

PROJECT OVERVIEW:

Title of Project: Manipulating miR156 to control vegetative phase change in *Camelina sativa*

Name of Student: Noir Tucker

Name of Supervisor: Jim Fouracre

Place where project took place: University of Bristol

Photograph:



Description: Leaf heteroblasty produced using ImageJ to highlight the phenotypical differences in leaf width and length based on genotype.

- **What was the aim of your project?** (please summarise in less than 50 words)

The aim of my project was to observe the genetic network between MiR156 and its target SPL (squamosa promoter binding protein like) factors, which in *Arabidopsis thaliana*, are responsible for the change from juvenile phase to adult phase in the vegetative phase change. This network was observed in *Camelina sativa*.

- **How did you address the aim?** (what sorts of methods did you use? Please summarise in 100-200 words)

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This aim was addressed from a phenotypical approach, which consisted of qualitative and quantitative components, and also a molecular approach.

The juvenile phase is characterised by oval leaves with petioles present and a high level of miR156, which actively repress SPL factors. As the plant ages, 156 levels naturally decrease and the SPL factors are able to act on their target genes, causing the change to adult phase. A characteristic phenotypical observation of the change from juvenile to adult phase is the presence of abaxial trichomes (AT) and a more serrated leaf shape.

The phenotypical approach in *C.sativa* included using 3 different genetic lines; a wild type line (WT), the 156 OE line and the mimic line (MIM). 156OE had an upregulated miR156 gene causing overexpression of 156. It was expected that there would be an elongation in the juvenile phase causing a prevalent oval shaped leaf with petioles and a lack of AT. MIM included an insertion of a sequence homologous to that of the miR156 target, allowing it to act as a sponge for 156 and reduce the repression of SPL's, thus reducing the length of the juvenile phase, causing earlier AT.

With these lines; a quantitative measure of the blade width:length ratio was done for leaves 1,3&5 of each genotype, an analysis of the first AT and a qualitative leaf heteroblasty of leaves 1-9 were done for visual comparison.

The molecular approach included RNA quantification and a semi-quantitative PCR of these lines. SD WT samples were harvested at weekly intervals for 3 weeks and LD 156 and MIM were also harvested and quantified.

- **What did you find out?** (Please include a figure and a description that summarises your key findings; 200-400 words)

Leaf Heteroblasty

As expected; there was a significant effect of the interaction between genotype and day condition on the leaf the first AT was present on (GLM, interaction genotype x day condition, $F[2,186] = 21.509$, $p < 0.01$). when it came to the presence of the first AT, MIM displayed them the earliest at leaf 1 in long day (LD) conditions and leaf 3 in short day (SD) conditions with 156OE displaying the latest emergence of first AT of all 3 genotypes at leaf 4 in LD and leaf 5 in SD (Fig.1). The difference in which leaf the first AT is seen on within the same genotype may be due to the day conditions affecting SPL transcriptional regulation, this could explain why there is a significant difference seen between LD and SDWT (Post-hoc comparisons with Benjamini-Hochberg correction for multiple testing: $t = -6.706$, $p < 0.01$), LD and SDMIM ($t = -8.812$, $p < 0.01$) but not LD and SD156OE ($t = -0.699$, $p = 0.48532$) as there is not a high level of SPL present.

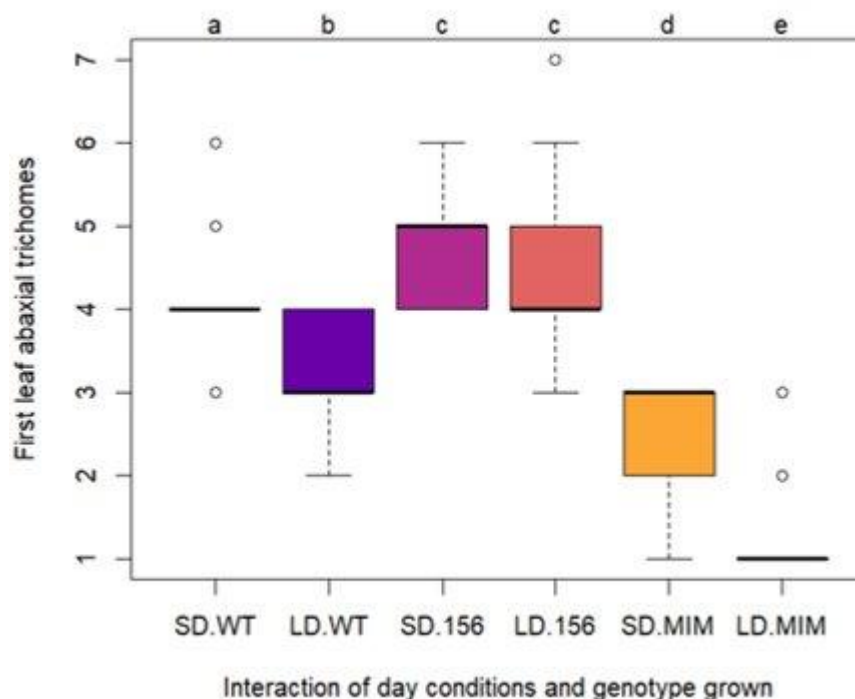


Figure 1. The interaction between day conditions and genotype and its effect on which leaf number abaxial trichomes are first observed on (GLM, interaction genotype x day condition, $F[2,186] = 21.509$, $p < 0.01$). Significant differences are indicated using letters (Post-hoc

comparisons with Benjamini-Hochberg correction for multiple testing. SD156 vs LD156: $t=-0.699$, $p=0.48532$, all other comparisons; $t \geq -156.5$ $p<0.05$).

The blade length:width ratio displayed a significant difference between the ratio and genotype and day condition (Kruskal-Wallis: $df=5$, $\chi^2=73.658$, $p<0.01$). However, upon post hoc analysis, the genotypes did not have any significant differences between the day conditions ($p>0.05$ for WT/MIM/LD) displaying that the results are solely due to the genotypes (SD156/SDMIM vs SDWT: $p<0.01$, LD156/LDMIM vs LDWT: $p<0.01$), this means that compared to the analysis of the first AT, the day length effect is not present here and the SPL transcriptional pathway may not be directly involved in leaf shape formation or blade measurements. MIM and WT were also significantly different from the 156OE line but not to each other (MIM: $p<0.01$, WT: $p<0.01$).

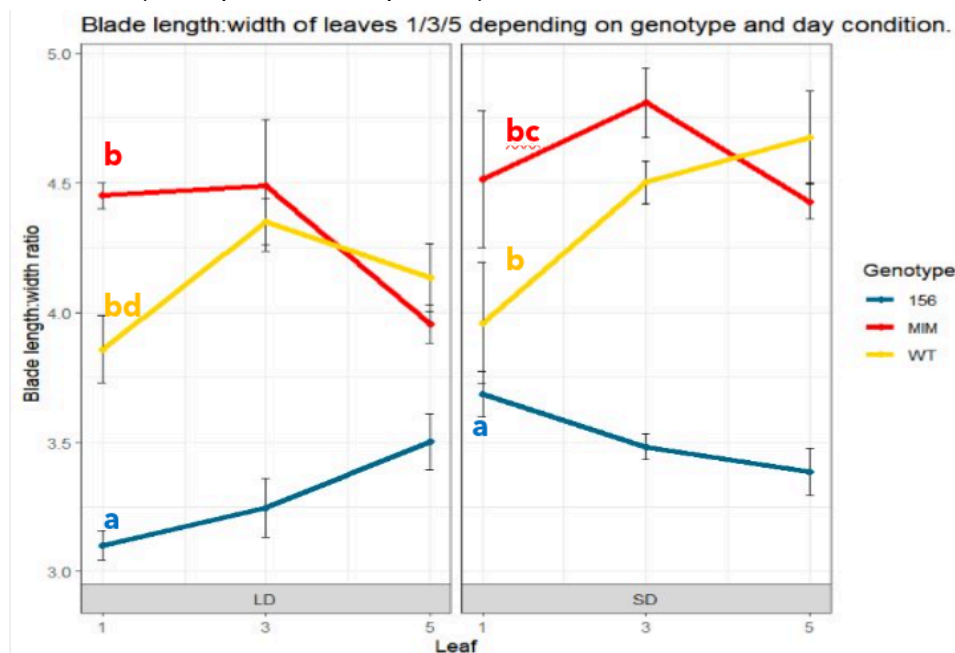


Figure 2. Blade length:width ratio of leaves 1/3/5 for WT, MIM and 156OE in both LD and SD, error bars indicate significant mean error. Significant differences are indicated using letters. (Post-hoc comparisons with Benjamini-Hochberg correction for multiple testing: SD MIM/SDWT vs SD156: $p<0.01$, LDMIM/LDWT vs LD156: $p<0.01$.)

Semi-Quantitative PCR

For the semi-quantitative PCR, it was split into primers used for the mature miRNA samples and primers used for the primary transcript samples. For both, general primers were used that were present in all the samples to act as standardised visual markers for the specific markers to be compared to. For the mature miRNA samples, Sno was used and for the primary transcripts, ACT and GAPC were used.

For the mature miRNA samples, none of the gels displayed clear enough bands, including Sno, so they are not included. For the primary transcript samples, the SPL9 and SPL13

samples displayed clear bands and displayed an expected increase in band strength as the weeks progressed for the WT SD samples (Fig. 3).

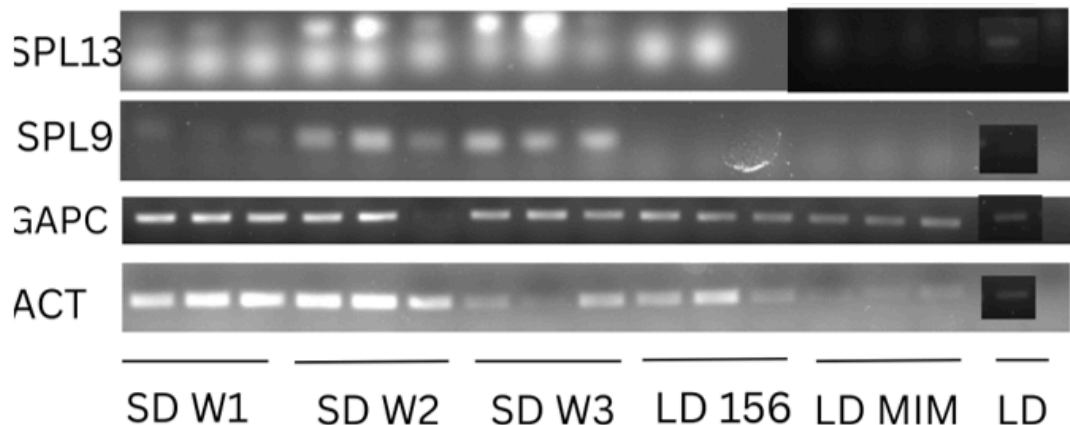


Figure 3. A semi-quantitative PCR of 9 WT *C.Sativa* samples, 3 taken per week, 3 LD 156 samples, 3 MIM and one WT LD control sample. ACT and GAPC are used as internal control genes. SPL9 and SPL13 primers were used to display the progressive level increase of SPL's in WT.

- **What did you learn from participating in this project?** (What did you enjoy, what skills did you learn, what did it teach you in terms of microscopy, imaging or flow cytometry? 100-200 words)

From this project, I was able to learn imaging techniques using the ImageJ software to create figures of leaf heteroblast that are of a high quality. I was also able to measure width and lengths of leaves from the varying genetic lines in order to analyse them to provide additional support for any subtle phenotypical differences between genetic lines.

I was also able to develop my molecular biology techniques as I was able to refine my DNA genotyping skills by learning how to conduct and alter PCR's if needed based on the polymerase or primers used, and I was also able to learn how to conduct refined RNA extractions, make cDNA and measure the levels via semi-quantitative methods.

- **How has this project affected your long-term goals?** (Will you do a PhD, or what career path will you choose, will you aim to do more microscopy in the future, 100-200 words)

This project gave me a very unique insight into what a career in research entails. Throughout this project, I was able to learn and see first hand how an experimental design can be created and shaped to answer and address a base hypothesis or question, what methods could be used to produce results and the reason these are chosen. This was extremely necessary for me to experience first hand as it was an area I previously felt was weak regarding the skillset I wanted to develop for my future career, however, I now feel more secure in it. Following this project, it has solidified my pursuit of doing a masters in research in order to gain more experience.