Expanding the genetic resources of lentils and *Rhizobium* to increase nitrogen fixation (BNF) in the lentil crop

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Outline

- Biological Nitrogen Fixation (BNF) in lentils
- Genetic Resources

Rhizobial strains Lentil genotypes/species

Methodology

Preliminary Results

- Future work

BNF in lentils

- Canada is the largest exporter/producer of lentils
- Symbiosis is the most important route to obtain N

  - inoculation

- %Ndfa (Nitrogen derived from atmosphere)

  - lentil: 0-87% (0-192 Kg/ha)

Modern varieties: usually selected under high fertility conditions.

FAO,2016. www.fao.org/faostat/en
Herridge *et al*., 2009. Plant and Soil 311:1-18.

Hypotheses

- The study of a broad group of cultivated and wild accessions within Lens species, will allow the identification of superior species/genotypes that can contribute positive N-fixation related alleles to modern lentil varieties.
- Exploring the genetic diversity of *Rhizobium* from the center of origin of lentils and other main production areas allows the incorporation of more efficient strains to the Saskatchewan cropping system, increasing N available in lentils.

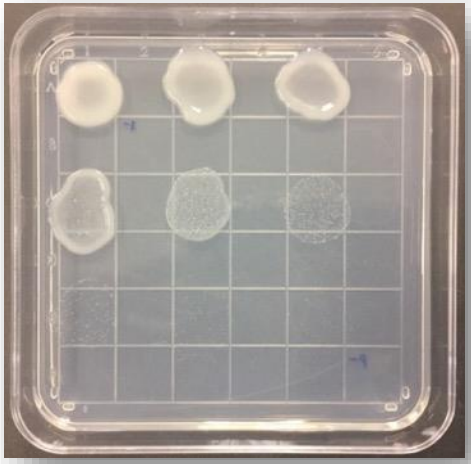
Genetic Resources

Lentils

- *L. culinaris, L. orientalis, L. tomentosus*
- *L. odemensis, L. lamottei*
- *L. ervoides*
- *L. nigricans*

Rhizobium strains

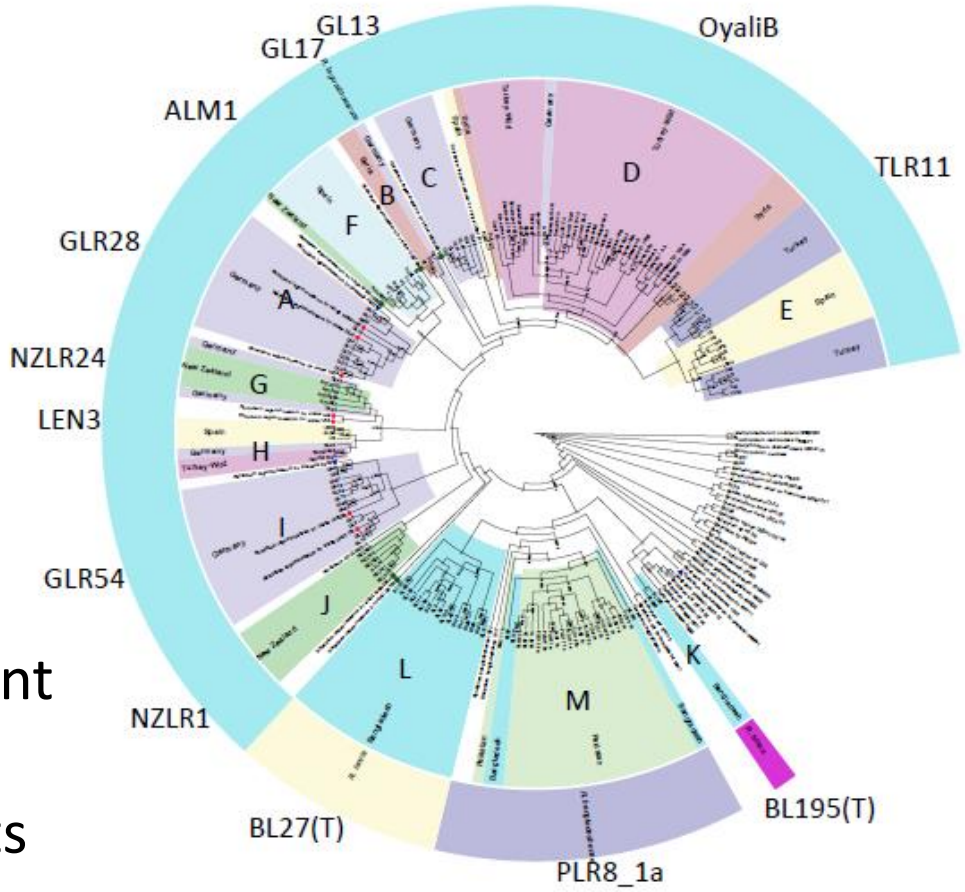
- *R. leguminosarum* bv. *viciae*
- *R. bangladeshense*
- *R. lentils*
- *R. binae*
- New species



Harun-on-Rashid *et al*., 2014. FEMS Microbiology Ecology 87:64-77
Podder *et al*., 2012. Plant Genetic Resources 11:26-35

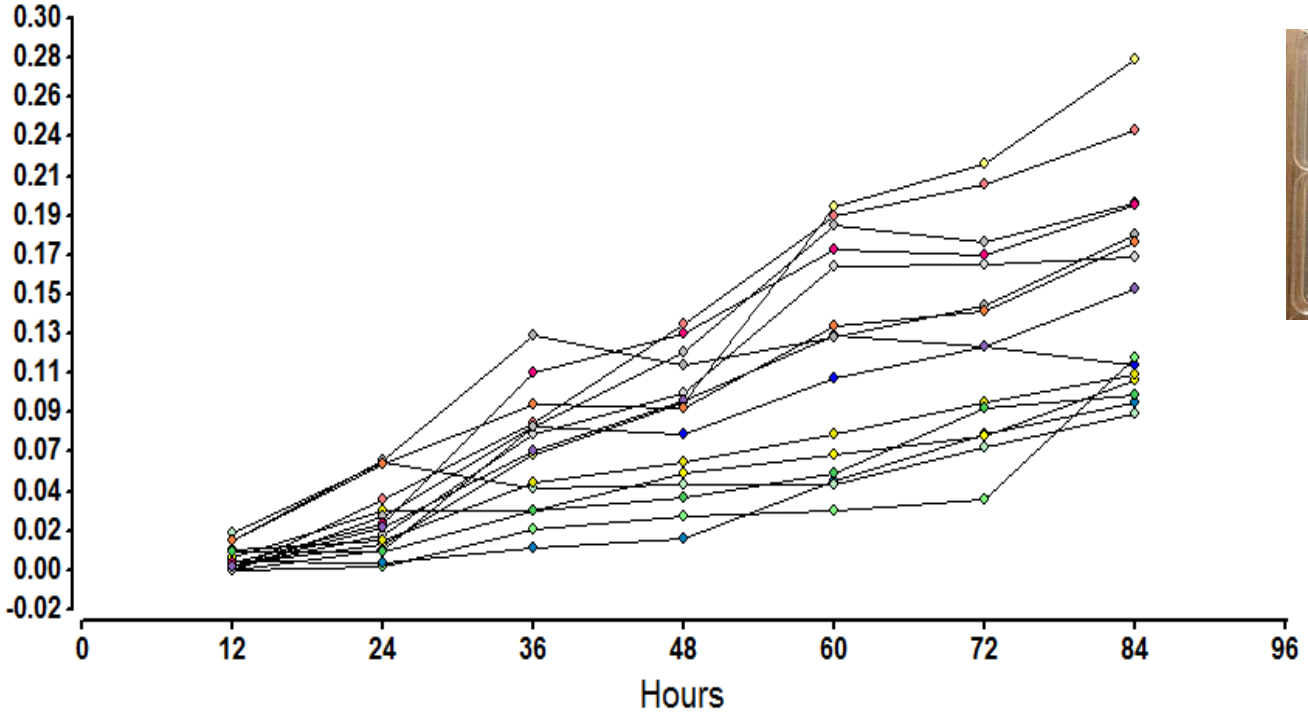
Methodology: strains

14 strains <-> CDC Maxim
BNF potential under local conditions
Controls: BASF 4035, Non inoculated
4 site-years: Sutherland, Rosthern (1-2017, 3-2018)
Soil analysis record for N content
RCBD: 8 repetitions
15 *Rhizobium* treatments
120 plots

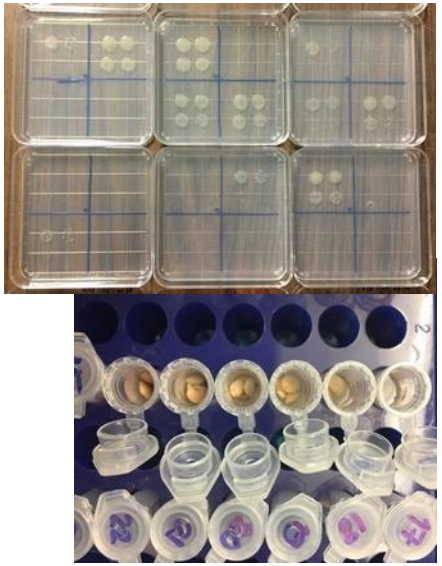


Riely *et al*., 2017. UC Davis

Methodology: strains



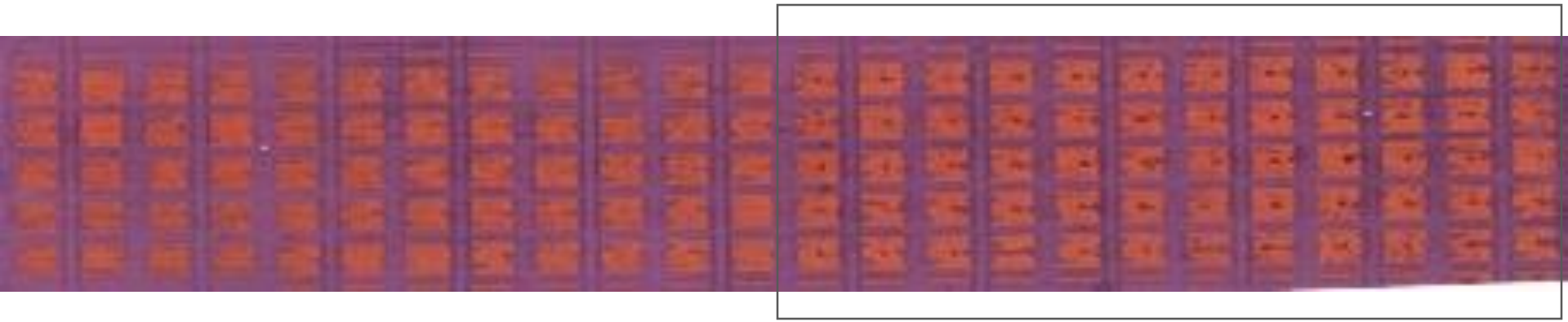
OD measurements of 15 strains at 600 nm during 84 hours

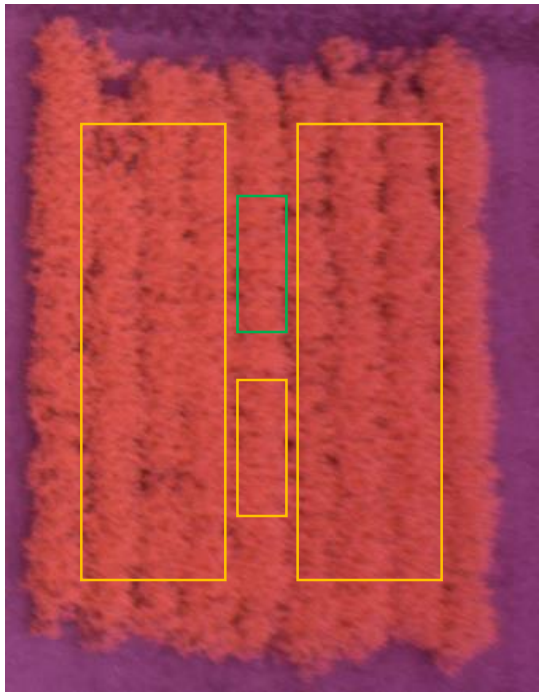


Survival on seed surface

Seed inoculation and drying

Methodology: strains





<u>At flowering (10 plants center row):</u>

Shoot area: N content, SDW (g)

Roots

-Presence and structure of nodules: number, position, color

-NDW

At maturity (10 plants+6 rows)

-yield traits: #pots/plant, 1000 SW

-yield

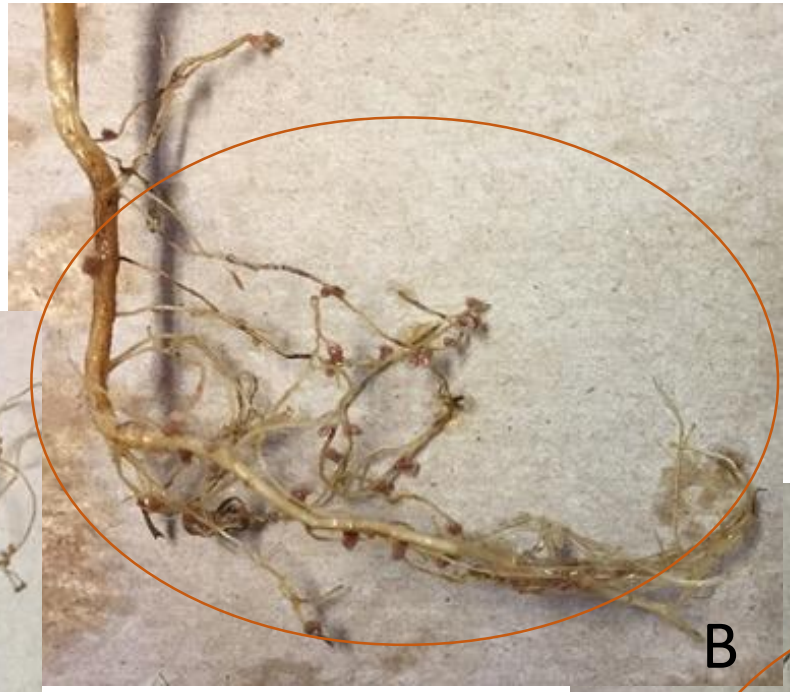
-Protein (seed)

Photos: Karsten Nielsen

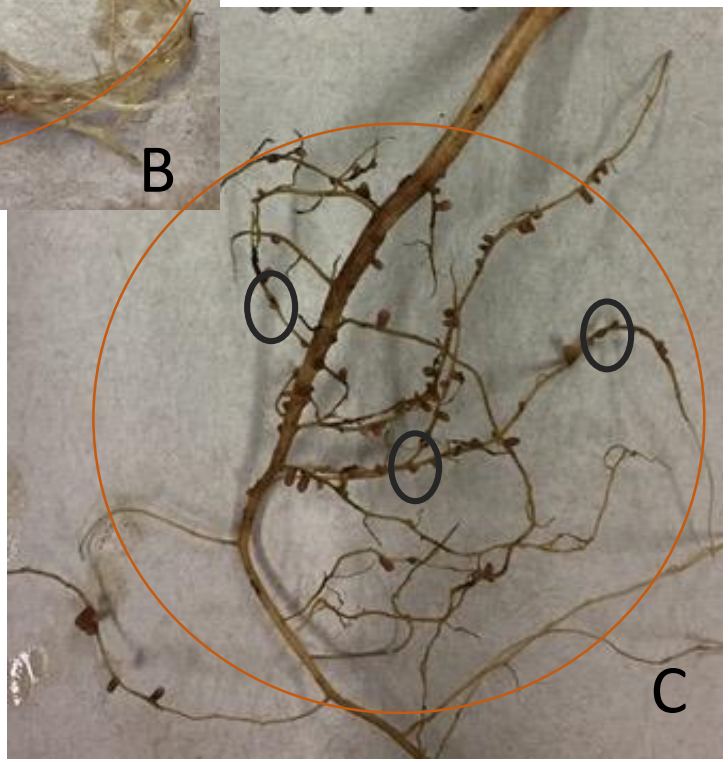
Preliminary Results

Nodulation in the field

OyaliB



NZLR1



GL17

Yield of CDC Maxim inoculated with 15 different *Rhizobium* strains. Sutherland, SK. 2017

Strain	Species	Yield(Kg/ha)	Protein (%)
NZLR24	*R. leguminosarum*	2587a	25.90a
NZLR1	*R. leguminosarum*	2409 b	25.38a
ALM1	*R. leguminosarum*	2377 bc	25.52a
BASF 4035	R. *leguminosarum*	2339 bcd	25.47a
GLR54	*R. leguminosarum*	2335 bcd	25.32a
PLR8_1a	*R. bangladeshense*	2333 bcd	24.51 b
Non-inoculated		2328 bcd	25.21ab
LEN3	*R. leguminosarum*	2307 bcde	25.61a
TLR11	*R. leguminosarum*	2275 bcde	25.64a
GL13	*R. leguminosarum*	2248 bcde	25.38a
BL27(T)	*R. lentils*	2245 bcde	25.55a
OyaliB	*R. leguminosarum*	2214 cde	25.44a
GL17	*R. leguminosarum*	2211 cde	25.49a
BL195(T)	*R. binae*	2180 de	25.57a
GLR28	*R. leguminosarum*	2145 e	25.77a
LSD,CV		174.9, 7.66	0.74,2.94

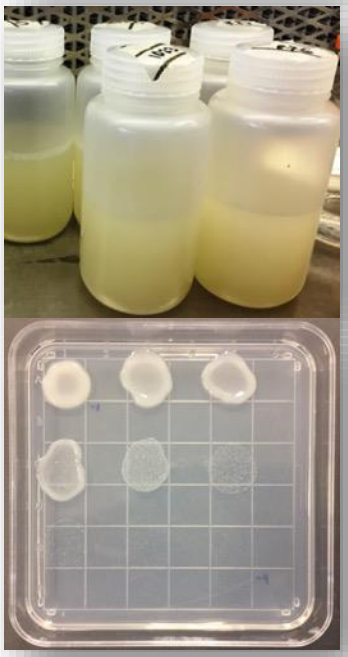
Methodology: genotypes

Sand:Sunshine (1:1)
pasteurized

N free nutrient solution

Seed
scarification/disinfection

36 genotypes
7 *Lens* species



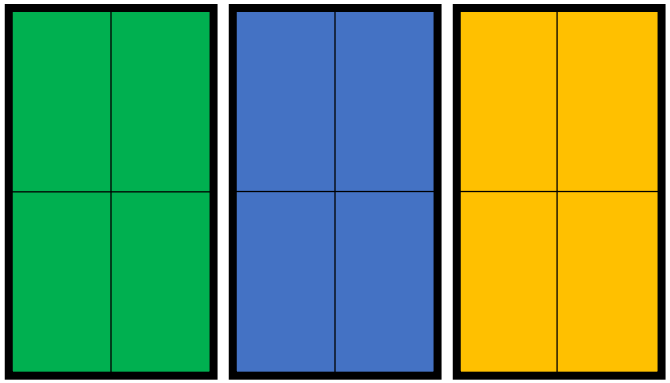
Inoculum
BASF 4035-YMB media

Quality control
1×10^9 cells/ml

Inoculation
1 ml/plant

Somasegaran and Hoben. 1991. Handbook for Rizobia

Methodology: genotypes



+N Rhizobium -N

Split plot design (432 exp.units)

Methodology: genotypes

Evaluation:

Photosynthesis related parameters

<u>MultispepQ with PhotosynQ app</u>

Chlorophyll Fluorescence

Relative Chlorophyll: SPAD

Leaf Temperature and differential from ambient temperature

<u>At flowering:</u>

Shoot area: N content, SDW (g)

Roots

-Presence and structure of nodules: number, position, color

-Efficiency (N_2fixed/nodule)

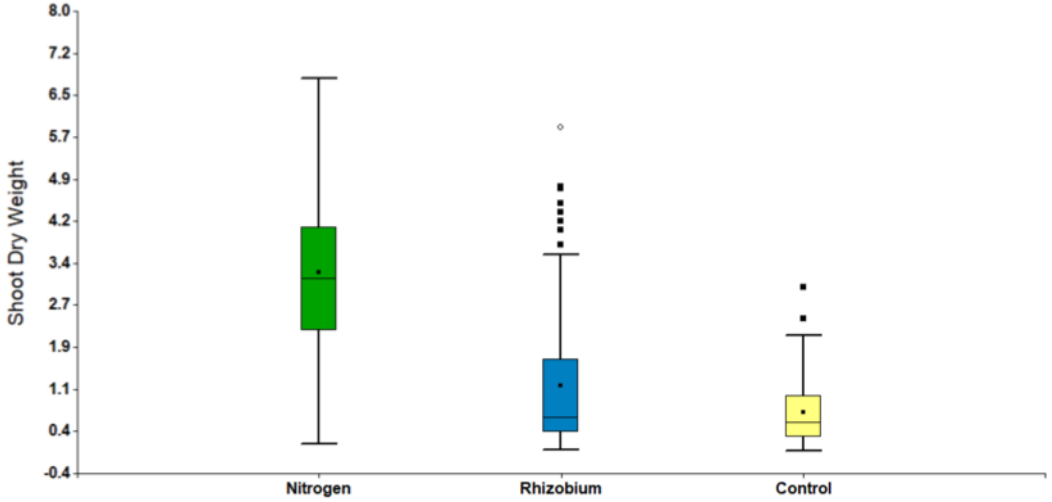
Nodulation in the GH

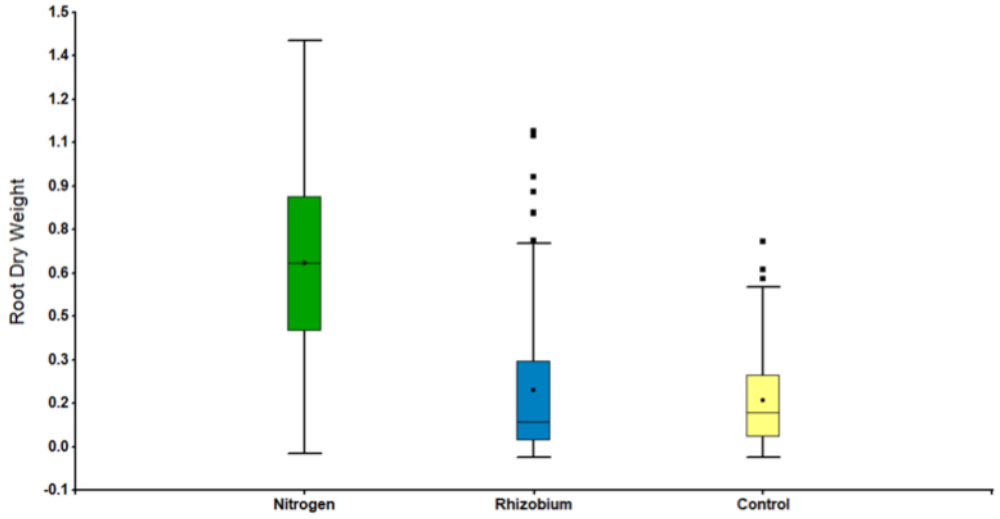
L. orientalis
IG 72643

L. orientalis
IG 72611

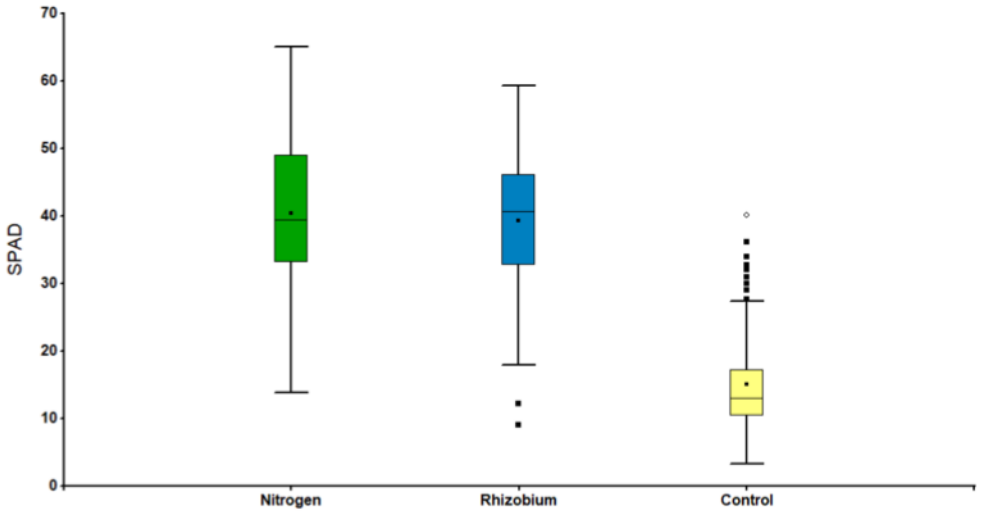
L. culinaris
CDC Maxim

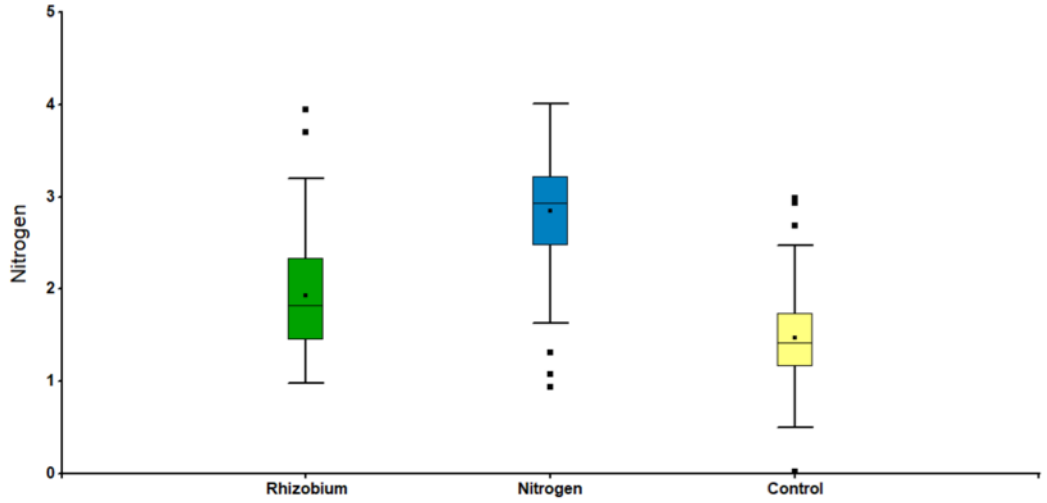
Shoot and root dry weight of 36 genotypes of lentils inoculated with *R. leguminosarum*



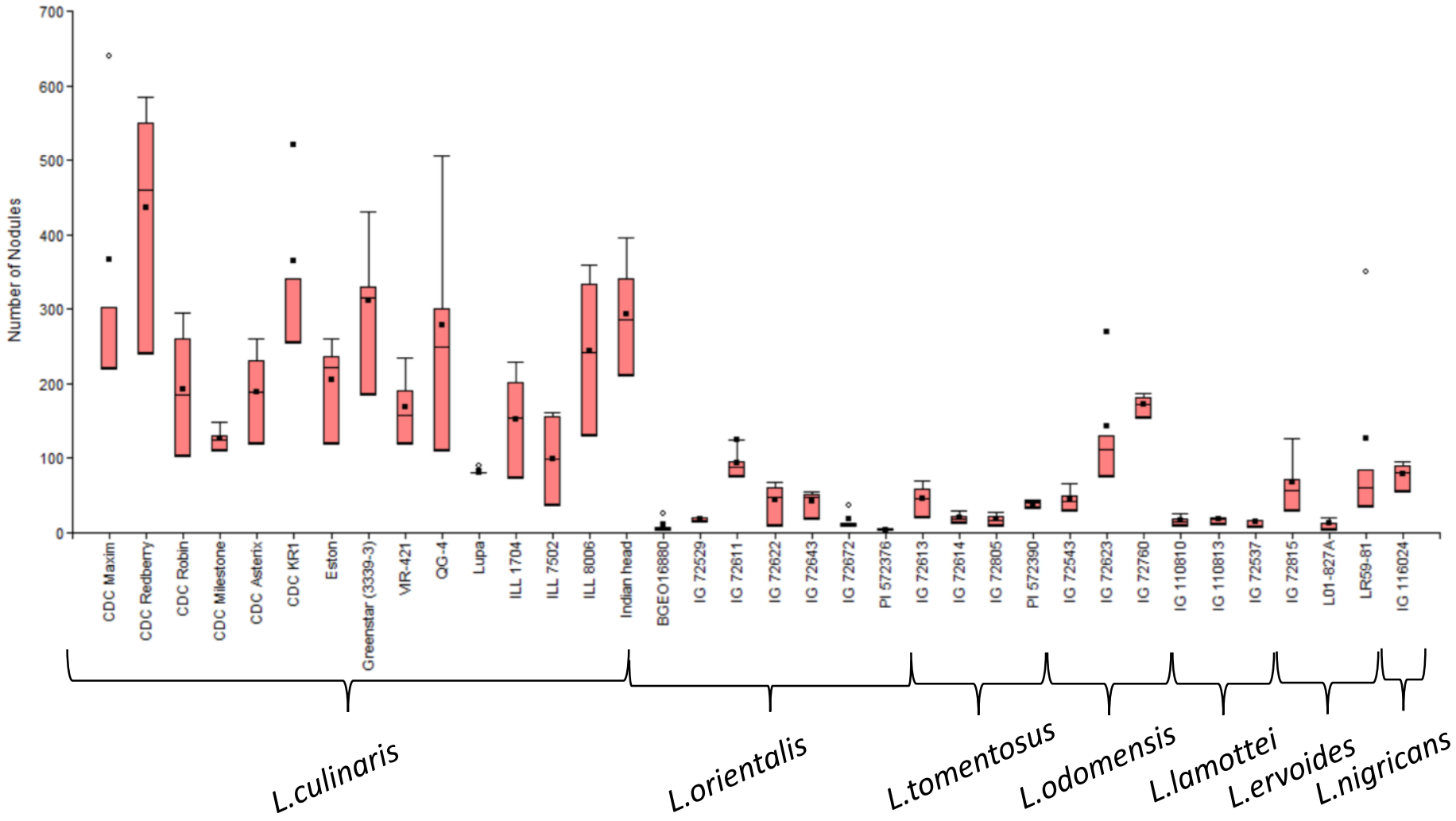


Relative Chlorophyll and Nitrogen of 36 genotypes of lentils inoculated with *R. leguminosarum*

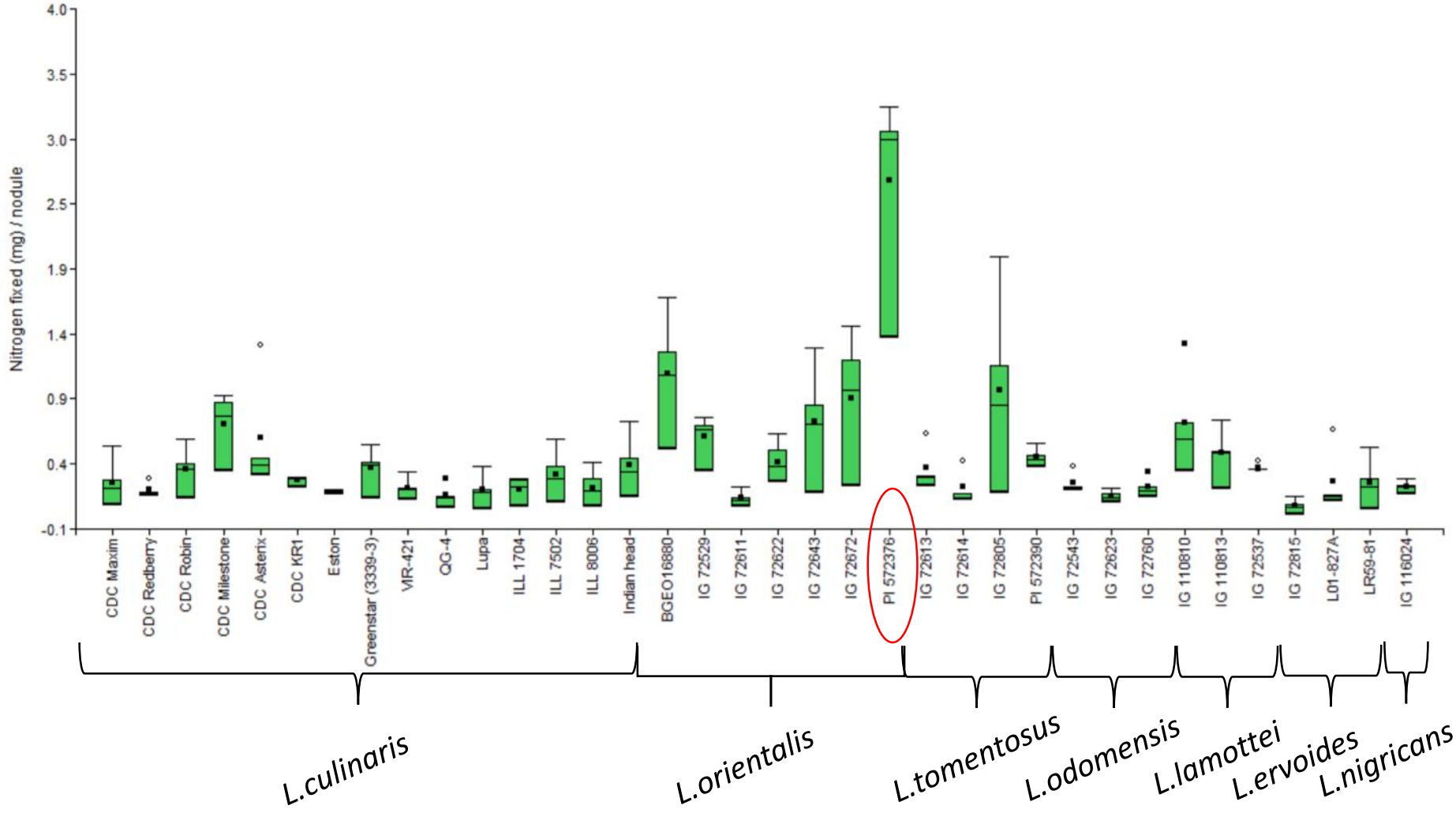




Number of nodules of 36 genotypes of lentils inoculated with *R. leguminosarum*

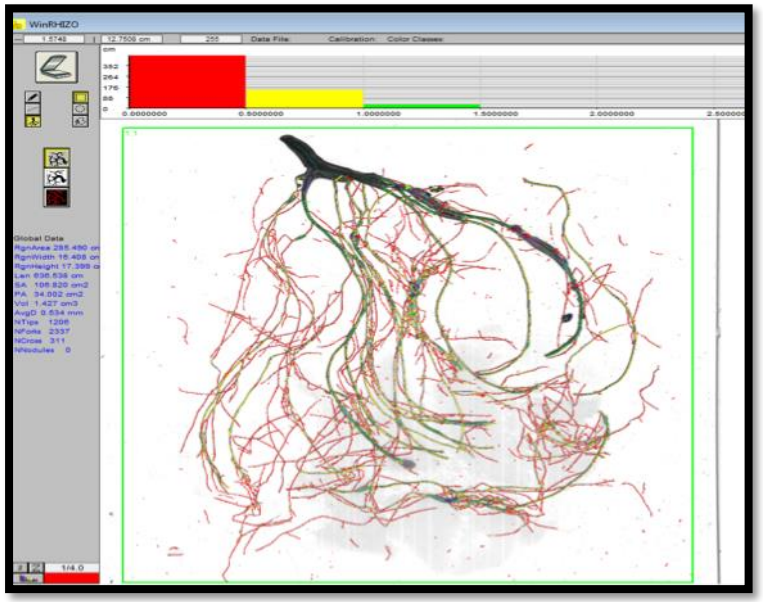


Nitrogen fixed (mg)/nodule of 36 genotypes of lentils inoculated with *R. leguminosarum*



What's next?

Image-based analyses



Study of interspecific populations

Genetic mapping of QTL's associated with higher BNF ability in interspecific populations

APPLICATION OF GENOMICS
TO INNOVATION IN THE LENTIL ECONOMY

GenomeCanada

GenomePrairie

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Brendan Riely
Douglas Cook
Brent Barlow
Devini Da Silva
Eldon Siemens
Linda Gorim
Crystal Chan
Robert Stonehouse
Akiko Tomita
Karsten Nielsen
Pulse Field Crew