Case Study Nr. 22

Elite inoculum - inc. yield & profit

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TRansition paths to sUstainable legume-based systems in Europe

Main Objective(s)

A
- Address the challenges of soil N-balance in organic agriculture.
- Investigate the contribution of legumes and rhizobia in N availability in soil through symbiotic nitrogen fixation under organic cultivation systems.
- Demonstrate new farming practices that optimize the production of organic common bean.
- Identify wider environmental effects of organic N-fertigation via green and animal manure.

B
- Isolation and characterization of rhizobia from nodules of various common bean varieties grown at different geographic locations of Greece.
- The assessment of genetic diversity of indigenous rhizobia using DNA fingerprinting techniques.
- Polyphasic characterization of representative isolates.
- The identification of the representative isolates and determination of their taxonomic position at species and symbiovar levels.
- Evaluation of Nodulation Efficiency (authentication test), Effectiveness and Competitiveness

Progress of the work during the second reporting period

A Yield characteristics

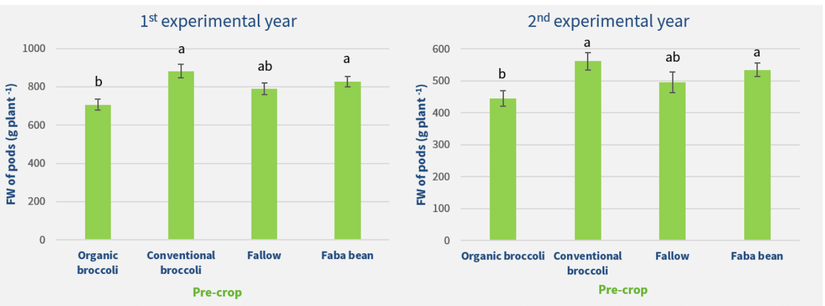

Figure 1. Impact of incorporation of plant residues of organic and conventional broccoli crop, fallow and green manure (faba bean) during the preceding winter.

- Green manure of faba bean crop in both rotation years enhanced the pod yield of the subsequent crop of organic common bean compared to organic broccoli as preceding crop.
- The yield of common bean obtained from plots with faba bean cultivated as green manure was similar with that obtained when the pre-crop treatment during the winter was fallow or conventional broccoli in both experimental years.

BNF activity of common bean

Treatment	Ndfa (%) 1st year	BNF (kg ha^{-1}) 1st year	Ndfa (%) 2nd year	BNF (kg ha^{-1}) 2nd year
Pre-crop				
Organic broccoli	12.61 ab	11.52 bc	34.94 a	12.62
Conventional broccoli	18,53 a	24.13 a	28.63 ab	13.85
Fallow	9,61 b	7.18 c	40.69 a	13.8
Green manure	18,47 a	17.89 ab	21.18 b	11.23
Inoculation with rhizobia				
Inoculated	16.99	18.52	32.28	14
Non-inoculated	12.62	11.83	30.44	11.78
Statistical significance				
Pre-crop	*	***	**	ns
Inoculation	*	*	ns	ns
Pre-crop* Inoculation	ns	ns	ns	ns

Table 2. Effects of inoculation of common bean with the strain *Rhizobium CIAT 899* on its BNF activity.

- The inverse impact of the different preceding crops on Ndfa(%) between the two experimental years is mainly ascribed to the inverse variability on N availability in soil.
- Unlike faba bean, no beneficial effects of re-inoculation were recorded on BNF activity of common bean.

B

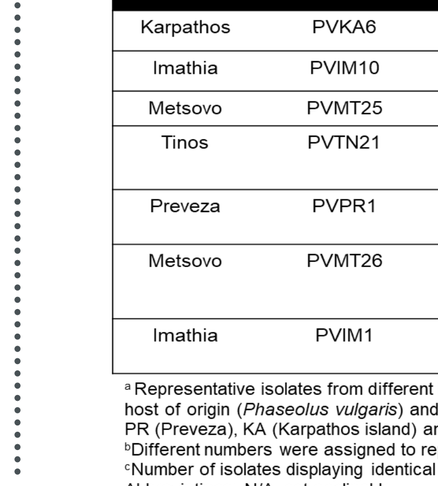

Figure 1: Simplified map of Greece showing the sampling sites of common bean-nodulating rhizobia. The coordinates (Latitude/Longitude) were as follows:
1: Imathia, N40°29'19.4"/E22°11'13.0";
2: Metsovo, N39°43'40.0"/E21°03'40.4";
3: Preveza, N39°16'07.8"/E20°51'18.7";
4: Tinos island, N37°34'26.8"/E25°10'05.7";
5: Karpathos island, N35°44'14.3"/E27°10'58.2".

➢ The genetic diversity of **fifty** rhizobial isolates was assessed by BOX-PCR and one representative isolate was chosen.

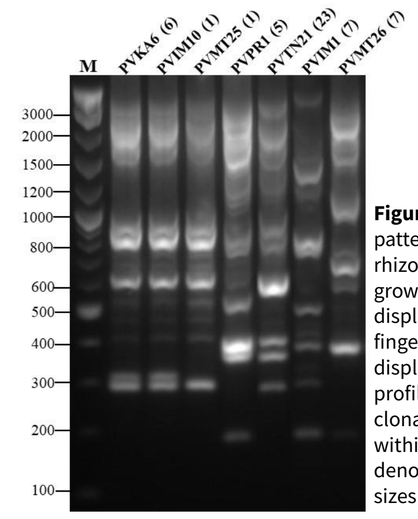

Figure 2: BOX-PCR fingerprinting patterns of 7 representative rhizobial isolates from field-grown common bean nodules displaying 6 distinct BOX fingerprints. Number of isolates displaying nearly identical profiles were considered as clonal isolates and are indicated within parentheses. Lane M denotes 1 kb DNA ladder and the sizes are indicated in base pairs.

➢ The phylogenetic affiliation of the representative isolates was assessed by multilocus sequence analysis (MLSA) of housekeeping (16S rDNA, *recA*, *atpD*, *gyrB*, *glnII*) and symbiosis-related genes (*nod*C).

Table 1: Characteristics of rhizobial strains obtained in this study and their phylogenetic relationships with the closest type species.

Geographic origin	Representative Isolates[a]	Box Type[b]	No isolates[c]	MLSA Clade	Strain definition	Symbiovars	nodC Allele
Karpathos	PVKA6	1	7	1	New lineage	phaseoli	α
Imathia	PVIM10	1	-	1	New lineage	phaseoli	α
Metsovo	PVMT25	2	1	1	New lineage	phaseoli	α
Tinos	PVTN21	3	23	3	*R. sophoriradicis*	phaseoli	γ-b
Preveza	PVPR1	4	5	2	*R. anhuiense*	phaseoli	γ-α
Metsovo	PVMT26	5	7	4	*R. hidalgonenseand*	phaseoli	α
Imathia	PVIM1	6	7	5	*R. azibense*	gallicum	N/A

[a] Representative isolates from different BOX-groups and geographic regions. The first two letters in the isolate name indicate the host of origin (*Phaseolus vulgaris*) and the latter two indicate their geographic origins, as follows: IM (Imathia), MT (Metsovo), PR (Preveza), KA (Karpathos island) and TN (Tinos island).
[b] Different numbers were assigned to represent each BOX-PCR pattern.
[c] Number of isolates displaying identical BOX-PCR pattern.
Abbreviations: N/A, not applicable.

➢ **Authentication test:** All strains were able to re-nodulate their original host, indicating that they are true microsymbionts of common bean.

Barriers remain to block or inhibit greater uptake of this approach

A
- Effects of organic farming practices and green manure applications appeared in long term.
- Morphological traits of faba bean plants inhibit the smoothly incorporation of plant biomass into the soil.
- The small percent of N originating from faba bean residues that is utilized for the subsequent crop.
- The beneficial effect of inoculation of green manure crop with rhizobia in total amounts of fixed-N did not enhance the N availability in soil for the subsequent crop.

B
- As a promiscuous legume host, Common bean (Phaseolus vulgaris) seeds usually carry rhizobia, which complicate the discrimination of inoculant and seed-borne rhizobia in experiments such as Authentication, Effectiveness and Competitiveness.
- In order to address this problem, the studied rhizobia are going to be fluorescently tagged with GFP to facilitate their discrimination from seed-borne rhizobia

Next steps

A
- To further investigate the impact of different organic farming practices in GHG's emissions.
- To explore and evaluate new competitive and efficient rhizobia strains.
- To delve into the responses of nitrogen fixing activity of legumes under different N fertigation schemes

B
- **Fluorescent labeling** → Fluorescently-tagged rhizobia with fluencense protein (e.g. green, cherry, scarlet) to study bacteria–bacteria, bacteria–host interactions.
- **Effectiveness** → The isolates will be assessed in order to evaluate the ability to fix N.
- **Competitiveness** → The Isolates will be assessed to evaluate the ability to nodule occupancy.

Innovations

A
- Organic farming practices that optimize the N availability and the yield of common bean.

B
- This is the first systematic analysis on the phylogenetic diversity of indigenous rhizobia nodulating P. vulgaris in Greece by identifying them at the species and symbiovar level.
- This is the first time that strains assigned to R. sophoriradicis and harbored the γ-b allele were found in European soils.
- Three representative isolates have been assigned to a new Rhizobium lineage which is provisionally named as Rhizobium sp. I.

Impact

A 'Comparative assessment of different crop rotation schemes for organic common bean production', which was submitted to Agronomy (Open Access Journal) on 4/30/2020.

B Part of the results originating from work package 2/Task 2.1 and Task 2.3 have been submitted for publication with the title " Genetic characterization at the species and symbiovar level of rhizobial isolates nodulating *Phaseolus vulgaris* in Greece " to Systematic and Applied Microbiology (Elsevier) .

Recommendations to realise this transition in practice

A
- To compare the contribution of faba plant residues in N availability in soil for the subsequent crop when they are cultivated either as green manure or for its fresh pods.
- To test other legumes cultivated as green manure crops with shorter life cycle.

B
- This research is enhancing our knowledge on the phylogenetic diversity of indigenous bean-nodulating rhizobia in Greece, and,
- is contributing to select novel strains adapted to the local environmental conditions and in other regions of the world with similar habitats.

Exploring the rhizobial biodiversity, the use of new strains that are best adapted to particular habitats and legume genotypes will contribute to the development of novel and more effective biofertilizers for the improvement of crop productivity with low environmental impact.

Policy Makers | Inputs | **Primary Production** | Aggregation | Processing | Distribution | Retailers | Markets | Consumers

WP1: Knowledge Exchange & Communication | **WP2: Case Studies** | WP3: Nutrition and Product Development | WP4: Markets and Consumer | **WP5: Life Cycle Assessments and Environmental Assessments** | WP6: Economic Assessments of Legume Production and Consumption | WP7: Policy and Regulations | WP8: Transition and Decision Support Tool

A MEFS:
1. Elite Inoculum and Use in Rotation with non-Legume Crops – incl. Yield & Profit (Reporting period 10/2018-3/2019)
2. Elite Inoculum and Use in Rotation with non-Legume Crops – incl. Yield & Profit (Reporting period 4/2019-9/2019)

B SOPs that have been used to TRUE WP2 (Tasks 2.1 and 2.3, Case Study 22)
SOP24-PCR sequencing analysis to test the presence of the inoculants in the nodules
SOP27-Establishment of protocol for nodule sampling
SOP28-Establishment of protocol for Rhizobia isolation and cultivation
SOP29-Establishment of protocol for polyphasic characterization
SOP48-Authentication of rhizobia (screening strains for nodulation)

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