

Short Term Scientific Mission - Report 2009

A short term scientific mission (STSM) within the COST863 (Euroberry Research: From Genomics to sustainable production, quality and health).

Temperature control of flowering genes in *Fragaria vesca*.

Reference code:

COST-STSM-863-04454

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Objectives of STMS:

As a new employed researcher in strawberry at the Norwegian Institute for Agricultural and Environmental Research I had several objectives for my STSM to the laboratory of Dr. Paulina Palonen and The small fruit research group at the University of Helsinki. **1**); to gain experience in laboratory work with *Fragaria vesca*, **2**); do genetic expression analyses for putative candidate genes involved in temperature regulation of flowering, and **3**); develop future project cooperation in strawberry research, with the aim of sending a joint application to a suiting funding Institution within 2009.

Introduction

The understanding of how temperature and day length regulates flowering in strawberry is important for both yield and berry quality. Numerous studies have shown that temperature and day length strongly interact to control flowering. The group of Paulina Palonen has now identified more than 90 putative flowering time genes (Mouhu, Hytönen *et al.*, submitted to BMC Plant Biology), and they are currently investigating how light control flowering. However, virtually nothing is known about how temperature affects flowering or how temperature interacts with light to control flowering at the molecular level. In short day (SD) genotypes of *F. vesca*, flowering is induced by SD and/or low temperature, and at least in some genotypes low temperature is more efficient floral stimuli. Hytönen *et al.* have shown that 3 weeks at 11°C is enough for flowering induction (LD or SD) in one Finnish SD genotype, but at least five weeks of SD is needed to induce flowering at 18°C. Two ever bearing (EB) genotypes, Hawaii-4 and Baron S, in contrast, show completely opposite flowering response in these conditions. The EB genotypes are induced to flower at a very early stage, and this trait is caused by one gene only (Seasonal Flowering Locus, *SFL*). To date, this gene has not been identified. The aim of my work in Finland was to explore temperature regulation of selected flowering time genes in different *F. vesca* genotypes and continue work on identifying the *SFL* gene.

Material and Methods

Beforehand, samples from plants grown at inductive (11°C) and non-inductive temperatures (18°C) as well as samples from long day (LD) and short day (SD) were collected, and when I arrived to Finland, we had more than 100 samples from where we could extract RNA for gene expression analysis. Tissue from both EB and short day SD genotypes of *F. vesca* was included in this material. Plant tissue (leaves and shoot apices) were harvested and immediately frozen in liquid nitrogen. The tissue was then stored at -80°C until homogenization in liquid nitrogen and subsequent RNA extraction. RNA was extracted using a pine tree method (Monte and Sommerville, 2002). cDNA was synthesized from total RNA using Superscript III RT kit (Invitrogen, Carlsbad, US) and dT18VN anchor primers. Light Cycler 480 SYBR Green I Master Kit (Roche Diagnostics, Indianapolis, US) was used to perform 15 uL real-time RT-PCR reactions in 384-well plates according to manufacturer's instruction by using Light Cycler 480-real time PCR system (Roche Diagnostics). PCR with T_m value of 60°C were used.

A putative *SFL* candidate was cloned from cDNA in 5 EB and 6 SD genotypes using Phusion™ High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, UK). Fragments were run on a gel to consider differences in length followed by sequencing on an ABI Prism 3100 16-capillary sequencer (Applied Biosystems, Foster City, CA, USA).

Results

In total, more than 20 genes were analyzed in 50+ samples using real-time RT-PCR during my stay. This generated a huge amount of data, and much work remains to analyze the expression patterns observed and consider plausible explanations and mechanisms. With the aim of establishing a molecular model for flowering in *F. vesca*, the work we did during my stay in Helsinki is a valuable starting point for future research on elucidation of such a model. In this future work the flowering model from *Arabidopsis* would likely prove to be very useful.

The samples analyzed contained both samples from time course studies and single time point studies (2 or 3 weeks after transfer to inductive or non-inductive conditions). In the figures below some putative flowering genes that likely are under circadian control are shown. One gene peaks in the dark period (Fig. 1) and one gene peaks in the light period (Fig.2). These genes seems to not respond to day length treatments by any phase shift, but maybe the amplitude seems to be affected in SD in the genotype Baron Solemacher (Fig. 2).

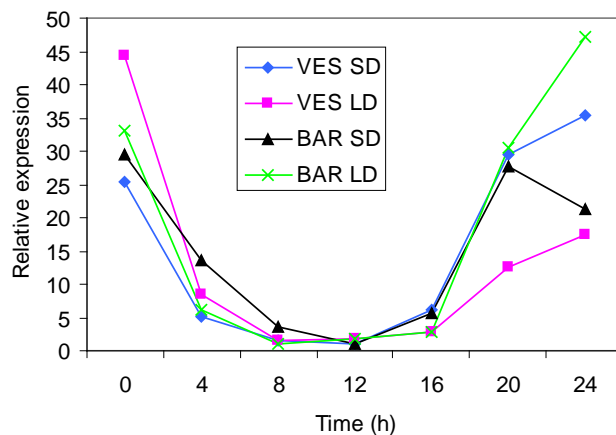


Figure 1. Gene expression of a putative flowering gene in short day (SD=0-8) and long day (LD=0-16). The expression of this gene peaks in the dark period.

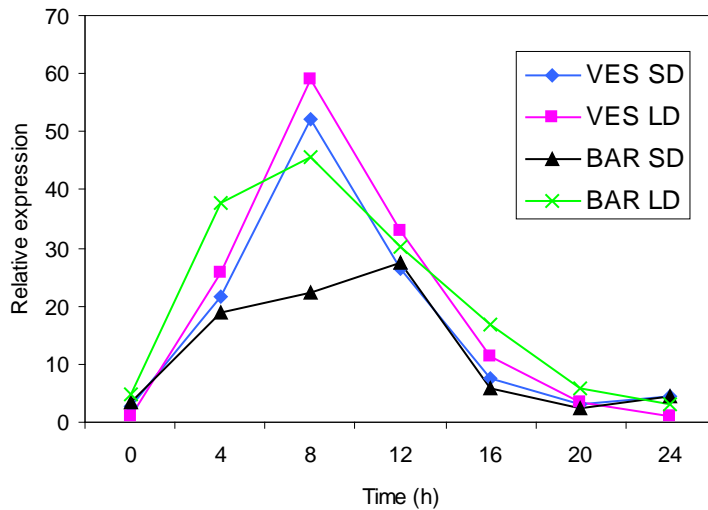


Figure 2. Gene expression of a putative flowering gene in short day (SD=0-8) and long day (LD=0-16). The expression of this gene peaks in the light period.

I also cloned a fragment of a putative SFL gene from cDNA. Initial analysis on agarose gels suggested that there were differences in fragment length between SD genotypes and EB genotypes. The fragments are now being sequenced and this will confirm whether there are any differences in the sequence of this gene between the genotypes.

Conclusions:

During my stay at Helsinki University I learned new methods for working with *F. vesca* and tried new equipments that will be valuable for my future scientific work and for my future collaboration with *Rocaceae* researchers. Dr. Timo Hytönen generously shared his deep knowledge about flowering in *Fragaria* and we had many fruitful discussions on the topic. In addition to the technology transfer several research tasks were completed within the four weeks duration of the STSM; getting a basis for understanding gene regulation of flowering in *F. vesca* by analyzing gene expression patterns of putative flowering genes and getting one step closer to a certain identification of the Seasonal Flowering gene.

Dr. Jon Anders Stavang - 18th of April 2009

STSM visit by Dr Jon Anders Stavang to the University of Helsinki and the laboratory of Dr. Paulina Palonen and Dr. Timo Hytönen

The visit by Dr. Jon Anders Stavang to the “The Small Fruit Research Group” at the University of Helsinki was very fruitful. In addition to achieving the goals of his mission and extending his repertoire of laboratory skills, Dr Stavang was also able to generate useful data on flowering in woodland strawberry, and we have extended our knowledge about molecular regulation of flowering. The data obtained will be incorporated in a joint publication on flowering. The visit was also an opportunity to discuss the potential for further collaboration between Dr Stavang and the Fruit and Berry group of the Norwegian Institute for Agricultural and Environmental Research and our group. The intention is that in the future, there will be an exchange of both students and information between the two groups and we plan to apply funding for joint projects to further

strengthen our research in *Fragaria*. I was pleased to host Dr Stavang's visit and would welcome the opportunity to continue our collaboration in the future through the COST and other programs.

Dr. Timo Hytönen - 18th of April 2009

Applied sum of money: 2410 €

Expenses for the STMS and sum of Budget:

Dietary Costs	1412 €
Accommodation	600 €
Travel expenses	398 €
Sum	2410 €