

Project title: A genetic approach to improving post-harvest quality (HAPI)

Project number: CP 150

Project leader: Prof. Dave Pink, succeeded by Dr James Monaghan

Report: Annual report, May 2016

Previous report: N/A

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Location of project: Harper Adams University, University of Warwick, Reading University and industry partner sites.

Industry Representative: Rijk Zwaan and G's Fresh are industry partners in the work.

Date project commenced: 4th May 2015

Date project completed 3rd May 2018

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

[Name]

[Position]

[Organisation]

Signature Date

Report authorised by:

[Name]

[Position]

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GROWER SUMMARY

This grower summary has been extracted from the report produced for BBSRC, with kind permission of Dr Jim Monaghan.

Headline

- Regions of chromosomes on the lettuce genetic map have been identified that control cut surface discolouration.
- In general, the cut leaves of lettuce lines either go pink or brown suggesting that these pathways differ at some stage.

Background

Minimal processing adds significant value to fresh produce, however, it also increases its perishability reducing shelf life and leading to waste of the produce and the resources used to grow it. This project is aimed at postharvest discolouration, a significant cause of quality loss in a wide range of fresh produce such as sliced apple, cut cabbage and lettuce. The main issue we are addressing is postharvest discolouration of lettuce in salad packs. UK lettuce production/imports are worth £240m farm gate but the retail value of UK processed salads is £800m. However, Tesco have recently reported that 68% of their salads are thrown away; the situation is similar for other retailers. There is therefore a need to improve postharvest quality to reduce waste and deliver consistently good quality products to consumers. Modified atmosphere packaging can provide control but once the pack is opened oxygen enters resulting in discolouration. Growing conditions also influence postharvest discolouration but are difficult to control in field crops. We are proposing breeding lettuce varieties with reduced propensity to discolour as a way to address the problem. To do this we need to understand the genetics and biochemistry of discolouration.

We are building on previous PhD research at Wellesbourne which identified genetic factors controlling the amount of pinking and/or browning that developed on lettuce leaves in salad packs 3 days after processing. However, we do not know what compounds or which genes are involved and we now intend to find this out by a multidisciplinary project involving three universities; Harper Adams, Reading and Warwick, a lettuce breeding company, a lettuce grower, a salads processor and AHDB Horticulture. We have produced a set of experimental lettuce lines which we know show differences in the amount of pink or brown discolouration they produce. We will grow and process these lettuces in a way that mimics commercial production. We will then assess the salad packs for the amount of discolouration developing over 3 days, which is the current best before date for supermarket salads. We can then link this information to the plant's DNA profile to identify genetic factors for discolouration and

DNA markers which can be used by plant breeders. The same lettuces will also be analysed for compounds produced by a biochemical pathway called the phenylpropanoid pathway. This is thought to produce the pigments that cause discolouration. We know from other studies in a plant called Arabidopsis the genes which control the phenylpropanoid pathway and we have found the same genes in lettuce. We will see how these genes behave in lettuce plants that produce a lot of discolouration and ones that don't discolour. We will also see how the genes behave under different growing conditions. We can link these gene expression patterns to the amount of pinking and browning to see which genes are the key ones. Once we have done this we can look for naturally occurring versions of the genes which give a reduced discolouration.

The compounds produced by the phenylpropanoid pathway influence other things such as pest and disease resistance, taste etc. We do not want to reduce the amount of discolouration by breeding but end up with lettuce susceptible to pests or with poor taste, so we will assess lines which show high discolouration or no discolouration for their resistance to aphids and mildew and for taste to see if there are any differences. There are some compounds produced by the pathway which are colourless but still provide some resistance so by knowing the genetics and biochemistry breeders will be able to carry out smart breeding.

We will see if the results for lettuce hold true for other crops by seeing how the key genes behave in apple, cabbage and babyleaf lines (*amended end of Yr 1, see below*) and whether this is related to the amount of browning that develops when they are processed and look for genetic differences in these crops.

Summary

Project Objectives:

1. Increase understanding of the genetics of pinking and browning in lettuce
2. Determine the role of phenylpropanoid (PP) pathway in lettuce discolouration
3. Determine whether non PP pathway genes have a role in lettuce discolouration
4. Test the robustness of a genetic approach to reducing discolouration.
5. Identify potential sources of beneficial alleles for key genes.
6. Assess the potential impact on pest and disease resistance and taste
7. Test the applicability of the findings from lettuce to cabbage and apple

Amended to

7. Test the applicability of the findings from lettuce *to babyleaf*, cabbage and apple.

Progress to date:

Year 1 was initially focused on field trials at HAU to grow 94 of the previously determined most informative lines for post-harvest discoloration from a set of F₈ recombinant inbred lines (RILs), derived from a cross of cv. Saladin x cv. Iceberg generated from the Defra VEGIN programme. These were sown in triplicate in randomised field trials at Harper-Adams University (HAU) along with the parental lines. Mature heads were harvested, chopped and sealed into pillow packs for storage. Pinking and browning symptoms were recorded at 0, 1 & 3 days post-harvest and samples subsequently flash frozen in liquid nitrogen and freeze dried. Dried material was sent to the University of Reading (UoR) for metabolite analysis. Assays have been developed for compound groups and enzymes associated with the phenylpropanoid and flavonoid synthesis pathway (potentially involved in discolouration based on previous work), e.g. total phenolic compounds, total quinones, polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activities. These assays were then employed on the milled material from the RILs.

Results to-date indicate lower levels of compounds and enzyme activity associated with lettuce heads with iceberg-type morphology compared to those with cos-type morphology (a phenotype which segregates within the RILs). This morphotype difference may also be associated with symptomatic differences, with browning symptoms being expressed more strongly and pinking less strongly in cos-type RILs than in the iceberg-type. This, coupled with data from some commercial lines selected for a reduced pinking phenotype that did not show a reduction in browning, suggest that the two symptoms may be the result of different pathways (although possibly sharing some genetic components). Quantitative Trait Loci (QTL) analysis of the phenotype data has initially identified 8 QTL for pinking, 12 for browning and 1 additional discoloration QTL. Further analysis to refine these QTL, map QTL for the morphotype differences in the RILs and compare the refined QTL with those produced in previous work is underway.

Year 2 work

We are growing more replicates of the RILs and meteorological data recorded during the year 1 field trials will be used to compare environmental effects with replicate trials in year 2 and with results from selected lines expressing extreme phenotypes (*i.e.* high and low levels of either pinking or browning) grown in controlled environments. These extreme lines will also

be used to investigate gene expression profiles and assessed for a number of quality traits and pest and pathogen resistance. The expression profiling results will be used to select further target genes, which will be screened in the lettuce genetic diversity set in year 2 and in other crop types, including lettuce and brassica baby leaf salad. Metabolic analysis (UoR) of these diversity set accessions will also be carried out which when combined with data from the RILs will allow us to understand what biochemical pathways are of greatest importance in causing discolouration of the cut surfaces.

To facilitate the screening of targeted genes in wider crop types, initial assays have been developed for apple and cabbage. This work has now been extended in year 2 to include babyleaf lines where the research will study the discolouration along the petiole and across cut leaves. This work will allow us to take the research from cut lettuce leaves to a wider range of minimally prepared fresh produce crops.

Work will continue in 2016/17.