Developing Breeding and Selection Tools to Reduce Spoilage of Soft Fruit and Wastage in the Supply Chain

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Introduction

In the UK, fruit softening in the berry fruits remains the main cause of waste and lost revenue with losses at the farm gate estimated to be up to £50 million in a poor season. However, just a one to two day improvement in shelf-life would increase the value of harvested fruit and reduce waste.

Softening of fruit is largely dependent on genes and their action under differing environmental conditions. Breeders currently select seedlings to provide good fruit firmness and shelf-life, but this can be a time-consuming and costly process.

Objectives

This Horticulture LINK-funded project aims to develop robust assisted breeding and selection tools using red raspberry (*Rubus ideaus* L.; Fig. 1) as a model crop that will enable breeders to accelerate development of new varieties having an extended shelf-life and reduced fruit spoilage.

Fig. 1 Red raspberry (*Rubus ideaus* L.)

Materials and Methods

The genetics of softening were investigated by using a 'Latham' × 'Glen Moy' mapping population of 188 progeny and Roche 454 sequencing of fruit-derived RNA. Phenotypic screening of raspberry fruit was carried out by a 'breeder score' of firmness on the bush and 16 quantitative measurements using a QTS-25 Texture Analyzer.

Candidate genes with expected roles in fruit softening were identified using the 454 transcriptome datasets and subsequently mapped onto one of the seven *Rubus* linkage groups (LG) (Woodhead *et al*., 2010) using JoinMap (v.4.0).

The trait data collected was also assigned to the Rubus linkage map by quantitative trait loci (QTL) mapping analysis using MapQTL (v.5.0).

In an effort to produce consistent and high quality data from qPCR studies, the *m*inimum *i*nformation for publication of *q*uantitative real-time PCR *e*xperiments (MIQE) guidelines (Bustin *et al*., 2009) were followed. Reference gene validation was carried out using the geNorm software (Vandesompele *et al*., 2002).

Different stages of raspberry fruit (immature green, mature green, white fruit, white/red fruit, ripe fruit) including leaf were harvested from three biological replicates of cultivars 'Glen Moy' and 'Latham' and RNA isolated.

References

Bustin,S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J. and Wittwer, C. T (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry* **55**:611–622.

Woodhead, M., Weir, A., Smith, K., McCallum, S., MacKenzie, K. and Graham, J. (2010). Functional Markers for Red Raspberry. *Journal American Society Horticultural Science* **135**:418–427

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A. and Speleman, F. (2002). Accurate Normalization of Real-Time Quantitative RT-PCR Data by Geometric Averaging of Multiple Internal Control Genes. *Genome Biology* **3**: RESEARCH0034.

Results

Analysis of variance revealed significant differences among the 'Latham' × 'Glen Moy' mapping progeny for the 'breeders score' of firmness ($p<0.001$), and QTS-25 measurements of the traits Hardness (Newtons, N; $p=0.005$), Rigidity (N; $p=0.052$), Final load (N; $p=0.005$), and Force/Mass_N/kg ($p<0.001$). The mass value (10 berry weights) also varied significantly between the progeny ($p<0.001$).

The positions of QTLs for Hardness, Rigidity, Final load, and Force/Mass_N/kg, together with the 'breeders score' and mass were predominantly located on LG3 (Fig. 3). Several candidate genes, including those implicated in cell wall metabolism (pectinmethylesterase, PME; β-1,4 xylan hydrolase, XL), ethylene biosynthesis (*S*-adenosylmethionine decarboxylase, SAMDC) and regulation of turgor pressure (aquaporin) are significantly associated with several of these QTLs (Fig. 2) and were chosen for further study.

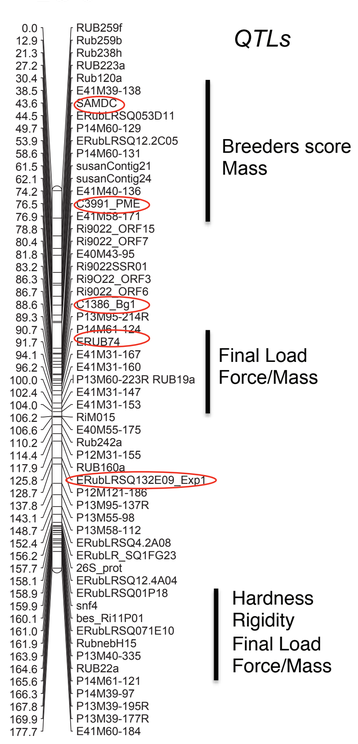

Fig. 2 Genetic linkage group 3 of red raspberry ('Latham' × 'Glen Moy') showing some of the candidate softening genes (circled) and associated softening QTLs under investigation

The geNorm software indicated that the novel reference genes Clathrin, YLS8, and TIP4 were the most suitable for accurate normalization with raspberry fruit samples. This is the first report of a systematic validation of reference genes for gene expression studies in *Rubus*.

Analysis of variance revealed several significant differences in normalized expression levels at various fruit stages between 'Glen Moy' and 'Latham' (Fig. 3).

The mean expression levels for aquaporin and PME show a similar steady rate decline during ripening, SAMDC shows the opposite trend of a steady increase up to the ripe fruit stage, and there is a significant rise in XL expression in ripe fruit (Fig. 3). The expression levels were always greater in the firmer variety 'Glen Moy' than 'Latham' although the patterns followed the same trend in both cultivars.

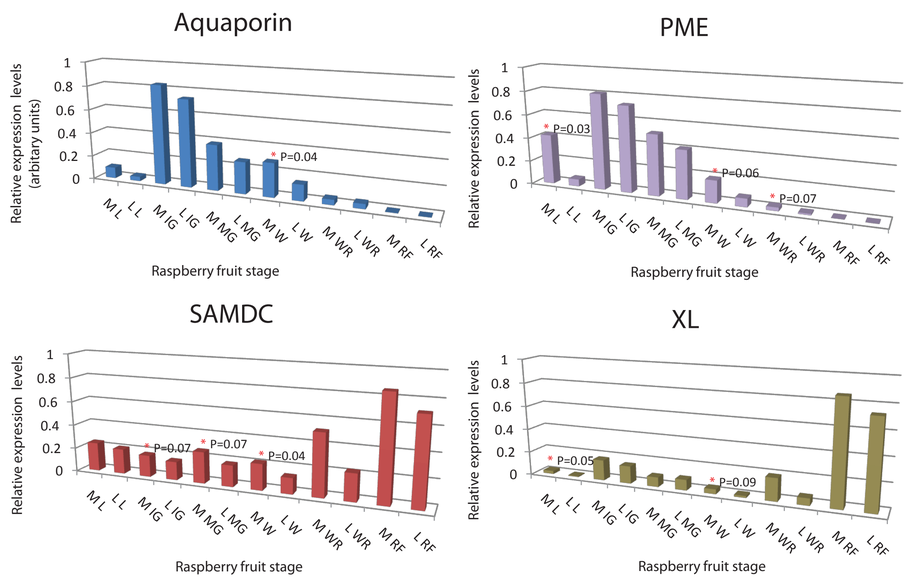

Fig. 3 Mean normalized relative gene expression levels obtained for each candidate gene in different developmental stages of fruit and leaf in 'Glen Moy' (M) and 'Latham' (L).

Key: L, leaf; IG, immature green; MG, mature green; W, white fruit; WR, white/red fruit; RF, ripe fruit. Significant differences in expression levels are indicated next to asterisk.

Correlations were also evident in gene expression levels when scatter plots (log10 scale) categorised by fruit stage are plotted (Fig. 4):

- Strong negative relationship between SAMDC and aquaporin, between SAMDC and PME, between XL and aquaporin, and between XL and PME.
- Very strong positive relationship between PME and aquaporin, and a positive relationship between XL and SAMDC.
- All of these correlations are driven by stage of fruit development, and within each stage the relationship is almost always positive.

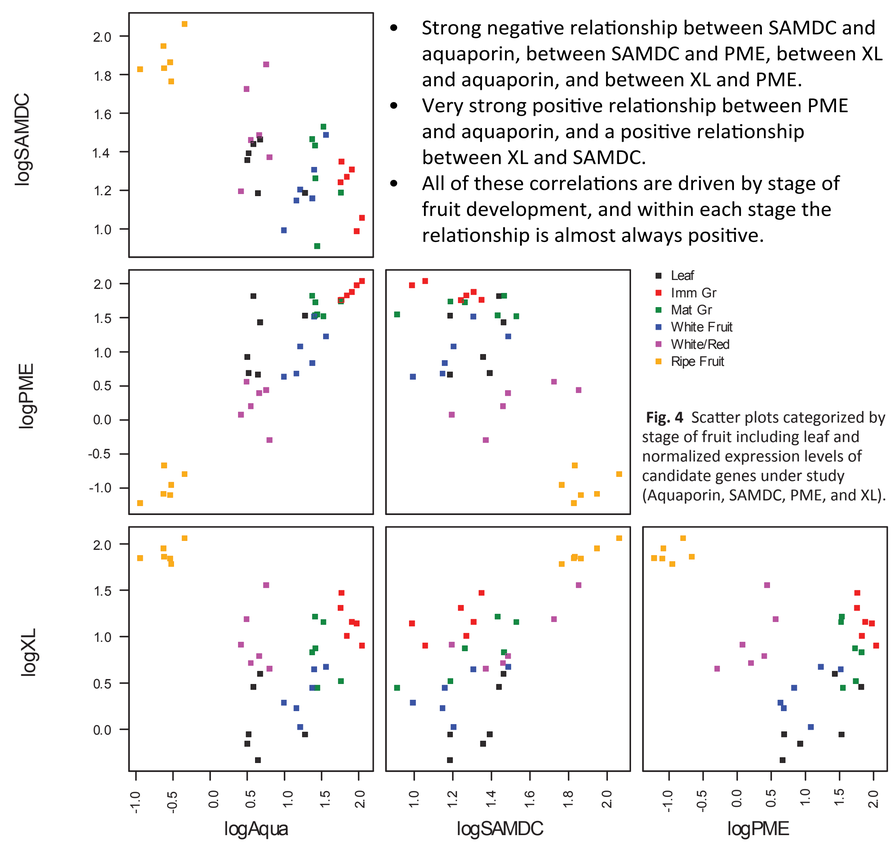

Fig. 4 Scatter plots categorized by stage of fruit including leaf and normalized expression levels of candidate genes under study (Aquaporin, SAMDC, PME, and XL).

Conclusions

The data indicates a co-ordinated expression between the candidate genes studied during fruit development and ripening whose differential expression could be related to the variation in fruit firmness, a major quantitative breeding trait.

Cell wall disassembly and the reduction of cell adhesion are the main factors that contribute to the overall fruit texture and firmness, and the largest changes in the cell wall occur in the pectin fraction. Work is now underway to investigate the gene expression profiles of other pectin degrading genes and inhibitors in other cultivars. In addition, an Agilent *Rubus* microarray has been developed to identify additional candidate genes, which altogether, should ultimately help provide a better understanding of the important fruit softening controls in *Rubus* and which may be applicable to other members of the Rosaceae.

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