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

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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⁻²s⁻¹
Seven weeks until flower initiation

Climate chamber for 24 h
Similar conditions
New leaf collected

27 °C

37 °C
Gene Expression

Agilent *Rubus* 60k microarray

- mRNA extraction
- cRNA labelling with cy3, cy5
- Hybridisation

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 = Glyceraldehyde 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.
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