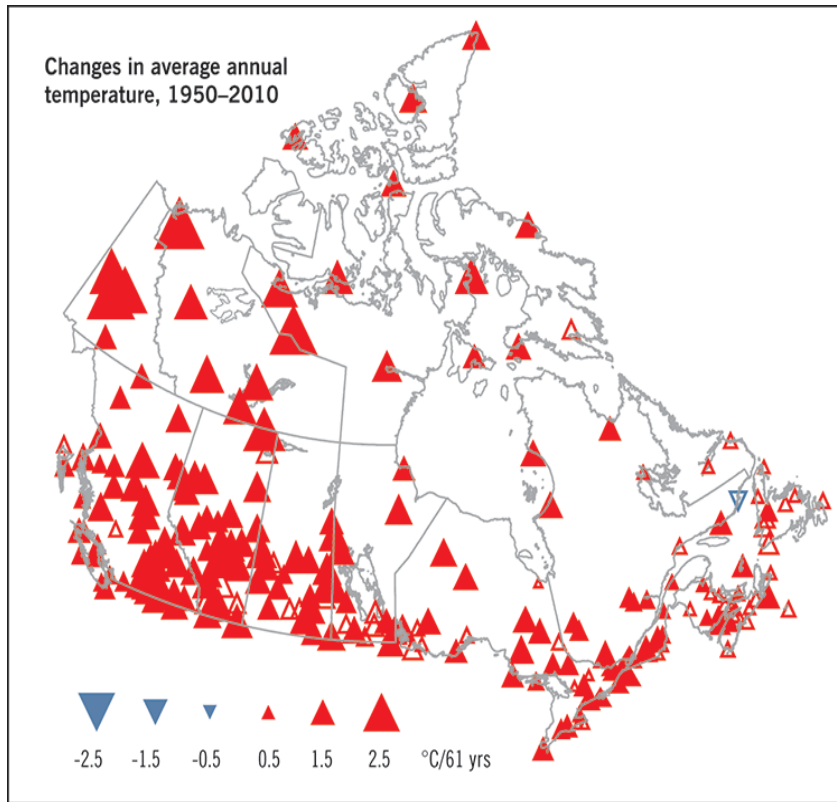# Pea Heat Stress-Responsive Transcriptome Analysis

Shaoming Huang,   PhD Candidate

Supervisor: Dr. Tom Warkentin

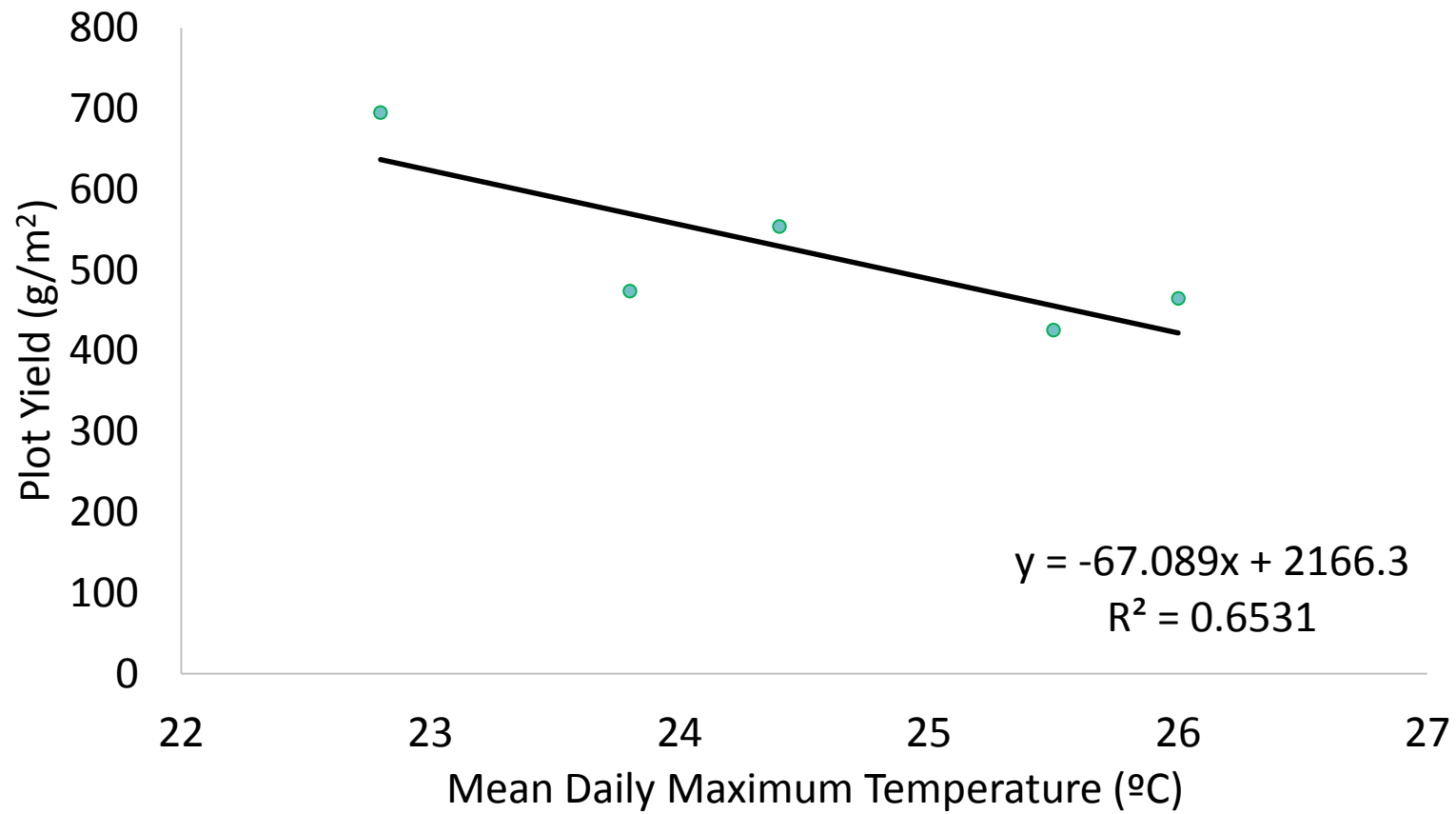# Warming Canada

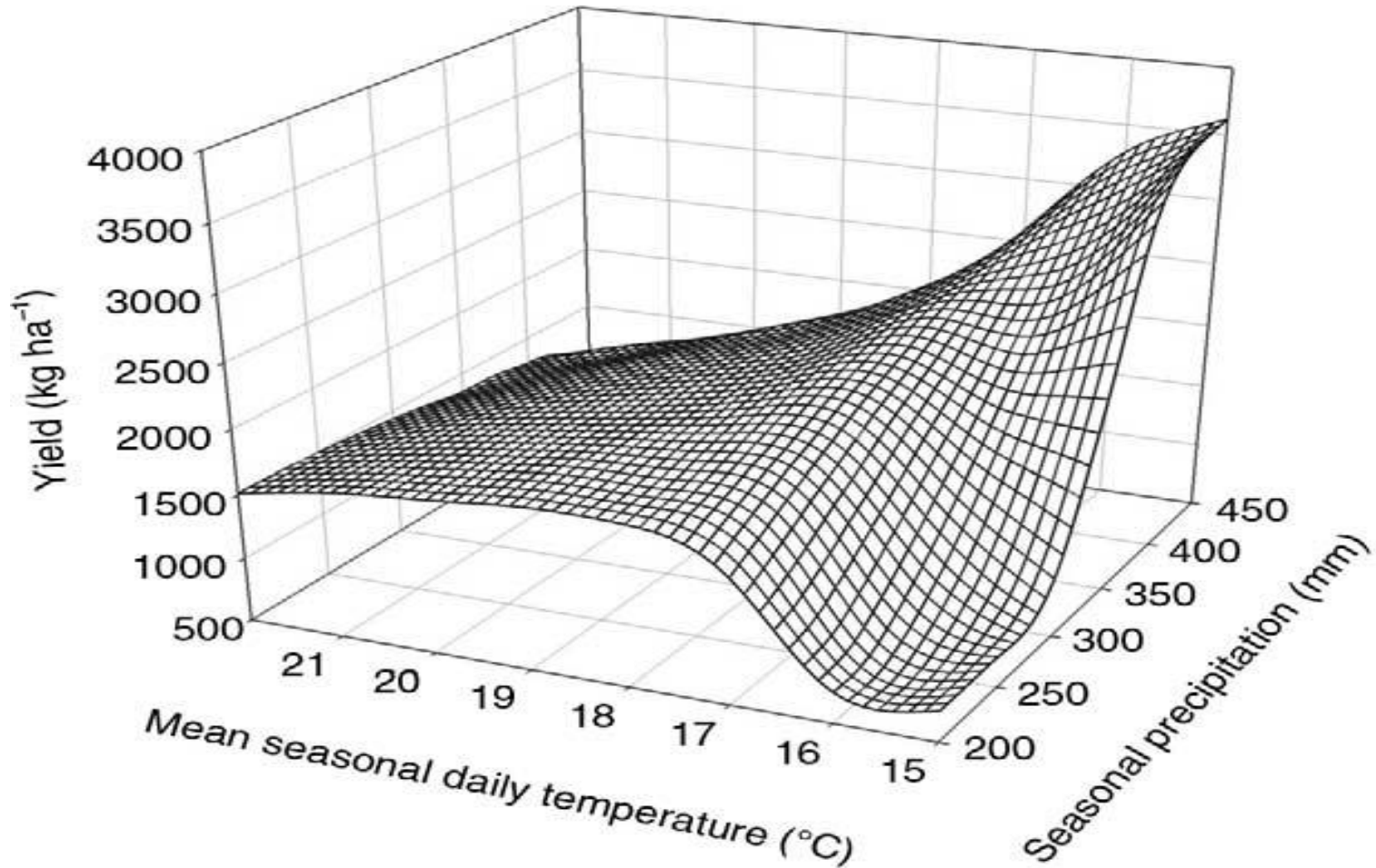Source: Vincent et al (2012) Environment Canada

Saskatchewan Pulse Growers

Negative Yield Response to Higher Temperature at Flowering

y = -67.089x + 2166.3
R² = 0.6531

Huang, 2016. MSc thesis, University of Saskatchewan.

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Pea Yield Response to Seasonal Temperature and Precipitation

Bueckert et al. 2015. *Can. J. Plant. Sci.* 95, 629-639.
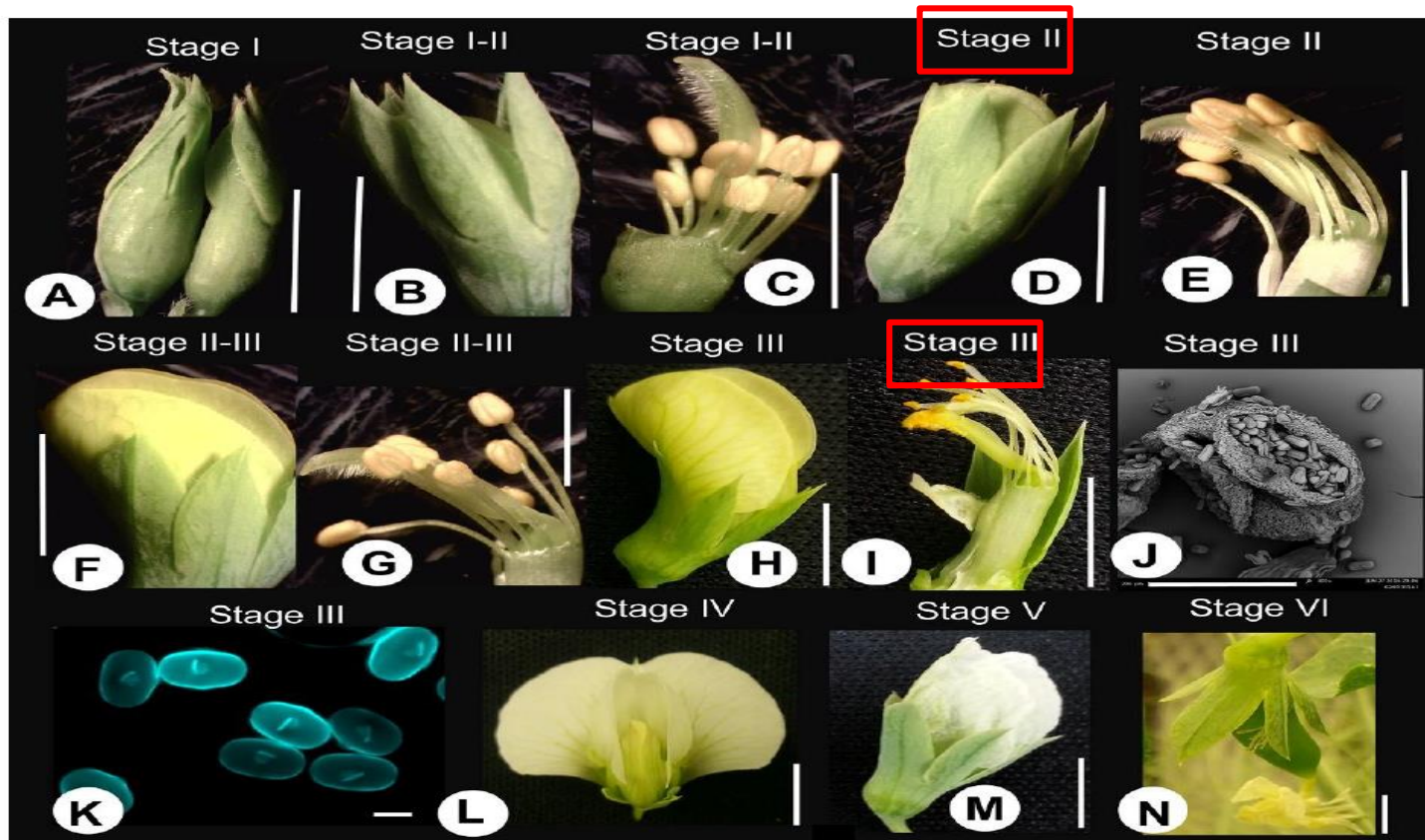
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Heat Stress on Pea

- Shortens life cycle

- Reduces pollen viability

- Induces flower, ovule and pod abortion

- Lowers individual seed weight

Jiang et al. 2015. *Plant Cell Environ*. 38, 2387-2397.
Huang et al. 2017. *Crop Sci.* 57, 1540-1551.

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Most Heat Sensitive Flowering Window

Jiang, 2016. Phd thesis, University of Saskatchewan.

Discovery of Heat Responsive Genes
via Transcriptome Profiling

- Heat responsive transcriptome profiling via microarray has been conducted in *A. thaliana*; wheat; barley; canola.

- The discovered genes are relating to heat shock protein, heat shock factor, reactive oxygen species etc.

- The expression of heat responsive genes varies among different species and among different organs within a species.

RNA-Seq in Pea – Reports so far

Roche 454 based RNA-Seq
- First transcriptome atlas (Franssen et al., 2011)
- Deep mining of SSR and SNP markers via transcriptome sequencing (Kaur et al., 2012; Duarte et al., 2014)
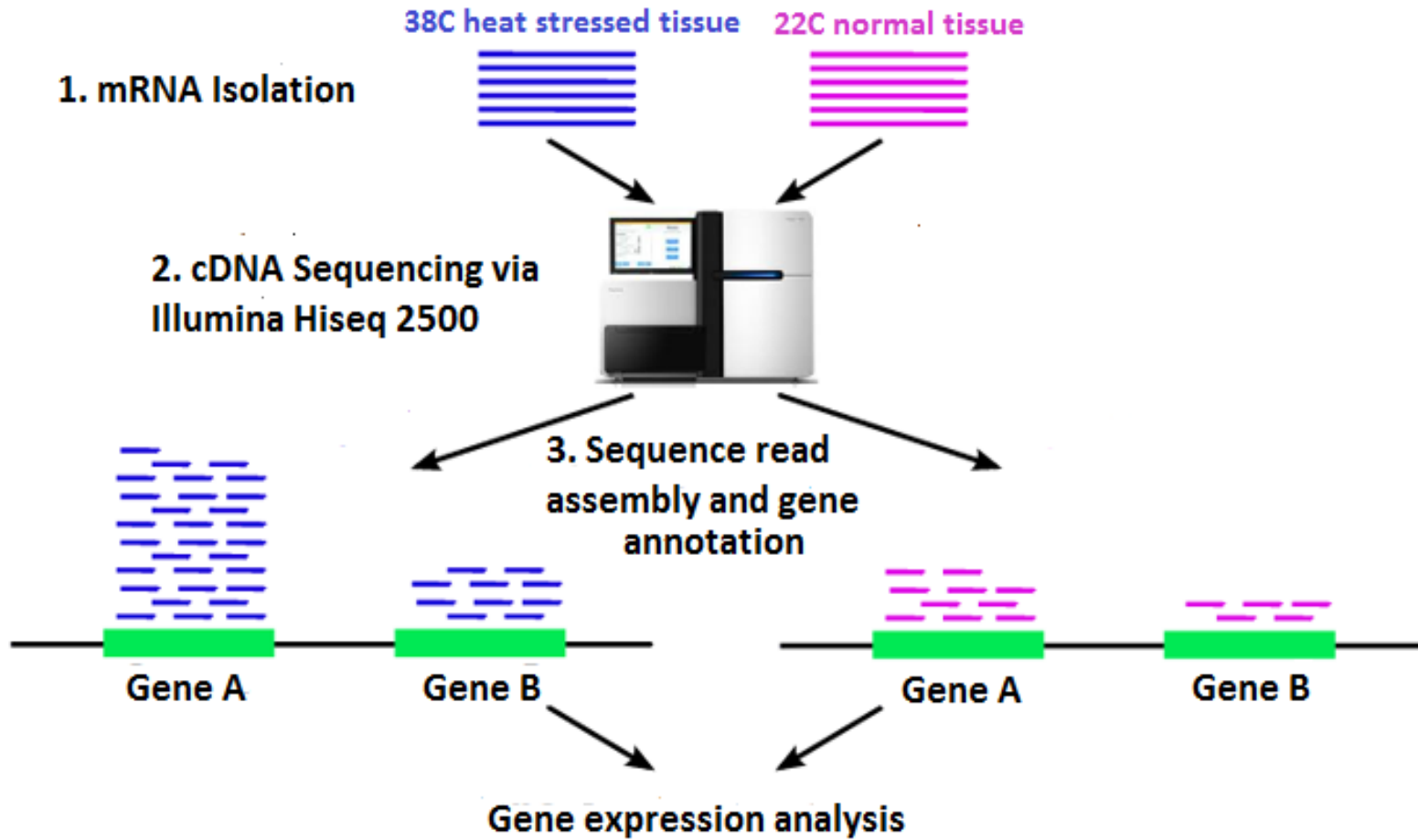
Illumina based RNA-Seq
- First *de novo* assembly of pea transcriptome sequencing data derived from Illumina platform (Sudheesh et al., 2015)
- Characterization of nodule transcriptome (Alves-Carvalho et al., 2015)

Objectives

1. Profile pea leaf and anther responses to high temperature at the transcription level via Illumina based RNA-seq

2. Compare the heat responsive gene expression between a heat tolerant and a heat sensitive pea variety

3. Build a heat responsive gene expression atlas in pea

Experiment Protocol

1. mRNA Isolation

38C heat stressed tissue   22C normal tissue

2. cDNA Sequencing via Illumina Hiseq 2500

3. Sequence read assembly and gene annotation

Gene A   Gene B   Gene A   Gene B

Gene expression analysis

Sequence Data Analysis

***De novo* assembly**

1. Assembly first method (softwares e.g. Oases, SOAPdenovo)

2. Bruijin graph method (softwares e.g. Trinity, Velvet)

Functional annotation of assembled contigs

BLASTN (threshold E-value <10^{-10}) against published pea transcript sequences as well as NCBI database of *M. truncatula*, chickpea and soybean

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Plant Material

Two pairs of heat tolerant and sensitive pea genotypes

Heat Tolerant: CDC Meadow   PR11-2

                          VS   VS

Heat Sensitive: Nitouche   PR11-90

Preliminary Validation based on RT-PCR of two genes encoding *PsHsp 18* and *70*

- Seeds planting

- Temperature treatment

- Tissue grinding

- RNA extraction

- cDNA synthesis

- RT-PCR (target genes *PsHsp 18* & *70*)

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Experimental Design

RCBD (4 genotypes X 4 time points X 2 temperature treatment X 3 reps=96 pots)

High temperature (38 /16 C, 16/8 h) vs normal temperature (22/16 C, 16/8 h)

Time points:

3h, 6h, 12h, 24h

Tissues: leaf and anther

Phytotron chamber, University of Saskatchewan

Some Symptoms of Heat Stress

38C   12h   24h   24h

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Preliminary Validation based on RT-PCR of two genes encoding *PsHsp 18* and *70*

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- cDNA synthesis

- RT-PCR (target genes *PsHsp 18* & *70*)

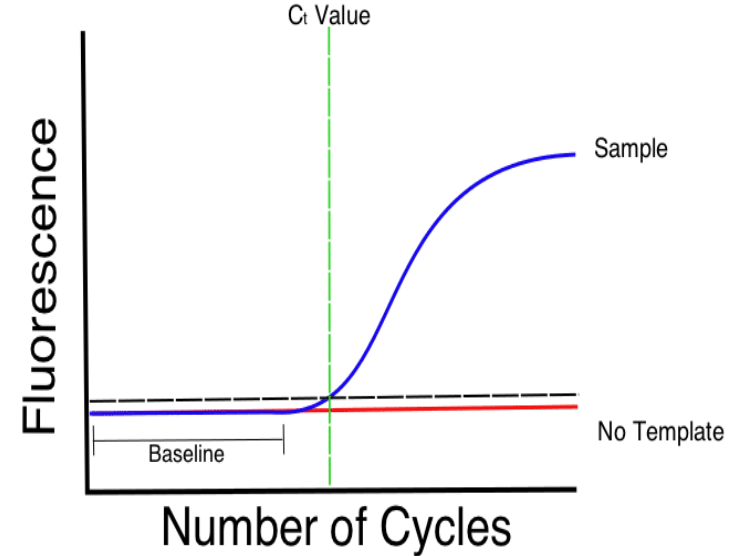# Data Analysis

Ct Value: threshold cycle

Fold Change Formula:

$2^{(-\Delta Ct)} = 2^{-(Ct\ gene\ of\ interest\ -\ Ct\ reference\ gene)}$

ΔCt = Ct gene of interest – Ct reference gene

Outlier Data Check:

Q1-1.5*(Q3-Q1) ≤ data ≤ Q3+1.5*(Q3-Q1)

Results

1. Selection of Reference Gene

GH720838 encodes transcription factor IIA

	Anther Sample	Leaf Sample
Average Ct Value	19.44±0.63	20.65±0.66
CV (100%)	3.31	3.25

2. Good Data Consistency between Technical Reps

Sample	Rep1	Rep2	Differential	Significant Level
Anther	19.47±0.61	19.41±0.66	0.06	0.56
Leaf	20.59±0.73	20.72±0.58	0.13	0.29

Results

- 3. Relative Low Expression of Hsp Genes on Non-heat Stressed Plant

Genotype	Leaf sample		Anther sample	
	Hsp18 gene	Hsp70 gene	Hsp18 gene	Hsp70 gene
PR11-2	0.18	0.07	0.41	0.16
PR11-90	0.11	0.05	0.26	0.05
CDC Meadow	0.19	0.05	0.25	0.07
Nitouche	0.30	0.12	0.41	0.11
Mean	0.20	0.07	0.33	0.10

Values are fold change, which are described as $2^{(-\Delta Ct)} = 2^{-(Ct\ gene\ of\ interest\ -\ Ct\ reference\ gene)}$

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Results

4. Up-regulation of Hsp Genes under High Temperature

Time points	Leaf sample		Anther sample	
	Hsp18 gene	Hsp70 gene	Hsp18 gene	Hsp70 gene
0h	0.20±0.11c	0.07±0.05c	0.33±0.11d	0.10±0.08c
3h	396.07±30.16a	154.68±12.84a	76.88±6.18a	31.77±3.06a
6h	130.94±30.51b	33.95±13.92b	58.43±6.18bc	24.20±2.98ab
12h	113.85±30.51b	30.60±13.62b	69.36±6.18ab	24.08±3.06ab
24h	77.91±31.18b	28.12±13.92b	47.79±7.05c	19.43±3.34b

Values are fold change, which are described as $2^{(-\Delta Ct)} = 2^{-(Ct\ gene\ of\ interest\ -\ Ct\ reference\ gene)}$
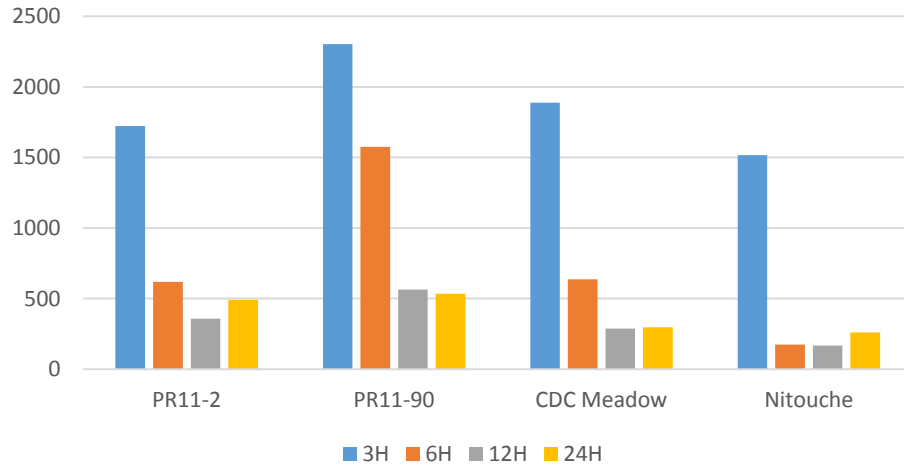
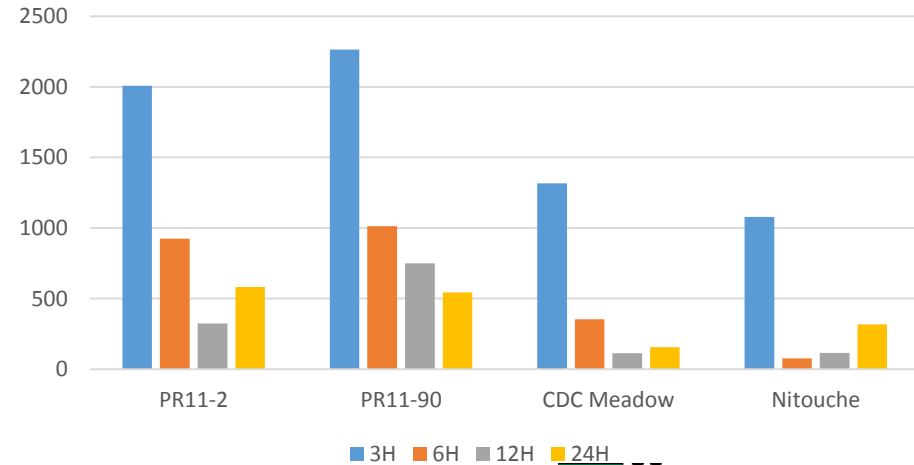# Results

Anther Hsp18 Gene Expression Fold Change
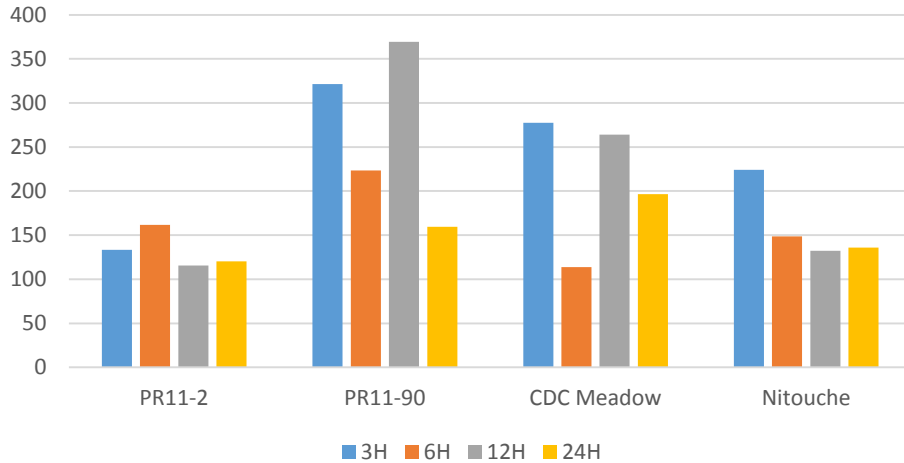
Anther Hsp70 Gene Expression Fold Change
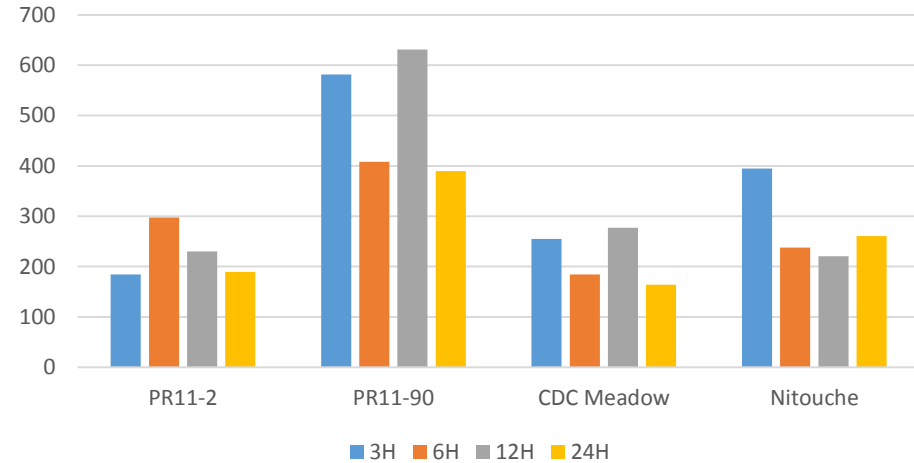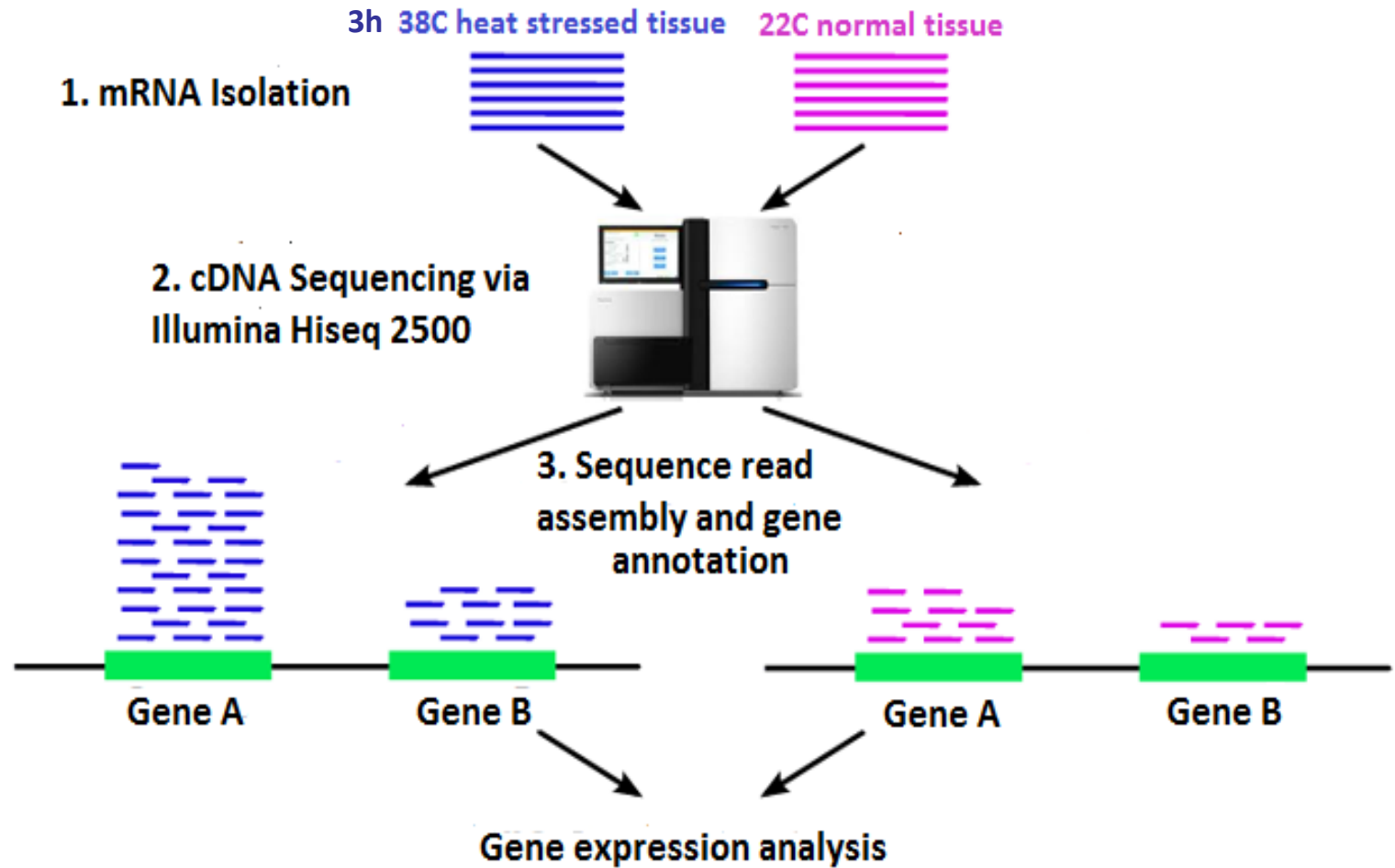
Leaf Hsp18 Gene Expression Fold Change

Leaf Hsp70 Gene Expression Fold Change

Summary

1. For leaf sample, 3-hour is most responsive timing; for anther sample, the most responsive timing seems to be genotype specific. In general, the expression of hsp genes are most up-regulated after 3 hours at 38C.

2. For both genes, the relative gene expression differential between PR11-2 & PR11-90 is bigger than the differential between CDC Meadow & Nitouche.

RNA-Seq Experiment Underway

Acknowledgement