364.84	76.15
30	406.69	3.35
31	464.14	2.09
32	440.39	3.87
33	448.34	5.11
34	385.99	2.95
35	439.49	2.19
36	485.04	8.81
37	459.34	2.30
38	455.39	4.41
39	488.09	3.17
40	458.54	6.36
41	470.44	4.85
42	423.94	2.37
43	441.84	3.09
44	472.64	2.45
45	483.54	2.34
46	464.74	3.54
47	545.19	5.71
48	559.14	10.57
49	527.54	4.55
50	573.14	2.17
51	514.79	3.08
52	507.89	10.87
53	513.89	6.61
54	561.44	21.36
55	563.44	2.55
56	519.94	2.54
57	527.94	2.84
58	599.24	11.36
59	588.44	19.21
60	572.64	2.01
61	599.44	2.88
62	557.89	4.69
63	574.74	4.73
64	587.79	5.47
65	591.79	2.72
66	597.89	15.47
67	633.54	4.55
68	679.09	3.38
69	632.69	2.16
70	616.89	11.76
71	720.09	7.29
72	724.09	3.10

73	674.69	2.35
74	738.19	3.17
75	680.79	3.68
76	754.09	8.24
77	669.94	3.77
78	733.39	7.62
79	726.49	2.05
80	773.04	2.67
81	710.74	13.91
82	730.64	3.60
83	720.84	5.57
84	845.09	48.35
85	853.54	2.19
86	863.84	2.35