SIRAM
KEY ACHIEVEMENT BOOKLET

Sustainable Innovations for Regenerative Agriculture in the Mediterranean Area

www.siram-prima.org

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Introduction

SIRAM (2022-2025) was a PRIMA project that involved 10 partners from all the Mediterranean area:

- Università Cattolica del Sacro Cuore (Italy)
- OpenTea srl (Italy)
- Agricultural Research Centre (Egypt)
- Institut de Recerca i Tecnologia Agroalimentaries (Spain)
- Ecological Finance Architectures P.C. (Greece)
- Ecole Nationale d'Agriculture de Meknès (Morocco)
- University Sidi Mohamed Ben Abdellah (Morocco)
- Universidade de Coimbra (Portugal)
- Regional Centre of Agriculture Research of Sidi Bouzid (Tunisia)
- Université Marie & Louis Pasteur (France)

SIRAM stands for "Sustainable Innovations for Regenerative Agriculture in the Mediterranean Area". Its aim was to develop smart, sustainable and resilient agricultural systems and economies in agreement with the UN Sustainable Development Goals and the EU Green Deal towards 2030 and beyond. In particular, the project worked for the achievement of the following main objectives:

Objective	SIRAM solution	Booklet pages
Development of tailored approaches to address climate change, desertification, pollution and low-income issues under different smallholder farming systems.	Design and implementation of 8 case studies (CSs) in 7 partner countries.	p. 5-12
Reduction of chemical inputs, restoration and regeneration of soil health.	Technological solutions combining SIRAM 4 pillars (microorganisms, biomasses, regenerative agriculture practices, resistant plant varieties).	p. 3-4
Understanding and exploitation of molecular mechanisms determining plant-microbiome interaction and induced systemic resistance.	Sequencing of environmental samples from case studies and targeted quantification of functional genes, genome mining of isolated microorganisms, RNA-seq of controlled experiments.	p. 5-12
Socio-economic evaluation of the tested methods.	Modelling structural change, i.e. shift from conventional practices to regenerative ones; performing lifecycle analysis of 3 reference SIRAM CSs with the Product Environmental Footprint (PEF) method; design of a prototype ecological finance instrument, tailored for small-scale regenerative agriculture farms.	p. 13
Improvement in knowledge, skills and competences of young scientists, professionals, and other interested parties through a dedicated training programme.	Production of a digital tool with e-Learning courses, and podcasts.	p. 14

SIRAM 4 PILLARS

1. Beneficial microorganisms for plant growth and pest control:

Microorganisms associated with plant roots and leaves can exert beneficial effects in terms of plant nutrition and defense, for example providing to the plant nutrients otherwise difficult to be adsorbed, favouring plant growth through the production of phytohormones, inducing in the plant systemic resistance mechanisms which can determine its ability to control biotic stresses. These beneficial microorganisms can be “isolated” by sampling soil strictly adherent to the plant roots (“rhizosphere”) or other plant organs (leaves, internal tissues) and culturing on microbiological specific media the bacteria and fungi living there. Throughout SIRAM project, 393 bacterial and fungal strains from 16 different cultivars have been isolated and characterized for their beneficial properties (i.e. Plant Growth Promoting traits and biocontrol abilities), both *in vitro* (through phenotypic tests, germination tests, shelf-life evaluation) and in pot experiments. The ones performing better have been applied in the cases studies, in combination with one or more of the other SIRAM pillars. Biomasses have been used in 3 case studies to formulate the selected microorganisms.

2. Crop varieties with resistance towards abiotic and biotic stresses:

Some crop cultivars can be resistant to biotic or abiotic (e.g. drought, heat, cold) stresses thanks to some genetic traits: the development and use of disease-resistant cultivars are among environment-friendly and cost-effective modern technologies that can be applied in an integrated approach. Throughout SIRAM project, 46 resistant varieties of 7 crops have been screened in 6 different case studies for their resistance to specific pathogens (i.e. *Phytophtora*, *Pythium*, *Verticillium*), sometimes combined with biotic stress. Greenhouse experiments under controlled conditions were performed to evaluate local lines of cultivated crops. At the end of the experiment, agronomic traits such as chlorophyll content, damage reduction of shoots, root dry weight, salt tolerance index, as well as the disease incidence of the main pathogenic fungi of each crop were determined to select the best performing varieties.

3. Agronomical practices to safeguard soil quality and fertility:

Practices such as reduced or no tillage, cover cropping, crop rotation, best practices for manure management and distribution have been assessed by previous research as important strategies for soil restoration and conservation, especially in areas such as the Mediterranean area, strongly impacted by climate change. While general advantages and/or disadvantages of those practices alone were already reported at the beginning of the project, through SIRAM case studies partners tried to understand the potential of practices combined together as a solution for Regenerative Agriculture within each specific pedo-climatic condition across the Mediterranean areas. These practices were also combined with the other SIRAM pillars. Main soil physical, chemical and biological parameters (e.g. texture, pH, organic carbon content, total nitrogen, exchangeable potassium and phosphorus) were evaluated at the beginning and at the end of each case study to evaluate the effects of the practices on soil health. Common methodologies for these measurements were established at the beginning of the project. Yield of the main crop was also evaluated to determine the economic efficacy of these practices, too.

4. Bioeconomy approaches to recycle and valorize waste biomasses that restore soil organic matter and have biostimulant properties:

Waste biomasses consisting in compost from crop residues, digestate from biogas plants and vermicompost were characterized for their chemical properties (nitrogen, potassium and phosphorus content, electrical conductivity, organic content) and biocompatibility through germination assays. In three case studies, these biomasses were used for the formulation of the selected beneficial microorganisms, promoting not only the growth of the crop, but also the survival of Plant Growth Promoting Rhizobacteria (PGPR).

SIRAM CASE STUDIES

Case study 1: Emilia Romagna, Italy. Regenerative forage farming and slurry use.

Crops: Maize and cover crops

Objectives:

1. No-till plus cover crops and beneficial microorganisms can enhance soil quality and soil C sequestration;
2. Fertilization strategies based on vermicompost and beneficial microorganisms can increase N-use efficiency and minimize N losses including N_2O emissions.

SIRAM pillars involved:

- *Trichoderma asperelloides* PV51, isolated from corn rhizosphere and with plant growth promoting properties, was applied formulated on vermicompost.
- Conventional tillage/no tillage + cover crops practices were applied in this site that is a long-term (>10 yr) field experiment.
- Vermicompost was used both as microorganism coformulant and to (i) improve N-use efficiency, (ii) reduce as much as possible the application of mineral fertilizers, (iii) ensure minimal risk of agricultural emissions.

Molecular focus: the genomes of two other fungal isolates of agricultural interest, *Talaromyces purpureogenus* ISA502 and *Metarhizium marquandii* ISA501 were sequenced, assembled and published for the first time.

Main results:

- No tillage determined an increase in the yield of the main crop.
- The application of beneficial microorganism *Trichoderma asperelloides* PV51 on vermicompost, combined with no tillage, further increased grain yield and nitrogen uptake of the main crop.
- All these practices determine an increase in soil quality as measured through molecular analyses focusing on the effects on microbial diversity and the expression of functional genes related to biogeochemical cycles.

Case study 2: Lombardia, Italy. Boosting organic farming via regenerative agriculture

Crops: Maize, sunflower, soybean, and cover crops

Objectives:

1. Cover crops (especially legumes) can enhance soil fertility and main crop productivity.
2. Grasses and grass-based mixtures may curb N_2O emissions from soil.

SIRAM pillars involved:

Cover crops are applied in this site since 2019 as monoculture (rye, hairy vetch, and radish), as 2-species, and as 3-species mixtures.

Residues of cover crops were incorporated into the soil as a green manure.

Main results:

- The rye-vetch mixture balanced high N input and moderate N_2O emissions, making it a promising option for sustainable production.
- Legume-based systems were essential to sustain main crop yield.

Case study 3: Fes-Meknès, Morocco. Resilient and climate-smart olive growing

Crops: Olive

Objectives:

1. Microbial characterization of olive soils and screening of potential antagonists to restore soil fertility and mitigate olive diseases.
2. Evaluating the efficacy of existing bacterial antagonists from the consortium against olive diseases.
3. Assessing the resistance of various olive varieties to *Phytophthora* root and crown rot and understanding the underlying resistance mechanisms.

SIRAM pillars involved:

- *Trichoderma* isolates (e.g., *T. harzianum* T-BM6, and *T. virens* T-KH4) were shown to have significant effect on olive diseases.
- Picual, Koroniki, and Haouziya olive varieties were found to be resistant to the pathogen causing root rot disease.

Molecular focus: Molecular tools were used to understand the genetic basis of resistance and identify up/downregulated genes in resistant/tolerant varieties.

Main results:

- Selected bacteria from the existing collection of the laboratory demonstrated a higher potential to decrease the severity of root rot disease in olive trees. Notably, the two strains *Alcaligenes faecalis* ACBC1 and *Bacillus amyloliquefaciens* SF14 both showed an 8.33% reduction in disease severity and were selected for further assays.

- In the greenhouse assay, out of the eight olive varieties tested, only three (Picual, Koroniki, and Haouziya) were found to be resistant to the pathogen causing root rot disease.

- Eight *Trichoderma* isolates (e.g., *T. harzianum* T-BM6, and *T. virens* T-KH4) were found to be effective in reducing *Pythium shmithenneri* growth both *in vitro* and *in vivo*. The genomes of the two most effective isolates were identified and submitted to NCBI.



Case study 4: Al Minya, An Nubariyah and Salheya regions, Egypt. Organic agriculture farm.

Crops: Potato and Citrus

Objectives:
1. Sustainable strategies for the management of plant-parasitic nematode diseases.
2. Novel biopesticides to enhance beneficial microorganism communities and minimize losses.

SIRAM pillars involved:

Trichoderma isolates (e.g., *T. asperellum*, and *T. longibrachiatum*) with different biochemical characteristics were selected for nematode disease management and plant growth.

Compost and rice bran formulations were utilized as coformulants for microorganisms, aiming to minimize the use of mineral fertilizers. This approach is designed to enhance soil quality, bolster plant resilience, and reduce the reliance on mineral fertilizers. Additionally, it seeks to lower the severity of nematode disease outbreaks.

Molecular focus: Molecular methodologies were employed to elucidate the gene's identity and the genetic foundations of resistance.

Main results:
- The capabilities of PGPR were noted, showing enhanced growth in both potato and citrus plant varieties.
- The best augmentation in potato growth parameters (plant length, total plant fresh weight, and shoot dry weight) and nematode reproduction was recorded with the application of *Trichoderma longibrachiatum* on compost.
- Rice bran formulation combined with both *Trichoderma* isolates gave better results than did dual and single applications after potato plantation.
- The assessment of the stimulating capacity of the defense mechanisms revealed a notable enhancement in the activity of oxidative enzymes, encompassing a marked improvement in peroxidase, catalase, and peroxidase. Furthermore, a significant rise in the concentrations of total phenols and proteins was recorded.

Case study 5: Berkane, Morocco. Experimental farm for Citrus root rot and cankers diseases and Phytophthora root rot

Crops: Citrus

Objectives:

1. Characterize Fungal Pathogens: Identify the specific fungal pathogens responsible for dry root rot, citrus cankers, and associated *Phytophthora* species in Berkane.
2. Assess Beneficial Bacteria: Test and develop strategies using beneficial bacteria to enhance soil fertility and control the major pathogens affecting citrus crops.

SIRAM pillars involved:

- Several beneficial bacteria (e.g., *Bacillus spp.)* that have different biochemical characteristics for disease management and plant growth were isolated.
- Citrus plant varieties treated with beneficial bacteria have shown promising results in terms of plant growth.

Molecular focus: ITS gene primers were applied for molecular identification.

Main results:

- Out of 20 bacterial isolates, 18 produced pectinase, 16 produced cellulose, and 8 produced chitinase.
- All isolates produced amylase and protease, while only 4 produced hydrogen cyanide.
- Fourteen isolates solubilized tricalcium phosphate, and ten produced indole-3-acetic acid (IAA).
- Production of antimicrobial substances varied greatly, with surfactin being the most common (14 isolates).
- PGPR capabilities were observed, with increased growth in citrus plant varieties treated with bacteria.
- *Bacillus subtilis* K4-4 and GH3-8 showed to be the most promising for disease control and bio-fertilizer applications.

Case study 6: Catalonia, Spain. Regenerative practices on rainfed and irrigated arable agriculture.

Crops: Wheat and barley (CS6.1), forage maize with different cover crops after the main crop (CS6.2).

Objectives:

1. Reducing soil tillage and applying cattle manure to enhance soil organic carbon and crop yields in rainfed systems.
2. Using cover crops and reduced soil tillage, under different fertilization strategies, to improve nutrient use efficiency and soil quality in irrigated arable cropping systems.

SIRAM pillars involved:

Conventional tillage/no tillage + cover crops practices in a long-term (>10 years) fertilization field experiment.

Manure, digestate, slurry and cover crops were used as amendments to improve soil quality and nutrient use efficiency and as substitutes to mineral fertilizers.

Different winter cereals (wheat and barley) varieties have been evaluated for water stress and disease tolerance/resistance.

Main results:

- Including legume as a cover crop -i.e. vetch, either pure or in mixture- can increase the yield of the subsequent maize crop.
- Maize and cover crop yields are higher under conventional tillage than under no-till conditions (without chemical weed control); weed growth was not well controlled on the no-till treatments, thus it may also negatively affect forage quality.
- Soil organic carbon stock is higher and soil bulk density is lower (meaning less compaction) in treatments where manure has been applied. Soil aggregates tend to be more stable in treatments with manure application.
- Few commercial varieties significantly tolerant to water stress have been found.

Case study 7: Sidi Bouzid, Tunisia. Experimental farm of Olive trees and intensive crop production areas

Crops: Olive trees

Objectives:

1. Compost and beneficial microorganisms can enhance soil quality and promote plant health. The goal is to prove that the combination of compost and beneficial microorganisms creates a healthier, more nutrient-rich, and disease-suppressive soil environment.
2. The second objective is to measure the effect of the improved soil on the olive trees. This includes monitoring agronomic and biochemical modifications.

SIRAM pillars involved:

- *Trichoderma spp.* and *Pseudomonas spp.* were applied formulated on compost.
- Compost was used both as a microorganism coformulant and to achieve the following objectives: (i) improve soil quality and plant resilience, (ii) reduce as much as possible the application of mineral fertilizers, and (iii) decrease disease severity of *Verticillium* wilt.

Main results:

- The combination of compost associated with *Pseudomonas spp.* and *Trichoderma spp.* showed a significant positive effect on the biological and physicochemical parameters of the soil.
- The compost + biostimulant combination demonstrated their effectiveness in improving physiological parameters.
- The combined treatment compost + *Pseudomonas spp*. + *Trichoderma spp*. significantly reduced the disease severity of *Verticillium* wilt on olive trees.
- The determination of the stimulating potential of the defense mechanisms revealed an increase in the activity of oxidation enzymes, including an improvement in peroxidase, catalase, and ascorbate peroxidase. In addition, an increase in the total phenols and proteins contents was observed. In contrast, a decrease in Malondialdehyde (MDA) was observed following treatment with microbial agents and compost. These effects were particularly remarkable when combining these two elements of compost and microbial consortium for all olive tree varieties (Arbequina, Arbosana, Chetoui).

Case study 8: North, Center and Center South, Portugal. Surveillance on potato growing soils

Crops: Potato and Tomato

Objectives:

1. To show the potential of native potato-growing soil bacterial strains to control plant-parasitic nematodes (PPN), in a more sustainable agriculture perspective.
2. To assess the microbiome of potato fields to identify the core microbiome of healthy (without PPN) and diseased soils (with PPN) and predict their functionality.

SIRAM pillars involved:

- Bacterial consortium composed of *Bacillus amyloliquefaciens* UC_2.4, *Pseudomonas capeferrum* UC_21.3 A.1, and *P. capeferrum* UC_21.30 A.1, isolated from potato-growing soils, was explored as plant growth-promoting bacteria (PGPB) and biological control agents.
- The bacterial consortium was evaluated for its growth over time to determine the amount and time required to test their potential as PGPB against PPN.
- The assays with bacterial consortium and resistant potato cultivars were performed in collaboration with Agricultural Research Center (ARC).

Molecular focus: the genomes of the three bacterial isolates with agricultural interest, UC_2.4, UC_21.3 A.1 and UC_21.30 A.1 were sequenced, assembled and published. The microbiomes of 450 soil samples were sequenced and analysed. The RNAseq from experience with *Pseudomonas* strain, *Trichoderma* and *Meloidogyne hapla* was sequenced and analysed.

Main results:

- The bacterial consortium showed PGPB traits, verified by genome mining and phenotypic assays *in vitro* and pot assays with plants.
- The bacterial consortium was able to act as nematicidal agents with 100% efficacy towards PPN but not against *C. elegans*, indicating a highly targeted action mechanism.
- The bacterial consortium reduced the infectivity of PPN in plants by threefold. This bacterial consortium was established for the first time and has the potential to serve as a new tool for managing Root-knot Nematode (RKN) in a more sustainable agricultural environment.

Socioeconomic value of SIRAM project results

The SIRAM socio-economic valuation modelling developed an original mathematical framework on Regenerative Agriculture (RA), filling a major gap in the academic literature and business practice. Specifically, SIRAM modelled by its 4 pillars (1) the shift from conventional to mixed and regenerative practices, (2) the lifecycle performance of selected CSs and (3) the Soil Organic Carbon (SOC) increase by a prototype ecological finance contract with compliance to the EU Taxonomy, as part of generated ecosystem services and of small-scale farms' income.

The SIRAM socio-economic valuation revealed multiple benefits that within integrated environmental-economic accounting place regenerative practices as profitable business options. The available annual income of RA farmers increased by 47%, deriving from the reduced insurance costs from resistant varieties and reduced lifecycle environmental footprint by 17-35%. The combination of better PEF and SOC increase from CO_2 sequestration in regenerative plots yields between ~8.97-10.4 times higher monetary value than conventional plots. Finally, from the more equal allocation of work between men and women in the CSs and the produced synergies, we observed a 21% increase in total productivity.

Fig.1: Soil organic carbon trend according to normal distribution fit for each condition (CT=Conventional Tillage, NTR=No-Tillage Rye, NTV=No-Tillage Vetch), calculated from CS1 observations.

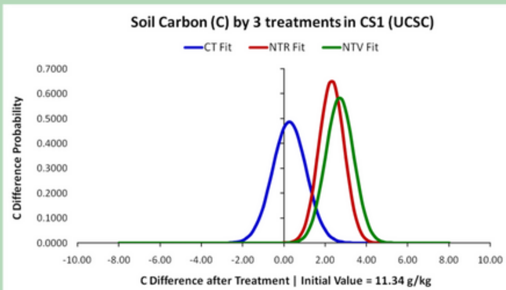

Fig.1

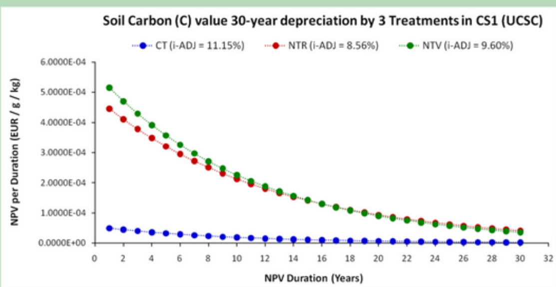

Fig.2: 30-year net present value of generated income at the first year from atmospheric CO_2 uptake through soil organic carbon formation in the same first year, for CS1 conditions (CT=Conventional Tillage, NTR=No-Tillage Rye, NTV=No-Tillage Vetch).

Fig.2

DIGITAL TOOL WITH E-LEARNING COURSES AND PODCASTS

SIRAM developed:

- a digital tool, which is hosted on **ICARUS AI e-Learning platform**, including different e-Learning courses, dealing with sustainable innovations for regenerative agriculture in the Mediterranean area. The video lessons can be watched with subtitles in 23 different languages.

Go to icarus.education/opentea and find SIRAM eLearning courses!

- Podcasts, which are available in Arabic, Italian and Catalan.

On **YouTube**, search for "OpenTea Project" and then select the "SIRAM Regenerative Agriculture" playlist!

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