

SPICY: Towards automated phenotyping of pepper plants

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EPPN workshop

Novel Sensor Technologies for Plant Phenotyping

13 September 2012



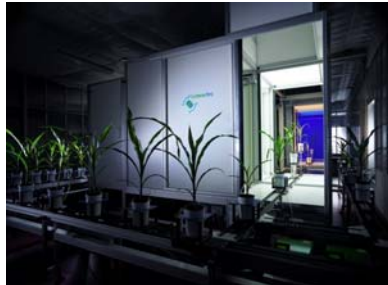
Introduction

Aim of the EU SPICY project:
Develop tools to predict phenotypic response of a genotype under a range of environmental conditions.

This requires characterisation of a large number of genotypes by measurements, preferably automated.



Phenotyping by Image Analysis



Scanalyzer 3D, LemnaTec



Sorting *Anthurium* cuttings,
Wageningen UR

- Most image analysis systems for automatic phenotyping bring the plants to the camera.



**Commercial tomato plants
in Almeria, Spain**



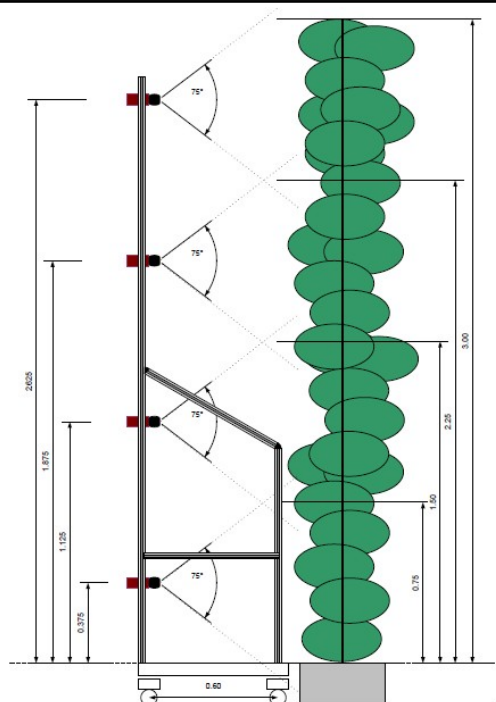
**Pepper plants in our
experiments**



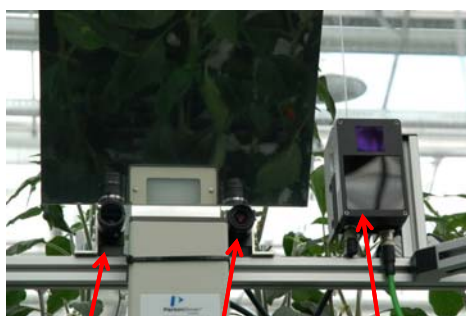
But for some crops, like pepper and tomato, this is not feasible ⇒ bring the cameras to the plants!



SPYSEE equipment



SPYSEE



4* IR, Colour, Range (ToF) cameras

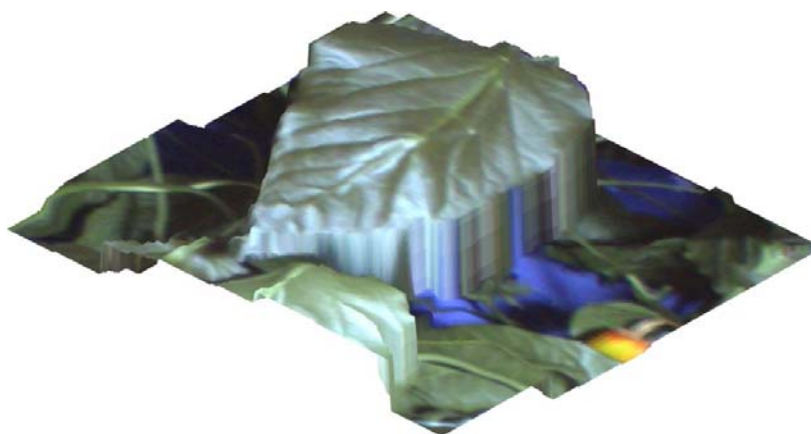


Automated phenotyping

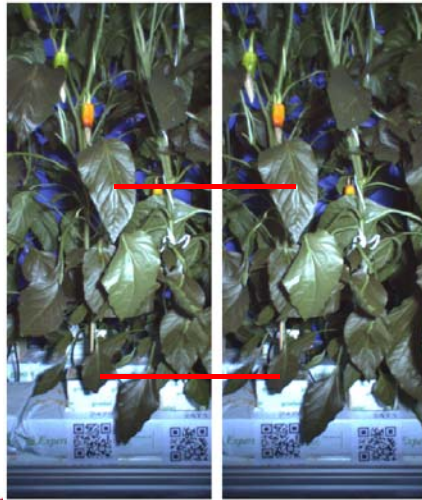
- We aim to :
 - Replace manual by automatic measurements
 - Find new features, which are not possible or too difficult for manual measurement
- Two approaches:
 - A. 3D
 - B. Statistical



A. 3D approach



Stereovision



3D information can be recovered from stereo pairs, because

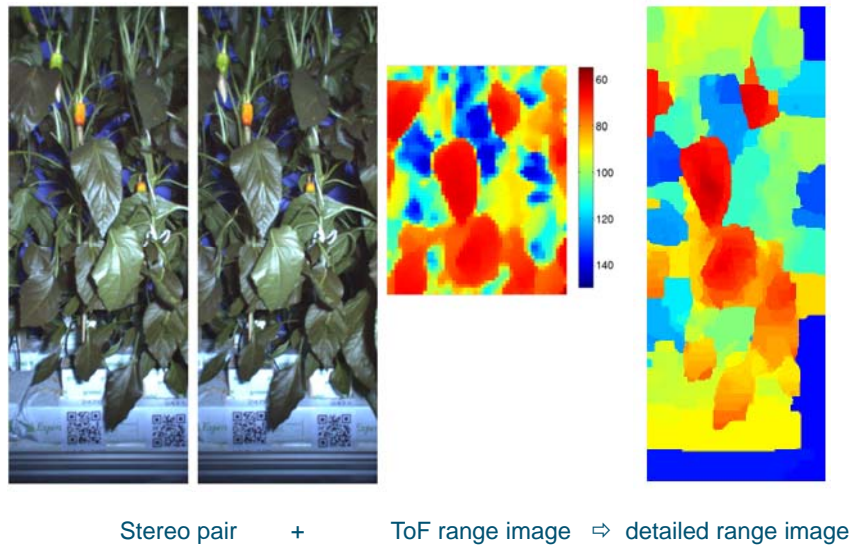
Depth = constant / disparity



Combining stereo and range imaging

- Combine stereo color images and ToF range image by mapping them to a single 3D reference coordinate system
- Start with ToF range image: (mixed) pixel represents a patch in 3D
- Integrate ToF range image with stereo images using graph cuts (GC).
- Method described in: Yu et al, Combining stereo and Time-of-Flight images with application to automatic plant phenotyping. SCIA 2011, Ystad, Sweden





Graph cuts / Energy Minimisation

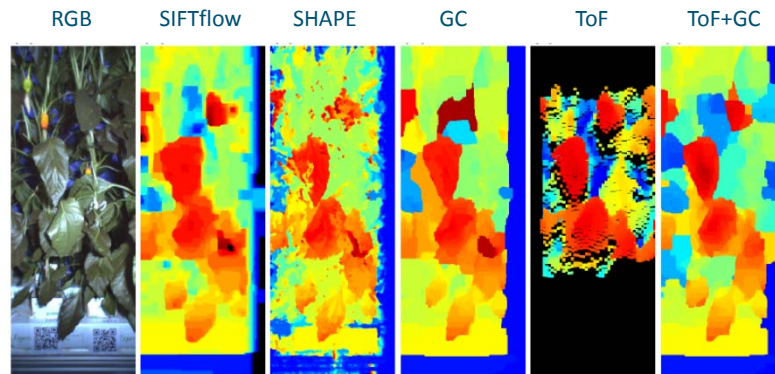
- A graph based energy minimization algorithm to solve the optimization problem:

$$E(d) = \underbrace{\sum D(d_{(x',y')})}_{\text{Data}} + \underbrace{\sum_{q \in N} V(d_{(x',y')}, d_{(x'_q, y'_q)})}_{\text{Smoothness}}$$

Established alpha-expansion technique, to find the parameter array d from all possible values that minimises the energy E (Boykov et al, 2001)



Results



Depth estimation using 3 state-of-the-art stereo algorithms (SIFTflow, Shape, GC), using only ToF and using our method, combining ToF and GC.



Evaluation of the methods

- No pixel-by-pixel ground truth.
- Identify some leaves by hand, then determine:
 - Do depth edges agree with intensity edges?
 - Is the surface smooth?
- Proposed evaluation is to quantify:

- Surface smoothness penalty
- Edge sharpness score

$$P_s = \overline{M(x'_s, y'_s)}$$

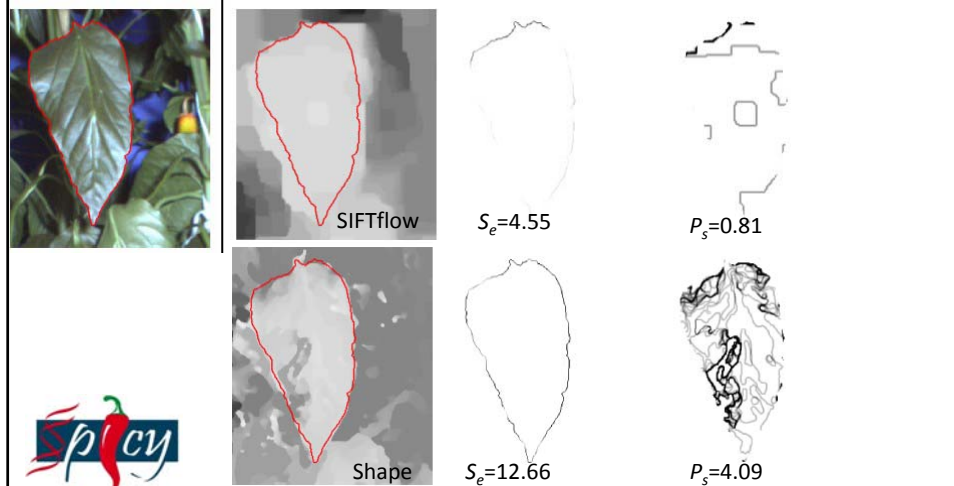
$$S_e = \overline{g(x'_e, y'_e)}$$

g is the Sobel edge magnitude M convoluted with a Gaussian filter

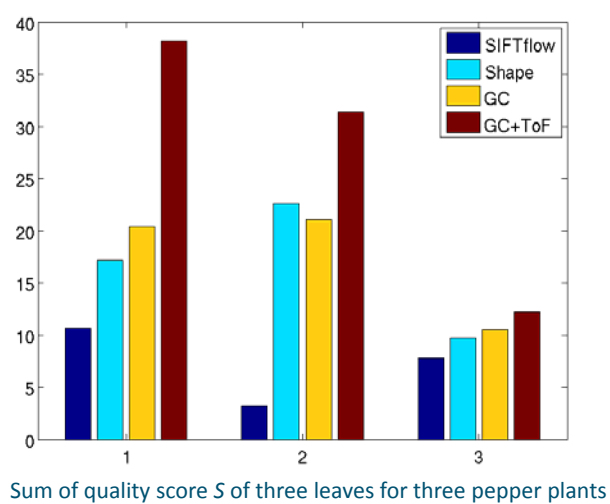


Evaluation using independent measure

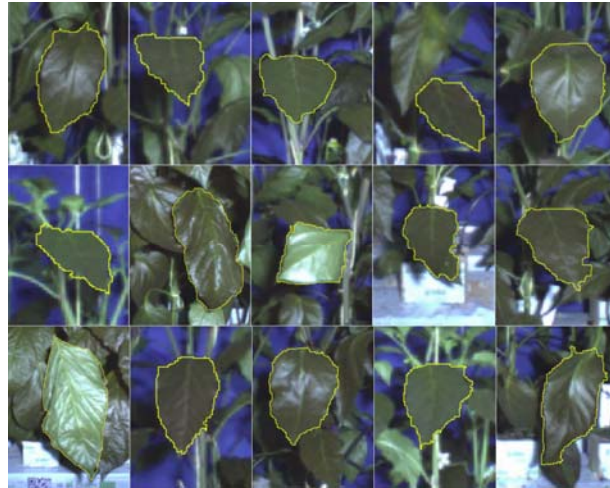
- Manually selected leaf edges vs. depth edges: S_e
- Leaf surface roughness (smoothness penalty): P_s
- Combined score $S = S_e - P_s$



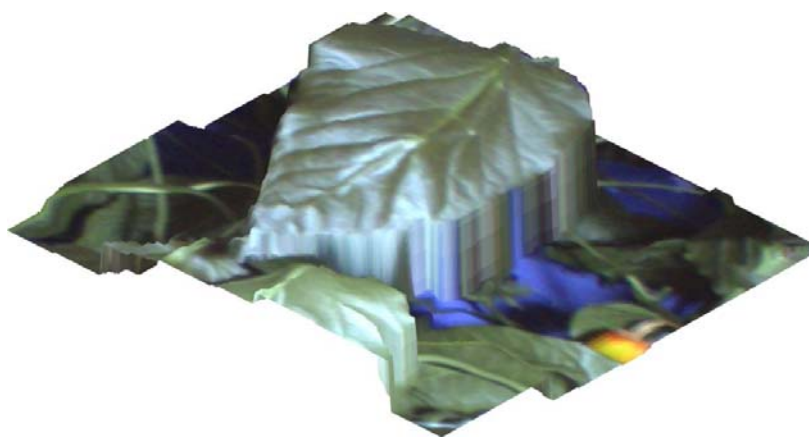
Evaluation



Examples of automatically extracted leaves

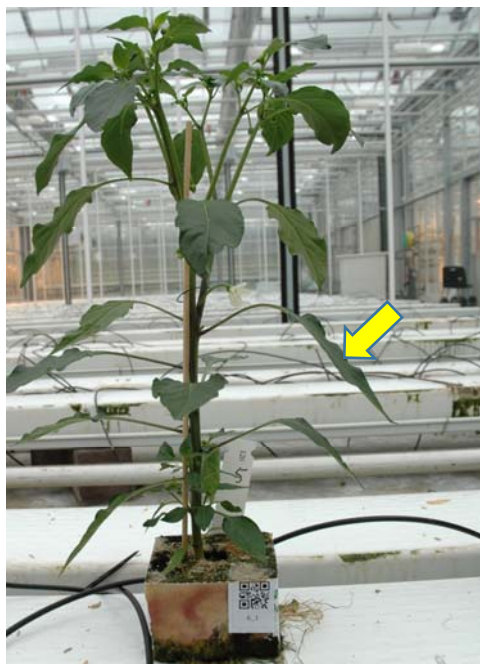
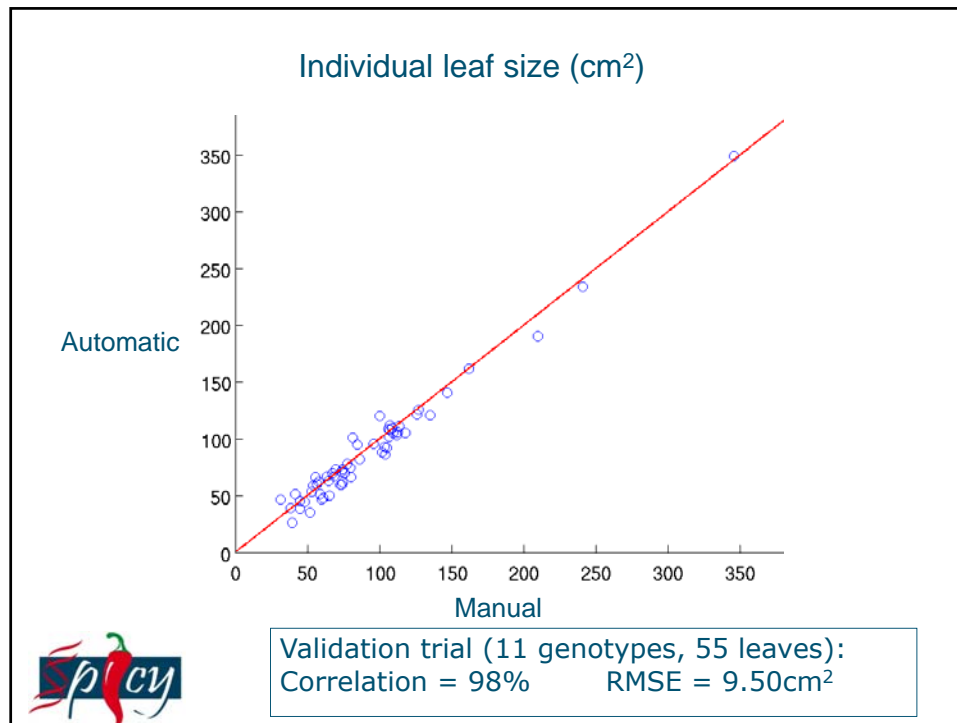


Calculating leaf size (area)



Leaf in 3D \Rightarrow automatic measurement of size, orientation, etc



**Leaf orientation:**

- Angle between the leaf and the vertical axis.

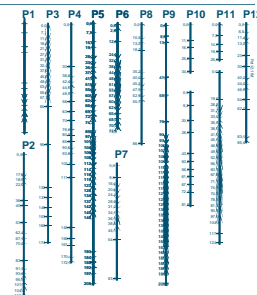


Leaf orientation:

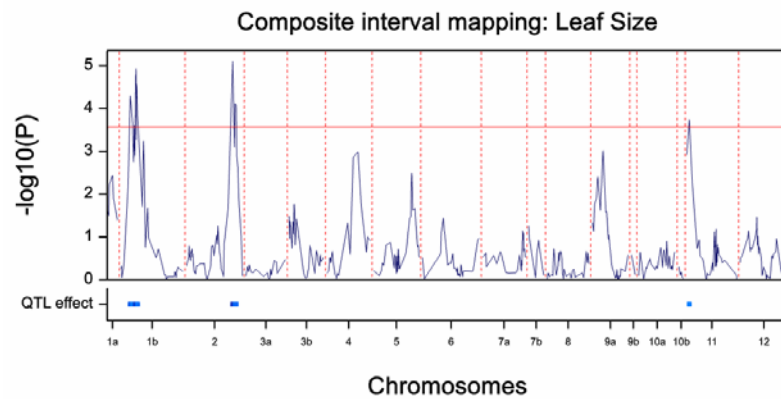
- Angle between the leaf and the vertical axis.

Genetic analysis (QTL)

- We want to find a genetic basis for the traits measured.
- The position of the markers on a genetic map is known
- Find markers/regions on the map, which show a correlation with the trait. This region is called Quantitative Trait Locus (QTL)
- We use a RIL population of 151 genotypes of pepper (cross between bell pepper cultivar 'Yolo Wonder' and chilli pepper 'Criollo de Morelos 334')



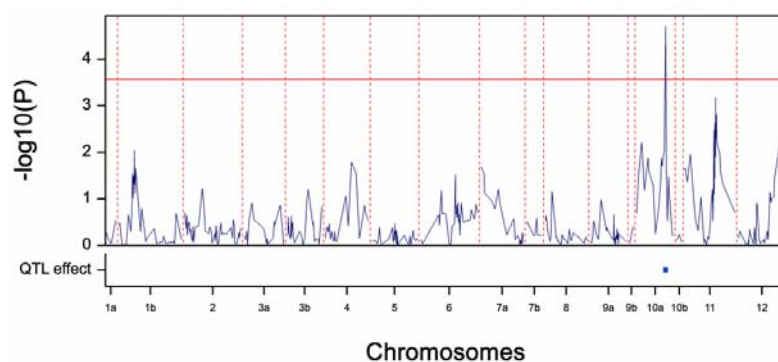
QTL analysis of automatically measured leaf sizes for 151 genotypes



Leaf size had a heritability of 0.70, three QTLs were found, together explaining 29% of the variation.



QTL analysis of leaf orientation for 151 genotypes

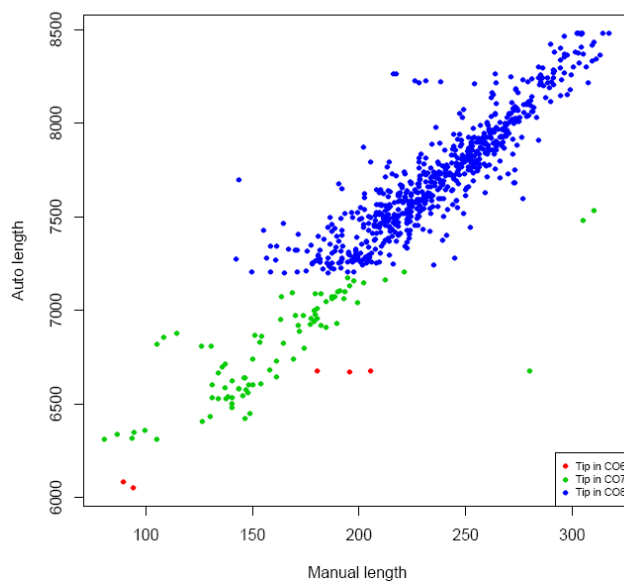


Heritability was 0.56, and one QTL explained 11% of the total variation



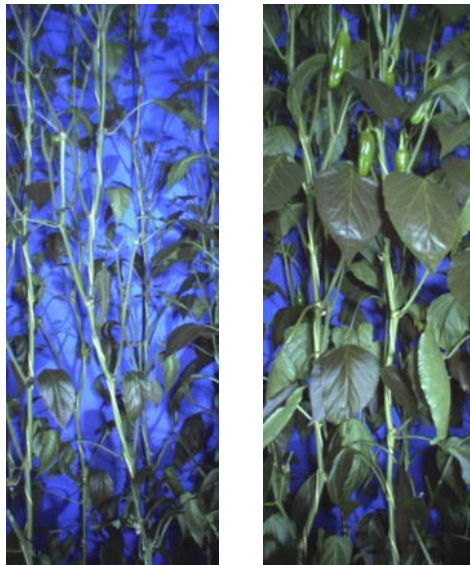
B. Statistical approach

Plant height estimated, from locations of 'green' pixels



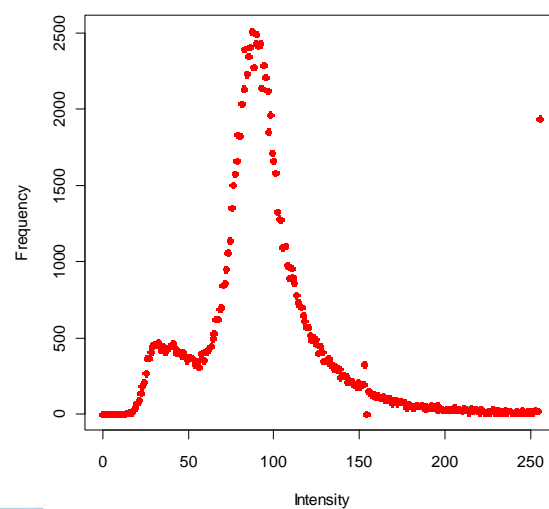
Correlation 93%
between automatic
and manual plant
heights





Total leaf area is a measure of how much solar radiation the plant can intercept

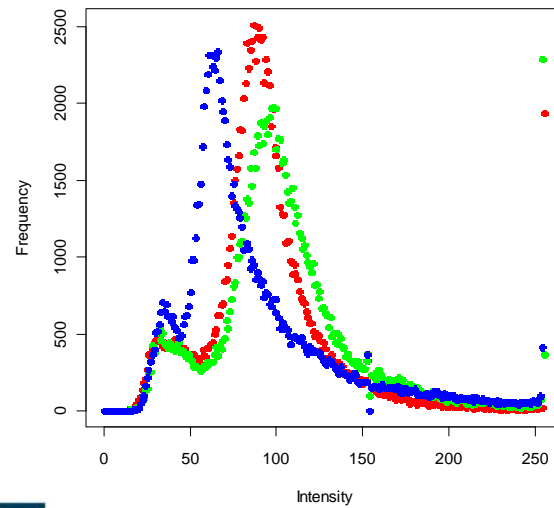
Colour distribution



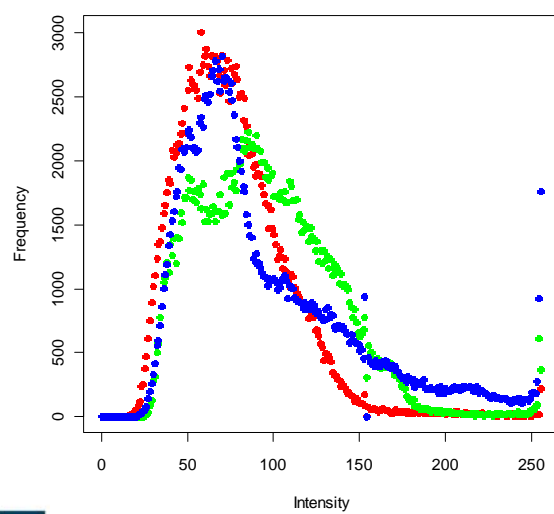
Counts how many pixels in the image have each red intensity



Colour distribution



Colour histograms



Another
example



Principal component regression

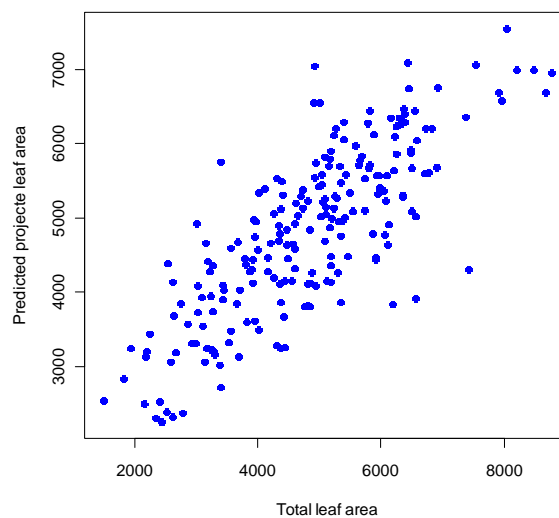
```
Call:
lm(formula = sep.leafarea ~ prl$x[, 1:6], na.action =
na.exclude)

Coefficients:
              Estimate      Std. Error t value Pr(>|t|)
(Intercept)  4.899e+03   6.288e+01   77.905  <2e-16 ***
PC1           5.282e-02   5.473e-03    9.651  <2e-16 ***
PC2           2.069e-01   1.875e-02   11.035  <2e-16 ***
PC3          -2.807e-01   2.339e-02  -12.002  <2e-16 ***
PC4          -5.750e-02   3.477e-02   -1.654   0.0997 .
PC5           1.038e-02   3.686e-02    0.282   0.7785
PC6           1.305e-01   5.607e-02    2.327   0.0209 *
---
Residual standard error: 867.1 on 209 degrees of freedom
(1334 observations deleted due to missingness)
Multiple R-squared:  0.6419,    Adjusted R-squared:  0.6317
F-statistic: 62.45 on 6 and 209 DF,  p-value: < 2.2e-16
```



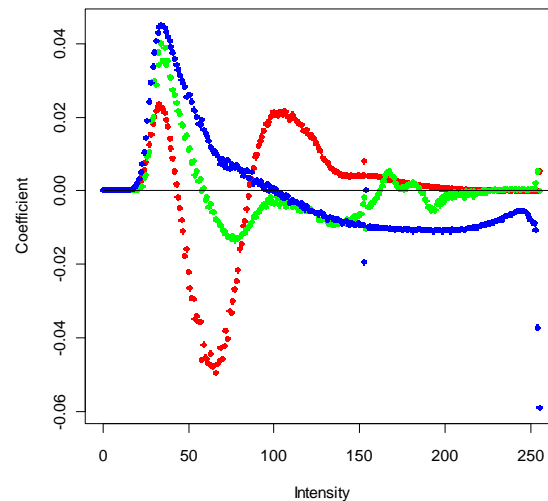
Number crunching to link colour histograms to manually measured total leaf area using PCR.

Prediction vs manual



Correlation 80%

Regression coefficients

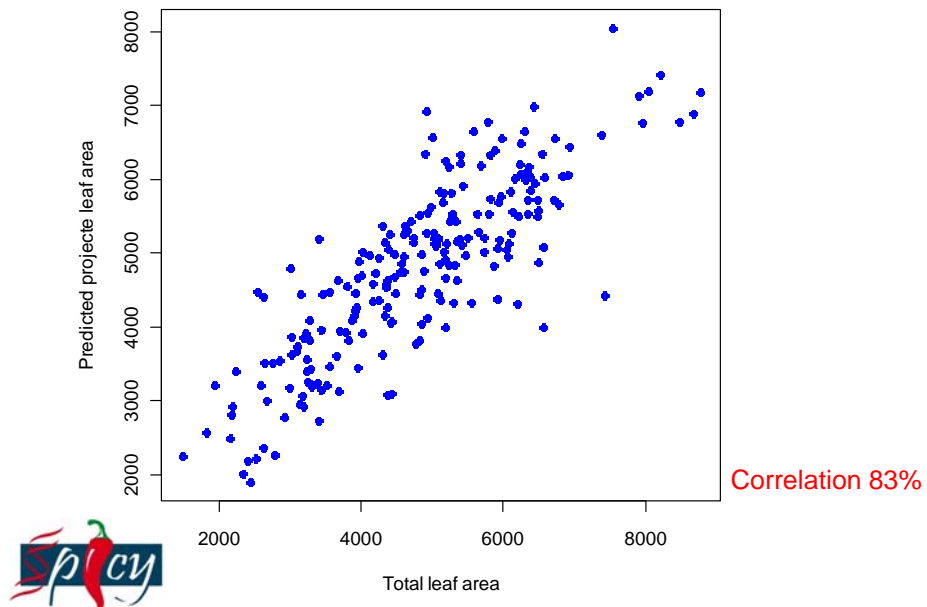


Multivariate histograms

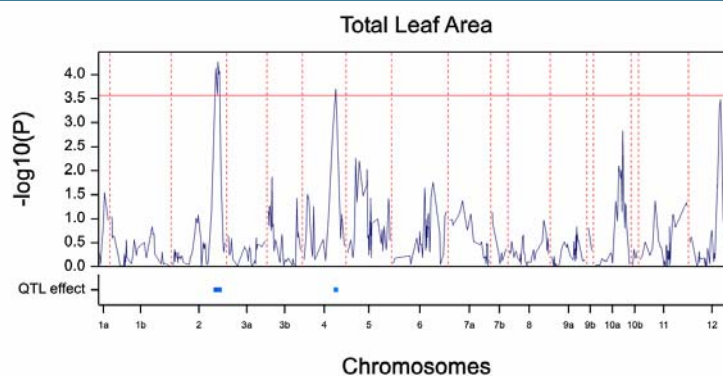
- Count the number of times each combination of the three colour components occurs
- Too many possibilities, so use bins of length 8 per component, leading to $16^3 = 4096$ variables
- Again do Principal Components Regression



Multivariate histograms

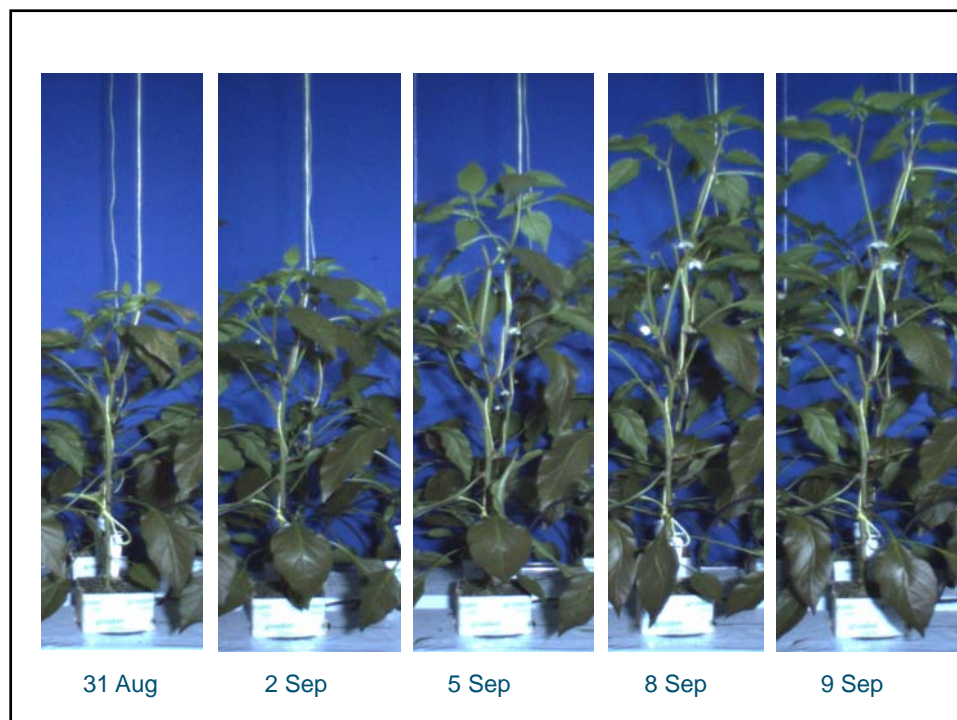
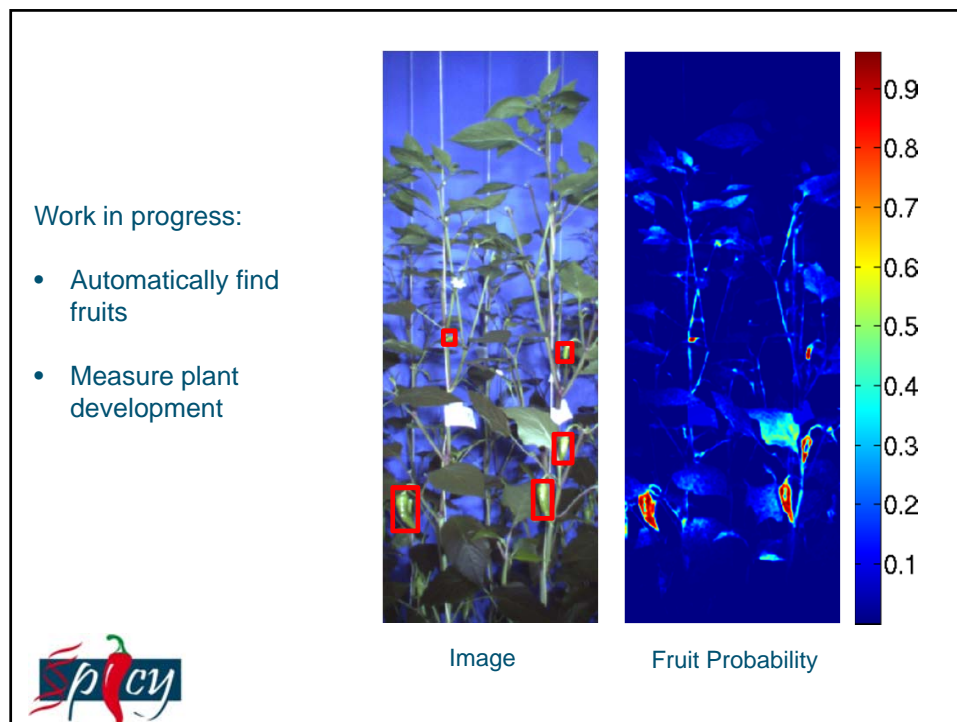


QTL analysis of total leaf area for 151 genotypes



The heritability of total leaf area was 0.55, and 20% of the variation was explained by QTLs. 2 QTLs agree with 2 of 3 found from manual measurements





Summary

- Two approaches to automatic phenotyping have been explored in SPICY:
 1. 3D approach for detailed measurements
 2. Use of statistical features
- The method has been tested on 151 genotypes of a RIL population of pepper
- For all features, QTLs have been found



Thank you for your attention.
Any questions?

This work is a collaboration of:

- Yu Song, Chris Glasbey, Graham Horgan (Bioss, UK)
- Gerie van der Heijden, Gerrit Polder (Biometris, NL)
- And many others in the consortium of EU-SPICY