# Analysis of genetic interaction between *CRY2* gene and floral meristem identity and actin polymerization pathways in *Arabidopsis thaliana* L. Heynh

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## ABSTRACT

*This work was carried out to detect the genetic interaction between Arabidopsis blue light photoreceptor cryptochrome 2 (CRY2) gene and the genes of the floral meristem identity pathway. The influence of Arabidopsis Actin Related Protein complex (ATARP2/3 complex) on flowering initiation and the kind of interaction with CRY2 gene was also studied and evaluated. Several parameters such as flowering time, E. coli ß-glucuronidase gene (GUS) activity assay, RNA and protein expression were used. Several monogenic and double mutant lines have been prepared and planted in different photoperiod conditions.*

*Data analysis showed indirect genetic interaction (additive) between CRY2 gene and the floral meristem identity pathway. Despite the negative regulation effect of DIS2 gene in the floral meristem identity pathway and GRL in the ARP2/3 complex on flowering time, there was no direct genetic interaction between CRY2 gene and the ARP2/3 complex. In future studies, work will be devoted towards the detection of a possible direct genetic interaction between ATARP2/3 complex and the different flowering pathways.*

***Keywords:*** *Arabidopsis, flowering time genes, CRY2, ARP2/3 complex.*

## INTRODUCTION

Flowering process is initiated by the transition of the apical meristem from a vegetative fate to a floral fate. Mechanisms that control the timing of floral initiation have been extensively studied in Arabidopsis by identification of mutations that flower earlier or later than the wild type (Koornneef *et al.,* 1998). These mutations are known as flowering-time mutations and the corresponding genes are known as flowering-time genes.

One of the most important environmental factors affecting flowering time is the daily duration of light, the photoperiod (Thomas and Vince-Prue, 1997). Plants in which flowering occurs or is accelerated in short days or long days are known as SD plants or LD plants, respectively. It is now proved that a late flowering mutation corresponds to a gene product that normally promotes floral initiation (Koornneef *et al*., 1998), while an early flowering mutation implies that the corresponding gene product is a suppressor of floral initiation.

Based on the genetic interactions of these mutations, flowering time genes have been grouped into several signal transduction pathways. They either suppress or promote the floral meristem identity genes, controlling the formation of the floral meristem (Koornneef and Peeters, 2000 and Schomburg *et al.*, 2001).

Photoperiodic pathway is one of the major signal transduction pathways regulating flowering-time (Levy and Dean, 1998). Mutations of genes in this pathway such as *CO* (*CONSTANS*; Putterill *et al.*, 1995) and *CRY2* (*Cryptochrome2*; Guo *et al.*, 1998) reduce plant responsiveness to photoperiod, and these genes encode either photoreceptors or circadian clock proteins (Simpson *et al.*, 1999).

*Cryptochromes* (*CRY1* and *CRY2*) are blue light photoreceptors and *CRY2* has been speculated to be the photoreceptor that perceives the LD photoperiod signal and reflects it to the down stream flowering pathways (Guo *et al.*, 1998). The *cry2* deficient mutants have been found to be allelic to the *fha1* mutant that flowers later than the wild type in LD but not in SD. In addition, a naturally occurring allele of *CRY2* in an accession from Cape Verdi Islands (Cvi), named as *EARLY DAYLENGTH INSENSITIVE* (*EDI*) has been identified. Plants carrying this allele flower early in both LD and SD (Alonso-Blanco *et al.*, 1998). The molecular analysis of this *CRY2*-Cvi *(EDI)* allele has shown that it is a gain of function allele that in SD conditions leads to a high level of CRY2 protein, which is maintained for longer time after the onset of the light period comparing with *CRY2*-L*er* allele (*CRY2*-wild type allele). This protein stability in SD correlates with early flowering in SD and therefore day length insensitivity (El-Assal *et al.*, 2001).

Plant cells employ the actin cytoskeleton to stably position organelles, as tracks for long distance transport, and to reorganize the cytoplasm in response to developmental and environmental cues. The Arabidopsis *Actin Related Protein complex (ATARP2/3)* controls actin cytoskeleton polymerization, through binding to the mother filaments and nucleates daughter filaments. Therefore, it is required for normal root and shoot development (Blanchoin *et al.,* 2000; Amann and Pollard, 2001; Le *et al*., 2003; El-Assal *et al.,* 2004 a, b).

In this work, four different genetic and molecular genetic parameters were used, including flowering time analysis, measuring the activity of the β-glucuronidase (GUS) reporter protein, detect the *CRY2*-RNA and CRY2-protein expression in the background of the several monogenic and the double mutant lines of *A. thaliana*.

The objectives of this study were; 1) detecting the type of genetic interaction between *CRY2* gene and genes of the floral meristem identity pathway, 2) the possibility of *ATARP2/3* complex involvements in flowering initiation, and 3) the type of genetic interaction between *CRY2* and *ATARP2/3* complex.

## MATERIALS AND METHODS

### *Construction of genotypes*

Seven monogenic lines of *A. thaliana* have been prepared in the Landsberg *erecta* (L*er*) genetic background. These lines included; *fha1* (Koornneef *et al*., 1991), *CRY2*-Cvi allele (*EDI*) (El-Assal *et al*., 2001), *LFY::GUS, AP1::GUS, CO::GUS, DIS2::GUS* and *GRL::GUS*. Monogenic lines; *LFY::GUS, AP1::GUS and CO::GUS* were obtained from Prof. Dr. M. Koornneef, Wageningen Univ., The Netherlands, while *DIS2::GUS* and *GRL::GUS* lines were prepared in the Agronomy Dept., Purdue Univ.Indiana, USA. In order to prepare these monogenic lines, the

promoters of *DIS2* (El-Assal *et al.*, 2004a) and *GRL* (El-Assal *et al.*, 2004b), genes were cloned into plasmid vector pBI121 in frame with *E. coli ß-glucuronidase (GUS)* gene.

Ten different double mutants between either *EDI* or *fha1* and each one of the five monogenic lines; *LFY::GUS, AP1::GUS, CO::GUS, DIS2::GUS* and *GRL::GUS* were selected from the F2 progenies using the PCR-based markers. The double mutants carrying either *EDI* or *fha1* alleles were identified using two dCAP's markers specifically designed for the *EDI* and *fha1* alleles (Neff *et al.*, 1998). *EDI* primers were 5'-CGGGGAAATAAGCGTCAGACACGT-3' and 5'-CATTTCCATGGAAGGAGAAGAA ACTTCC-3'. The PCR product was cleaved using *BfaI* restriction enzyme, while the *fha1* primers were 5'-GACAGTT TTATCCTGGAAGAGCTTCACCAT-3' and 5'-GCTTTGCACAGAGATCCCAC GTTCC-3'. The PCR product was digested with the *NcoI* restriction enzyme.

### *Growth conditions and light treatments*

Seeds were sown on filter paper and planted in soil. Fifteen plants were used for each genotype / treatment combination. Individual pots were grown in LD (25°C, 16 hr light) and SD (25°C, 8 hr light) cabinets.

### *Measurement of flowering*

The number of rosette and cauline leaves in the main inflorescence were counted on the day when the first flower became visible. Total leaf number (TLN) and days to flowering were used as flowering-time parameters.

### *Measurement of GUS activity*

GUS activity was measured by performing a quantitative GUS-assay on apical meristems. The amount of converted substrate (MUG, 4-methyl umbelliferyl glucuronide) into 4-MU, correlates with the enzyme activity, and consequently with the promoter activity of the genes under study. In order to quantify the GUS activity every 7 days after germination, 2 - 4 apical meristems per line were harvested. The apical meristems were stained in GUS staining solution (0.5 M $NaH_2PO_4$; Triton X-100; 0.2 M $K_3Fe(CN)_6$; 100 mM X-gluc), overnight at 37°C. The Hoefer TKO 100 fluorometer was calibarated with 0.2 M $NaCO_3$ (set 0) and 0.05 µM 4-MU (in 50% GUS-extraction buffer / 50% $NaCO_3$) (set 250). Calibaration was checked with 0.2 µM 4-MU (in 50% GUS extraction buffer / 50% $NaCO_3$) (set ± 1000).

### *RT-PCR*

Total RNA was isolated using the RNeasy Mini Kit (Qiagen). For the first strand cDNA synthesis, 1 µg of the total RNA was primed using a mixture of random hexanucleotides at a final concentration of 20 ng / µl , and then reverse transcribed with 8 units / µl of M-MLV (Invitrogen). Thereafter, 1/25 volume of the cDNA was used as a template for PCR. For the *CRY2* gene expression, two primers intervals 200 bp were used; CRY2-Forward, 5'-TTGGCGGTTGATGCCAAT-3'; CRY2-Reverse, 5'-TCCAGCCCTAGTTCTTCA-ATCG-3'. Primers specific to glyceraldehydes-3-phosphate dehydrogenase (GAPC) were used as a control.

### *Protein expression*

The polyacrylamide / SDS mini gel was prepared according to Laemmli (1970). Electrophoresis was carried out at 80 volts for approximately 3 hr and the gel was carefully removed from the glass plates and placed in 100 ml pre-cooled transfer buffer (20 mM Tris-HCl, 137 mM NaCl, 0.1% v/v Tween-20). Proteins were blotted onto the nitrocellulose paper and electrotransferred for 2 hr at 100 V. Confirming the transfer of proteins to the

membrane was conducted using Ponceau-S stain (Sambrook *et al.*, 1989). The CRY2 expression was detected using anti-CRY2 antibodies. Immuno-detection was done using the HRP system (Amersham, ECL).

## RESULTS AND DISCUSSION

### *Flowering time analysis*

A double mutant between the *CRY2* gain-of-function allele (*EDI*), and a mutant of the *CO* gene made and analyzed. The double mutant flowered as late as the monogenic *co* mutant, in both LD and SD. These results imply that *CO* acts directly downstream of *CRY2* and, therefore, *CRY2* need the product of *CO* gene to promote flowering through the photoperiod promotion pathway (El-Assal *el al.,* 2003). Therefore, new monogenic and double mutants between *CRY2* and *CO* genes were used in this study as controls for the type of genetic interaction.

Since it is known that both *LFY* and *AP1* are up-regulated by the photoperiod pathway (Levy and Dean, 1998, and Koornneef *et al.*, 1998), the type of genetic interaction between *CRY2* and both *LFY* and *AP1* genes was studied. The monogenic parents; *EDI, fha1*, *LFY::GUS and AP1::GUS*, and the double mutants; *EDI / LFY::GUS, fha1 / LFY::GUS, EDI / AP1::GUS* and *fha1 / AP1::GUS* were planted in both LD and SD. The flowering time was estimated for all genotypes using two parameters, the days to flowering and the total rosette leaf number until the flowering initiation. These two parameters showed a linear relationship (Fig. 1-A). The monogenic lines *LFY::GUS and AP1::GUS* flowered earlier than the wild-type L*er*, but later than *EDI* under LD and SD. The double mutants *EDI / LFY::GUS; EDI / AP1::GUS* showed intermediate flowering-time between the parental monogenic lines, also *fha1 / LFY::GUS* and *fha1 / AP1::GUS* double mutants flowered in a number of days between the monogenic parents (Fig. 1A, B and C). These results may suggest that *CRY2, LFY* and *AP1* genes do not interact with each other. In other words, *CRY2* controls flowering independently from the floral meristem identity pathway.

At the same time, it has been demonstrated that both *DIS2* and *GRL* genes belong to the Arabidopsis *ATARP2/3* complex, and are involved in trichome cell development. Mutations in the genes, *dis2-1* and *grl-6*, showed trichome distortion phenotype and defect in cell shape (El-Assal *et al.*, 2004 a and b). Preliminary experiments recorded that *dis2-1* and *grl-6* mutants flowered earlier than the wild type L*er* (data not shown). These data might suggest that the wild-type alleles of these genes act as flowering suppressors and, therefore, interact with one of the flowering initiation pathways. In order to detect the type of genetic interaction between *CRY2* and the *ATARP2/3*, the monogenic lines; *EDI*, *fha1*, *DIS2::GUS and GRL::GUS,* and the double mutants; *EDI / DIS2::GUS, fha1 / DIS2::GUS, EDI / GRL::GUS* and *fha1 / GRL::GUS* were planted in both LD and SD. The monogenic lines *DIS2::GUS* and *GRL::GUS* showed day length insensitivity, when flowered later than the wild-type in both LD and SD (Fig. 1, B and C). The double mutants; *EDI / DIS2::GUS*, *EDI / GRL::GUS, fha1 / DIS2::GUS,* and *fha1 / GRL::GUS* flowered in between the monogenic parental lines. As was the case with *LFY* and *AP1* genes, the obtained results showed intermediate genetic interaction between *CRY2* and either *DIS2* or *GRL* genes Therefore, it was clear that there are some other genes that separate the direct interaction between *CRY2* gene and *ATARP2/3* complex.

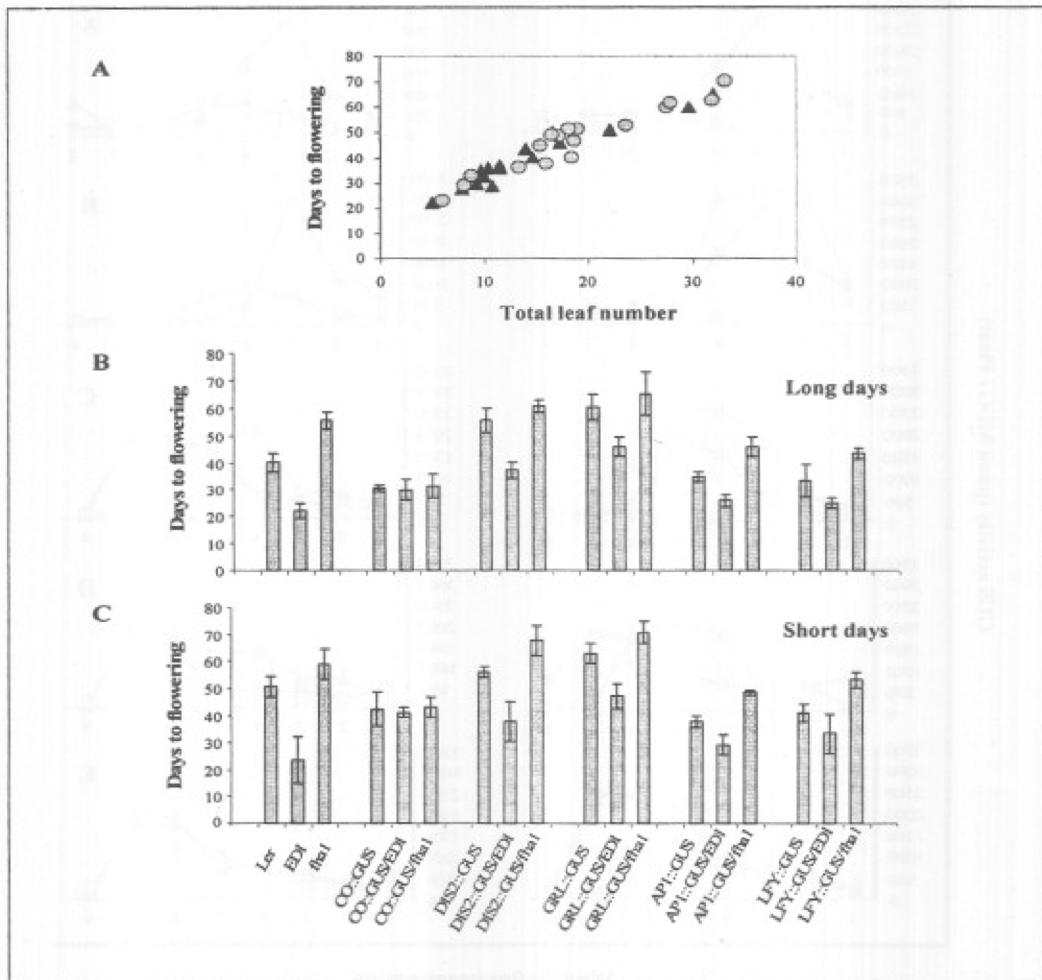


***Fig. (1): Day-length effects on flowering-time. (Panels A and B): Wild type (Ler), EDI, fha1, monogenic lines (CO::GUS, LFY::GUS, AP1::GUS, DIS2::GUS, GRL::GUS) and Double mutants EDI or fha1 and either one of the five monogenics, were grown in LD (Long days) and SD (Short days) climate chamber conditions respectively. The standard error of the mean of 15 plants is indicated on each bar. (Panel C): The correlation between days to flowering and total leaf number for all used lines in LD (black triangels) and SD (gray circles).***

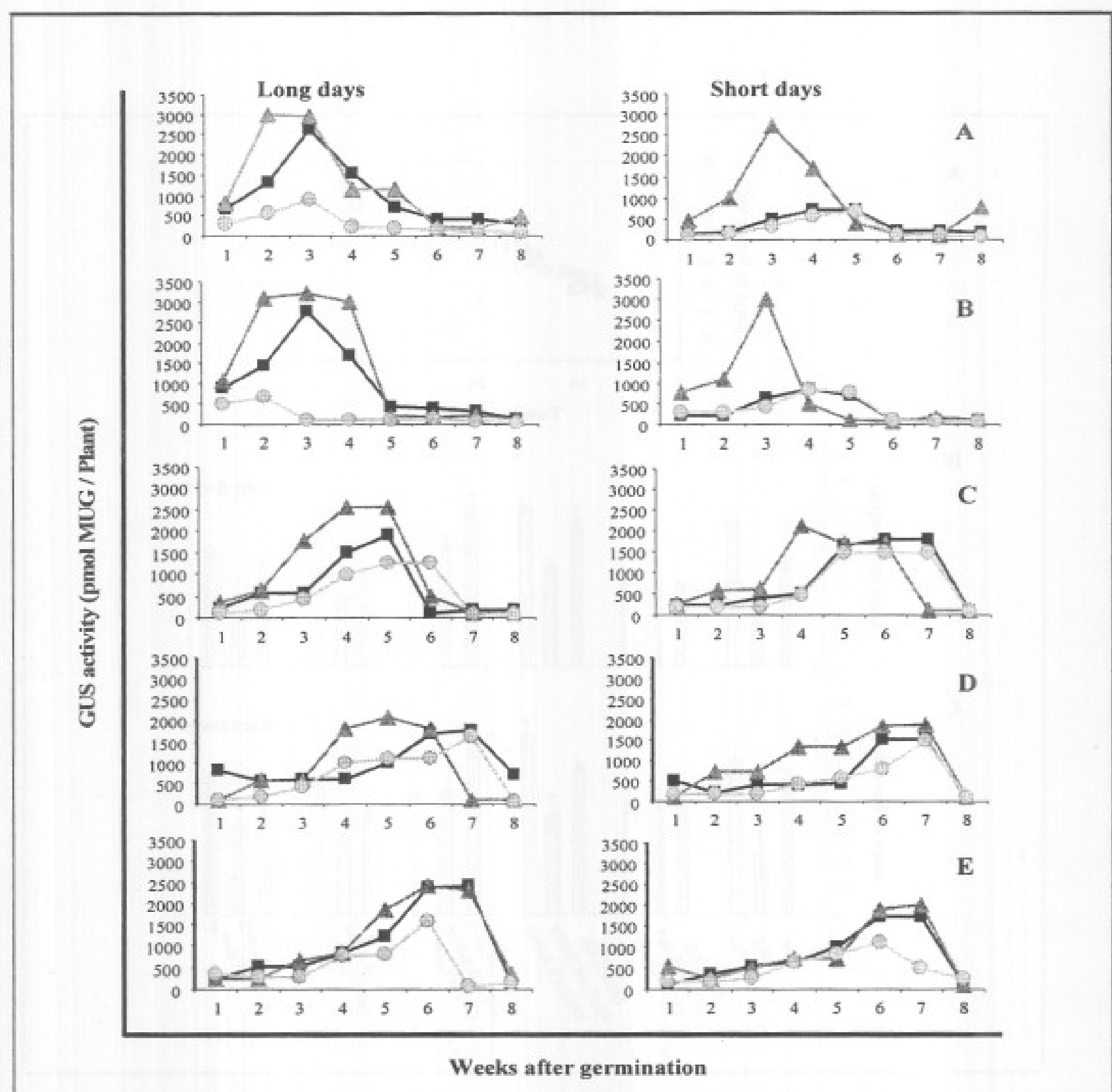


***Fig. (2): GUS expression assay in LD and SD. Symbols; (black boxes refer to the monogenic lines), (dark gray triangles refer to the double mutant between EDI and one of the genes under study) and (light gray triangles refer to the double mutant of fha1 and one of the genes under study). (A): The monogenic and the double mutants (LFY::GUS; EDI / LFY::GUS and fha1 / LFY::GUS). (B): Plant lines (AP1::GUS, EDI / AP1::GUS and fha1 / AP1::GUS). (C): Plant lines (DIS2::GUS, EDI / DIS2::GUS and fha1 / DIS2::GUS). (D) Plant lines (GRL::GUS; EDI / GRL::GUS and fha1 / GRL::GUS). (E): Plant lines (CO::GUS; EDI / CO::GUS and fha1 / CO::GUS).***

***ß-glucuronidase gene (GUS) activity***

One of the powerful tools to measure the gene expression is to fuse the promoter of the gene under study in a frame with *E. coli* ß-glucuronidase gene (GUS), and the GUS enzyme will reflect the gene activity (Levy and Dean, 1998).

In order to detect the expression of *CO; LFY*; *AP1*; *DIS2* and *GRL* genes, the monogenic lines; *CO::GUS, LFY::GUS, AP1::GUS, DIS2::GUS* and *GRL::GUS* were prepared. To study the effect of *CRY2* on the expression of these genes as a tool to recognize the type of genetic interaction between them and *CRY2*, a set of double mutant lines was prepared between either of the two *CRY2* alleles and each one of these five monogenics (as described in materials and methods), and studied in both LD and SD. The GUS activity was measured in the apical meristems of these lines, every seven days after germination until the latest plant flowered.

The monogenic *LFY::GUS* showed a high GUS activity in LD during the 3rd week, while the GUS activity was very low in SD (Fig. 2, panel A, black boxes). In the double mutant *EDI / LFY::GUS*, the maximum GUS expression was recorded during the 2nd week in LD and during the 3rd week in SD (Fig. 2, panel A, dark-gray triangle). These data reflect that the GUS expression in *EDI / LFY::GUS* was higher than *LFY::GUS* in both LD and SD. While the GUS activity in the double mutant *fhal / LFY::GUS* was lower than that of the monogenic *LFY::GUS* (Fig. 2, panel A, light-gray circles). The obtained results of the double mutants might suggest that both of *LFY* and *CRY2* genes are acting independently, and confirm the flowering time data (Fig. 1).

The monogenic *AP1::GUS* and the double mutant *EDI / AP1::GUS* showed a high expression of GUS enzyme during the 3rd week in LD, but the *EDI / AP1::GUS* started accumulating the GUS expression earlier than *AP1::GUS* line. The highest accumulation of GUS expression was found in *EDI / AP1::GUS* plants, more than one week earlier than *AP1::GUS*. In SD, the line *EDI / AP1::GUS* showed markedly higher GUS activity compared with the *AP1::GUS* line (Fig. 2, panel B, dark gray angles and black boxes). On the contrary, the double mutant *fhal / AP1::GUS* showed a delay and reduction in GUS expression especially in LD, compared with the monogenic *AP1::GUS* (Fig. 2, panel B, light gray circles).

As the case between *CRY2* and *LFY* genes, the shifting in GUS expression between the monogenic and the double mutants might indicate that *AP1* gene is not interacting directly with the *CRY2* gene. These results are expected because both of *AP1* and *LFY* genes belong to the floral meristem identity pathway (Levy and Dean, 1998).

To study the type of genetic interaction between *CRY2* and the members of *ATARP2/3* complex, the GUS activity was measured in the monogenics *DIS2::GUS* and *GRL::GUS* and the double mutants *EDI / DIS2::GUS; fhal / DIS2::GUS; EDI / GRL::GUS* and *fhal / GRL::GUS* (Fig. 2, panels C and D). The double mutants with *EDI* plants showed GUS expression earlier and much higher than the monogenic lines *DIS2::GUS* and *GRL::GUS* in LD and SD, while the double mutants with *fhal* allele exhibited much slower and less efficient GUS expression compared with the monogenic parents. These data showed that the double mutants *EDI / DIS2::GUS; fhal / DIS2::GUS; EDI / GRL::GUS* and *fhal / GRL::GUS* recorded the highest GUS expression two weeks before the flowering initiation. Although the flowering time data showed that *ATARP2/3* complex interacted with flowering time pathways, the interaction was not directly through *CRY2* gene, and probably through some other flowering-time genes.

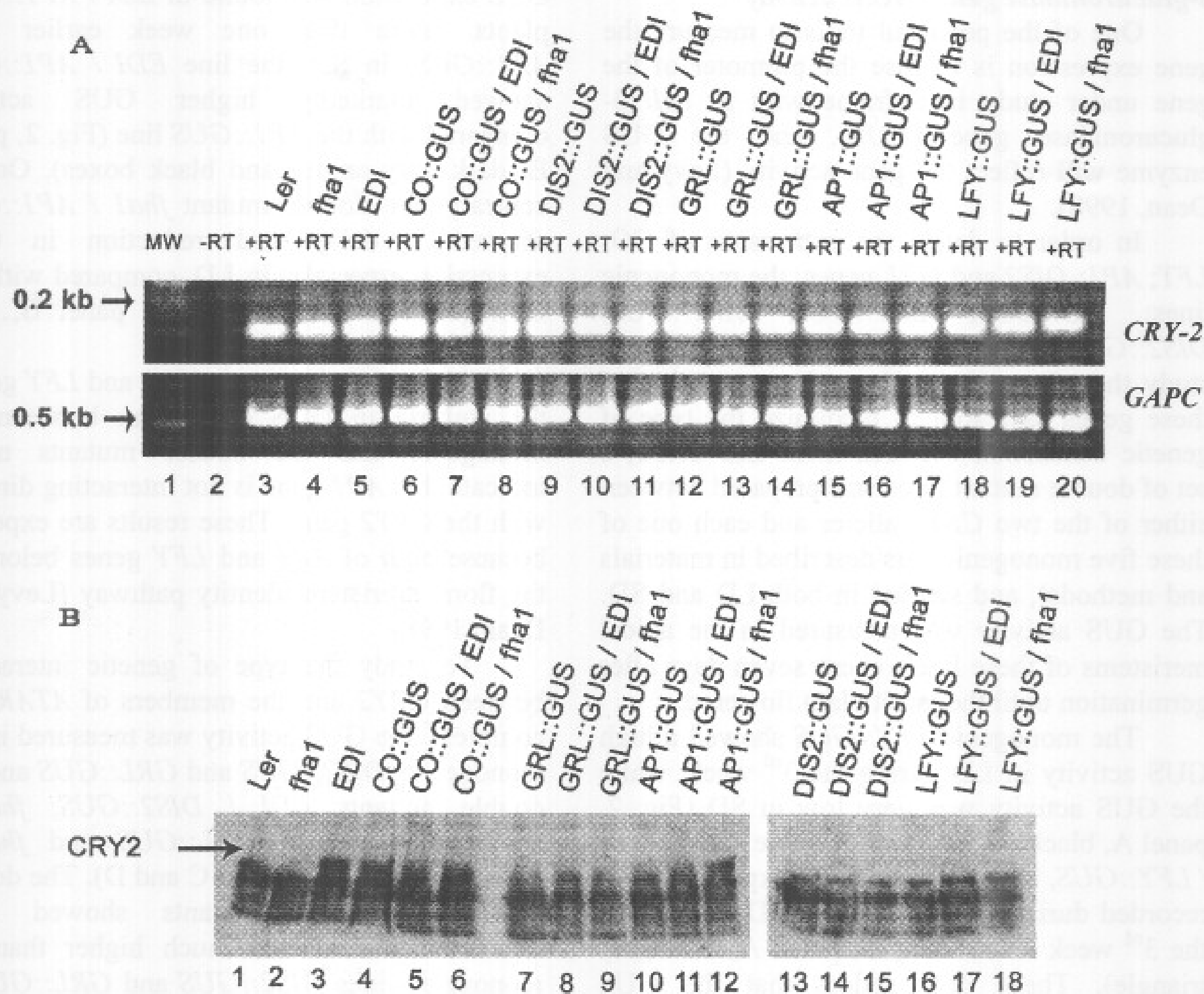


***Fig. (3): Analysis of CRY2 expression. (A): RT-PCR analysis of CRY2 gene expression in different Arabidopsis lines. Upper samples are CRY2 expression using 20 cycles of PCR, while lower samples are GAPC (glyceraldehydes-3-phosphate dehydrogenase) expression. Lane-1 is a 1 kb ladder, lane-2 is a negative PCR (-RT), lane- 2 to 18 is CRY2 expression (+RT) in different plant lines. (B): CRY2-Protein expression is several Arabidopsis lines. Negative controls, Ler plants after 7 hours in light and fha1. Lane- 3 to 18 showed CRY2-protein expression in different plant lines. Anti-CRY2 antibodies were developed in rabbit blood. The background bands were used as a loading control.***

The earlier accumulation of GUS protein in the *EDI* double mutants compared with flowering-time might suggest that the flowering time genes start contributing different signals at least two weeks before flowering initiation (Fig. 1 and 2).

As expected, the control double mutants *CO::GUS / EDI* and *CO::GUS / fha1* flowered as late as the monogenic *CO::GUS* in LD and SD conditions (Fig. 1), and accumulated GUS expression as low as *CO::GUS* (Fig. 2-panel C).

### *CRY2-RNA and protein expression*

It is well known that the LD conditions have a determined effect on promoting the photoperiod pathway because most photoperiod gene mutations flower late in LD (Putterill *et al.*, 1995). Therefore, the plant lines of this study were planted in LD, and three-week-old plants were used to study the RNA and protein expression.

In order to detect any differences in *CRY2*-RNA expression among the monogenic and the double mutant lines under study, only 20 PCR cycles were applied (non saturated PCR reaction). *CRY2*-RNA expression analysis in the monogenics backgrounds (*LFY::GUS; AP1::GUS; CO::GUS; DIS2::GUS* and *GRL::GUS*) and their related double mutants with *EDI* or *fha1* mutants, showed no marked difference among these lines (Fig. 3-A).

The CRY2-protein expression analysis using anti-CRY2 antibodies was applied in order to confirm the flowering time and the GUS assay, because RNA expression analysis does not show the *fha1* loss-of-function phenotype. The plant lines, *LFY::GUS; AP1::GUS; EDI / LFY::GUS; EDI / AP1::GUS; fha1 / LFY::GUS* and *fha1 / AP1::GUS* showed no differences in CRY2-protein expression, confirming that there is no direct interaction between *CRY2* and either *LHY* or *AP1* genes (Fig. 3-B).

CRY2-protein expression was reduced in plant lines *DIS2::GUS; GRL::GUS; EDI / DIS2::GUS; fha1 / DIS2::GUS; EDI / GRL::GUS* and *fha1 / GRL::GUS* (Fig. 3-B), confirming that both genes *DIS2* and *GRL* are reacting negatively (suppressors) with flowering time pathways (Fig. 1). But these data did not support a direct genetic interaction between *ATARP2/3* complex and *CRY2* gene. Finally it can be suggested that in order to detect the exact type of genetic interaction between *ATARP2/3* complex and the flowering time genes, several double mutants between the *DIS2* or *GRL* mutants and different flowering time pathway mutants should be prepared.

## REFERENCES

**Alonso-Blanco, C., El-Assal, S.E-D., Coupland, G. and Koornneef, M. (1998).** Analysis of natural allelic variation at flowering time loci in the Landsberg *erecta* and Cape Verde Islands ecotypes of *Arabidopsis thaliana*. Genetics 149, 749-764.

**Amann, K., and Pollard, T. (2001).** Direct real-time observation of actin filament branching mediated by Arp 2/3 complex using total internal reflection fluorescence microscopy. PNAS 98, 15009-15013.

**Blanchoin, L., Amann, K.J., Higgs, H.N., Marchand, J.B., Kaiser, D.A., and Pollard, T.D. (2000).** Direct observation of dendritic actin filament networks nucleated by Arp2/3 complex and WASP/Scar proteins. Nature 404, 1007-1011.

**El-Assal, S.E-D., Alonso-Blanco, C., Peeters, A.J.M., Raz, V. and Koornneef, M. (2001).** A QTL for flowering time in Arabidopsis reveals a novel allele of CRY2. Nature Genetics 29, 435-440.

**El-Assal, S.E-D., Alonso-Blanco, C., Peeters, A.J.M., Wagemaker, C., Weller, J. L., and Koornneef, M. (2003).** The role of

cryptochrome 2 in flowering in Arabidopsis. Plant Physiology 133, 1504-1516.

**El-Assal, S.E-D., Le, J., Basu, D., Mallery, E.L., and Szymanksi, D.B. (2004 a).** DISTORTED2 encodes an ARPC2 subunit of the putative Arabidopsis ARP2/3 complex. Plant J. 38, 526-538.

**El-Assal, S.E-D., Le, J., Basu, D., Mallery, E.L., and Szymanksi, D.B. (2004 b).** Arabidopsis GNARLED encodes a NAP125 homologue that positively regulates ARP2/3. Curr. Biol. 14, 1405-1409.

**Guo, H., Yang, H., Mockler, T.C. and Lin, C. (1998).** Regulation of flowering time by Arabidopsis photoreceptors. Science 279, 1360-1363.

**Koornneef, M., Alonso-Blanco, C., Peeters, A.J.M. and Soppe, W. (1998).** Genetic control of flowering time in Arabidopsis. Annu. Rev., Plant Physiol., and Plant Mol. Biol. 49, 345-370.

**Koornneef, M., Hanhart, C.J. and van der Veen, J.H. (1991).** A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana.* Mol. Gen. Genet. 229, 57-66.

**Koornneef, M. and Peeters, A.J.M. (2000).** The late flowering phenotype of *fwa* mutant is caused by gain-of-function epigenetic alleles of a homeodomain gene. Mol. Cell 6, 791-802.

**Laemmli, U. K. (1970).** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680.

**Le, J., El-Assal, S.E., Basu, D., Saad, M.E., and Szymanksi, D.B. (2003).** Requirements for *Arabidopsis ATARP2* and *ATARP3* during epidermal development. Curr Biol 13, 1341-1347.

**Levy, Y.Y, and Dean, C. (1998).** The transition to flowering. Plant Cell 10, 1973-1989.

**Neff, M.M., Neff, J.D., Chory, J. and Pepper, E. (1998).** dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: experimental applications in *Arabidopsis thaliana* genetics. Plant J. 14, 387-392.

**Putterill, J., Robson, F., Lee, K., Simon, R. and Coupland, G. (1995).** The *CONSTANS* gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factor. Cell 80, 847-857.

**Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989).** Molecular cloning: A laboratory Manual (Cold Spring Harbor laboratories, Cold Spring Harbor, NY).

**Schomburg, F.M., Patton, D.A., Meinke, D.W. and Amasino, R.M. (2001).** *XSFPA*, a gene involved in floral induction in Arabidopsi*s*, encodes a protein containing RNA-recognition motifs. Plant Cell 13, 1-11.

**Simpson, G.G., Gendall, A.R. and Dean, C. (1999).** When to switch to flowering. Ann. Rev. Cell Dev. Biol. 99, 519-550.

**Thomas, B., and Vince-Prue, D. (1997).** Photoperiodism in plants. Academic Press, New York.

## الملخص العربي


### التحليل الوراثي للتداخل بين جين الكريبتوكروم CRY2 المستقبل للضوء الازرق وكل من مسارات المرستيمات الزهرية و متبلمرات الأكتين فى نبات الأرابيدوبسيس



**صلاح الدين العسال**

جامعة القاهرة ، كلية الزراعة ، قسم الوراثة ، الجيزة ، مصر



أجرى هذا البحث من أجل التعرف على نوعية العلاقة الوراثية بين أحد الجينات التى تتوسط فى إستقبال طيف الضوء الأزرق فى نبات الأرابيدوبسيس (كريبتوكروم-2) و بعض الجينات التى تنتمى للمسار الجينى المتحكم فى تخليق المرستيمات الزهرية . و بالاضافة تهدف هذه الدراسة إلى معرفة إذا ما كان المعقد البروتينى الذى يتحكم فى عملية تخليق ألياف الأكتينات فى الأرابيدوبسيس (ATARP2/3) يلعب دوراً فى عملية التزهير ، كما وقد تم دراسة نوعية التداخل الوراثى بين هذا المعقد البروتينى وجين الكريبتوكروم-2 . استخدم العديد من الطرق فى هذا البحث ، والتى تضمنت بعض الدراسات المورفولوجية وكذا بعض القياسات البيوكيماوية والجزيئية من أجل تقدير نشاط جين الكريبتوكروم-2 كوسيلة لدراسة نوعية التداخل الوراثى بين هذا الجين و الجينات الأخرى فى هذه الدراسة.

جهزت بعض الطفرات المزدوجة المختلفة و التى تجتمع فى خلفيتها الوراثية طفرتين إحداهما خاصة بالجين المستقبل لطيف الضوء الأزرق (كريبتوكروم-2) و الأخرى تخص أحد الجينات الأخرى المراد دراسة نوعية التداخل الوراثى معها . دلت النتائج المتحصل عليها على أن نوعية العلاقة الوراثية بين الجين (كريبتوكروم-2) و بعض الجينات المتحكمة فى تخليق المرستيمات الزهرية من نوع العلاقة الوسطية . أما بالنسبة لدراسة نوعية العلاقة الوراثية بين جين الكريبتوكروم-2 و معقد (ATARP2/3)، فبالرغم من أن الدراسة أظهرت أن الجينات التى تنتمى لهذا المعقد البروتينى لها تأثير على موعد التزهير فى نبات الأرابيدوبسيس ، إلا أنها لم تحدد علاقة وراثية مباشرة مع جين الكريبتوكروم-2 . إ ن اجراء دراسة مستقبلية سوف تحدد بالضبط أى جين من المجموعة التى تنتمى للمسارات الوراثية المتحكمة فى عملية التزهير يمكنه أن يتداخل وراثياً بصورة مباشرة مع المعقد البروتينى (ATARP2/3) .