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Effect of heat stress on gene expression profile of annual fruiting rasberries

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The Interreg IVB North Sea Region Programme

Hypothesis:

Heat stress regulates the gene expression profiles in raspberries

Objective:

To study the effect of heat stress on gene expression in annual fruiting raspberries by microarray and qRT-PCR

Materials and Methods

Four cultivars:
Autumn Bliss, Autumn Treasure, Erika and Polka

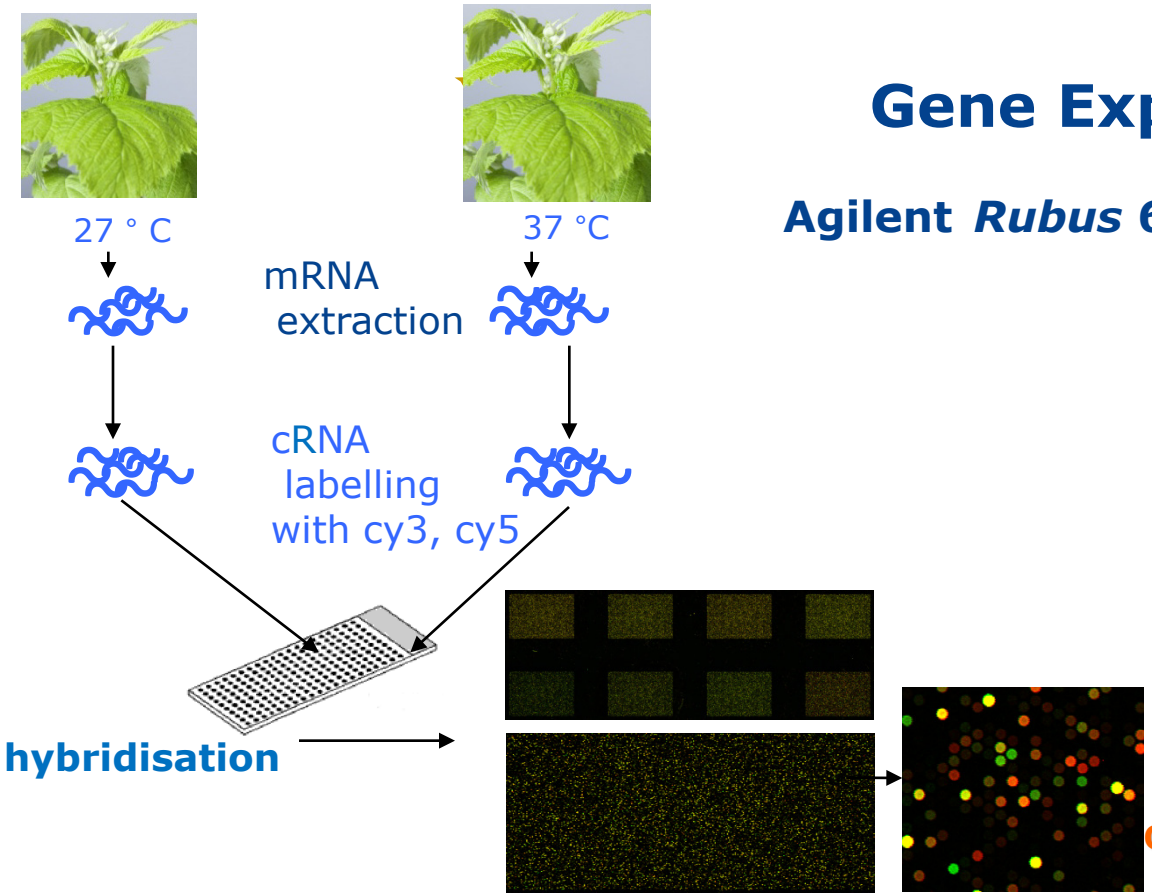
Greenhouse conditions:
20 ±2 °C (D/N), 14 h light and 10 h dark
50-60% RH, 350 µmol $m^{-2}s^{-1}$

Seven weeks until flower initiation

Climate chamber for 24 h
Similar conditions
New leaf collected

Gene Expression

Agilent *Rubus* 60k microarray

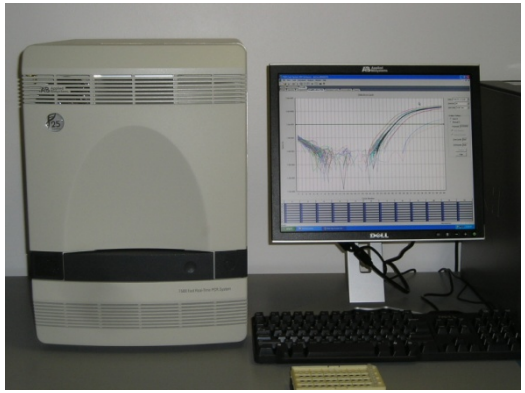


Red : active in 27 °C

Green : active in 37 °C

Orange : active in both

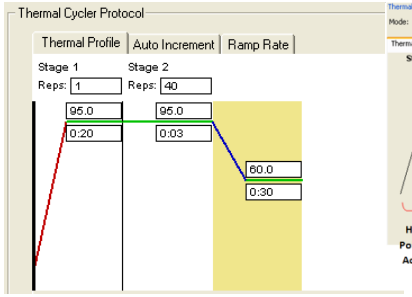
Validation
AB7500 Fast Real-Time qPCR

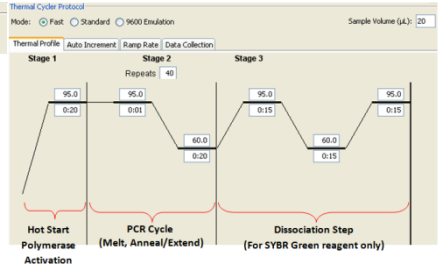


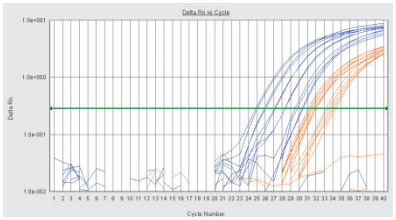
UPL probe/primers designed for 4 candidate genes:
CysP, MLP, PMP and Aquaporin

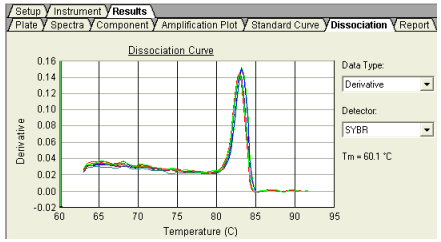
Efficiency (E) for each assay was calculated from standard curves

Normalization
Reference gene- *Rubus_GAPDH*
27 °C as control





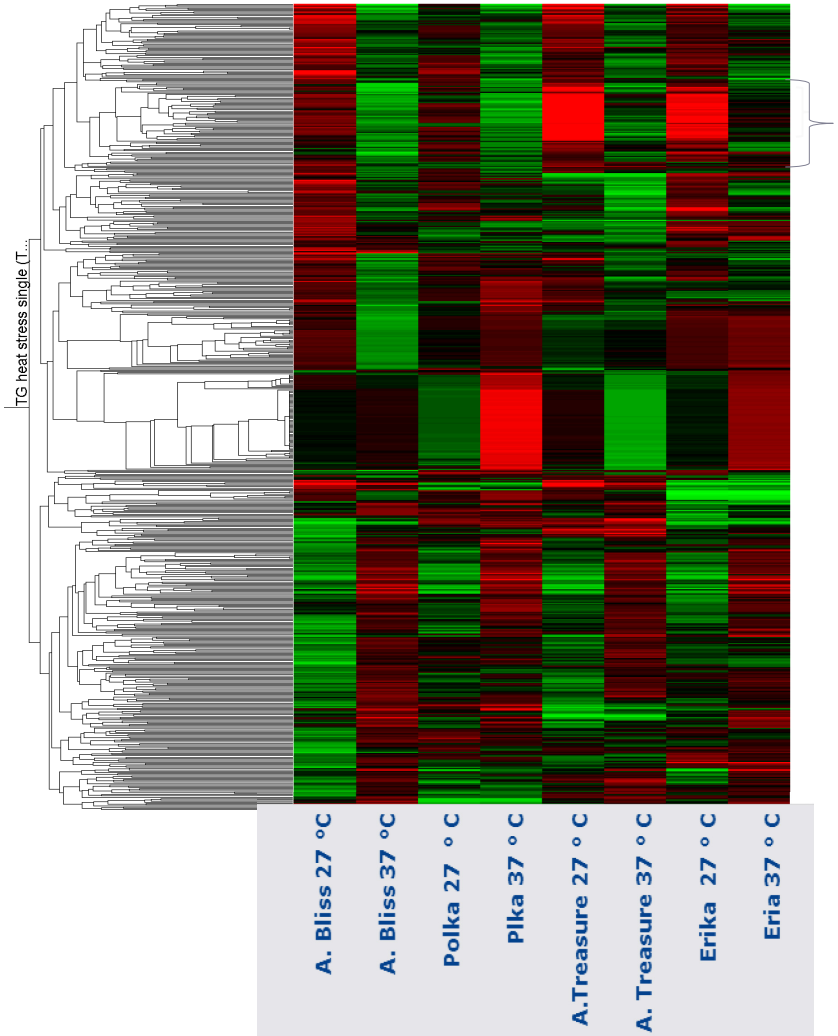




CysP=Cysteine protein; MLP= Major Latex like protein; PMP=Plasma membrane protein;

UPL=Universal probe library; GAODH = Glyceraldehayde 3-phosphate dehydrogenase

Results so far..



Selected probe -38 gene sets

These potential candidates include genes encoding major latex-like & stress related proteins

Figure-Heatmap of volcano filtered probes generated from cDNA microarray data reflecting gene expression at 27 and 37 °C

Statistical tests was done by using **Volcano Plots** which combined Student's T-test (p-value <0.05). For Autumn Bliss this identified 427 probes, and for Erika 229 probes.

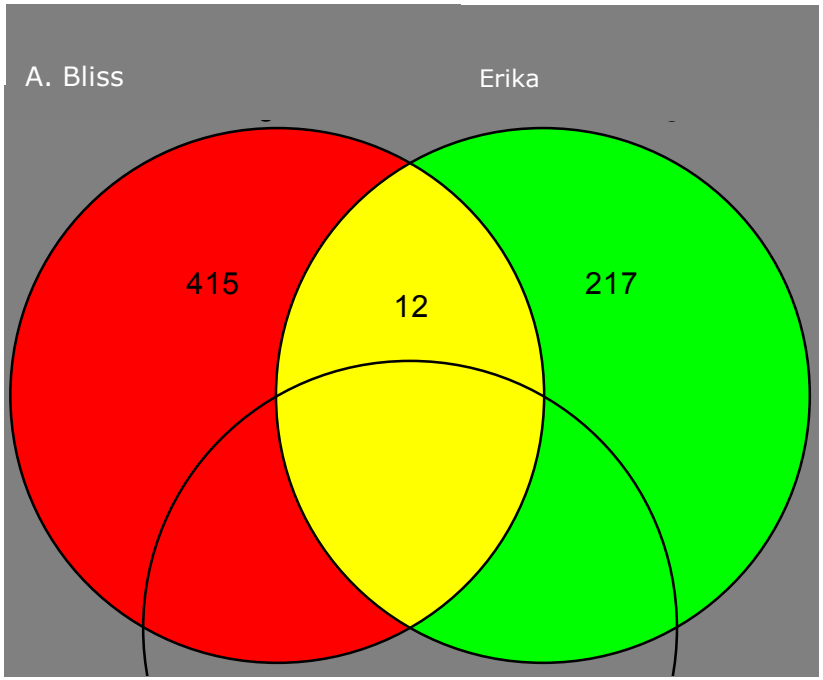
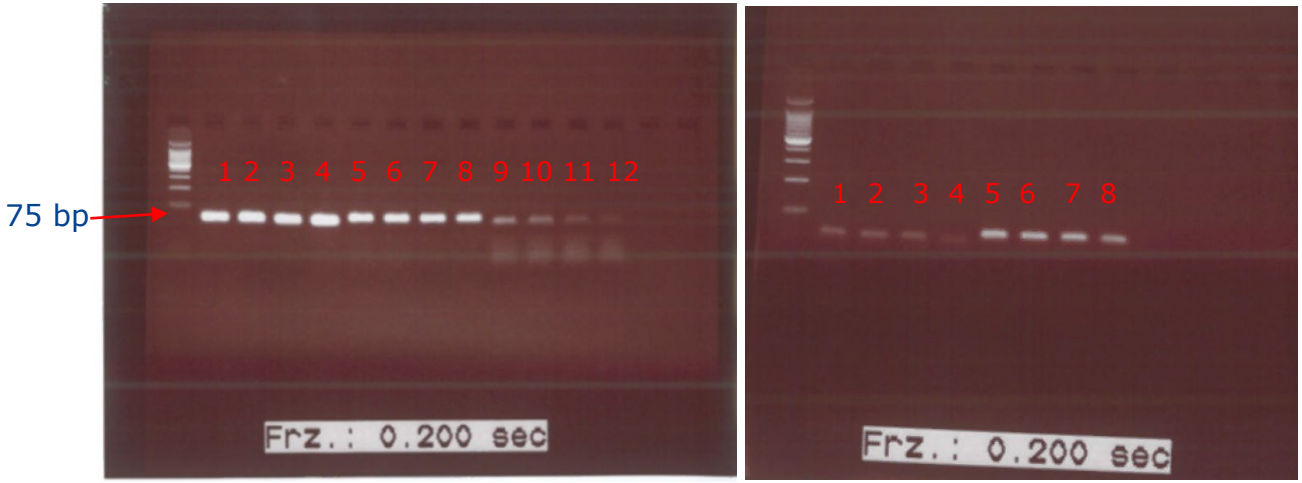


Figure- Venn diagram showing the overlapping and non overlapping probes in 'Autumn Bliss' and 'Erika ' at 27 and 37 °C

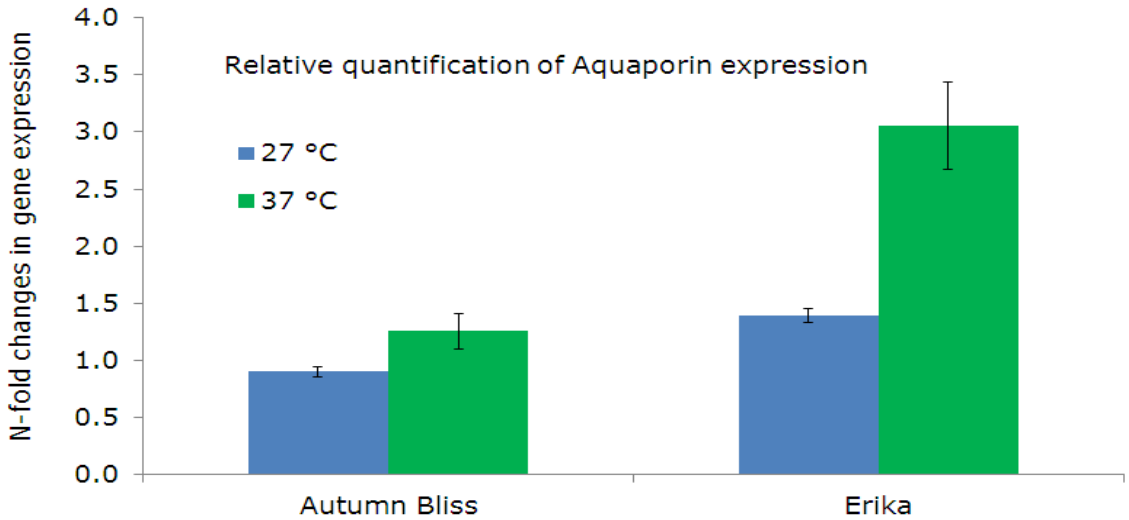
From 38 gene sets, 4 genes from '**Autumn Bliss**' and '**Erika**' were selected for validation using qRT-PCR

Cysteine protein ↓
Major latex like protein ↓
Plasma membrane protein ↓
Aquaporin ↑

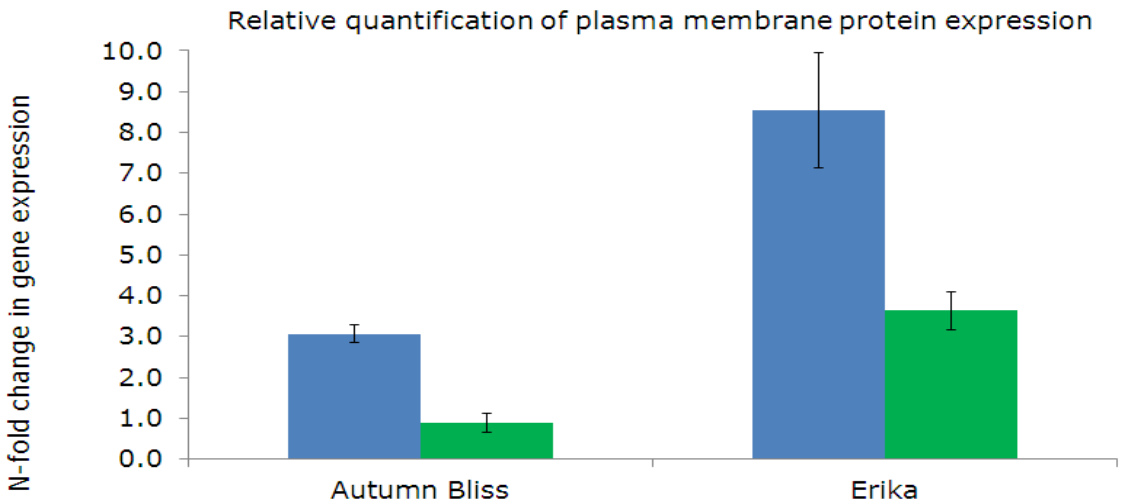


Aquaporin (1-4; *Rubus*_AQUA),
GAPDH (5-8; *Rubus*_GAPDH)
Plasma membrane protein, PMP (9-12; *Rubus*_PMP)

PMP (1-4) and GAPDH (5-8) using SYBR GREEN as detector



UPL Probe



SYBR Green

Figure- N-fold changes in expression of an Aquaporin and Plasma Membrane Protein in 'Autumn Bliss' and 'Erika' at 27 and 37 °C.

Conclusions

- The expression of aquaporin gene **increased** by 1.5-fold in 'Autumn Bliss' and 3-fold in 'Erika' grown at 37 °C as compared to 27 °C
- The expression of plasma membrane protein gene **decreased** by 2-fold in 'A. Bliss' and 3-fold in 'Erika' grown at 37 °C as compared to 27 °C

Acknowledgement

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Future-proofing berryfruit
ClimaFruit