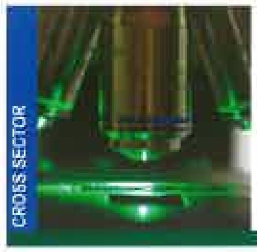




# Well-bred for a longer life

**New insights into lettuce genetics being gained in two projects funded through the Horticulture and Potatoes Initiative may lead to new varieties more resistant to post-harvest pathogens and discolouration, as Adam Talbot explains**

Most shoppers want crisp, clean-looking lettuce which stays that way for as long as possible after they've bought it. There are two reasons why that can't always be achieved in the supply chain, leading to wastage which erodes profitability.

First, there are a number of fungal pathogens, such as sclerotinia and botrytis, which attack at harvest or during storage after most of the costs of producing the crop have been incurred. Fungicides can be effective against them but the harvest intervals associated with some, and the increasing prevalence of fungicide resistance in pathogen populations, means they're not always an option. Growers therefore often drill a bigger area than they are contracted to do, which leads to waste in years when disease levels turn out to be low.


Preparing samples for analysis in work on post-harvest discolouration at Harper Adams University. Inset: the genes of varieties which show particularly high and low levels of pinking or browning are being compared

The second issue is post-harvest pinking and browning, particularly in cut and bagged salads. Modified atmosphere packaging reduces the incidence of discolouration in packs but once opened, it then tends to develop rapidly, moving the waste burden onto consumers with potential implications for their future perception of the product.

Both issues have attracted the attention of plant breeders over many years but the very fact that both affect crops late in the production and supply cycle has made them particularly difficult to deal with using traditional plant breeding methods.

When looking for tolerance to these late-striking pathogens, or resistance to post-harvest discolouration, breeders have to grow plants to maturity and, probably, process and pack them to commercial standards before they can analyse them to identify individuals with the right traits – and that goes for each of the many generations of breeding lines required to end up with a commercial variety. It takes 10 to 15 years to develop a new variety that way, so breeders are looking to save time and costs by finding ways to screen lettuces at an earlier stage. And that's now possible, using genetics-based approaches to pinpoint plants with desirable genes early in their growing cycle.

The principles are similar for most crops – AHDB has already co-funded some of this type of work in soft fruit, for example. First, a population of plants is screened to identify those with traits you want. Then the DNA from each member of the population is extracted and compared for differences known as genetic markers. Finally, the genetic markers from the candidates with the desirable traits are compared to the overall population, which highlights correlations between a small number of genetic markers and the traits.

Once breeders have this information they can look for the same genetic markers in other populations – the seedlings resulting from a cross, for example. The advantage of using markers is that potential candidates for use in a breeding programme can be analysed at a young stage, without needing to grow them to maturity before testing for desirable traits.

Of course, it is important that breeders don't select for one trait, such as disease resistance, at the expense of another, such as an aspect of taste. So researchers need to understand the mechanisms that result from the genetic differences between plants in a breeding population. They need to know precisely how a particular marker is linked to enhanced resistance to fungal pathogens or development of pinking and browning symptoms.

For example, the overall flavour of lettuce is a result of the balance between levels of bitter and sweet-tasting compounds. But some of the compounds that are involved in pest and disease resistance are the same as those that are responsible for the bitter flavours. By understanding the molecular and biochemical processes underpinning the traits the industry wants, breeders can select for production of compounds that enhance pest resistance but avoid those that spoil the flavour.

To help breeders, two projects funded through the government's Horticulture and Potato Initiative are attempting to clarify the biochemical mechanisms that lead to post-harvest discolouration and identify sources of tolerance to sclerotinia and botrytis. They are also working to find genetic markers linked to these mechanisms that can be used in lettuce breeding programmes to reduce the impact of disease and enhance shelf-life.

## DISCOLOURATION

Jim Monaghan is leading the work on discolouration at Harper Adams University. He and his team started with a population of lettuce from a cross between Saladin (an Iceberg type) and Iceberg (actually a Batavian type). They have found variation for both types of post-harvest discolouration – pinking and browning – in this population and have been able to identify specific groups of genes that are correlated with this variation – a process known as quantitative trait loci, or QTL, mapping.

These QTL regions are still large numbers of genes on the chromosomes so to narrow down the relevant ones Monaghan's team is now exploring how they differ between individual plant lines which show particularly high and low levels of pinking or browning. This is done by analysing to what extent each gene is active – or 'expressed'. Genes whose expression correlates with the appearance of pinking or browning may be responsible in some way for those symptoms.

**PROJECT PROFILES**

**CP 150 A genetic approach to improving post-harvest quality**
**Term:** May 2015 to May 2018
**Project leader:** Jim Monaghan
**Location:** Harper Adams, Reading and Warwick universities
**Industry partners:** G's Fresh, Rijk Zwaan

**CP 152 A systems approach to disease resistance against necrotrophic fungal pathogens in lettuce**
**Term:** May 2015 to May 2018
**Project leader:** Katherine Denby
**Industry representatives:** Frances Gawthorp and Mel Miles
**Location:** Reading, Warwick and York universities

Meanwhile, researchers at the University of Reading are using various laboratory analysis techniques to find differences in plant compounds associated with differences in post-harvest symptoms. By combining the genetic locations identified from all these strands of evidence the project team aims to pinpoint the specific genes responsible for controlling the appearance of post-harvest pinking and browning. They can then develop genetic markers for these traits for breeding.

A 'diversity set' – a group of around 100 lettuce types sourced from around the world, including commercial varieties and cultivated and wild plants – will then be screened for the presence of the appropriate genes to provide new sources of breeding material.

## DISEASE RESISTANCE

This population of varieties was also the starting point for the second project, on botrytis and sclerotinia resistance, being led at the University of York. Plants of each type were challenged with each disease and a small number proved highly resistant to one or both pathogens. These included a number of wild relatives of lettuce which have previously been sources of useful traits such as aphid resistance. Now the aim is to incorporate this disease resistance into a breeding programme.

Lettuces from the Saladin x Iceberg cross growing at Harper Adams University, Shropshire

Two lettuce lines with varying resistance levels have been crossed and groups of genes are being identified that appear to correlate with differences in disease resistance among the offspring. Genetic markers associated with these can then be mapped.

As in the work on post-harvest discolouration, the disease resistance project is also investigating gene expression to help it analyse the processes underpinning disease resistance. The researchers at York are also looking at the genetics responsible for the 'power balance' between pathogen and host.

Modern techniques enable every gene within the plant to be simultaneously analysed during infection to see how it's switched on or off as the pathogen attacks, allowing researchers to track how the plant activates its immune system. This will help them to find which genes are the most critical to the plant immune system. If successful, this approach should be broadly applicable not only across most lettuce varieties and types, but to other closely related crops and diseases.

The Reading University team is contributing, too, by investigating the compounds that lettuce produces to fight off pathogens, and establishing which ones are also linked to other traits such as flavour or shelf-life.

By running these projects in parallel the research teams are able to work together and hope to identify genetic markers that can be used to select for both delayed post-harvest discolouration and disease resistance.

Once the genetic markers are proven, within the next year, breeders from the projects' industry partners will be able to start their work to bring the useful genes into commercial varieties designed to help reduce waste in the lettuce supply chain.

**ABOUT THE AUTHOR**
Adam Talbot is a post-doctoral research assistant at the University of York. Government funding for the Horticulture and Potatoes Initiative is provided through the Biotechnology and Biosciences Research Council.