Analysis of genes differentially expressed during fruit ripening in raspberry (*Rubus idaeus* cv. Glen Clova)

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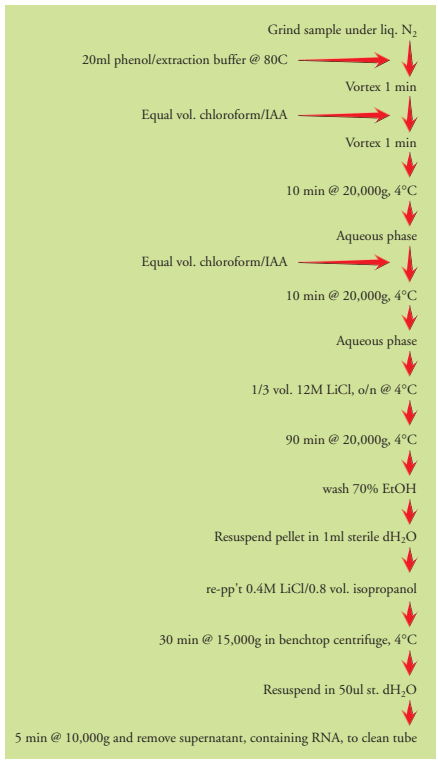
Introduction

In common with many soft fruits, the potential exists to improve the post-harvest and processing characteristics of raspberry fruit using a transgenic approach. Such an approach requires an understanding of the alterations in gene expression, occurring during ripening which give rise to changes in fruit texture, flavour and appearance. This poster describes the development and application of techniques, that will lead to improved raspberry fruit quality through genetic manipulation.

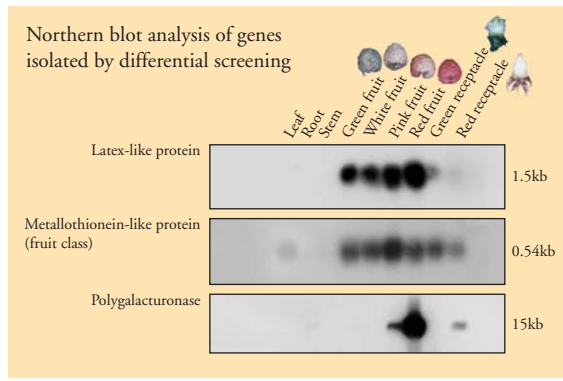
Results

Extraction of RNA from raspberry fruit.
A pre-requisite for the successful cloning of fruit genes is the availability of good quality RNA. We have developed a method for the isolation of RNA from raspberry fruits at all stages of development, receptacle, leaf, root and stem tissue (Jones *et al.*, in press). This protocol may be suitable for the extraction of RNA from other tissues containing high concentrations of RNases and polysaccharides.

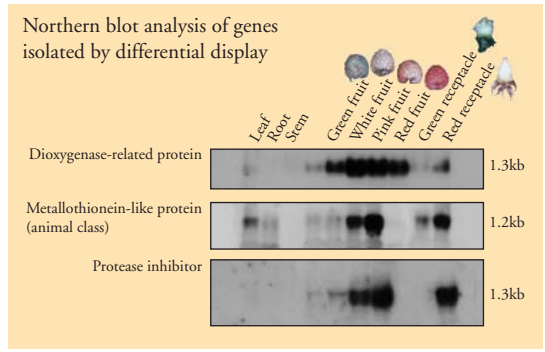


Isolation of differentially expressed genes.
A number of techniques have been reported for the isolation of differentially expressed genes. Each have advantages and disadvantages associated with them. We have applied three such methods to the ripening raspberry fruit.

(1) Conventional differential screening.
Approximately $5x10^3$ plaques were screened with cDNA probes from early and late fruit ripening stages, yielding three differentially expressed genes.
Latex-like protein With sequence similarity to the wound-responsive major latex protein gene from opium poppy. The raspberry gene also shows similarity to genes up-regulated in ripening fruits of muskmelon and bell pepper.
Metallothionein-like protein Metallothioneins are heavy metal binding proteins involved in metal ion detoxification and have been implicated in protecting against oxidative stress. Sequence similarity has been demonstrated to genes up-regulated in the ripening fruit of kiwi, blackcurrant, papaya, apple and banana.
Polygalacturonase (PG) The most intensively studied cell-wall hydrolase with a key role in the softening of ripening fruits. Sequence similarity has been demonstrated with the endoPGs of apple and peach. EndoPG enzyme activity in the peach is correlated with the melting-flesh phenotype. PG enzyme activity has been correlated with the softening of ripe raspberry fruit (P. Iannetta *et al.*, submitted).



(2) Differential display.
A comparison of the mRNA profiles of green, white, pink and red fruit has been made, using the HIEROGLYPH mRNA profile system (Genomyx/Beckman). This technique has so far yielded clones representing sixteen genes up-regulated in the ripening raspberry fruit. Sequence similarity has been demonstrated with several genes, including:
Dioxygenase-related protein With greatest sequence similarity to ***1-aminocyclopropane-1-carboxylic acid oxidase*** (***the ethylene forming enzyme***). As in the tomato, ethylene is intimately involved in raspberry fruit ripening.
Metallothionein-like protein A cysteine rich sequence with no similarity to the gene isolated by differential screening. The cloning of two metallothionein-like genes increases the potential significance of these metal binding proteins in the developing fruit.
Protease inhibitor With sequence similarity to isoaprotinin, a protease inhibitor with antibacterial activity from bovine lungs. Protease inhibitors have an insecticidal role in plant tissues.



(3) cDNA AFLP.
cDNA-AFLP analysis has produced twenty six sequence tags apparently particular to the ripe fruit. Sequence similarity searches have to date enabled the identification of one:
Pectin methyl esterase (PME) A second important cell-wall hydrolase. The activity of this enzyme increases with raspberry fruit ripening (P. Iannetta et al.). RNA dot blot analysis indicates that the gene is expressed throughout the ripening stages.

Summary

A catalogue of genes that are up-regulated during raspberry friut ripening is being assembled. So far, tags representing twenty up-regulated genes have been isolated. From these, several targets have been selected which will be used for studies into the transgenic improvement of raspberry fruit quality.

Acknowledgements

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