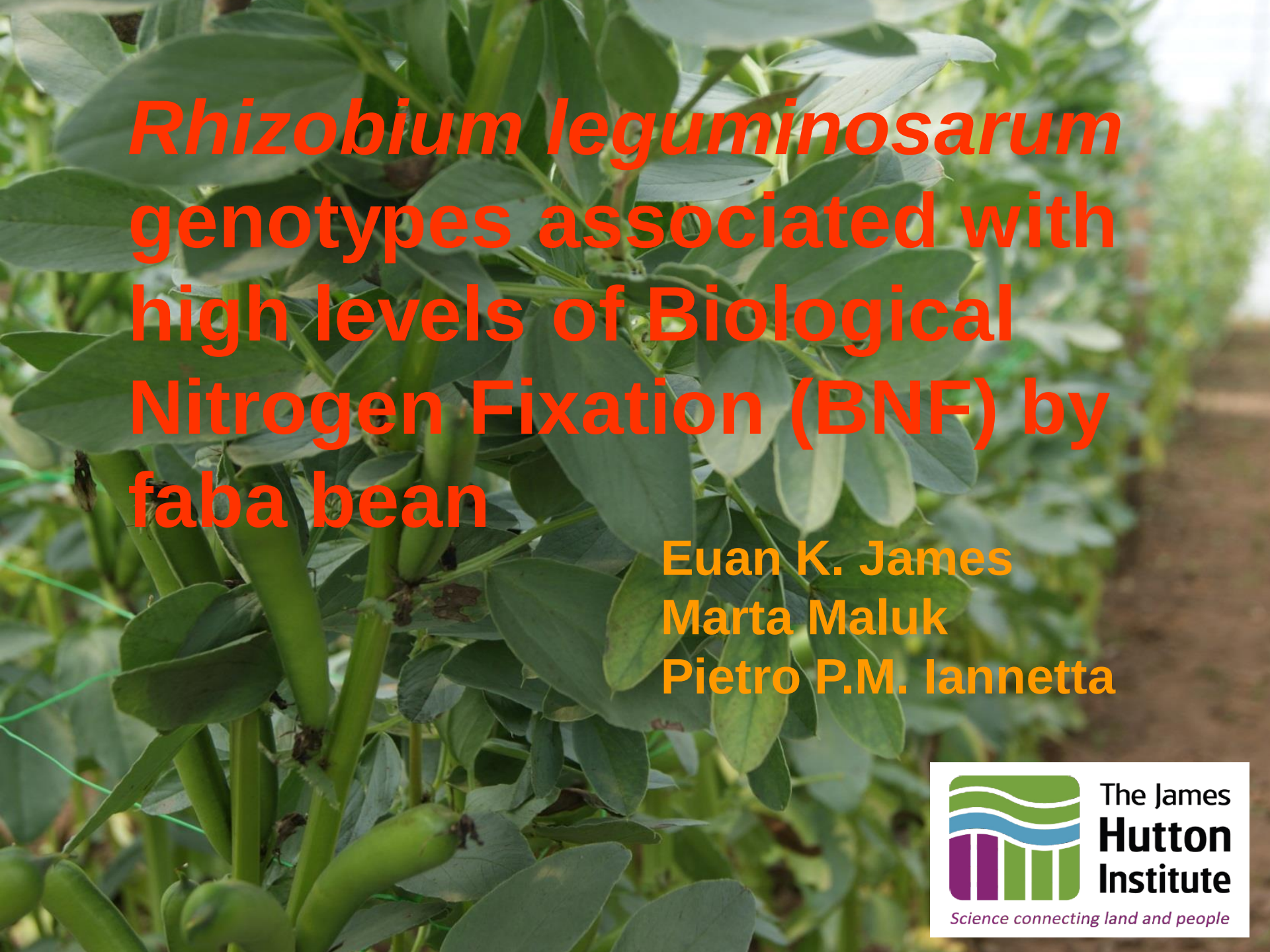

# *Rhizobium leguminosarum* genotypes associated with high levels of Biological Nitrogen Fixation (BNF) by faba bean


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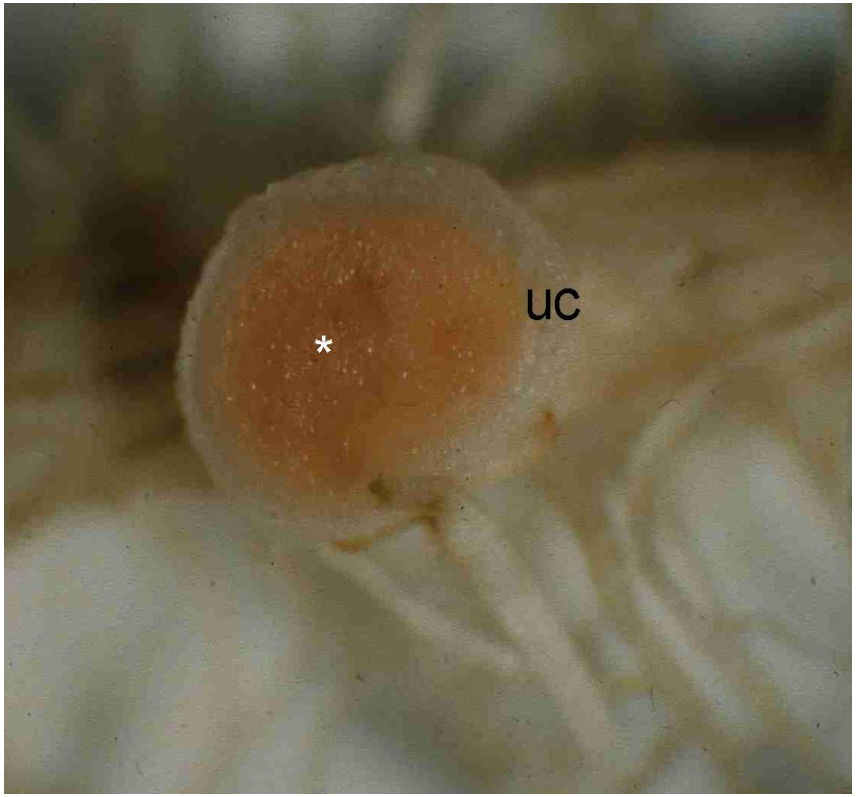
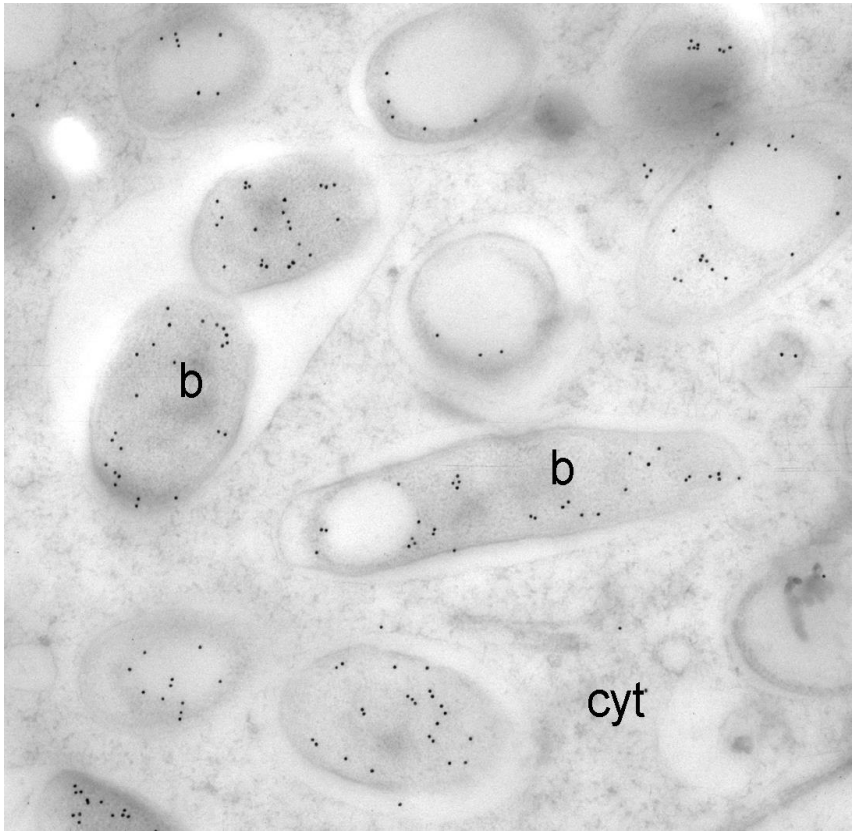
The James Hutton Institute
Science connecting land and people

# Biological Nitrogen Fixation (BNF)

$N_2 + 10H^+ + 8e^- \rightarrow 2NH^{4+} + H_2$




Root nodule on *Sesbania rostrata* cut open to reveal the $N_2$-fixing cells (*)




Immunolocalisation of nitrogenase Fe-protein in bacteroids (b)

## BNF “Green Manures” in action

No fertiliser necessary, as you get your Nitrogen for free!


Paddy field at IRRI, Manila

Stem nodulated *Sesbania rostrata*

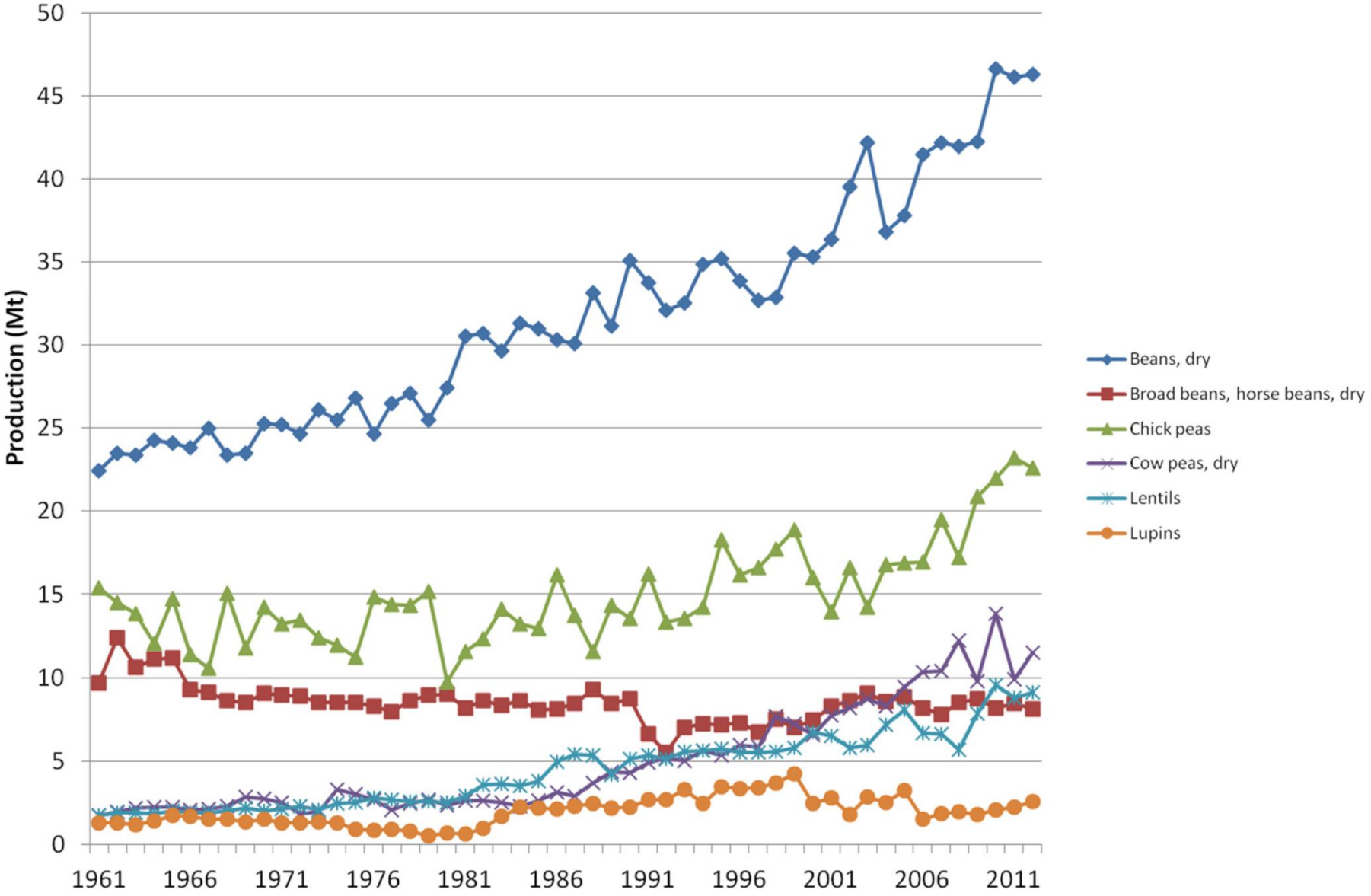
# Non-nodulated legumes are N-deficient

# Economical importance of BNF

- Australia (soybean, peas, f. beans)
- New Zealand (pasture legumes for dairy)
- Brazil/Argentina (soybean, French bean)
- USA (soybean, peas)
- Worth billions of $ to the economies of these countries
- Assisted by large research programmes into rhizobial inoculants and their application (in partnership with industry)
- $10 m Bill Gates-funded programme aims to bring this technology to Africa ("$N_2$ Africa"): soybeans, common beans, cowpeas

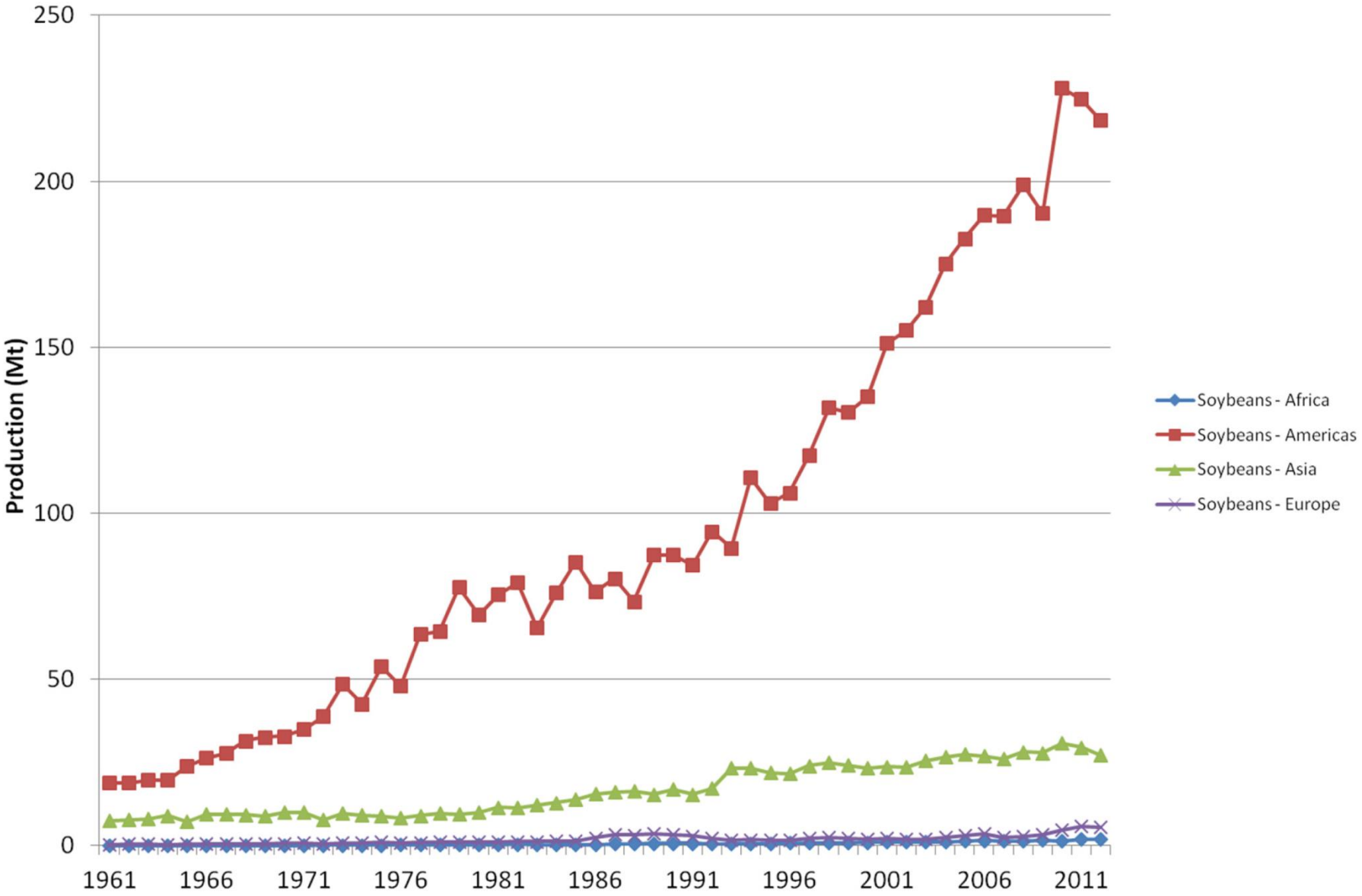
# Global grain legume production

## FAO STAT 2013



# Soybean production

## FAO STAT 2013

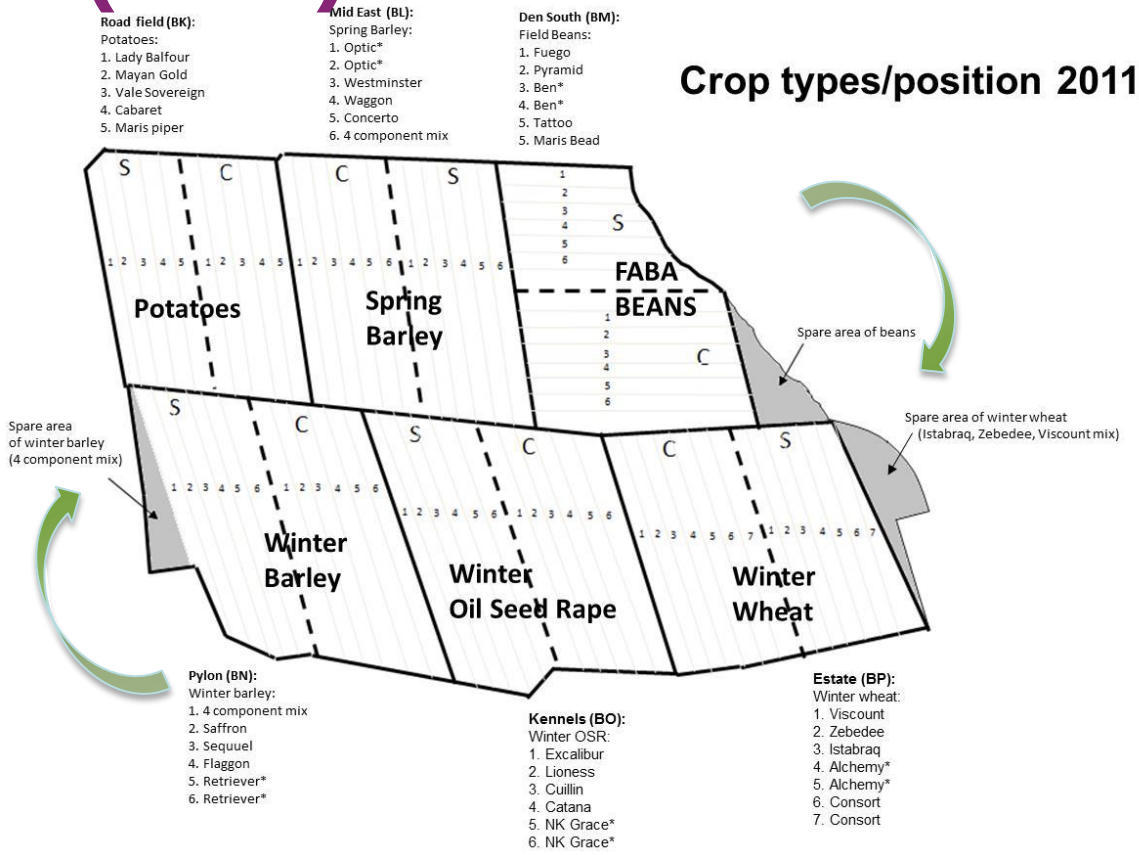


# Faba beans and peas

- Faba beans (*Vicia faba*) and peas (*Pisum sativum*) are the UK's main grain legumes, and are of great economic and agricultural importance as a source of protein for humans and animals (Prices: £250 & £350 per tonne, respectively).
- Like many legumes they can have all their N-requirements supplied by forming symbioses with a common soil bacterium called *Rhizobium*.
- Both are widely grown in temperate regions, such as East Scotland, often in rotation with non-legume crops, in which their capacity for BNF is utilised.
- However, they are NOT inoculated with rhizobia and farmers simply rely on native rhizobial populations.

# Experimental trials of Faba beans at JHI (Balruddery CSC)

# Centre for Sustainable Cropping (CSC)



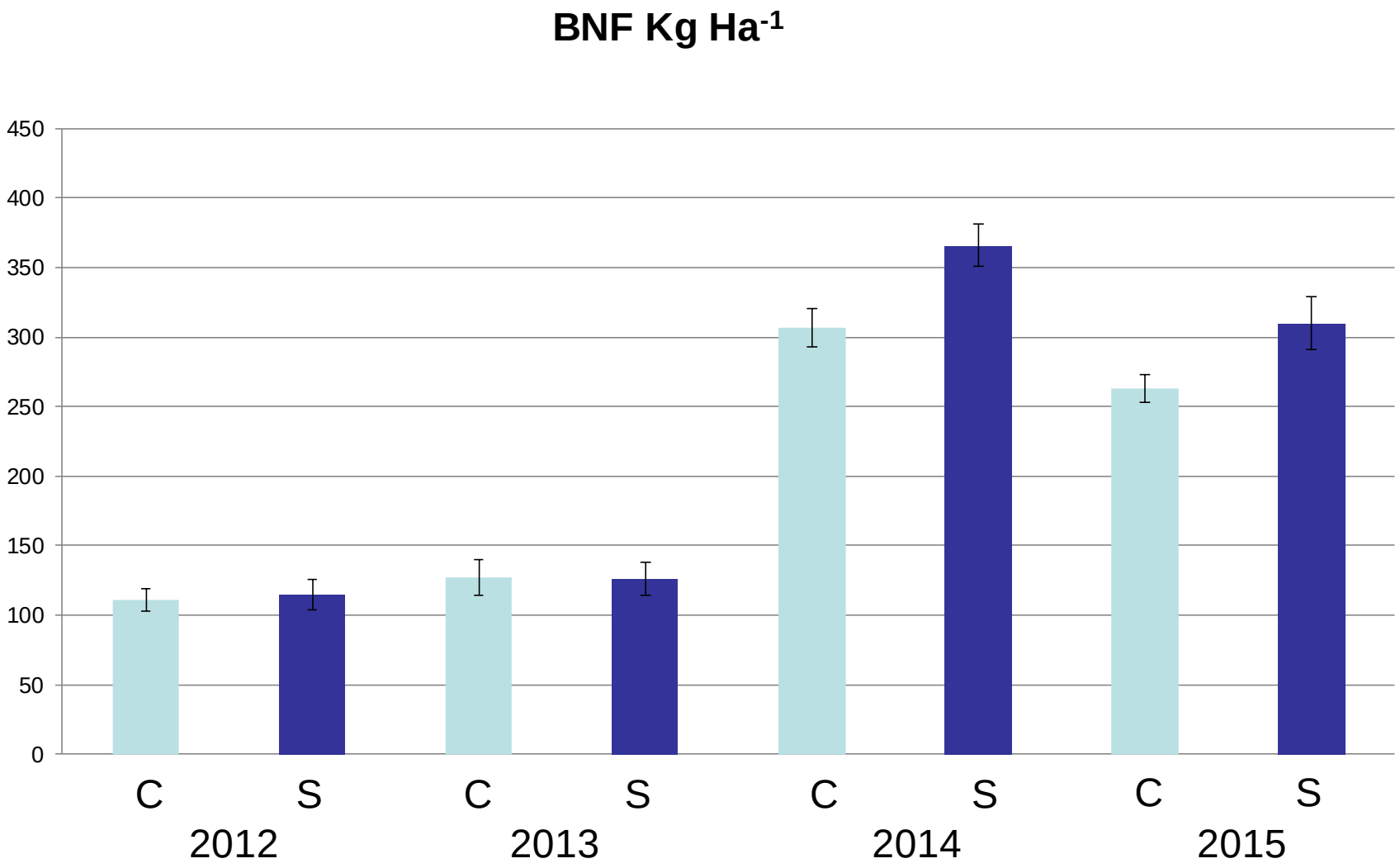

- At Balruddery Farm
- Experimental research platform
- Large scale and long-term
- 6-course rotation
- Split field design
- Conventional (“**C**”)
- Sustainable (“**S**” = compost only)

- **Crop yield and quality**
- **Economics**
- **Nutrient budgets**
- **Soil structure**
- **GHG emissions**
- **Above and below ground diversity**

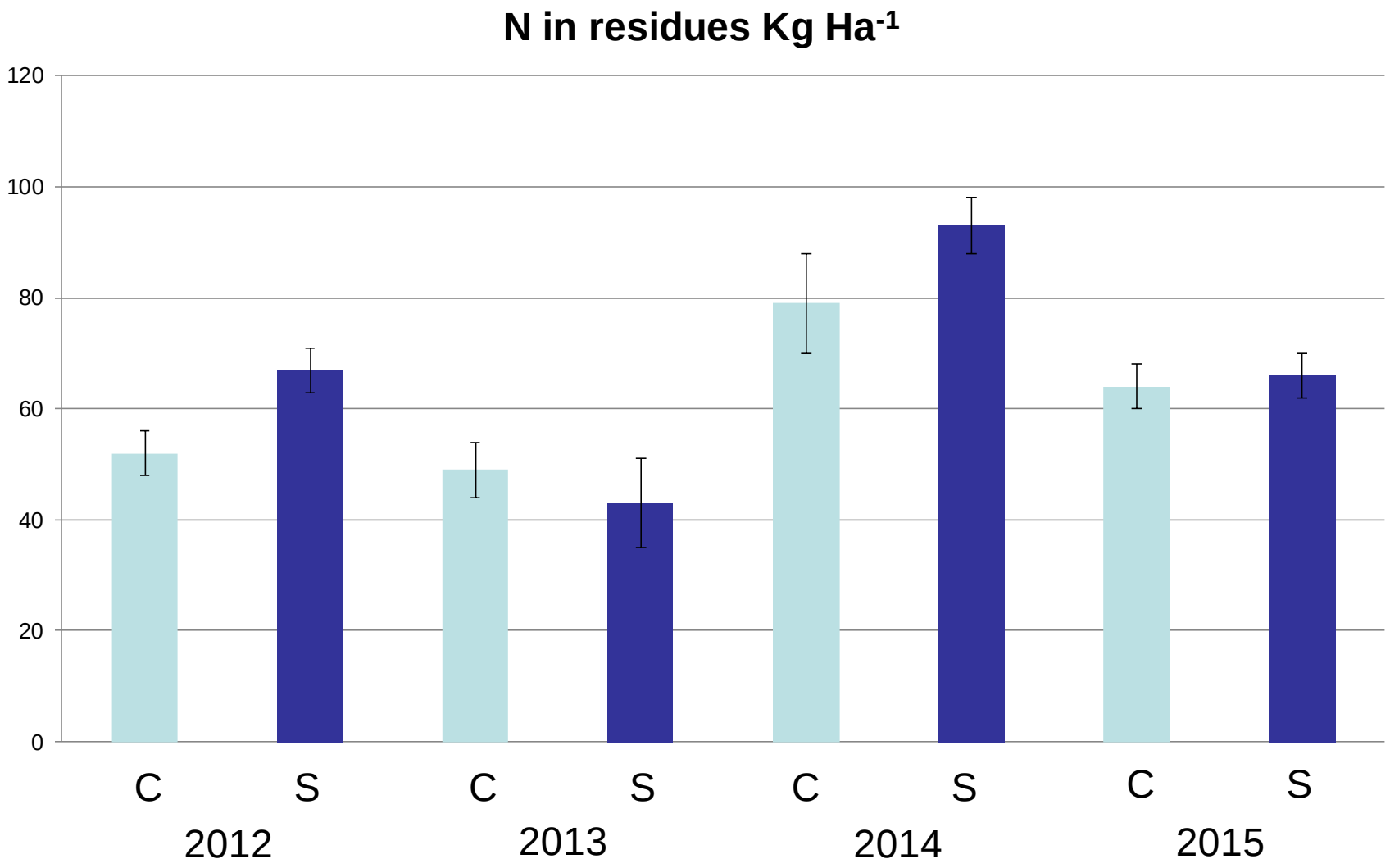
# Measuring BNF by Faba beans

- BNF measured at early pod fill using the (delta) $\delta^{15}N$ natural abundance technique.
- Total N removed in the grains at final harvest, and the N remaining in the field (shoots & roots) also estimated.
- Rhizobia isolated from nodules and genotyped.
- Strains tested on peas in greenhouse and field trials

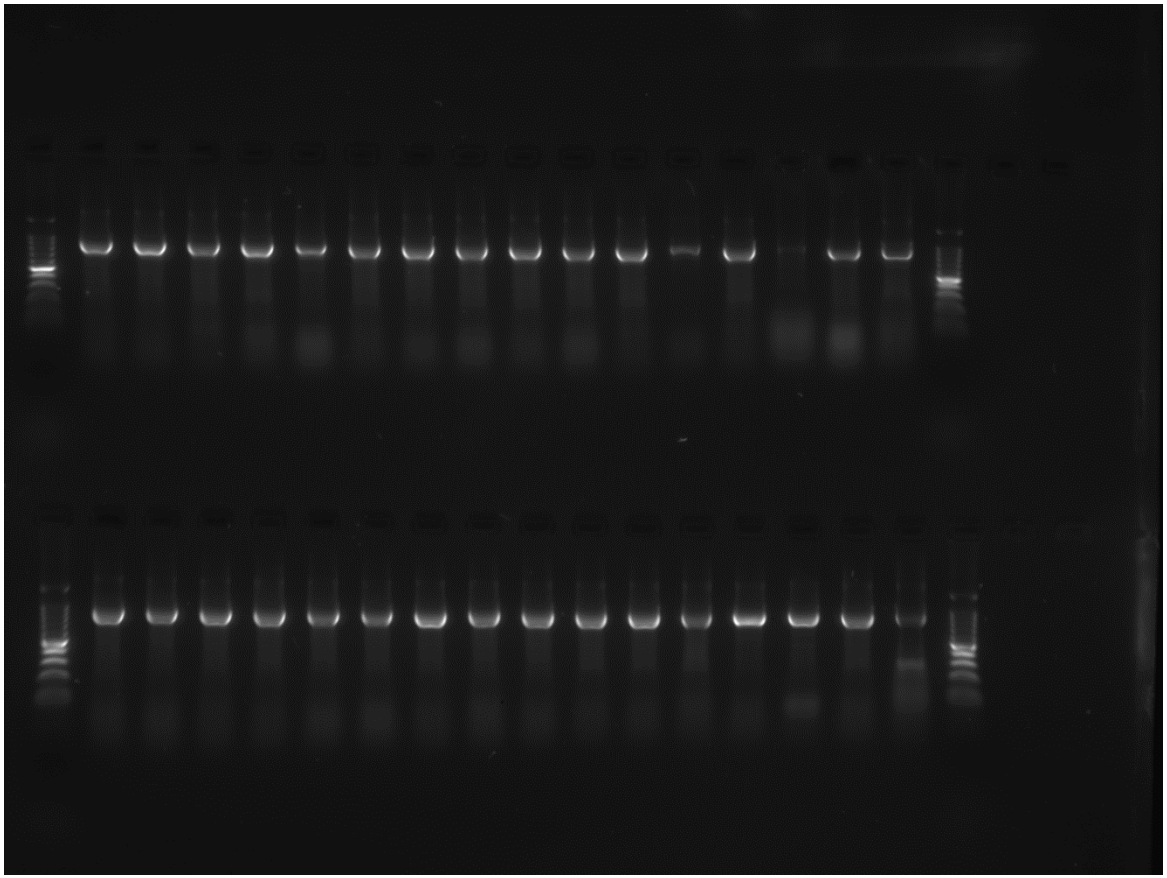
# Fixed N at mid podfill (2012 - 2015) Estimated using $^{15}N$

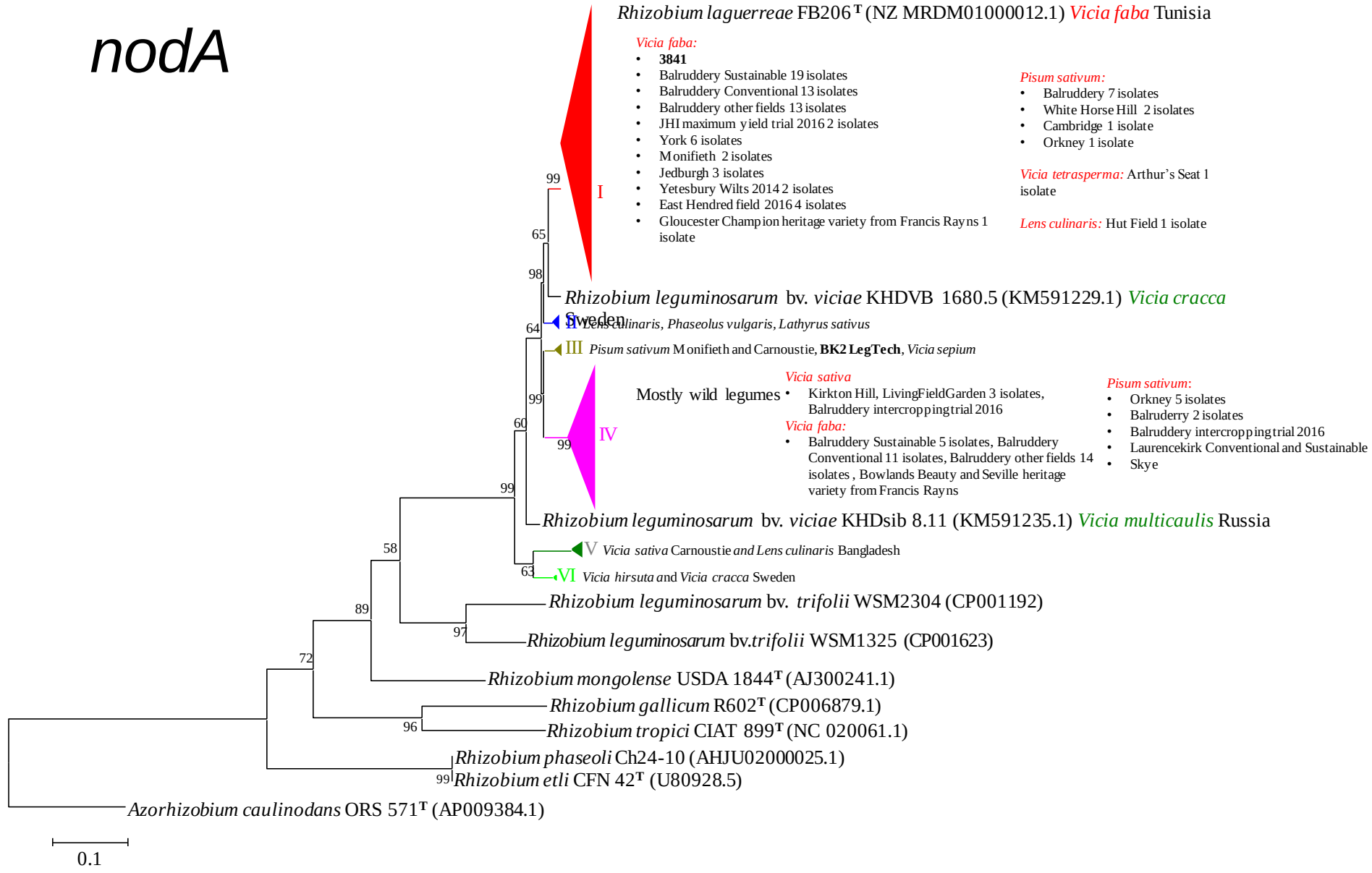


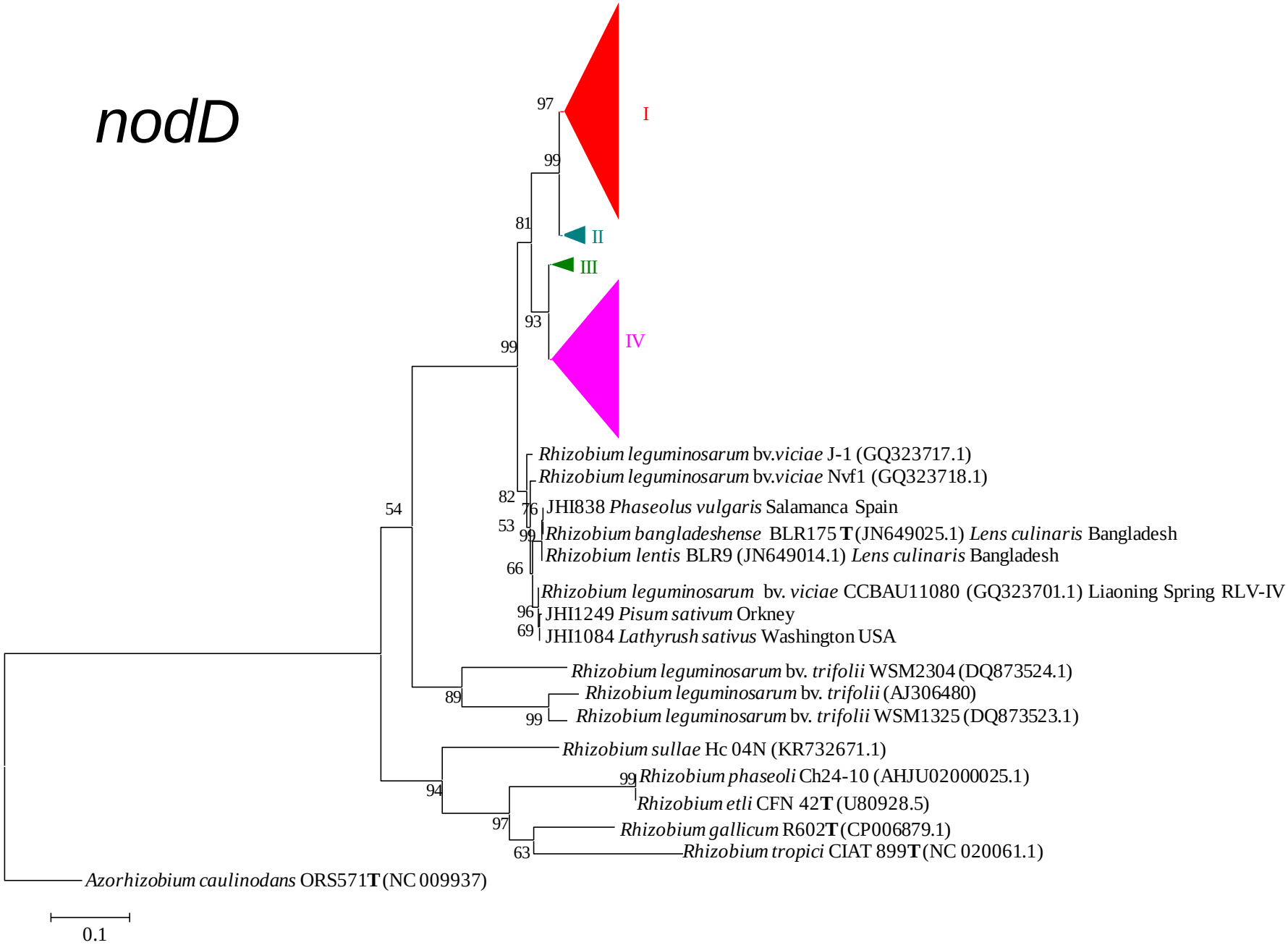
# Fixed N in crop residues (shoots + roots)



# Genetical markers to identify rhizobia (*nodD*)




nodA
Rhizobium laguerreae FB206T (NZ MRDM01000012.1) Vicia faba Tunisia
Vicia faba:
3841
Balruddery Sustainable 19 isolates
Balruddery Conventional 13 isolates
Balruddery other fields 13 isolates
JHI maximum yield trial 2016 2 isolates
York 6 isolates
Monifieth 2 isolates
Jedburgh 3 isolates
Yetesbury Wilts 2014 2 isolates
East Hendred field 2016 4 isolates
Gloucester Champion heritage variety from Francis Rayns 1 isolate
Pisum sativum:
Balruddery 7 isolates
White Horse Hill 2 isolates
Cambridge 1 isolate
Orkney 1 isolate
Vicia tetrasperma: Arthur's Seat 1 isolate
Lens culinaris: Hut Field 1 isolate
I
99
65
98
Rhizobium leguminosarum bv. viciae KHDVB 1680.5 (KM591229.1) Vicia cracca Sweden
II Lens culinaris, Phaseolus vulgaris, Lathyrus sativus
64
III Pisum sativum Monifieth and Carnoustie, BK2 LegTech, Vicia sepium
99
60
99
IV
Mostly wild legumes
Vicia sativa
Kirkton Hill, LivingFieldGarden 3 isolates, Balruddery intercropping trial 2016
Vicia faba:
Balruddery Sustainable 5 isolates, Balruddery Conventional 11 isolates, Balruddery other fields 14 isolates , Bowlands Beauty and Seville heritage variety from Francis Rayns
Pisum sativum:
Orkney 5 isolates
Balruderry 2 isolates
Balruddery intercropping trial 2016
Laurencekirk Conventional and Sustainable
Skye
99
Rhizobium leguminosarum bv. viciae KHDsib 8.11 (KM591235.1) Vicia multicaulis Russia
58
V Vicia sativa Carnoustie and Lens culinaris Bangladesh
63
VI Vicia hirsuta and Vicia cracca Sweden
89
Rhizobium leguminosarum bv. trifolii WSM2304 (CP001192)
97
Rhizobium leguminosarum bv.trifolii WSM1325 (CP001623)
72
Rhizobium mongolense USDA 1844T (AJ300241.1)
Rhizobium gallicum R602T (CP006879.1)
96
Rhizobium tropici CIAT 899T (NC 020061.1)
Rhizobium phaseoli Ch24-10 (AHJU02000025.1)
99
Rhizobium etli CFN 42T (U80928.5)
Azorhizobium caulinodans ORS 571T (AP009384.1)
0.1

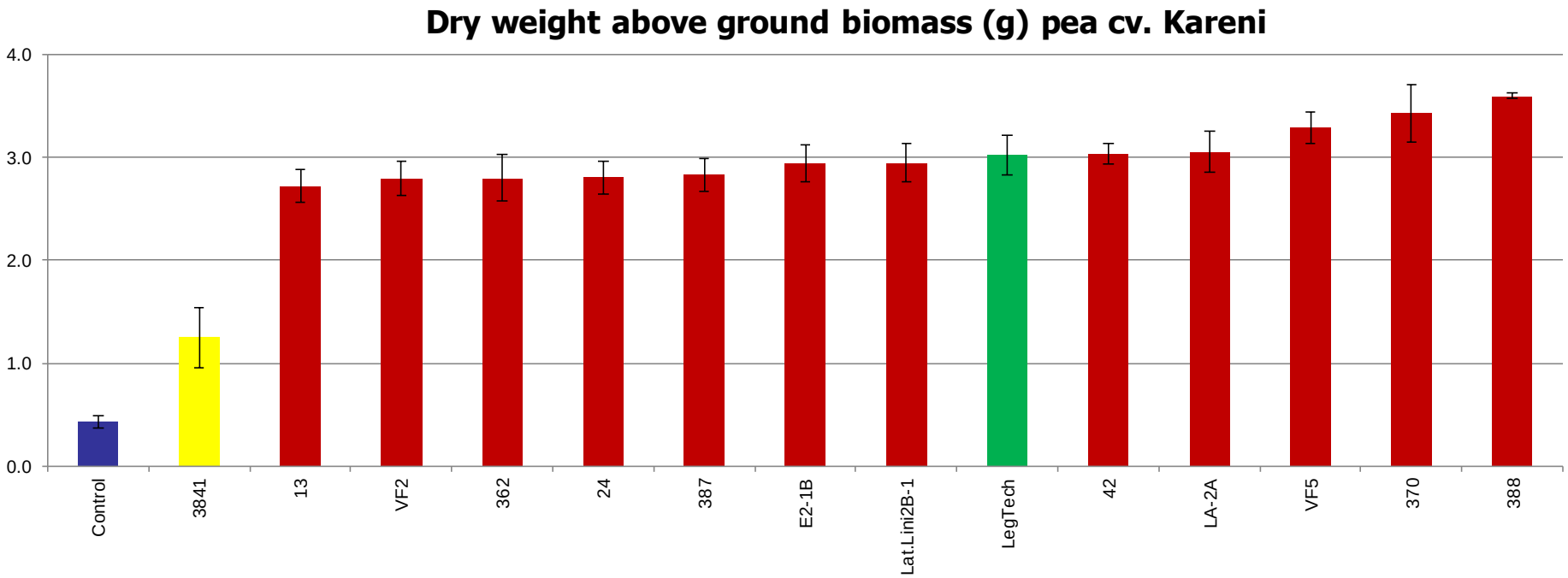

nodD
I
II
III
IV
97
99
81
93
99
54
82
76
53
99
66
96
69
89
99
94
99
97
63
Rhizobium leguminosarum bv.viciae J-1 (GQ323717.1)
Rhizobium leguminosarum bv.viciae Nvf1 (GQ323718.1)
JHI838 Phaseolus vulgaris Salamanca Spain
Rhizobium bangladeshense BLR175 T (JN649025.1) Lens culinaris Bangladesh
Rhizobium lentis BLR9 (JN649014.1) Lens culinaris Bangladesh
Rhizobium leguminosarum bv. viciae CCBAU11080 (GQ323701.1) Liaoning Spring RLV-IV
JHI1249 Pisum sativum Orkney
JHI1084 Lathyrush sativus Washington USA
Rhizobium leguminosarum bv. trifolii WSM2304 (DQ873524.1)
Rhizobium leguminosarum bv. trifolii (AJ306480)
Rhizobium leguminosarum bv. trifolii WSM1325 (DQ873523.1)
Rhizobium sullae Hc 04N (KR732671.1)
Rhizobium phaseoli Ch24-10 (AHJU02000025.1)
Rhizobium etli CFN 42T (U80928.5)
Rhizobium gallicum R602T (CP006879.1)
Rhizobium tropici CIAT 899T (NC 020061.1)
Azorhizobium caulinodans ORS571T (NC 009937)
0.1

# Screening rhizobia

Greenhouse

Field

# “Top 12” rhizobial strains

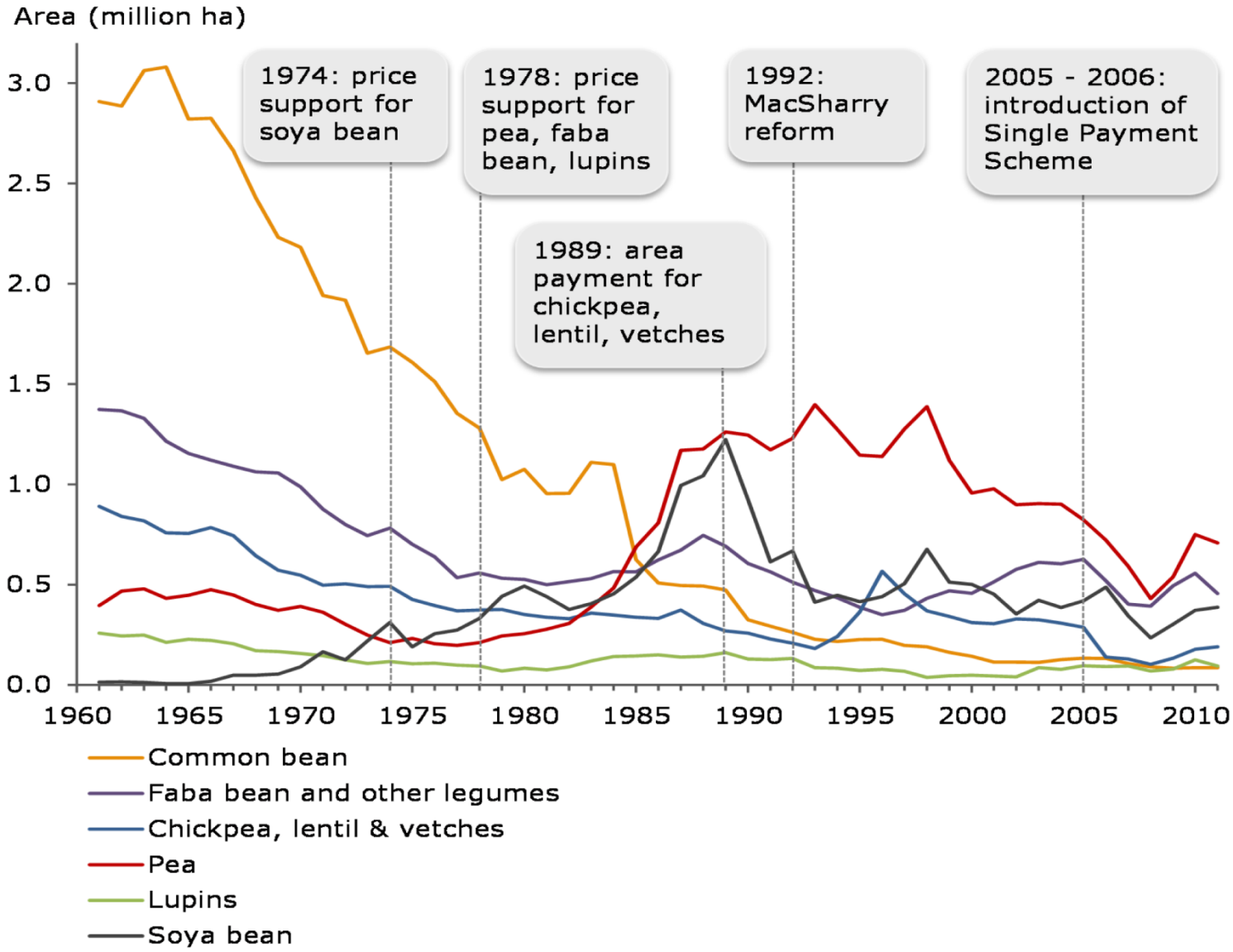


# BNF work at JHI

- Faba beans can fix >300 kg N $ha^{-1}$
- After grain harvest up to 100 kg N $ha^{-1}$can be left in the soil for the use of the following (non-legume) crop.
- With fertiliser at a price of £300 $t^{-1}$ this BNF amounts to a considerable cost saving.
- Dependent on the presence of good rhizobia in the soil.
- These are a source of potential elite inoculants for commercial exploitation.

# Area change for European pulse crops

## FAO STAT 2013



# Problems…
# and solutions…

- Domestically-grown f. beans suffer from massive competition from imported soybean.
- F. beans suffer from “yield instability”, which makes them unreliable.
- In part, this is linked to erratic BNF performance in the field and it can be corrected.

# BNF is adversely affected by:

- Lack of suitable rhizobia in the soil leading to low nodulation and low BNF.
- Inappropriate use of N-fertiliser (legumes will not fix N if fertiliser is present).
- Inappropriate crop rotation/sequences.

# Solutions

- Inoculation with elite strains of rhizobia
- We have a collection of >150 genotypes
- These have been screened for their BNF ability in the greenhouse
- So far, 12 genotypes have performed significantly better than industry standards
- These 12 are being tested in the field

# Participating organisations

- Genomia Fund
- Producers & Growers Research Organisation
- Legume Technology



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