

## Novel approach to breeding resistance to fungal pathogens in lettuce

*Botrytis cinerea* and *Sclerotinia sclerotiorum* are two of the most important pathogens of field grown and protected lettuce crops in the UK causing grey mould and lettuce drop respectively. However, breeding of resistance against these two pathogens has remained an intransigent problem for breeders, partly due to the variation in disease pressure each year and the need to retain texture, taste and quality in resistant varieties. In this project we combine a novel big data approach with a more traditional quantitative genetics strategy to identify alleles and markers for resistance to these fungal pathogens which can be exploited in a commercial breeding program. Resistance against these pathogens is not an all-or-nothing trait but we aim to identify genes that can quantitatively reduce the impact of infection.

We have used a lab-based pathogen infection assay to enable us to rapidly screen large numbers of lettuce lines in a reproducible and quantitative way. Size of lesions on lettuce leaves after inoculation with fungal spores is used as a measure of susceptibility/resistance. We have screened a diversity set of wild and cultivated lettuce, of varying type and geographical origin, and demonstrated there was genetic variation within this set for resistance to *B. cinerea* and *S. sclerotiorum*. We have used this information to generate mapping populations by crossing lines with differing resistance. The progeny of these crosses have different combinations of the parental genes (and hence different levels of pathogen resistance) and are being used to map regions of the genome conferring quantitative resistance (Quantitative Trait Loci). In one population we have identified several such QTL and work is ongoing to narrow down these regions and determine markers tightly linked to resistance for use in breeding strategies.

We have tested our lab-based infection assays with trials in polytunnels and in the field. We used direct inoculation of whole plants in a polytunnel with spores of *B. cinerea* and *S. sclerotiorum*, and monitored the progression of disease symptoms. Disease levels in different lettuce lines in the polytunnel were strongly correlated with the results from inoculation of detached leaves. We also carried out a field trial to look at resistance to lettuce drop. Sclerotia from *S. sclerotiorum* were buried in the soil prior to lettuce being transplanted. Inoculation of the field in this way led to a high disease level enabling us to determine differences in susceptibility between different lines. The field trial will be repeated next season to ensure we are seeing reproducible differences.

At the same time we are using a big data approach to test whether this can speed up identification of alleles and markers enhancing resistance of lettuce to fungal pathogens. We have inoculated lettuce leaves with *B. cinerea* and separately with *S. sclerotiorum* and measured how the expression of lettuce genes changes over time in infected leaves compared to uninfected leaves (sampling every 3 hours). With up-to-date sequencing techniques, we can analyse expression of all the genes in lettuce at the same time and with analysis from multiple time points after inoculation and replicates, we have generated over 7 million measurements. We have used computational techniques to mine this big data set, determining genes that are responding to pathogen infection, and predicting which genes play a key role in activating the plant defence response, and hence the resistance of the plant.

We are now starting to test whether our predictions from the big data set are correct. We are using new genome editing techniques to knock out the gene and see if this reduces the ability of lettuce to resist infection. We are looking in the diversity set to see if the level of expression of these key genes differs between lines that are more or less resistant to the fungal pathogens, and looking at the genome sequence to see if our predicted key genes are located within regions that confer increased resistance (QTL). If we find that the predictions from our big data approach do confer enhanced disease resistance, we can focus on identifying markers to enable these genes to be bred into cultivated varieties.

If we are successful in breeding lettuce with enhanced resistance to *B. cinerea* and *S. sclerotiorum* these varieties will reduce losses from grey mould and lettuce drop, as well as enabling reduced chemical control. Given the increasing restriction on chemical control products and fungal resistance, as well as the cost of applications, we aim to reduce reliance on chemical control to promote cheaper, more sustainable leafy salad production in the UK.



Image from the field trial showing a resistant lettuce variety next to lettuces which have succumbed to lettuce drop (*Sclerotinia sclerotiorum* infection).