SOUTH AFRICAN SUGARCANE RESEARCH INSTITUTE

©2012

# Shades of orange and brown: Towards exploiting quantitative rust resistance in sugarcane.

T Mhora, RS Rutherford and SA McFarlane

# BIOSECURITY ALERT

**Orange rust has spread south from West Africa towards the DRC. A previously unknown rust species also appears to be emerging in South Africa. Please look out for unusual symptoms.**

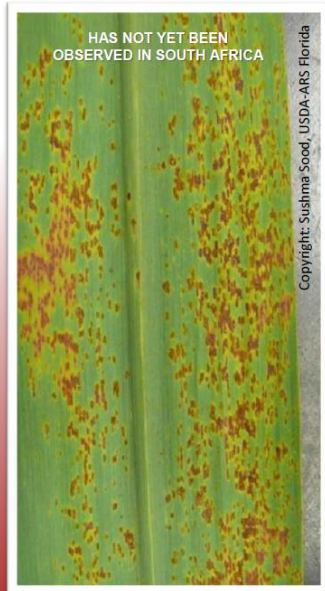
## ORANGE RUST

**Lesions (marks) on leaf:**
- orange to reddish brown
- up to 4 mm long
- more severe towards leaf base

**Spores:**
- orange when fresh
- usually only on the lower leaf surface

Favoured by humid, warm conditions

**Tends to occur on older crops (>6 months)**




## BROWN RUST

**Lesions (marks) on leaf:**
- cinnamon brown to dark brown
- up to 20 mm long
- more severe towards leaf tip

**Spores:**
- brown when fresh
- usually only on the lower leaf surface

Favoured by cool nights, warm days

**Tends to occur on young crops (< 6 months)**




## NEW RUST

**Lesions (marks) on leaf:**
- orange to reddish-brown
- similar in size to brown rust
- more severe towards leaf tip

**Spores:**
- orange when fresh, profuse
- also occur on upper leaf surface

Favoured by cool, moist weather

**Has been observed on young and older crops of up to 10 months**




**If you see symptoms that you are unsure of, please inform your local Extension Specialist or contact us via the Biosecurity Hotline.**

South African Sugarcane Research Institute
www. sugar.org.za
Biosecurity Hotline: +27-31 508 7459

Oct 2011

# Qualitative vs Quantitative

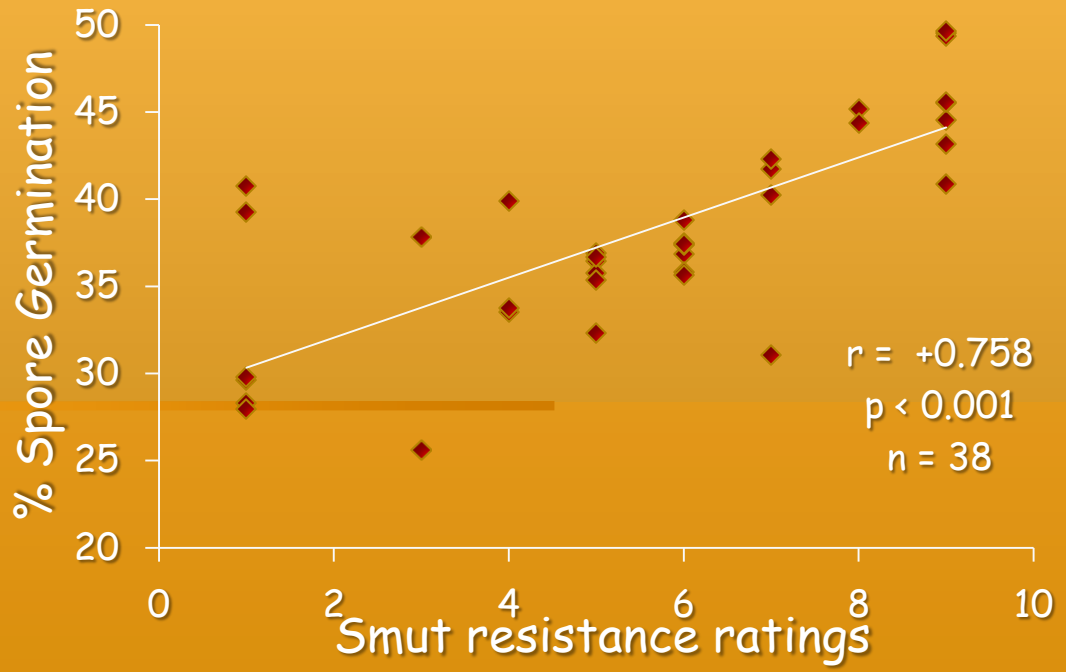
| Qualitative | Quantitative |
| --- | --- |
| Mono-genic | Mono- to poly-genic |
| Race-specific/ Vertical/ Complete | Mostly non race-specific/ Horizontal/ Partial |
| R-genes ~ NB-LRR | Other than R-gene |
| Large/major effect ~ HR, often not durable | Small/minor to large effects ~ non-HR, durable |

# Quantitative resistance:

- Quantitative disease resistance could be conditioned by genes…
- •… involved in basal defense, –LRR-Kinases
- •… that are weak or defeated R-genes

# Quantitative resistance:

- Quantitative disease resistance could be conditioned by genes…
- •… encoding constitutive chemical and morphological traits

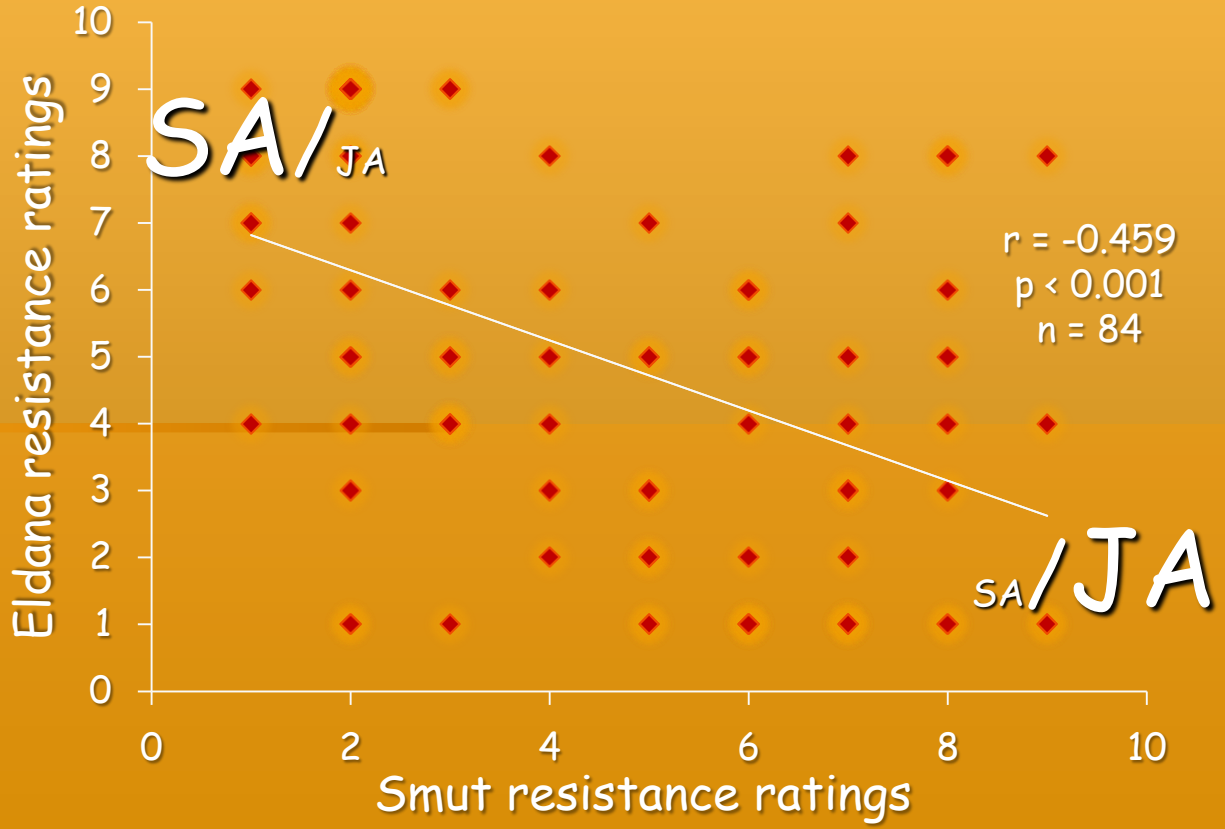



- James, G.L. (1973) Smut spore germination on sugarcane internode surfaces. Proceedings of the South African Sugar Technologists Association, 47, 1-2.

# Quantitative resistance:

- Quantitative disease resistance could be conditioned by genes…
- •… encoding components of the SA and JA pathways



# Quantitative resistance could be effective against multiple diseases.

- If the resistance mechanism is somewhat general then it might be expected to be effective against a set of similar pathogens.

- Mitchell-Olds, T., et al. (1995) Genetics of Brassica rapa (syn. campestris). 2. Selection for multiple disease resistance to three fungal pathogens: *Peronospora parasitica*, *Albugo candida*, and *Leptosphaeria maculans*, Heredity, 75, 362-369 .

- Wisser, R.J., et al. (2011) Multivariate analysis of maize disease resistances suggests a pleiotropic genetic basis and implicates a GST gene. PNAS USA, 108, 7339-7344.

# The wheat gene Lr34/Yr18/Pm38 provides resistance against leaf rust (*Puccinia triticina*), stripe rust (*Puccinia striiformis*) and powdery mildew (*Blumeria graminis*).

Leaf tip necrosis associated with *Lr34*




- Resistance results from senescence-like processes?
- or from the export of metabolites that affect fungal growth?


- Krattinger, S.G., (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science, 323, 1360-1363.

# Objectives & Approach

- Develop markers for quantitative resistance and alternative qualitative resistance to brown rust for use in MAB.
- Develop rapid screening methods for detecting resistance phenotype in progenies.
  - LD2 mapping population
  - Existing AFLP data
  - Inoculation ~ whorl/detached leaf
  - Phenotyping ~ Visual/ Chl fluorescence/ NIR

# LD2 mapping population: Brown rust ratings by whorl inoculation

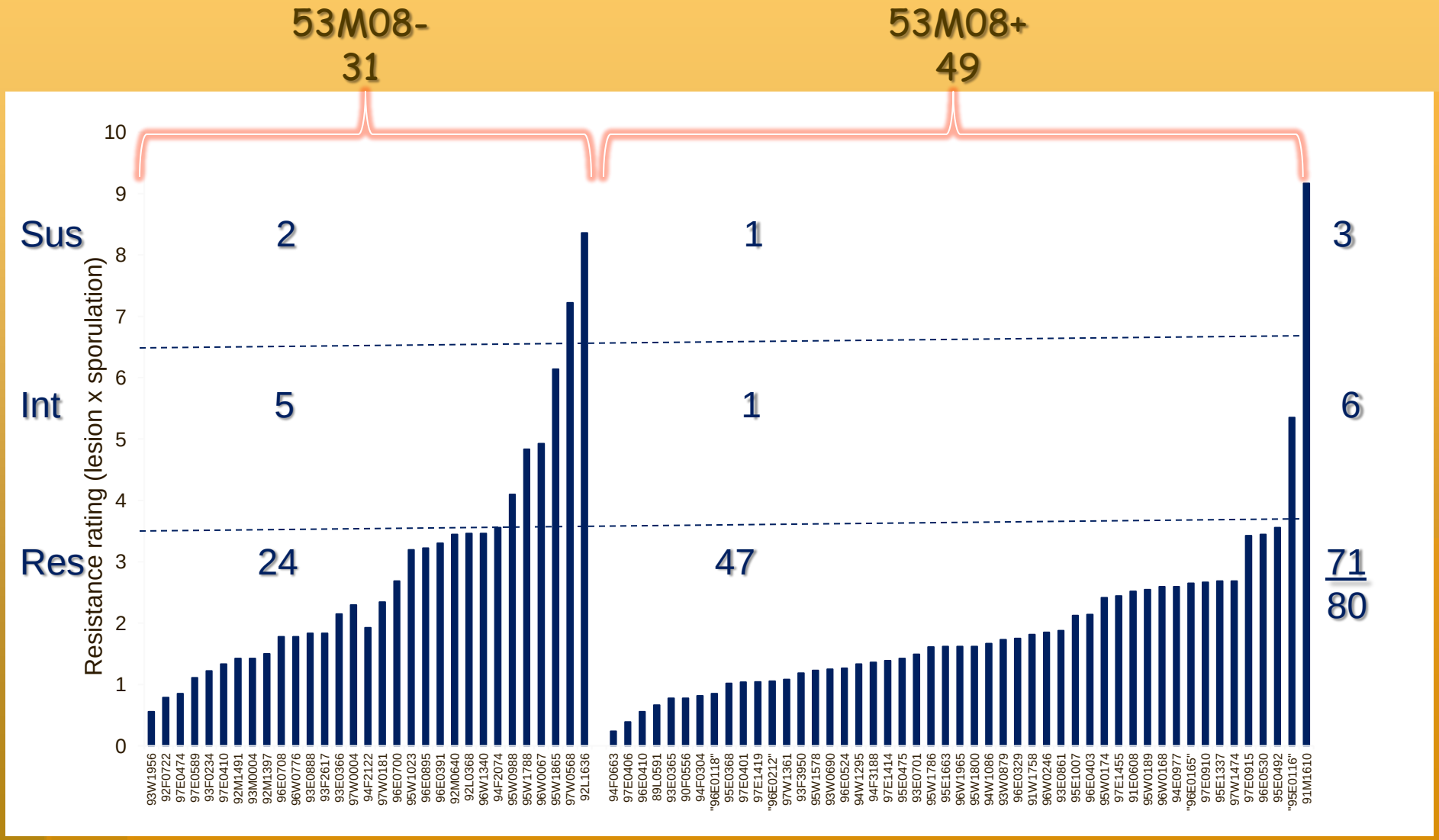
Jack Comstock USDA-ARS




| Lesion rating scale | Symptoms |
|---|---|
| 0 | no lesions/ chlorotic flecks |
| 1 | one to a few lesions |
| 2 | more than a few lesions |
| 3 | numerous lesions |
| 4 | numerous coalescing lesions |
| 5 | severe numerous coalescing lesions and necrosis |

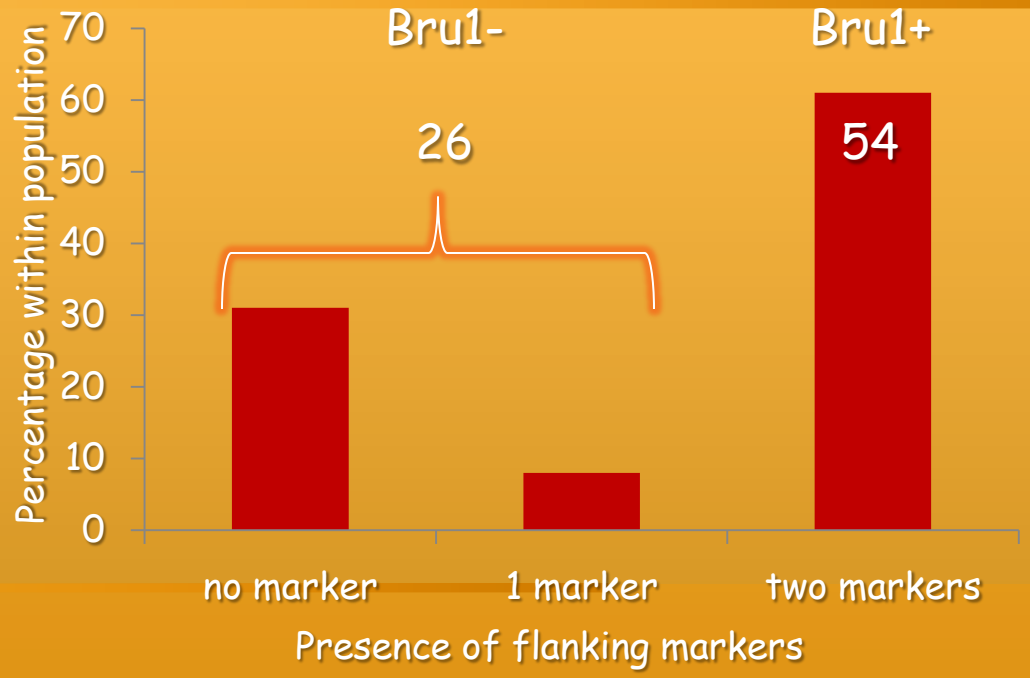
| Sporulation rating scale | Symptoms |
|---|---|
| 0 | no sporulation |
| 1 | slight sporulation |
| 2 | medium sporulation |
| 3 | heavy sporulation |

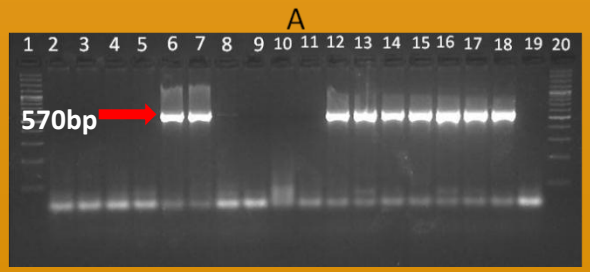
# One AFLP marker was strongly associated with resistance

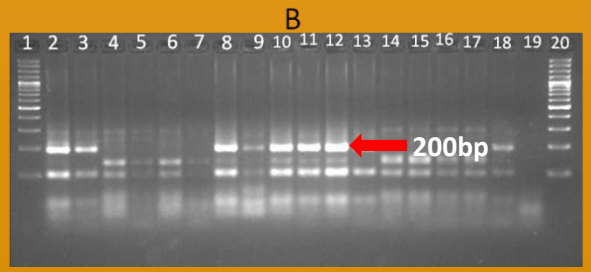


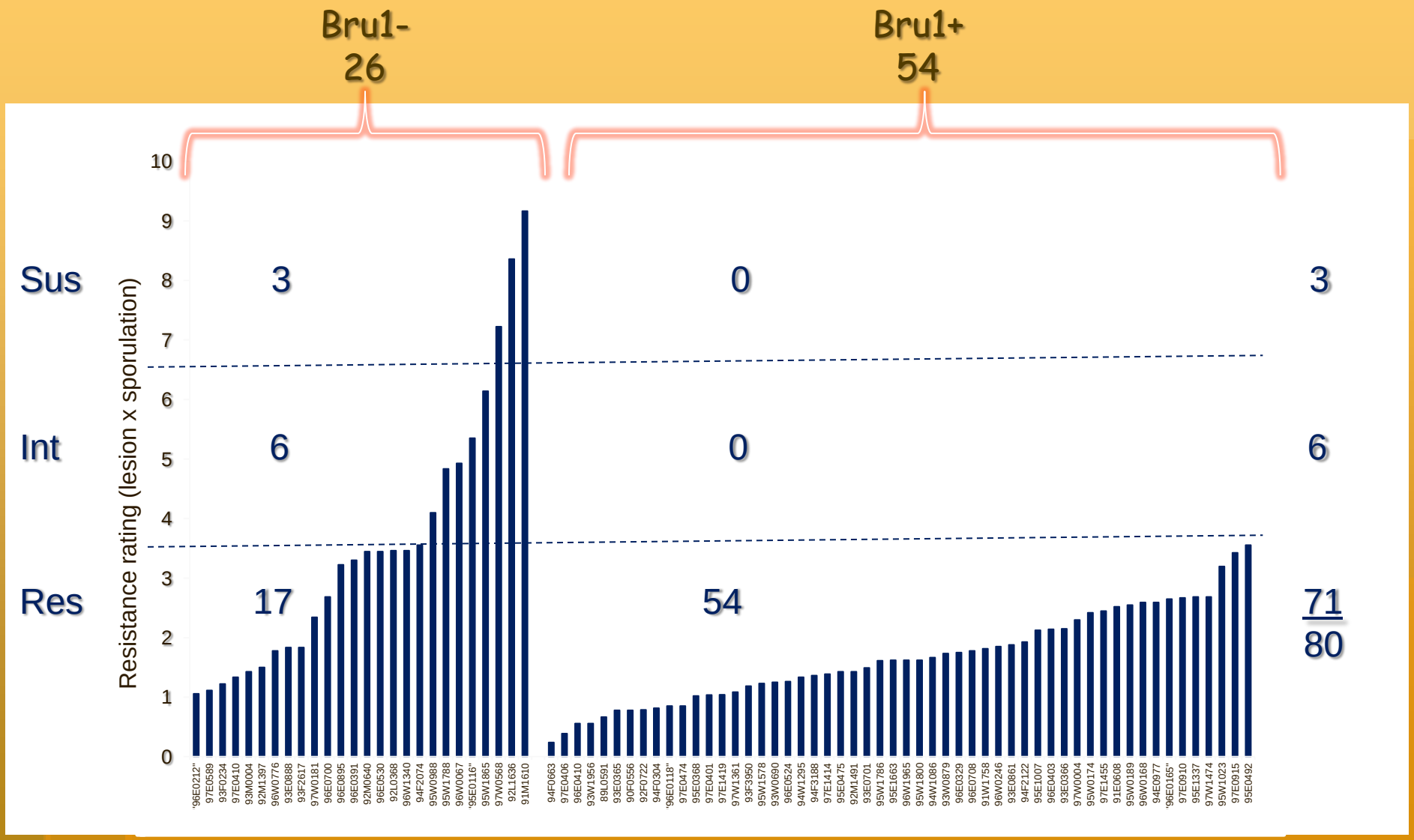
# LD2 mapping population: Presence of the Bru1 gene (80 genotypes)

Angelique d'Hont CIRAD




Bru1-
26
Bru1+
54
Sus
Int
Res
3
6
17
0
0
54
3
6
71
80
Resistance rating (lesion x sporulation)
10
9
8
7
6
5
4
3
2
1
0
"96E0212"
97E0589
93F0234
97E0410
93M0004
92M1397
96W0776
93E0888
93F2617
97W0181
96E0700
96E0895
96E0391
92M0640
96E0530
92L0388
96W1340
94F2074
95W0988
95W1788
96W0067
"95E0116"
95W1865
97W0568
92L1636
91M1610
94F0663
97E0406
96E0410
93W1956
89L0591
93E0365
90F0556
92F0722
94F0304
"96E0118"
97E0474
95E0368
97E0401
97E1419
97W1361
93F3950
95W1578
93W0690
96E0524
94W1295
94F3188
97E1414
95E0475
92M1491
93E0701
95W1786
95E1663
96W1965
95W1800
94W1086
93W0879
96E0329
96E0708
91W1758
96W0246
93E0861
94F2122
95E1007
96E0403
93E0366
97W0004
95W0174
97E1455
91E0608
95W0189
96W0168
94E0977
"96E0165"
97E0910
95E1337
97W1474
95W1023
97E0915
95E0492

# AFLP markers associated with alternative qualitative rust resistance

| | Bru1 negatives | | | | Bru1 positives |
|---|---|---|---|---|---|
| Rating Range | 0- 3.5 | | 3.51- 6.5 | 6.51- 10 | |
| Marker | Number of resistant genotypes with marker (out of 17) | Mean phenotypic rating for resistants with marker | Number of intermediate genotypes with marker (out of 6) | Number of susceptible genotypes with marker (out of 3) | Number of genotypes with marker (out of 54) |
| 18M30 | 13 | 2.19 | 0/5 | 0 | 22/51 |
| 87M02 | 9 | 2.02 | 0 | 0 | 27 |
| 67M15S | 7 | 2.69 | 0 | 0 | 16 |
| 43M44 | 6 | 1.97 | 0 | 0 | 12 |
| 45M45 | 6 | 2.09 | 0 | 0 | 12 |
| 12M58 | 6 | 2.18 | 0 | 0 | 18 |
| 48M35 | 6 | 2.56 | 0 | 0/2 | 20/50 |
| 54M08 | 6 | 2.67 | 0 | 0 | 16 |
| 71M14S | 6 | 2.78 | 0 | 0 | 9 |
| 74M09S | 5 | 1.93 | 0 | 0 | 6 |
| 55M27 | 5 | 1.63 | 0 | 0 | 7 |
| 54M14 | 5 | 2.09 | 0 | 0 | 4 |
| 57M38 | 5 | 2.10 | 0 | 0 | 11 |
| 58M40 | 5 | 2.26 | 0/5 | 0 | 9 |
| 53M08 | 2 | 2.25 | 1 | 1 | 42/51 |

Rule: marker should be present in resistant genotypes and absent in intermediate and susceptible genotypes

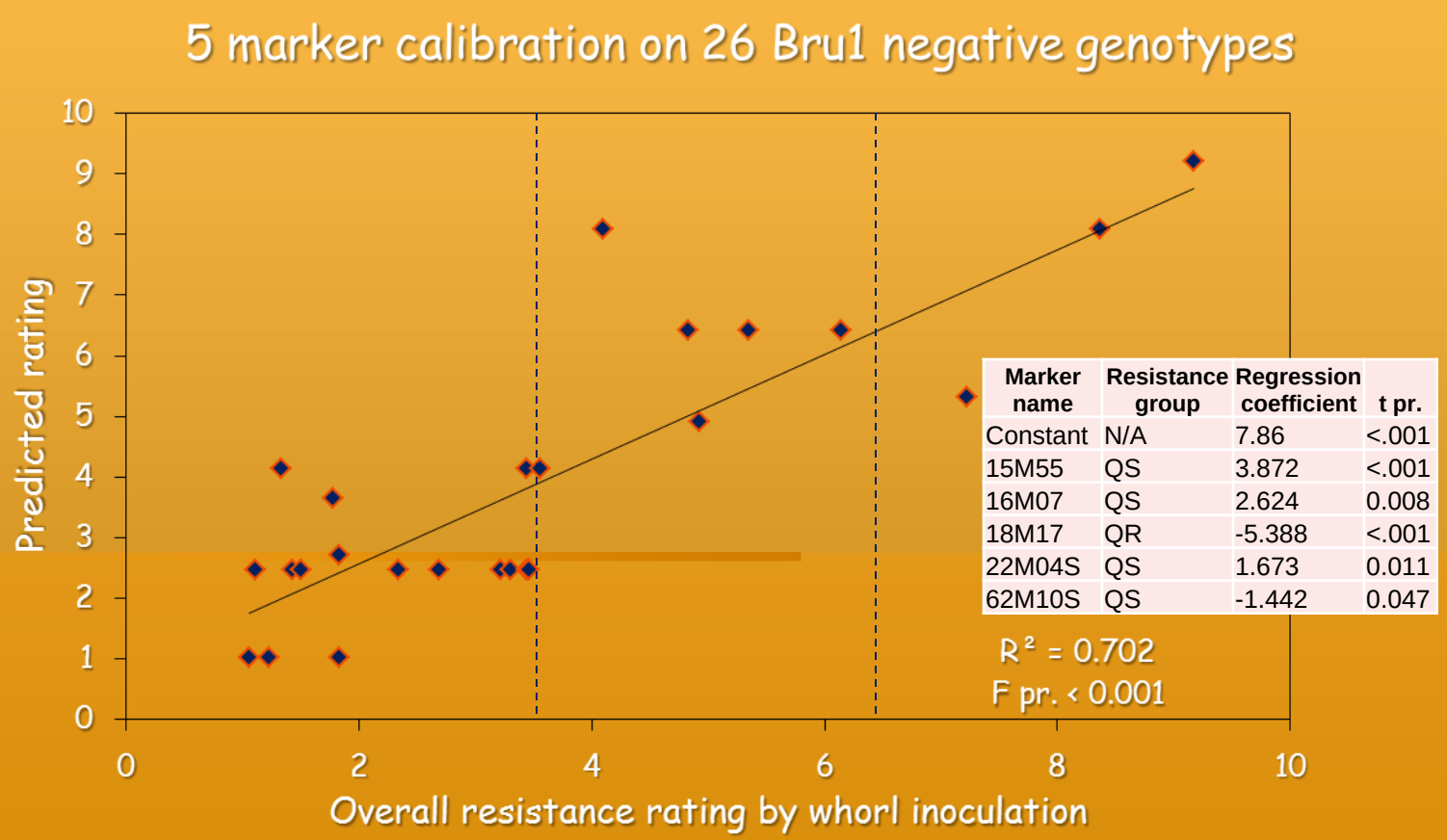
# AFLP markers associated with quantitative rust resistance

| Marker | Pearson correlation coefficient | P-value | Number of Bru1 negatives with marker | | | Number of Bru1 positives with marker (out of 54) |
|---|---|---|---|---|---|---|
| | | | Resistant (out of 17) | Intermediate (out of 6) | Susceptible (out of 3) | |
| 53M31 | -0.651 | 0.0002 | 14 | 2 | 0 | 26 |
| 34M24S | -0.651 | 0.0002 | 14 | 2 | 0 | 23 |
| 24M17S | -0.625 | 0.0006 | 14 | 2 | 0 | 35 |
| 86M24 | -0.538 | <0.001 | 13/16 [a] | 4 | 0/2 | 30/48 |
| 55M39 | -0.476 | 0.0011 | 14 | 3 | 1 | 30 |
| 34M01S | -0.540 | 0.0026 | 11 | 3 | 0 | 22 |
| 71M13S | -0.480 | 0.0041 | 15 | 15 | 1 | 32 |
| 82M56 | -0.574 | 0.0042 | 13 | 1 | 0 | 24 |
| 18M30 | -0.604 | 0.0047 | 13 | 0/5 | 0 | 22/51 |
| 35M12S | -0.556 | 0.0049 | 14 | 3 | 0 | 36 |
| 62M12S | -0.613 | 0.0059 | 17 | 6 | 1 | 51 |
| 88M37 | -0.544 | 0.0059 | 16 | 4/5 | 1 | 42 |
| 86M04 | -0.537 | 0.0059 | 16 | 5 | 1 | 28/50 |
| 26M16S | -0.506 | 0.0059 | 16 | 6 | 1 | 47 |
| 18M39 | -0.484 | 0.0059 | 16 | 5/5 | 1 | 39/51 |
| 38M01S | -0.512 | 0.0075 | 17 | 3 | 2 | 6 |
| 87M02 | -0.506 | 0.0084 | 9 | 0 | 0 | 42 |
| 43M58 | -0.398 | 0.0106 | 8 | 4 | 0 | 33 |
| 76M07S | -0.461 | 0.013 | 10/16 | 3/4 | 0 | 29/51 |
| 16M54 | -0.457 | 0.014 | 14/16 | 4 | 1 | 47/53 |
| 55M37 | -0.394 | 0.0154 | 12 | 4 | 1 | 38 |
| 18M17 | -0.473 | 0.0169 | 17 | 2/5 | 2 | 32 |
| 46M47 | -0.382 | 0.0188 | 15 | 4 | 2 | 39 |
| 26M07S | -0.451 | 0.0208 | 17 | 3 | 2 | 2 |
| 17M37 | -0.458 | 0.0209 | 13 | 2 | 1 | 33 |

# AFLP markers associated with quantitative rust susceptibility

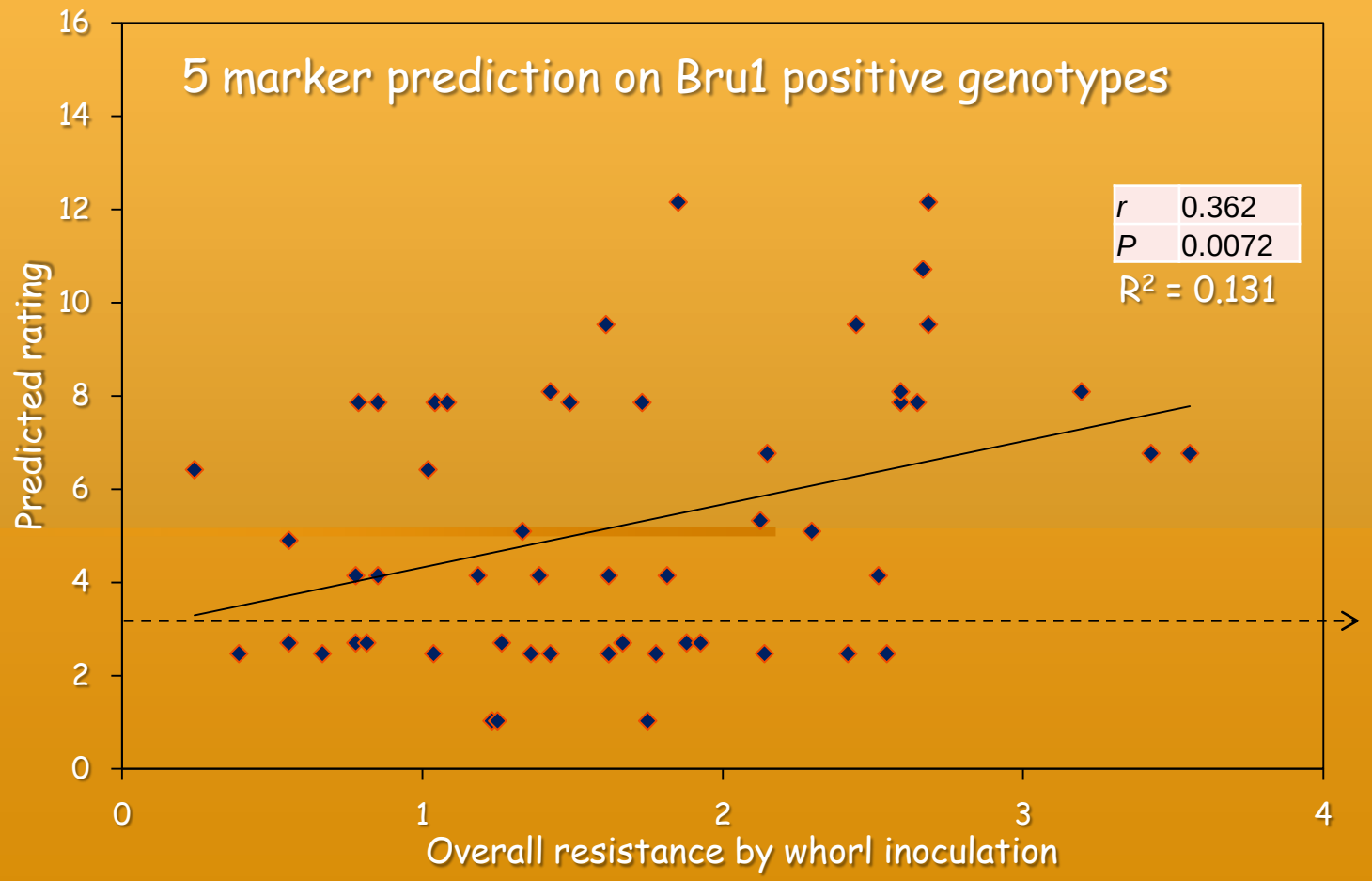
| Marker | Pearson correlation coefficient | P-value | Number of Bru1 negatives with marker | | | Number of Bru1 positives with marker (out of 54) |
|---|---|---|---|---|---|---|
| | | | Resistant (out of 17) | Intermediate (out of 6) | Susceptible (out of 3) | |
| 47M04 | 0.531 | 0.0006 | 5 | 3 | 3 | 17/51 |
| 18M20 | 0.491 | 0.0006 | 6 | 2/5 | 3 | 25/51 |
| 72M16S | 0.490 | 0.0028 | 3 | 2 | 3 | 21/50 |
| 47M12 | 0.492 | 0.0047 | 7 | 3 | 3 | 24/51 |
| 45M52 | 0.435 | 0.0047 | 4 | 1 | 3 | 20 |
| 43M32 | 0.552 | 0.0052 | 3 | 2/5 | 3 | 16/53 |
| 25M02S | 0.474 | 0.0057 | 7 | 3 | 3 | 27 |
| 54M03 | 0.512 | 0.0075 | 0 | 1 | 2 | 28 |
| 85M23 | 0.494 | 0.0082 | 3 | 3 | 2 | 12 |
| 11M43 | 0.443 | 0.0115 | 3 | 2 | 2 | 17 |
| 82M52 | 0.408 | 0.0149 | 4 | 2 | 3 | 21 |
| 31M02S | 0.468 | 0.0160 | 6 | 4 | 3 | 27 |
| 15M55 | 0.462 | 0.0175 | 0 | 1 | 1 | 3/29 |
| 16M30 | 0.495 | 0.022 | 4/14 [a] | 1/4 | 3 | 11/50 |
| 82M49 | 0.447 | 0.0222 | 1 | 1 | 1 | 37/51 |
| 15M36 | 0.389 | 0.0237 | 8 | 2 | 3 | 24/53 |
| 22M04S | 0.406 | 0.0265 | 3 | 2 | 3 | 27 |
| 34M21S | 0.432 | 0.0277 | 7 | 4 | 3 | 47 |
| 41M10 | 0.474 | 0.028 | 6/16 | 5 | 2 | 39/52 |
| 84M29 | 0.427 | 0.0294 | 6 | 4 | 2 | 45 |

# MLR model for the prediction of quantitative resistance



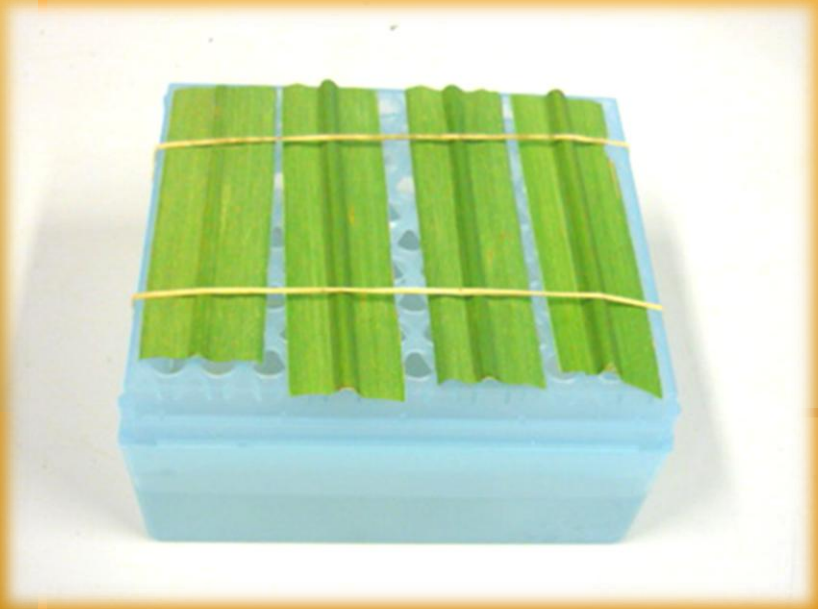

| Marker name | Resistance group | Regression coefficient | t pr. |
|---|---|---|---|
| Constant | N/A | 7.86 | <.001 |
| 15M55 | QS | 3.872 | <.001 |
| 16M07 | QS | 2.624 | 0.008 |
| 18M17 | QR | -5.388 | <.001 |
| 22M04S | QS | 1.673 | 0.011 |
| 62M10S | QS | -1.442 | 0.047 |

# The model suggests that QR contributes towards reducing lesion severity in Bru1+ genotypes



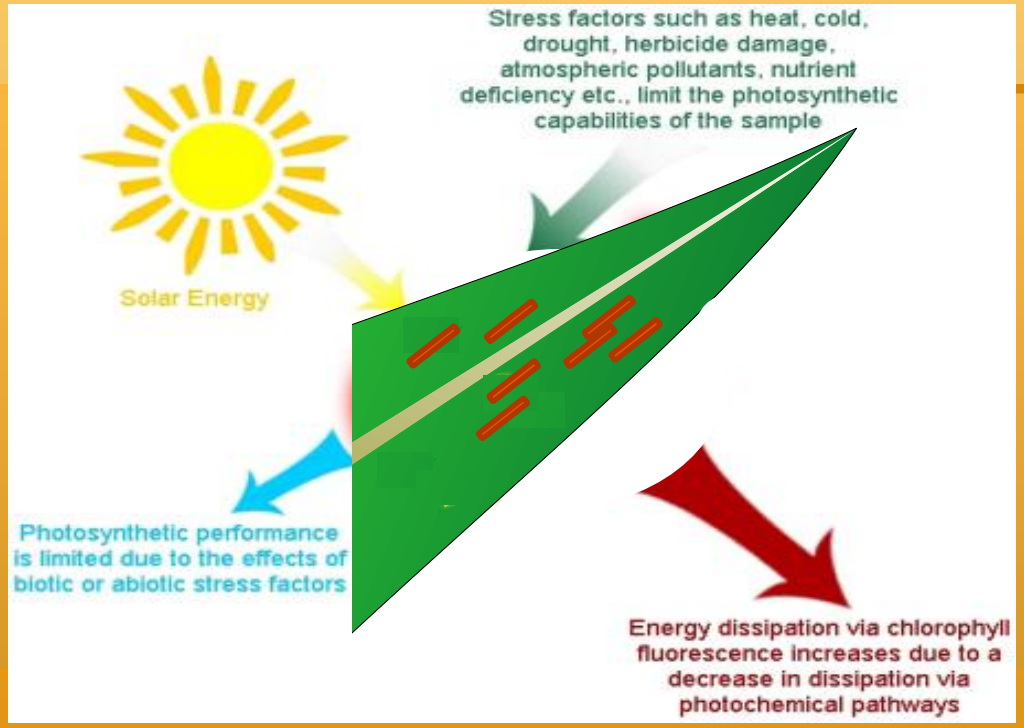
# Rapid Phenotyping: Detached Leaf Assay

Kathy Braithwaite ~ BSES





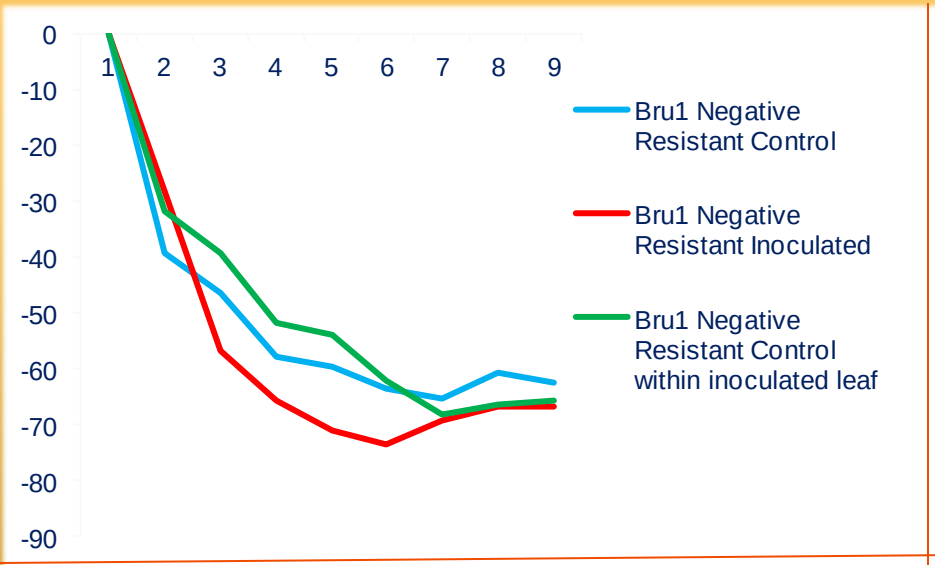
# Chlorophyll fluorescence for the early detection of reaction phenotype

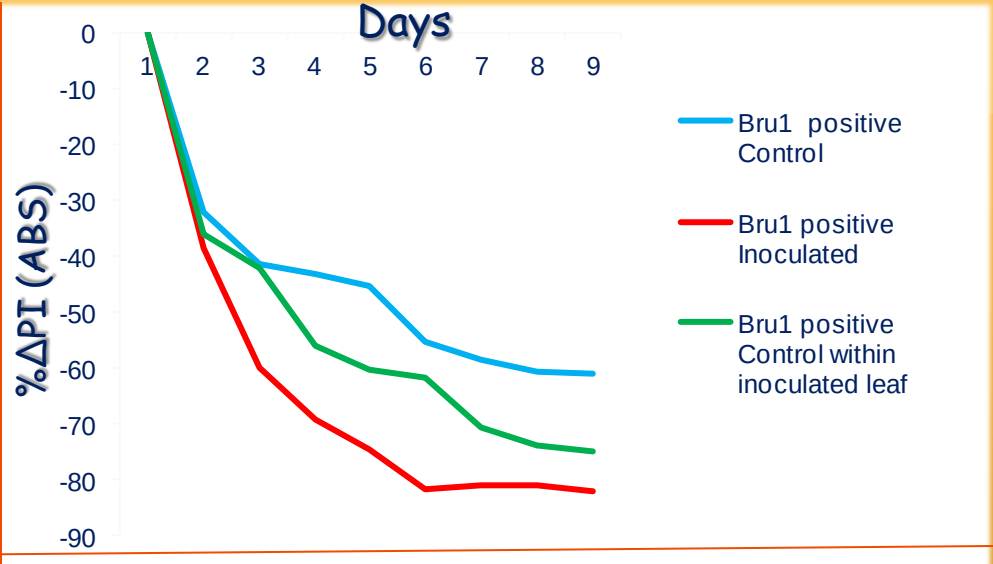


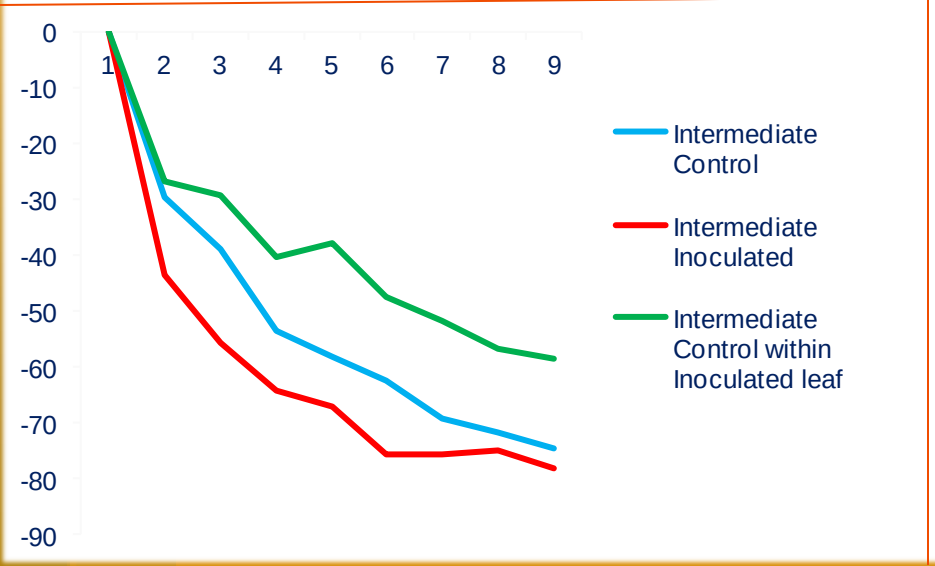


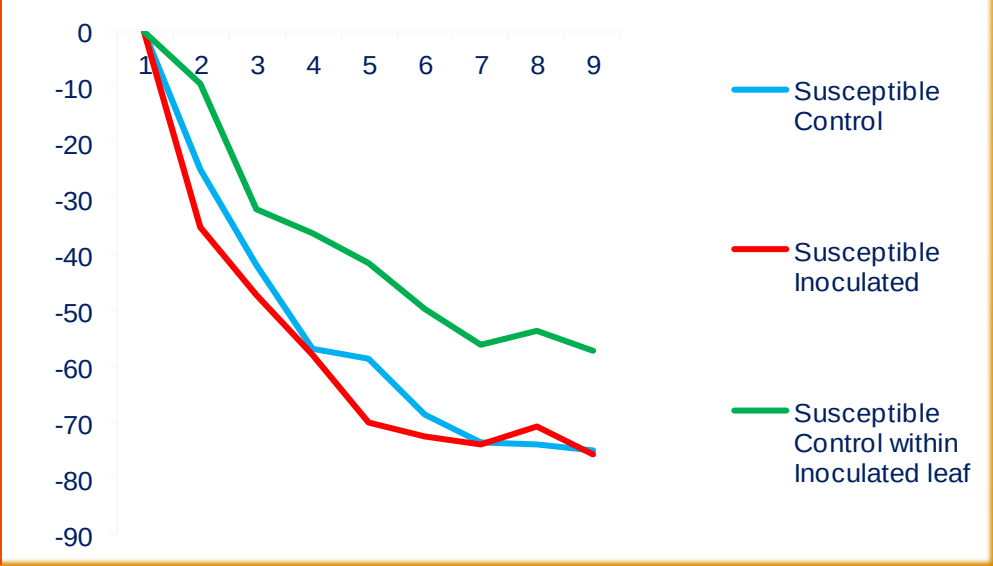
- Chaerle, L., et al. (2007) Chlorophyll fluorescence imaging for disease-resistance screening of sugar beet. Plant Cell Tiss Organ Cult 91: 97–106
- Rolfe, S.A. & Scholes, J.D. (2010) Chlorophyll fluorescence imaging of plant–pathogen interactions. Protoplasma 247: 163–175
- Stefanov, D., et al. (2012) Accelerated leaf senescence takes part in enhanced resistance in cucumber mosaic virus inoculated pepper leaves. Acta Physiologiae Plantarum 34: 181-190,

# Chlorophyll fluorescence for the early detection of reaction phenotype

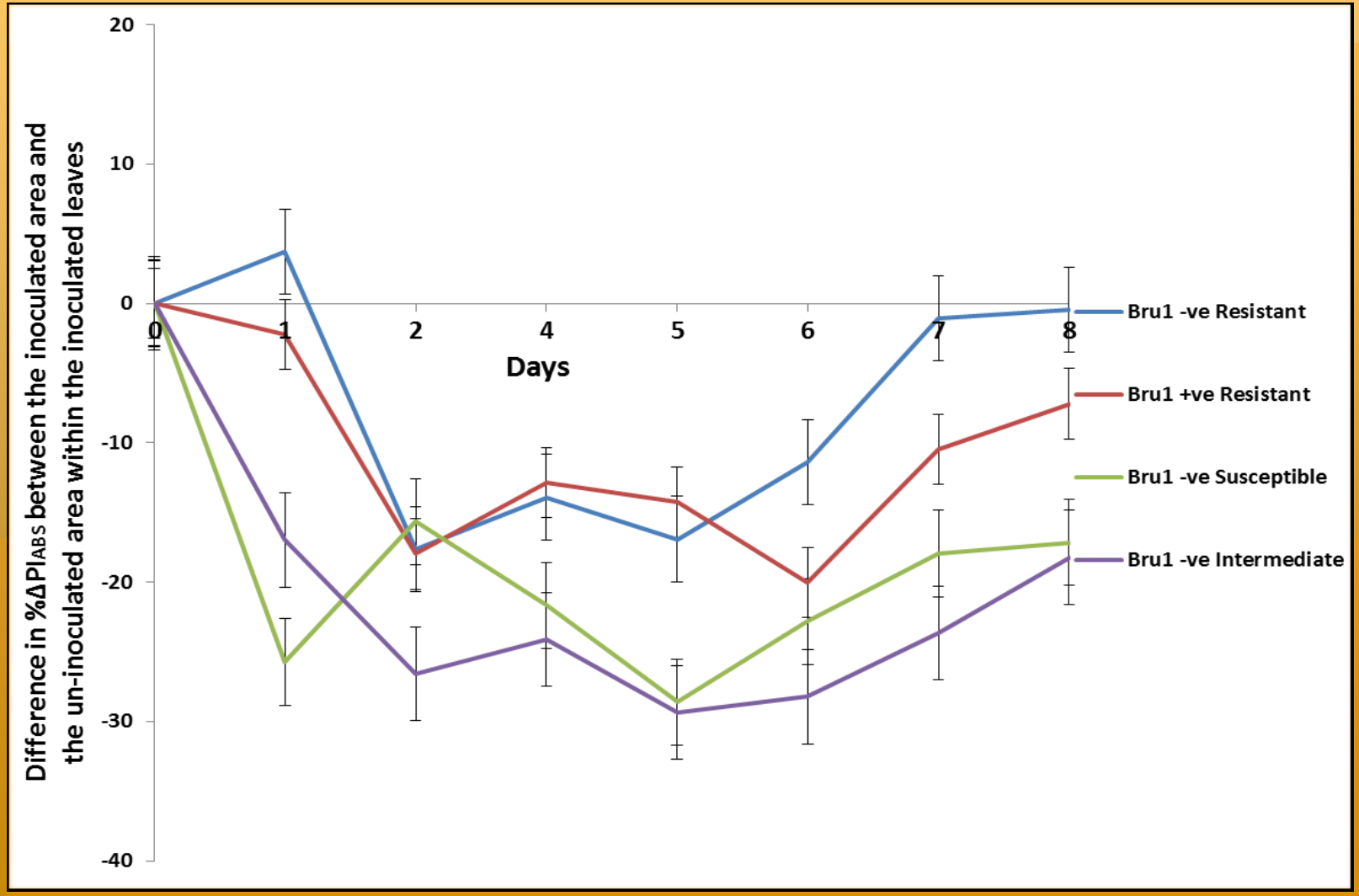


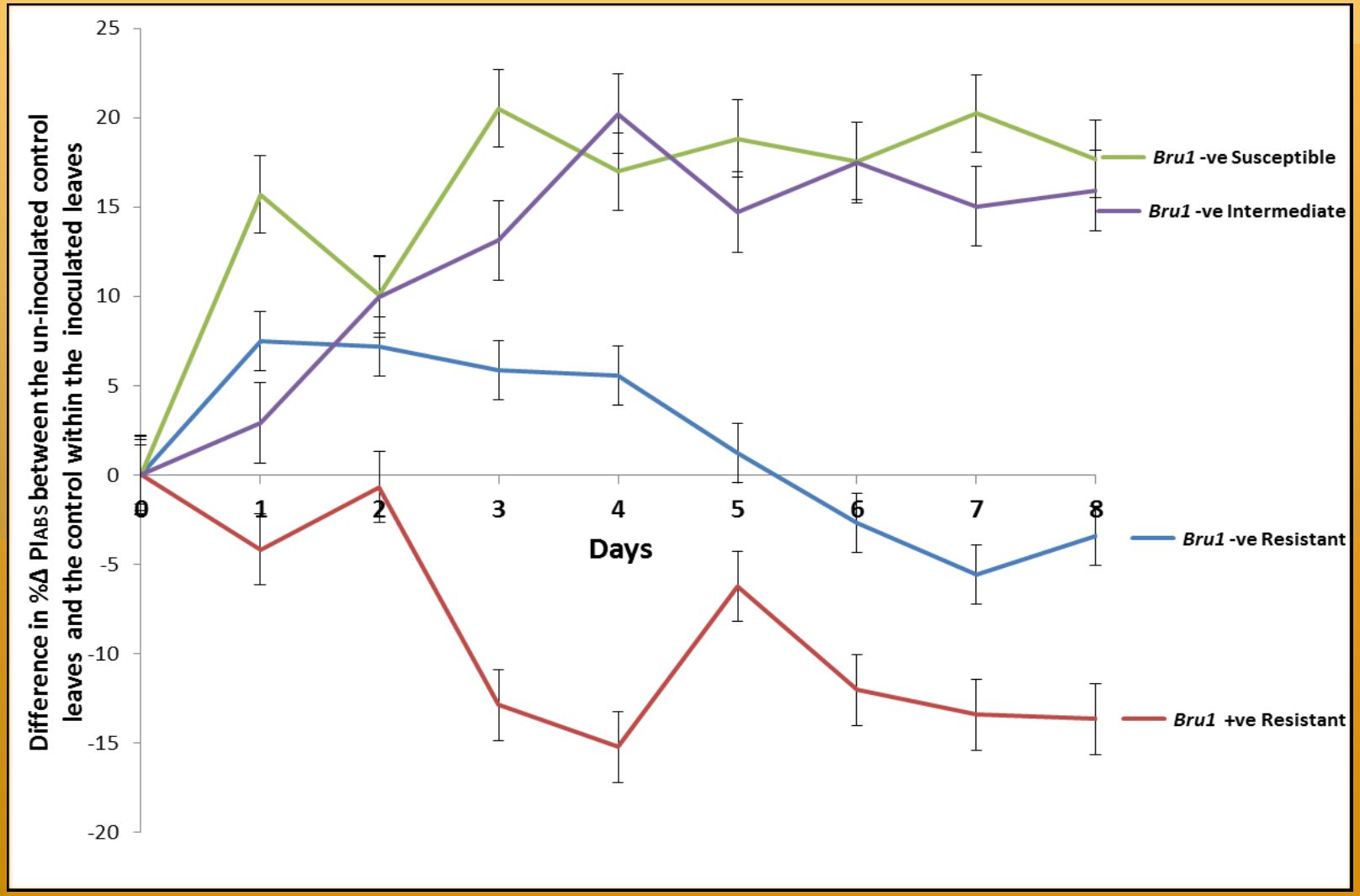



# Differences in %ΔPI(ABS) when the inoculated areas on leaves were compared to the un-inoculated area on the same leaf



# Differences in %ΔPI(ABS) when un-inoculated leaves were compared to the un-inoculated areas of inoculated leaves



# Summary

- 71 of the 80 LD2 genotypes are resistant
  - Only 6 intermediate and 3 susceptible genotypes were identified ~ sub-optimal for marker identification
- 54 of 71 resistant genotypes were Bru1+
  - Putative markers for alternative qualitative resistance were identified
  - A quantitative resistance model suggests that QR underlies Bru1 in many cases, contributing towards reducing lesion severity
- Chlorophyll fluorescence can be used to detect differences in reaction to rust inoculation

# Current & Future

- Increasing the number of Bru1- genotypes
  - equal numbers of resistant, intermediate and susceptible genotypes
- Use of NIR to detect resistance phenotype

  NIR could distinguish between …….
  - Constitutive resistance,
  - CWA,
  - HR,
  - Slow rusting and
  - Susceptibility…. within a few days after inoculation