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Understanding the genetic mechanism of *Brassica napus* resistance to Cabbage Stem Flea Beetle

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Background:

The Cabbage Stem Flea Beetle (CSFB) is a major pest of winter oilseed rape (OSR; *Brassica napus*) in Europe. The ban of neonicotinoid pesticides by the European Union has caused an increase in CSFB pressure on winter oilseed rape, posing a significant challenge for farmers and growers in managing the crop. CSFB adults cause visible shot-holing damage on the leaves of young seedlings affecting crop establishment, while larvae mine and damage the petiole and stems, reducing plant survival and yield. Plant resistance offers a component of an integrated pest management (IPM) approach to controlling CSFB, but there is limited knowledge of OSR resistance against CSFB. Additionally, the mechanisms of plant-insect interactions between CSFB and OSR is not well understood.

Objective:

Overall, we aim to understand the genetic mechanism underlying *B. napus* resistance against CSFB. Previously, we identified *B. napus* lines showing variation in adult feeding in controlled laboratory experiments using 96 diverse lines (Hughes *et al.* unpublished).

Within this study we aim to:

1. Confirm that feeding variation observed between selected extreme lines is consistent and reproducible.
2. Conduct comparative transcriptomic analysis to identify changes in gene expression that are associated with CSFB feeding.
3. Identify genes that are involved in controlling feeding variation by CSFB in OSR.

Methods:

We conducted a controlled laboratory experiment to compare the feeding behaviour of CSFB on selected 'resistant' and 'susceptible' lines. One sexed beetle, controlled for age, was introduced to a non-choice assay chamber containing a seven-day-old seedling and left to feed for 24 hours. We assessed damage by calculating the percentage of area eaten.

We then used RNA-seq to investigate gene expression changes in response to beetle feeding in both lines. We subjected young seedlings of each genotype to either beetle feeding, no feeding (as control), and mechanical damage (to determine damage related changes in gene expression independent of beetle feeding). Samples were collected at 2-, 4-, and 8-hours post-treatment. Following Illumina sequencing, differentially expressed gene (DEG) analysis was performed using edgeR/Limma package in R to identify genes associated with beetle feeding and differences between genotypes.

Results:

Our results showed that the feeding damage caused by adult CSFB on young seedlings was significantly lower in the 'resistant' line compared to the 'susceptible' line, as shown by a lower percentage area damaged. This result is consistent with the findings of the previous experiments. In addition, preliminary results of our RNAseq experiment, analysed by PCA, revealed distinct gene expression clusters that were affected by both beetle feeding and mechanical damages across time in the two lines. Furthermore, we identified differentially expressed genes that were specific to plant response to CSFB attack.

Further analysis will be performed to investigate these changes and the role they play in OSR-CSFB interactions and variation in feeding.

Conclusions:

This research will increase our understanding of the interaction between OSR and CSFB, as well as the genes involved in defence responses against CSFB. This knowledge can be utilised to reduce the susceptibility of OSR to CSFB attack through genetic improvement strategies, such as the development of CSFB-resistant cultivars.