EXPLOITATION AND CHARACTERIZATION OF RESISTANCE TO THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* IN SOYBEAN

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Production of soybean, which is an important oilseed crop in South Africa, is increasing due to a rising demand for protein rich food. Parasitism by the root-knot nematode *Meloidogyne incognita*, however, damages the crop in local production areas. The use of host plant resistance, is a useful and cost-effective tool for optimization of soybean yield in nematode infested areas. Therefore, results of traditional screening assays were compared with those for endogenous catalase (CAT) enzyme levels in seedlings of *M. incognita*-infected and non-infected resistant as well as a susceptible cultivar. Subsequently, 25 local cultivars were evaluated for resistance to *M. incognita* race 2 in separate, but concurrent greenhouse trials. These cultivars were screened for resistance using an inoculum density of 5000 *M. incognita* eggs and J2/seedling. Root system gall ratings, eggs and J2 counts/root system as well as reproduction factor values were used as indicators of the level of plant resistance/susceptibility to *M. incognita*. Significantly lower gall rating indices for LS5995 and the commercially available cultivar PHB95Y20 were obtained when compared to those for the other cultivars. Cultivar LS5995 was also classified as the poorest host (Rf value < 1), indicating resistance and poor population development of *M. incognita.* Comparison of CAT enzyme levels and traditional nematode parameters exploited to identify resistance will also be elaborated. Cost-effective and quick methods to identify *M. incognita*-resistance in local cultivars can play a significant role in soybean breeding programmes and sustainable production of this crop in South Africa.

Exploitation and characterization of resistance to the root-knot nematode *Meloidogyne incognita* (Nematoda, Tylenchida) in soybean (*Glycine max* L. Merr)

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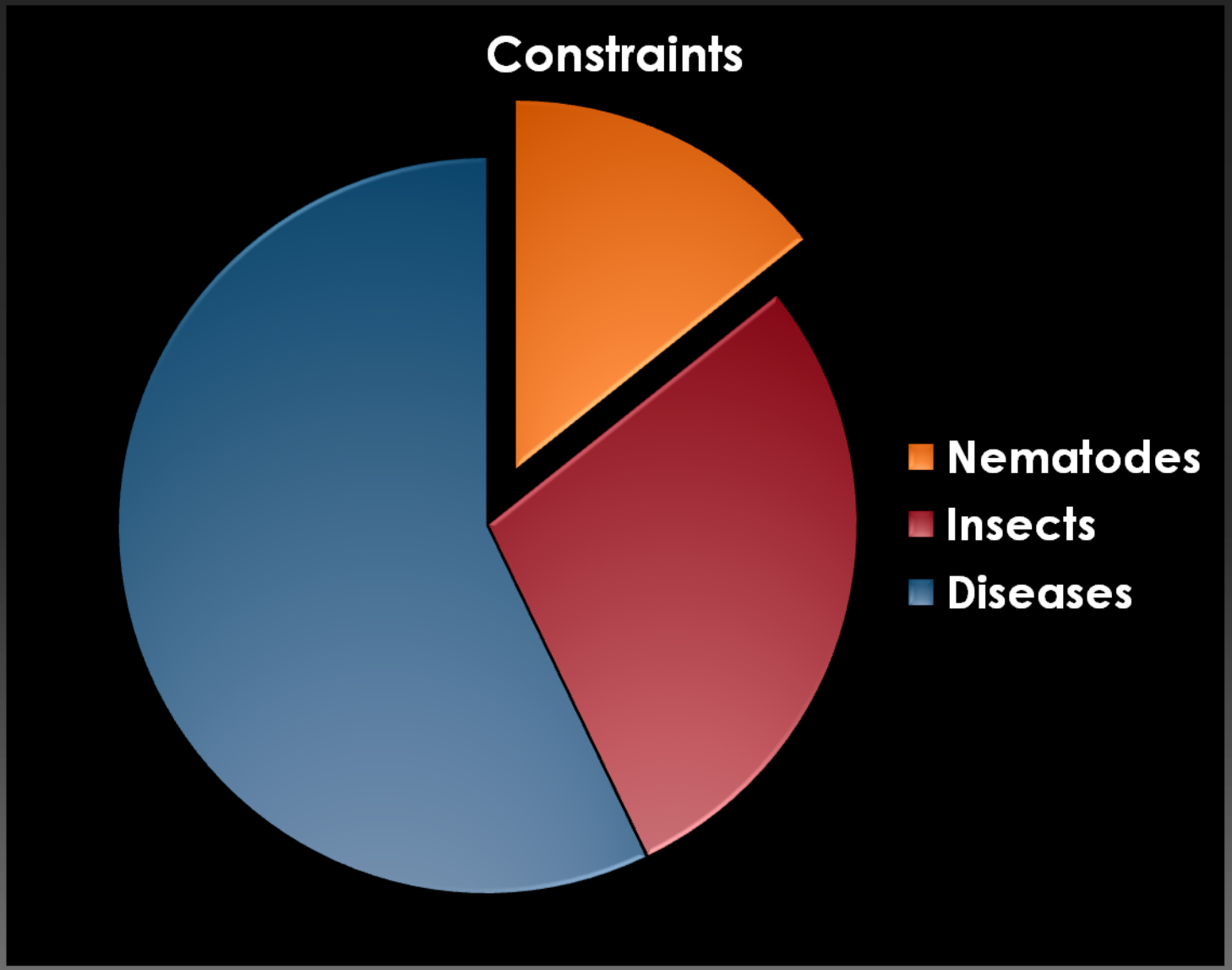
Introduction

Predominant pests and diseases of soybean In South Africa



Introduction

Study has dual purpose:

Commercial

- Annual evaluation to supply farmers and industry with most recent host status of cultivars to RKN

Scientific

- To optimize screening process

Introduction

NEED

- Evaluation of soybean genotypes on an annual basis - determine their host suitability to root-knot nematodes:
- threatens production of the crop in local soybean growing areas

THREAT

- Trend in local soybean production - expansion of production to lighter textured soils where maize was traditionally the dominant crop
- Frequent occurrence of both *M. javanica* and *M. incognita* as the predominant spp. in these maize-producing areas often results in significant maize yield losses

Introduction

INFECTION LEVELS

- Exceptionally high levels of RKN infection experienced in soybean cropping systems:
- 4 252 – 11 401/50g roots in the Bothaville area

MANAGEMENT

- Host plant resistance to RKN - currently one of the few cost-effective and environmentally-friendly management strategies for reducing yield losses caused by these pathogens
- No nematicides (synthetic and/or biological) is currently registered for use on soybean in South Africa

Introduction

TRADITIONAL TECHNIQUES

- Use of traditional greenhouse screening techniques = laborious, time consuming and expensive

BIOCHEMICAL TECHNIQUE

- Use of enzymes such as endogenous catalase activity = quick, accurate and cost-effective to screen genotypes for resistance
- Detection of catalase inhibition in roots of young crop seedlings resistant to RKN can be performed only a few days after nematode inoculation

Objectives

To exploit and enhance the screening of soybean genotypes for their host status to RKN by means of a comparative study using various approaches, namely:

TRADITIONAL TECHNIQUES

- RKN ratings – 30 DAI
- Final population levels (Pf)/root system & Reproduction factor (Rf) values

BIOCHEMICAL TECHNIQUE

- Catalase activity (CAT)

Methodology

Traditional/Classical techniques

Greenhouse trials:
23 Commercially available cv's. (2012) +
LS5995 (R to *M. incognita*)

Cultivar number	Cultivar name
1	Sonop
2	LS 6444 R
3	PAN 1454 R
4	LS 6146 R
5	LS 6248 R
6	PAN 1583 R
7	Highveld Top
8	Knap
9	PHB 95 Y 20
10	PHB 95 Y 40
11	A 5409 RG
12	PHB 95 B 53
13	PAN 1666 R
14	PAN 1664 R
15	LS 6164 R
16	Dundee
17	Marula
18	LS 6161 R
19	LS 6150 R
20	PAN 737 R
21	Egret
22	Heron
23	Ibis 2000
24	LS 5995
25	GCI 1
26	GCI 2
27	GCI 3
28	GCI 4
29	GCI 5
30	GCI 6
31	GCI 7

Methodology

Traditional/Classical techniques

Trial 1:

- Gall ratings 30 DAI
- Histopathology (Light microscopy)

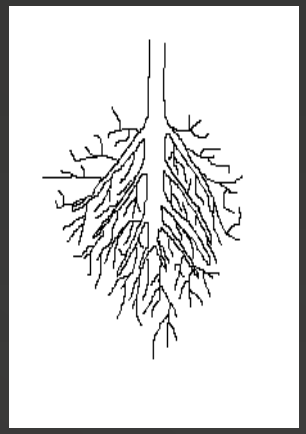
Trial 2:

- Egg & J2 counts
- Rf values

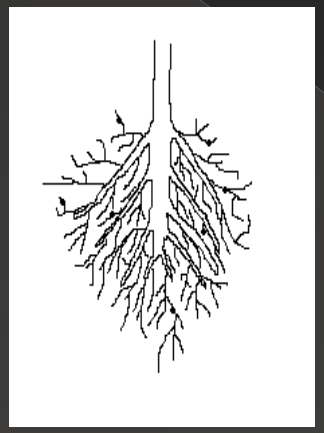
Methodology

Traditional/Classical techniques

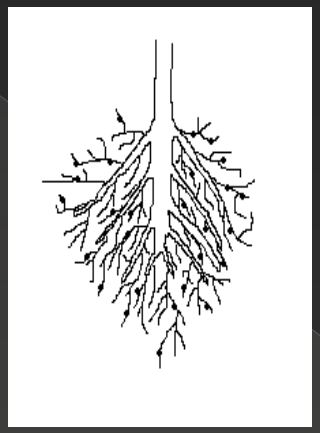
Gall ratings:



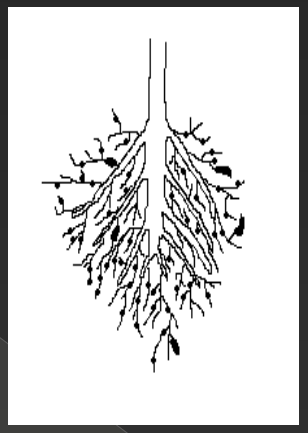
0 - No knots



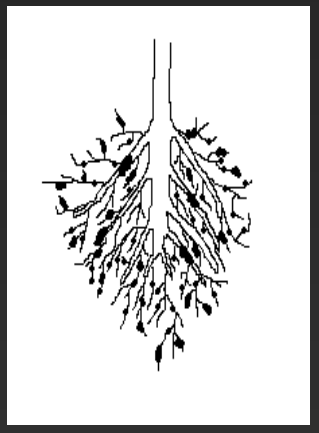
1 – Few knots, difficult to find



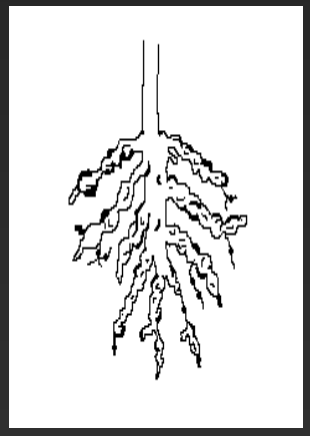
2 – Small knots more visible and abundant, main roots clean



3 – Many small knots visible with some larger knots appearing, main roots clean



4 – Many small and large knots, some larger knots appearing on main roots



5 – Root system reduced, very large knots appear on main roots

Egg & J2 counts:

extracted using adapted NaOCl method (Riekert, 1995)

Rf values:

Pf (final *Mi* population) /Pi (initial *Mi* population)

Methodology

Traditional/Classical techniques

Histopathology (30 DAI)

- Root material - fixated in Todd's and prepared for light microscopy
- Three cultivars used
  - 1 resistant , 1 poor host
  - 1 susceptible

Methodology

Biochemical technique

Cultivars

24 cultivars – greenhouse assay

- identify 2 resistant or non-hosts, 1 highly susceptible

Innoculation

Inoculate 30 day old seedlings with 5 000 *M. incognita* J2

- Six replicates
- Split-plot design - half inoculated
  - half uninoculated (control)

Material

Leaves and roots – obtained 48h after inoculation

- Preserved in liquid nitrogen

Methodology

Biochemical technique

CAT

CAT activity was determined by following the disappearance of H_2O_2 in the enzyme reaction mixture, thus **Higher levels of H_2O_2 indicate lower levels of catalase**

Absorbance

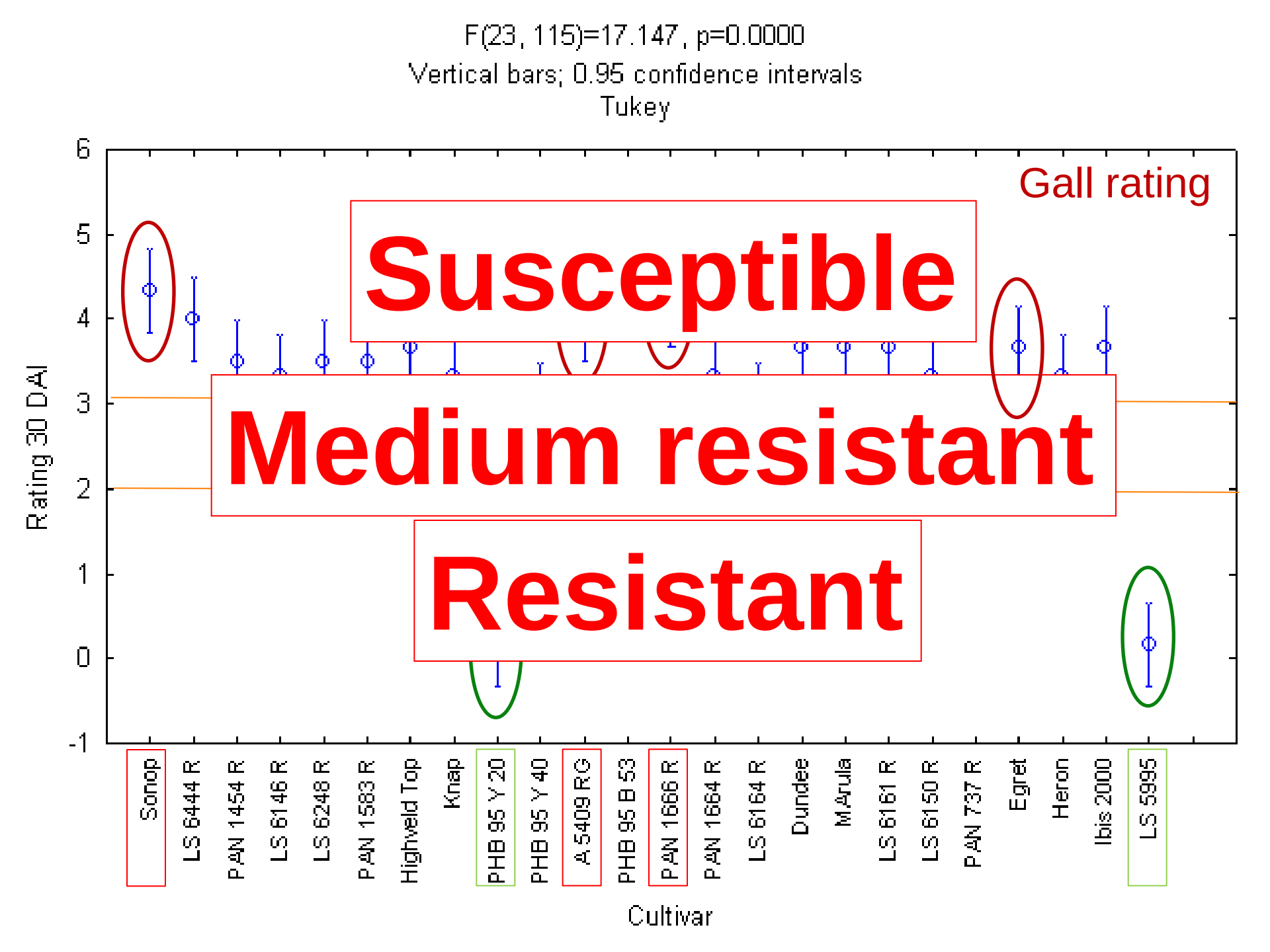
The absorbance of the reaction mixture was read at 415 nm against a distilled water control

Standard curve

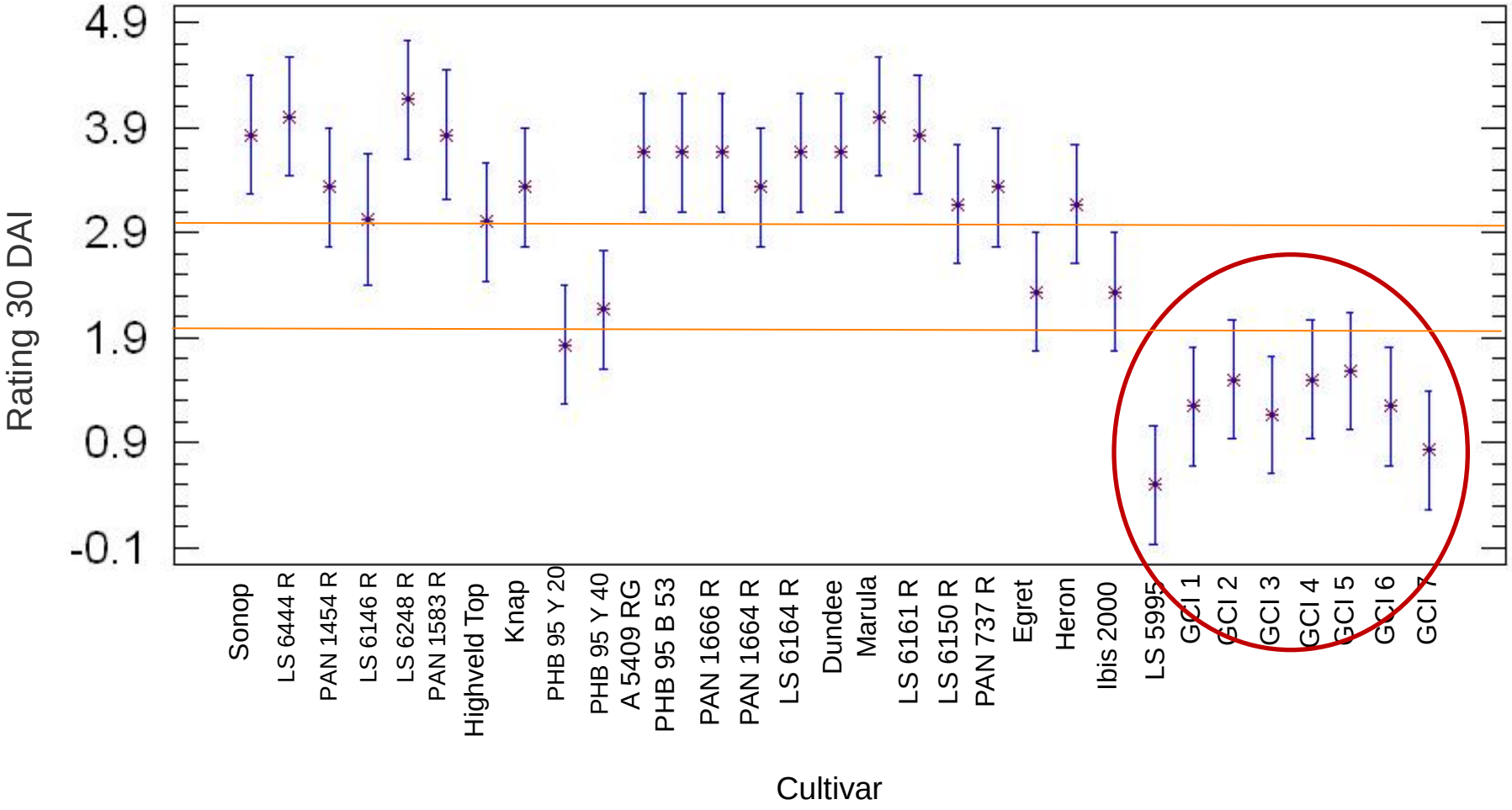
CAT activity was determined by comparing absorbance against a standard curve of H_2O_2 from 0.25 to 2.5 mM. The activity of CAT was presented as H_2O_2 mM /min /mg protein (Gong *et al.*, 2001)

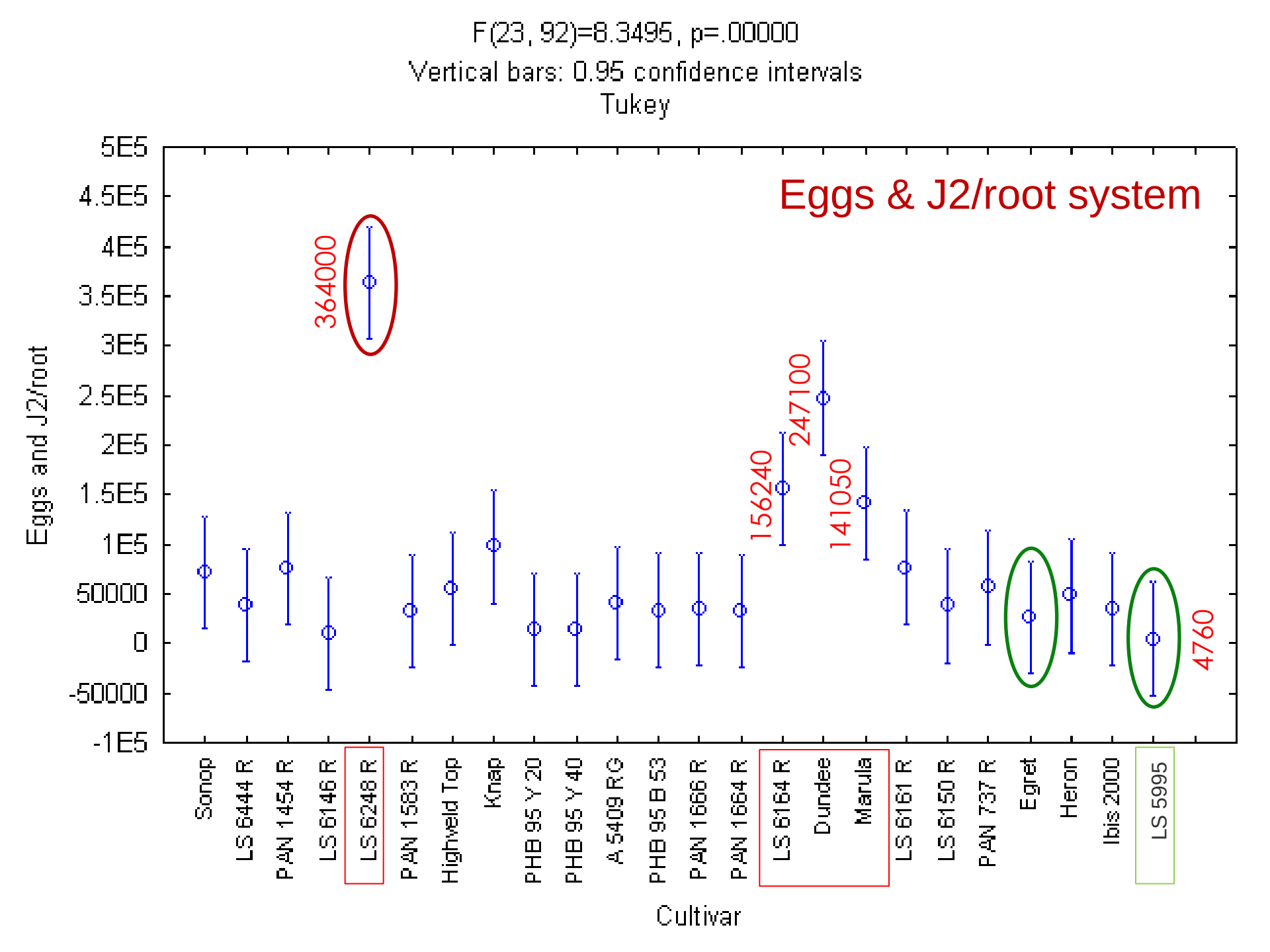
Traditional techniques

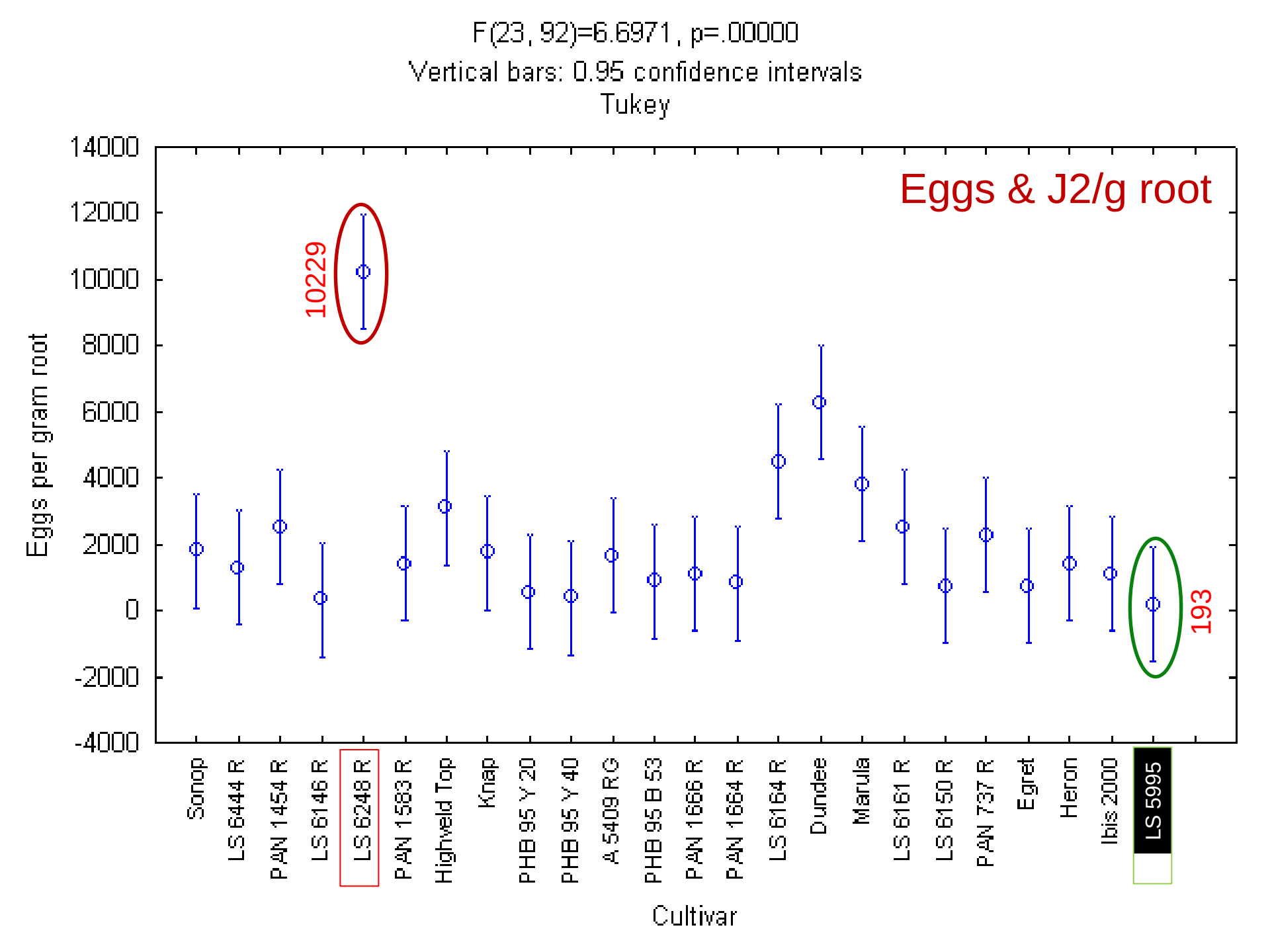
Results

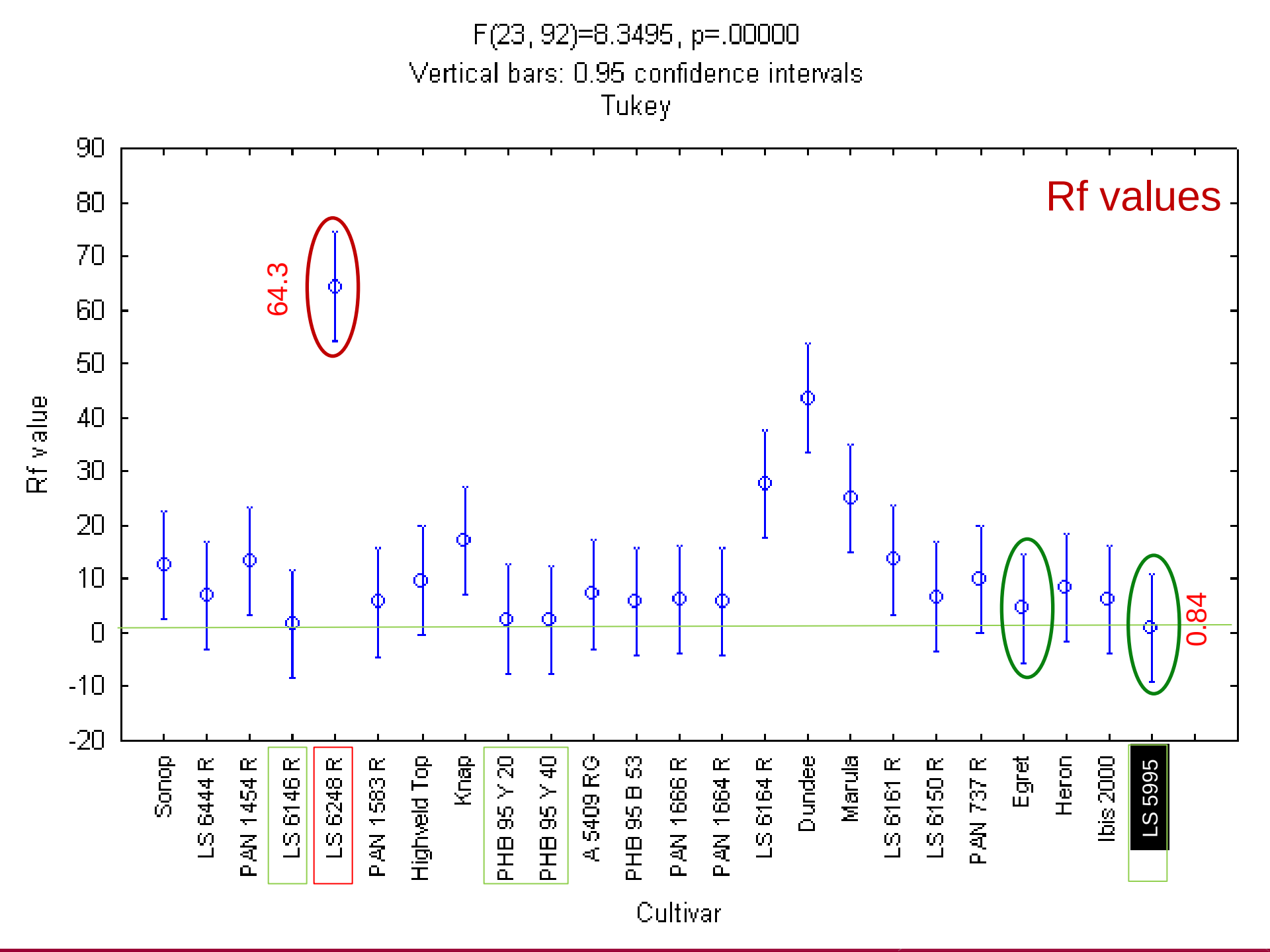

F(23, 115)=17.147, p=0.0000
Vertical bars; 0.95 confidence intervals
Tukey
Gall rating
Susceptible
Medium resistant
Resistant
Rating 30 DAI
6
5
4
3
2
1
0
-1
Sonop
LS 6444 R
PAN 1454 R
LS 6146 R
LS 6248 R
PAN 1583 R
Highveld Top
Knap
PHB 95 Y 20
PHB 95 Y 40
A 5409 RG
PHB 95 B 53
PAN 1666 R
PAN 1664 R
LS 6164 R
Dundee
MArula
LS 6161 R
LS 6150 R
PAN 737 R
Egret
Heron
Ibis 2000
LS 5995
Cultivar

F (31, 148) = 28,03, P=0.0000
Vertical bars; 0.95 confidence intervals
Tukey




F(23, 92)=8.3495, p=.00000
Vertical bars: 0.95 confidence intervals
Tukey
Eggs & J2/root system
Eggs and J2/root
5E5
4.5E5
4E5
3.5E5
3E5
2.5E5
2E5
1.5E5
1E5
50000
0
-50000
-1E5
364000
156240
247100
141050
4760
Sonop
LS 6444 R
PAN 1454 R
LS 6146 R
LS 6248 R
PAN 1583 R
Highveld Top
Knap
PHB 95 Y 20
PHB 95 Y 40
A 5409 RG
PHB 95 B 53
PAN 1666 R
PAN 1664 R
LS 6164 R
Dundee
Marula
LS 6161 R
LS 6150 R
PAN 737 R
Egret
Heron
Ibis 2000
LS 5995
Cultivar


F(23, 92)=6.6971, p=.00000
Vertical bars: 0.95 confidence intervals
Tukey
Eggs & J2/g root
Eggs per gram root
14000
12000
10000
8000
6000
4000
2000
0
-2000
-4000
10229
193
Sonop
LS 6444 R
PAN 1454 R
LS 6146 R
LS 6248 R
PAN 1583 R
Highveld Top
Knap
PHB 95 Y 20
PHB 95 Y 40
A 5409 RG
PHB 95 B 53
PAN 1666 R
PAN 1664 R
LS 6164 R
Dundee
Marula
LS 6161 R
LS 6150 R
PAN 737 R
Egret
Heron
Ibis 2000
LS 5995
Cultivar


F(23, 92)=8.3495, p=.00000
Vertical bars: 0.95 confidence intervals
Tukey
Rf values
64.3
0.84
Rf value
Cultivar
90
80
70
60
50
40
30
20
10
0
-10
-20
Sonop
LS 6444 R
PAN 1454 R
LS 6146 R
LS 6248 R
PAN 1583 R
Highveld Top
Knap
PHB 95 Y 20
PHB 95 Y 40
A 5409 RG
PHB 95 B 53
PAN 1666 R
PAN 1664 R
LS 6164 R
Dundee
Marula
LS 6161 R
LS 6150 R
PAN 737 R
Egret
Heron
Ibis 2000
LS 5995

Summary of previous results

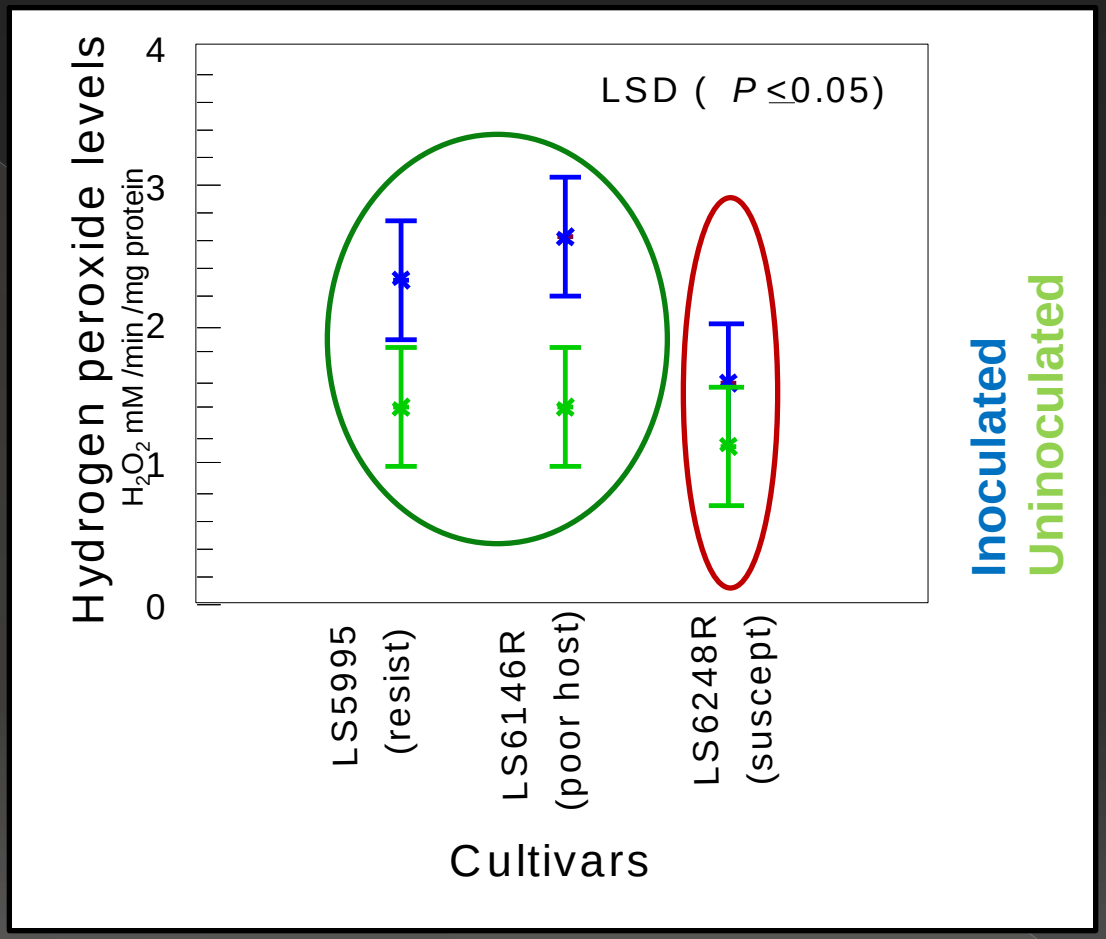
Technique	Highly resistant cv (s)
Rating 30 DAI	LS5995; PHB95Y20; GCI 1 to 7
Eggs and J2/root system 56 DAI	LS5995
Rf value	LS5995

Gall ratings – indication of R but not as reliable as eggs and J2 or Rf values

- many galls may be present but low numbers of eggs produced by female due to sub-optimal development within resistant/poor host cv's – histopathology results

Biochemical technique

Results

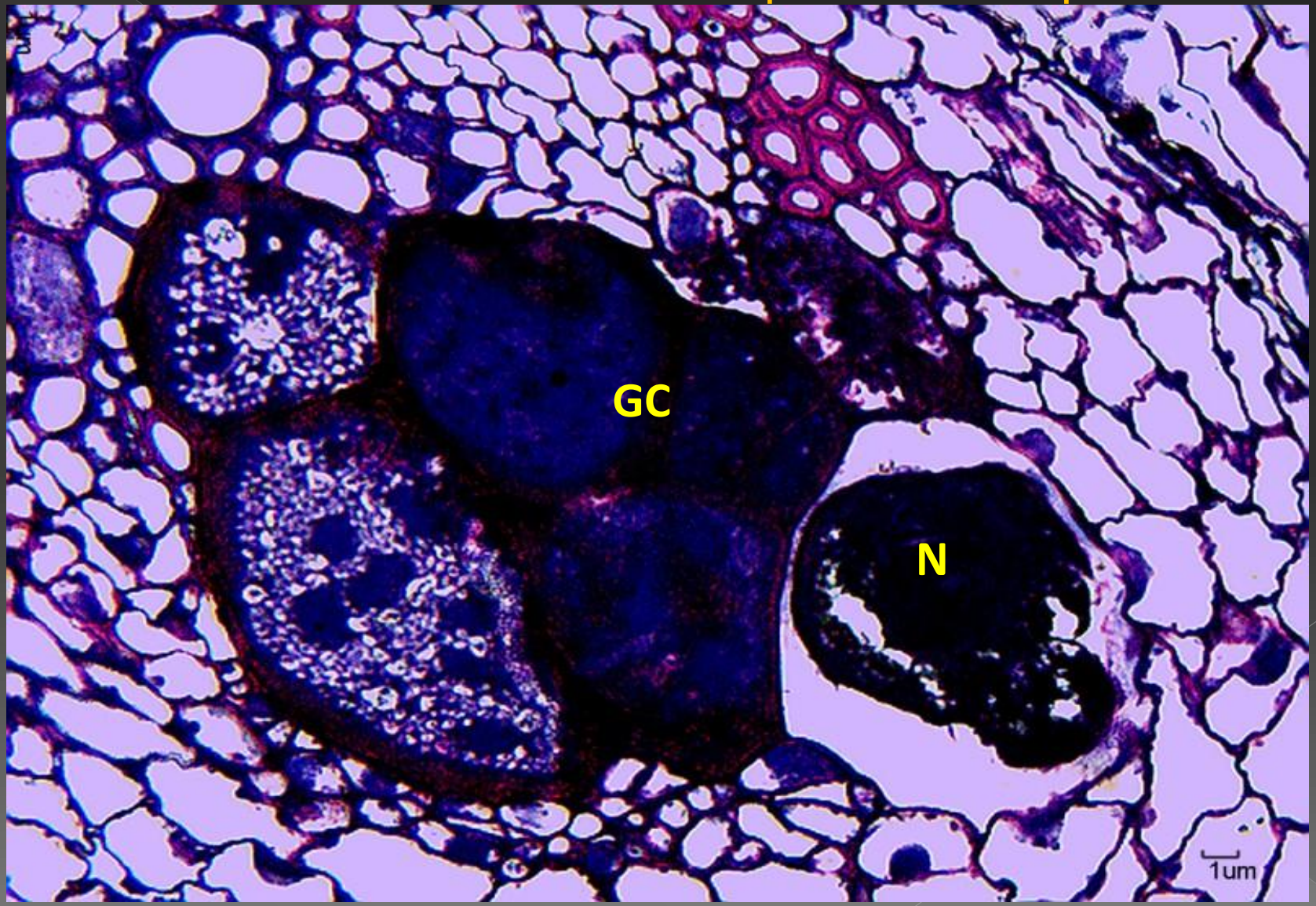


Leaf data - root data inconsistent at this stage

Light microscopy

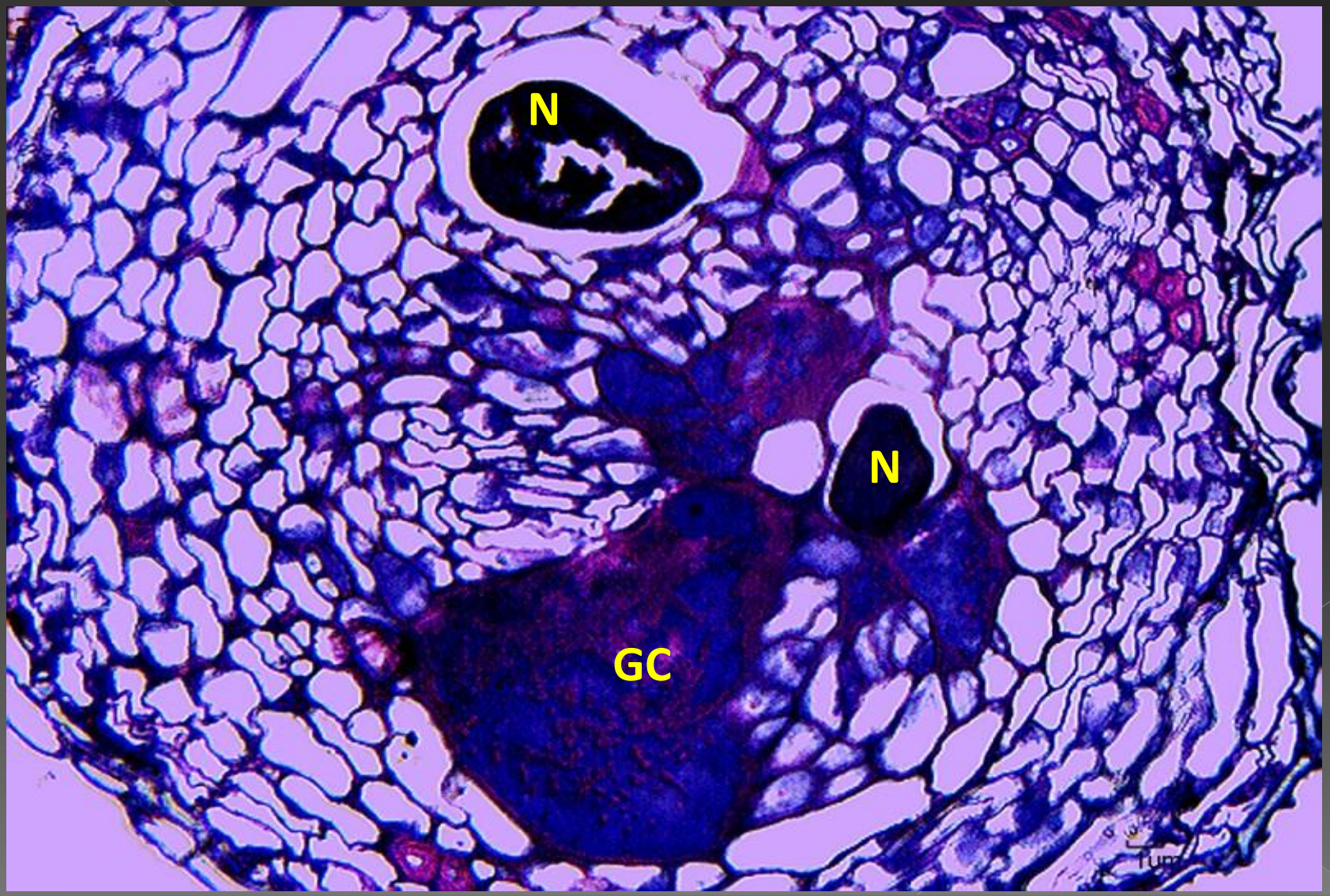
Susceptible: LS6248R – many GC, large with thick walls
= OPTIMAL for nematode development and reproduction

Results



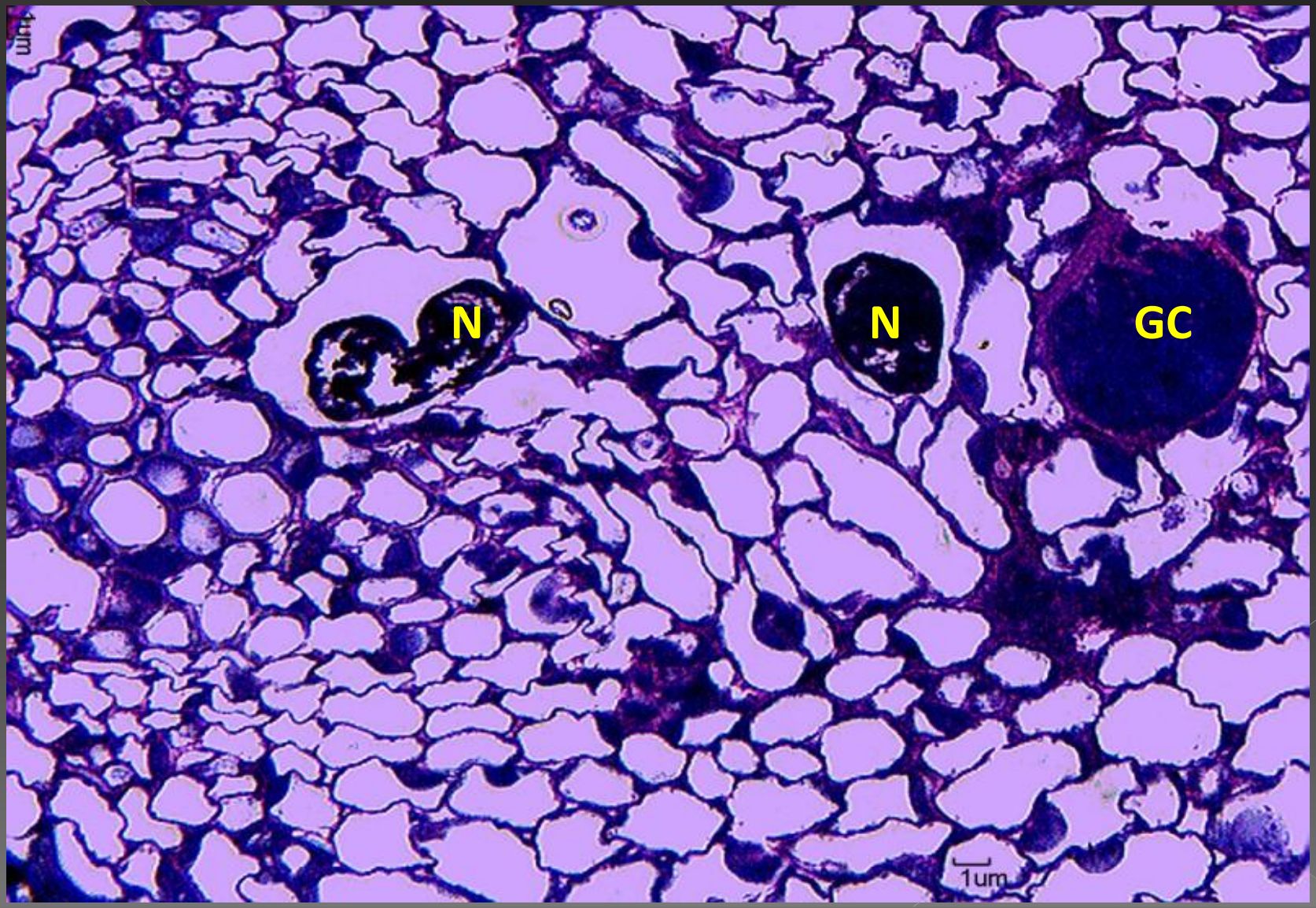
Results

Resistant: LS5995 – few GC, weak with thin walls = NON-OPTIMAL for nematode development and reproduction



Results

Resistant: GCI7 – one/few GC, weak with thin walls = NON-OPTIMAL for nematode development and reproduction



Conclusive remarks

According to results obtained using the traditional screening assays:

Gall ratings:

differences in results in terms of gall ratings at 30 DAI and eggs and J2 counts/root system, eggs and J2 counts/g root and Rf values 56 DAI

- egg and J2 counts & Rf values still the best parameters

CAT – still in early stages but a definite trend already visible

– higher CAT inhibition in R cv's

– still refinement

Light microscopy – giant cell formation in susceptible cv = optimal and in resistant cultivar = non-optimal and retarded

The way forward

Annual screening of commercially-available cultivars – identification of resistant and poor-host cv's

Further testing for CAT activity and refinement of the protocol

Detection of CAT activity through O^2 generation – 782 Oxygen Meter (Strathkelvin Instrument) together with zoologist (physiologist)

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NWU for availability of Infrastructure

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OPDT OPOT

Olie- & Proteiensade Ontwikkelingstrust

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Everyone present this afternoon
Thank you

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