

**cost**  
EUROPEAN COOPERATION  
IN SCIENCE & TECHNOLOGY

Towards zer0 Pesticide AGRiculture : European Network for sustainability (TOP-AGRI-Network)

# Biofertilizers and cropping systems for a sustainable and resilient agriculture

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**CATOLICA**  
CBQF - CENTRE FOR BIOTECHNOLOGY  
AND FINE CHEMISTRY ASSOCIATE LABORATORY  
CBQF  
PORTO

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## Agriculture - main challenges



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**The dominant form of agriculture is non-sustainable....**

Monoculture, tillage, excessive use of pesticides and fertilizers



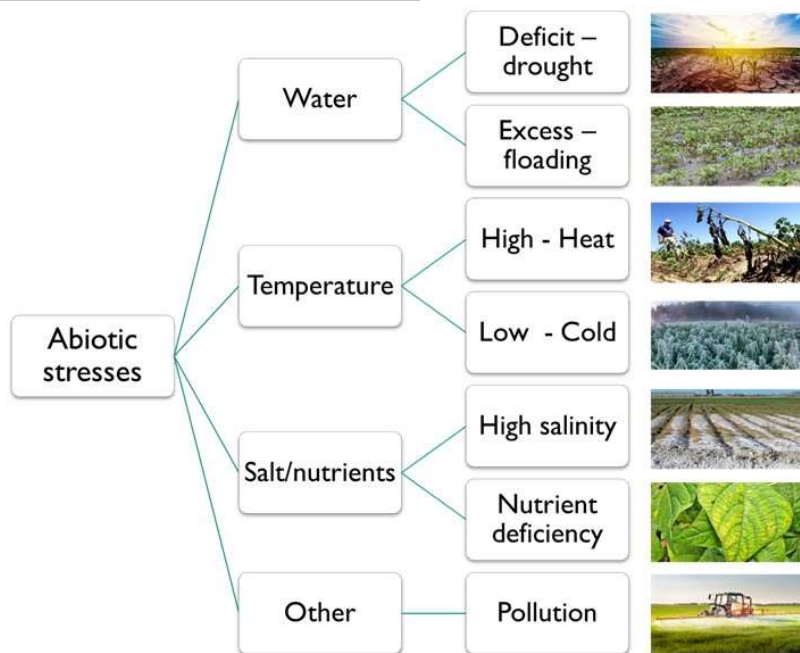
Compaction, erosion....



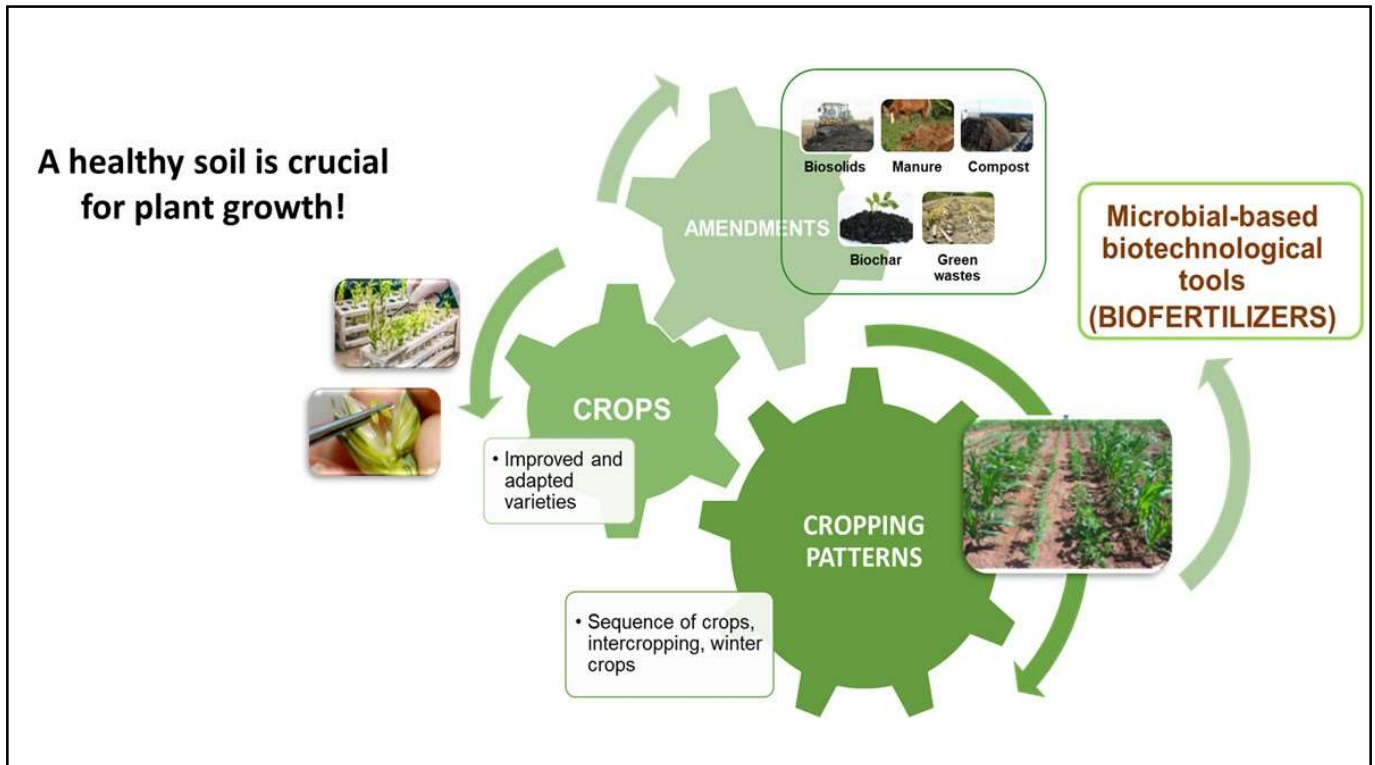
.....loss of fertility and reduced crop growth.

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**Climate change - impact on agriculture**



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## Biofertilizers – What are they?

**Biofertilizers** (i.e. microbial inoculants) may be defined as “substances which contain living microorganisms that colonize the rhizosphere or the interior of the plants and promote growth by increasing the supply or availability of primary nutrients to the target crops, when applied to soils, seeds or plant surfaces”. (Mazid et al. 2011)

The term **biofertilizer** can be defined “as formulations comprised of living microbial cells, either a single strain or multiple strains (mixed or consortium), that promote plant growth by increasing nutrient availability and acquisition” (Riaz et al., 2020)

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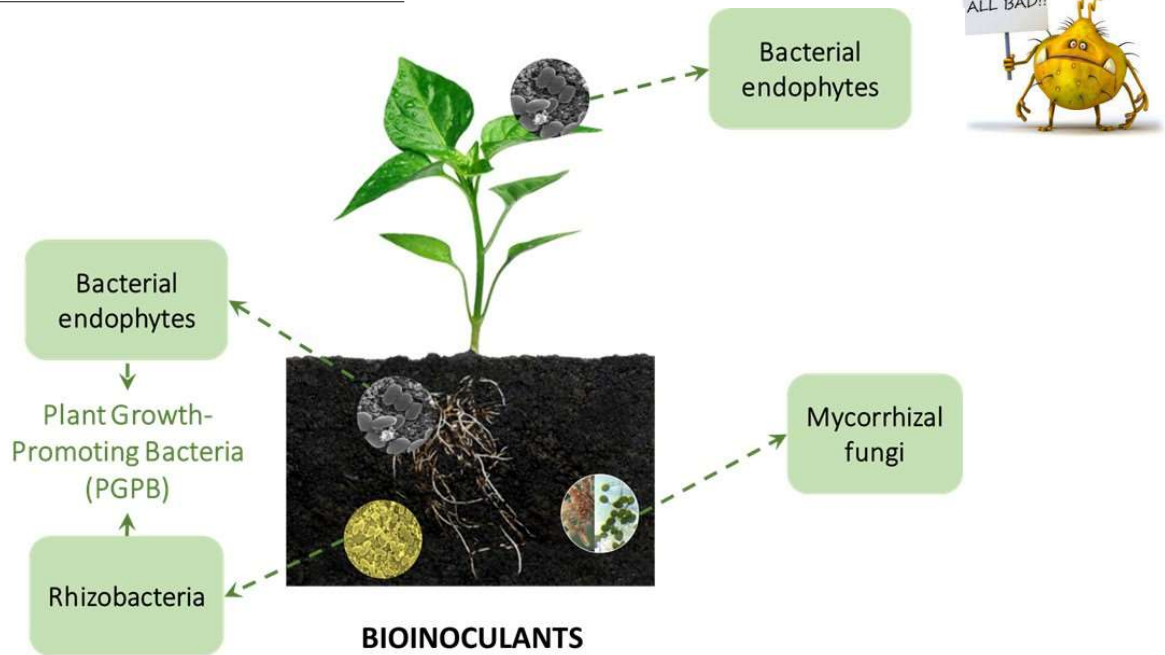
**It is very hard to crop on poor soil...**

Belowground biodiversity plays a key role in sustaining plant life... Yet it goes mostly unrecognized



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**Beneficial Soil Microorganisms**



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## Beneficial Soil Microorganisms

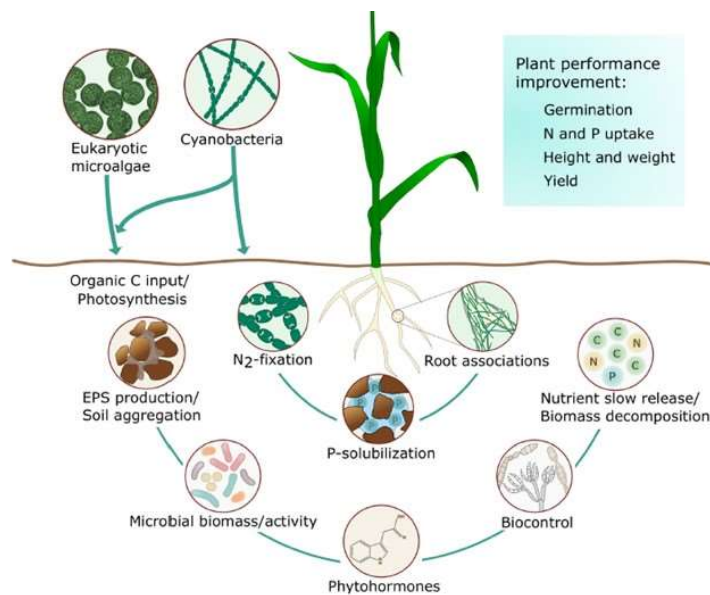


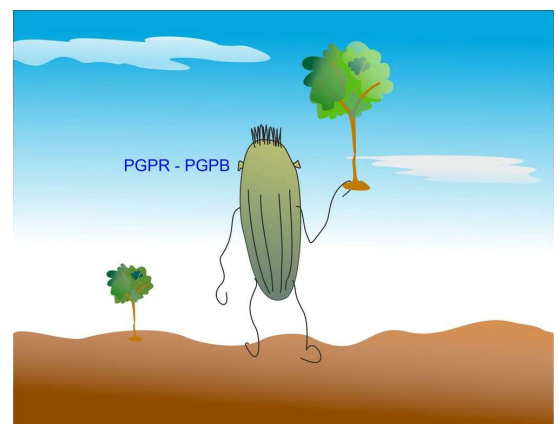
Fig. 1. Selected beneficial effects of microalgae on soil and plants.

Alvarez et al., 2021. *Algal Research* 54: 102200

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## What are Plant Growth-Promoting Bacteria (PGPB)?

- a group of beneficial bacteria capable to establish a symbiotic or nonsymbiotic association with plants (rhizosphere, inside tissues)
- able to increase plant growth and yield
- wide range of species belonging to several genera (e.g. *Pseudomonas*, *Enterobacter*, *Arthrobacter*, *Bacillus*, *Azospirillum*, *Alcaligenes*, ect)



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## Plant Growth-Promoting Bacteria - PGPB

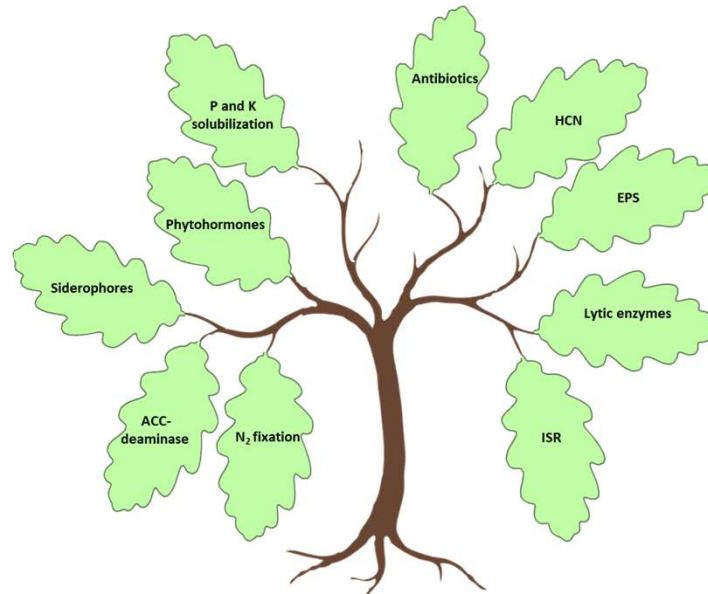
Direct mechanisms

Indirect mechanisms

Nutrient acquisition



Growth promotion



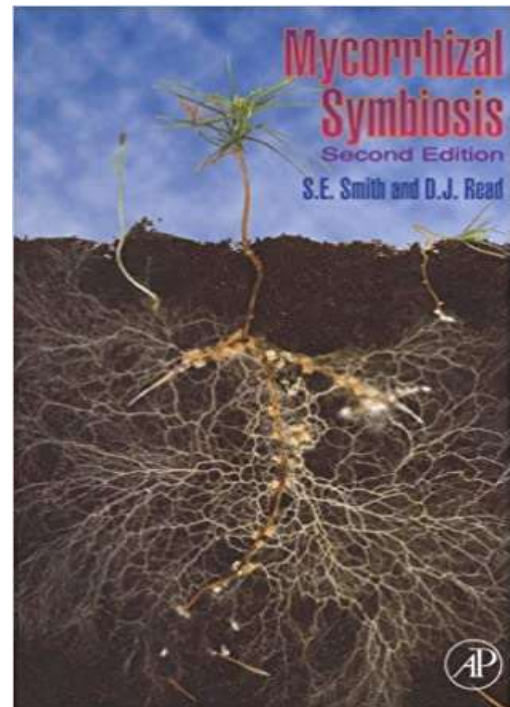
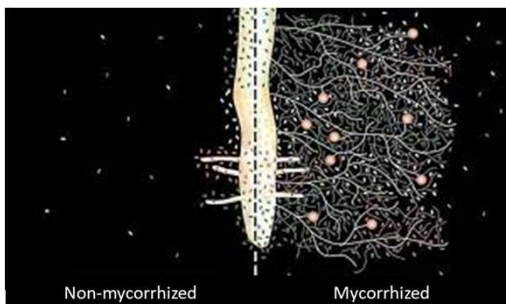
Biocontrol

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## Mycorrhizal fungi

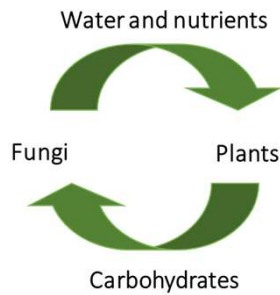
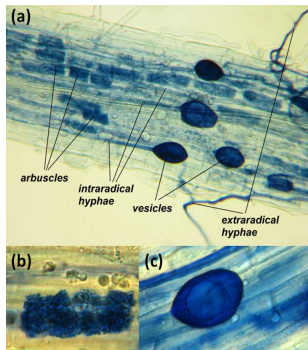
**Mycorrhiza** "root of fungi"

- Symbiosis between fungi and plant roots
- 80% of vascular plants form symbiosis with fungi



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## Arbuscular Mycorrhizal Fungi (AMF)



Higher plants' protection under adverse conditions:

- water and nutrient deficiency
- extreme temperatures and pH
- metals

- Not possible to culture without a host plant
- Dependent on host plant to obtain carbon and energy

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## Arbuscular Mycorrhizal Fungi (AMF)

### Maintenance of trap cultures



### Massive production

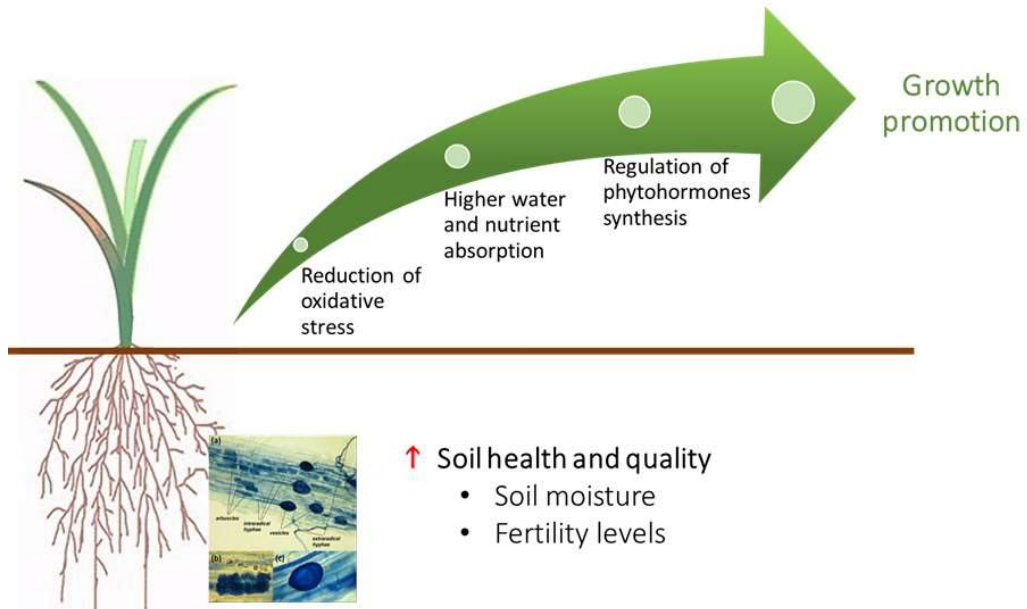


### Commercial products



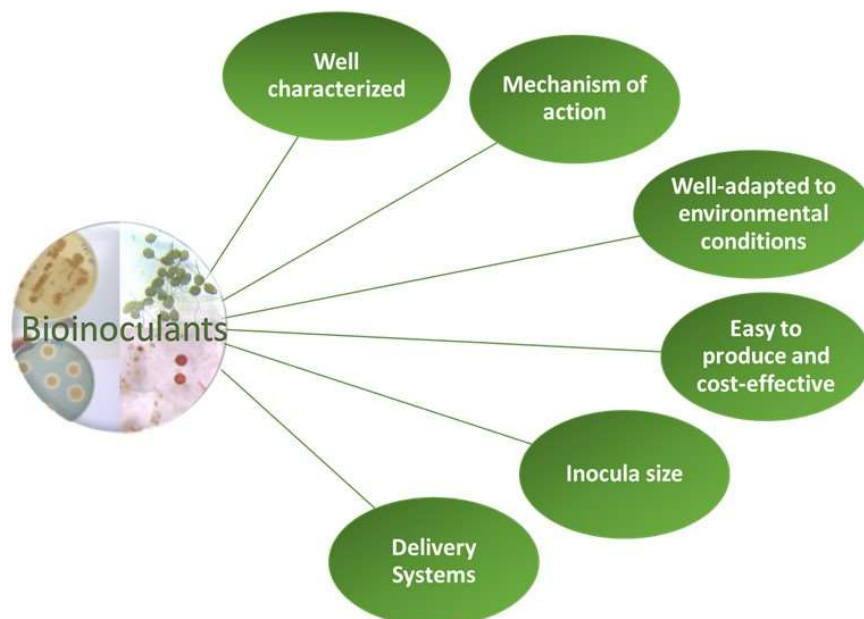
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## Arbuscular Mycorrhizal fungi (AMF)



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## Biofertilizers – Challenges



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## Biofertilizers - Challenges

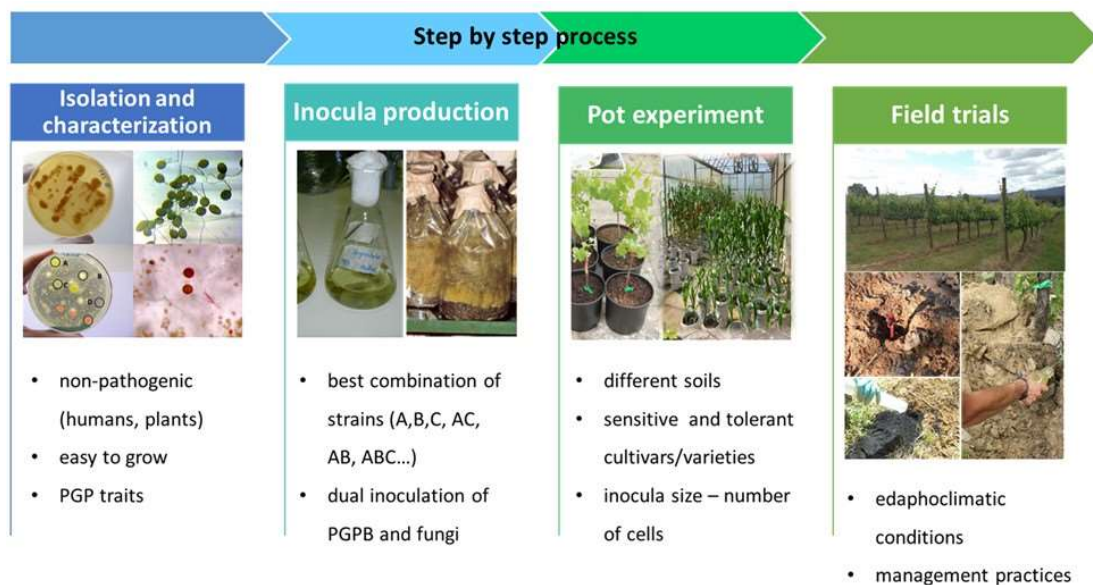
Challenges		
Edaphic and Environmental	Biotic	Negative interactions with the resident microbiome (e.g., competition, predation, and antagonism).
	Abiotic	High variability in soil physicochemical properties (e.g., nutrient levels, pH, organic matter content, moisture, temperature, salinity).
	Agricultural practices	Interaction with other agricultural practices (e.g., organic amendments, fertilizers, pesticides, tillage, crop diversification strategies).
Plant-related	Plant genotype and physiological status	Different outcomes depending on plant genotype due to different degrees of specificity or indirect selection via plant rhizodeposition and root architecture. Variability in different plant growth stages and overall physiological status.
Inocula-related	Genetic and physiological traits	Microbes with poor ecologically relevant traits affecting their establishment, colonization, persistence and tolerance to abiotic stresses (e.g., osmotic and temperature).
	Formulations	Insufficient physical and chemical protection to maintain cell viability and prevent desiccation/contamination.
Practical Aspects	Costs	Economic feasibility at a commercial scale (bioprospecting, testing, scaling up, storage, and application).
	Farmer accessibility	Products with limited versatility, reproducibility, shelf-life, practicality (handling and application), adaptability to different agricultural practices. Insufficient collaboration and communication between researchers, industry, and farmers.
	Regulations	Lack of standardized and universal testing protocols and evaluation guidelines.
	Intellectual property	Disregard or negligence to protect intellectual property (patent development) and technology transfer.

Mitter et al., 2021

- Inconsistent responses over different soils, crops and environmental conditions
- Practical aspects related to mass production, shelf-life, appropriate recommendations and ease of use for farmers
- Lack of awareness and low adoption rate of biofertilizers
- High Initial Investment

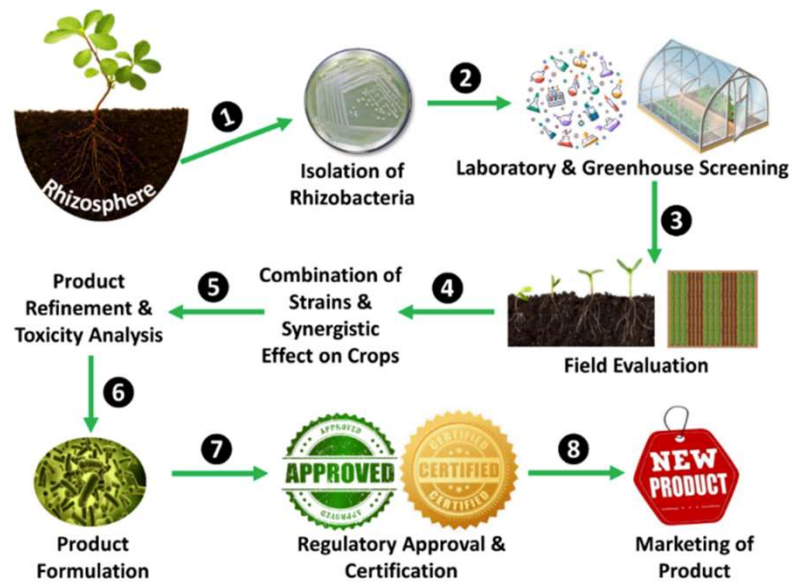
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## Biofertilizers - Challenges



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## Roadmap for the comercialization of biofertilizers



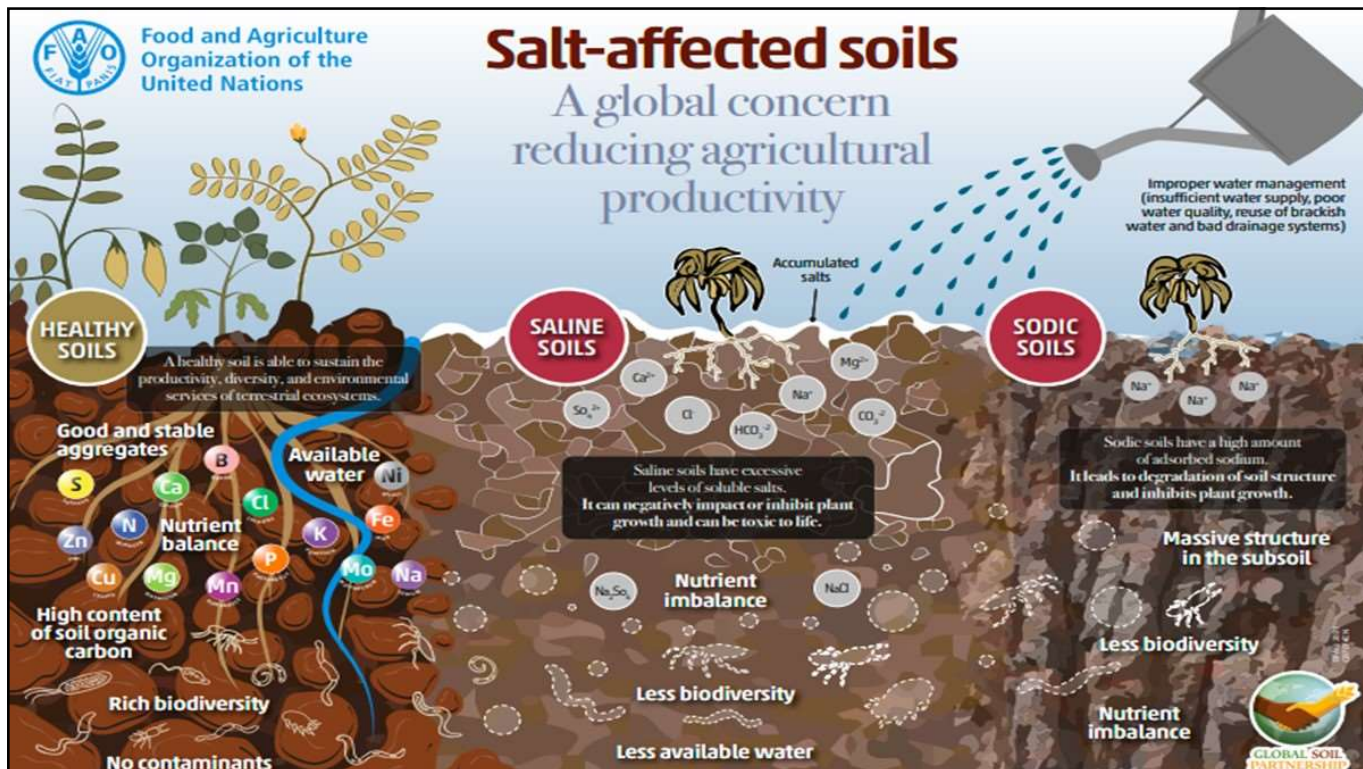
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## Promotion of sunflower growth under saline water irrigation by the inoculation of beneficial microorganisms

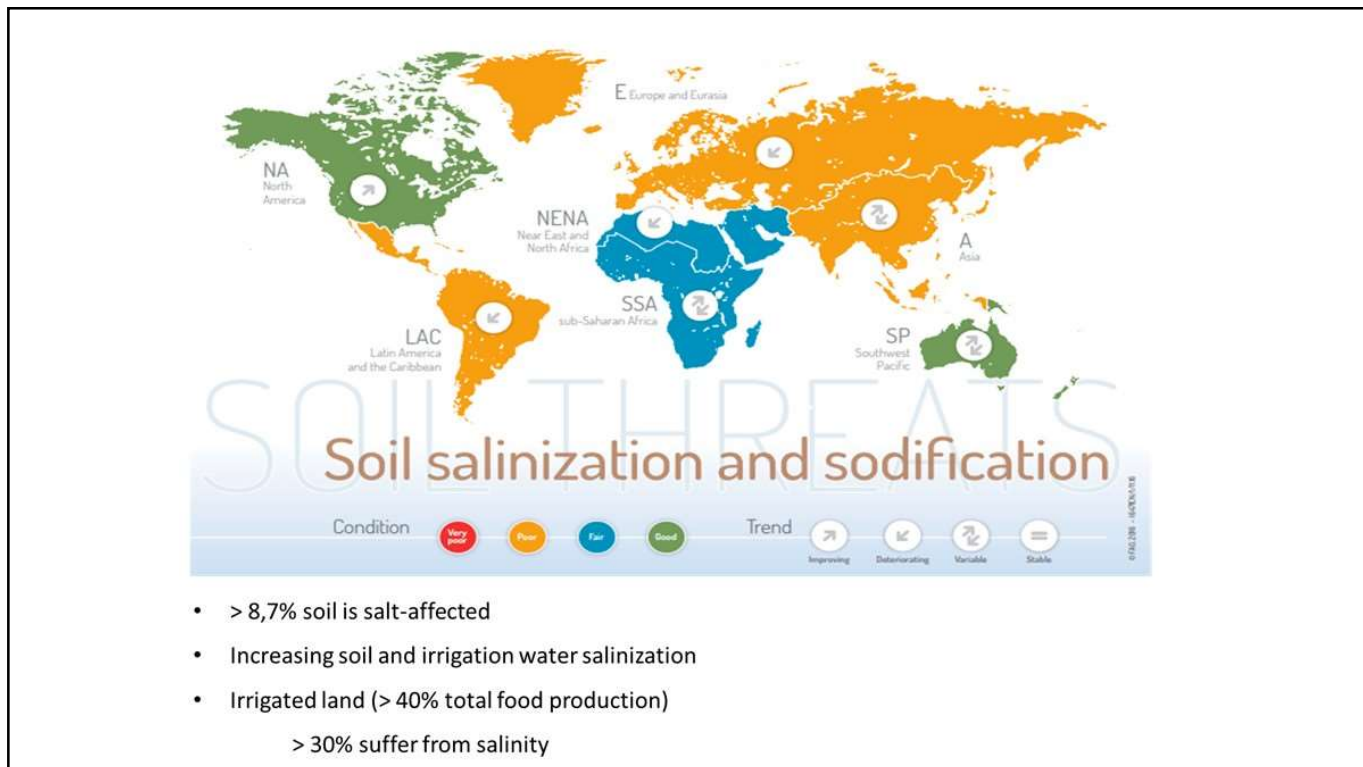


Pereira et al., 2016. Applied Soil Ecology. 106: 36-47

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## Soil salinity

Soil Salinity Class	Conductivity of the Saturation Extract (dS/m)	Effect on Crop Plants
Non saline	0 - 2	Salinity effects negligible
Slightly saline	2 - 4	Yields of sensitive crops may be restricted
Moderately saline	4 - 8	Yields of many crops are restricted
Strongly saline	8 - 16	Only tolerant crops yield satisfactorily
Very strongly saline	> 16	Only a few very tolerant crops yield satisfactorily



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## Effects of salt stress in plants



### Mitigation of adverse effects by bioinoculants' application



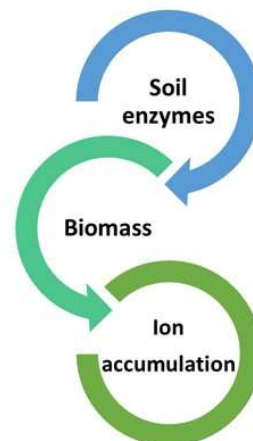
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## Experimental design

To assess the contribution of bioinoculants to improve sunflower growth in soils irrigated with saline water



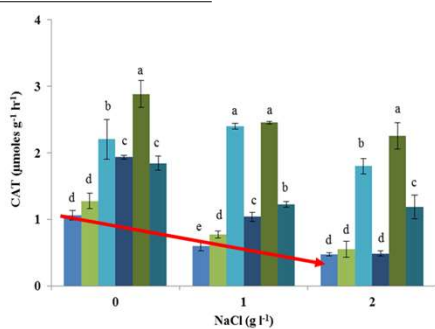
10 weeks



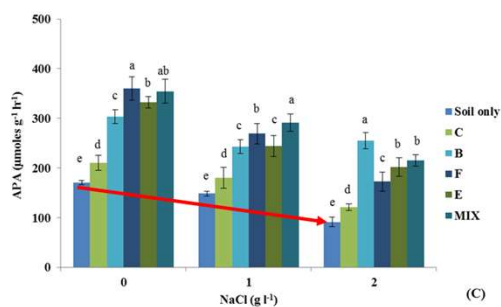
C- Control  
 PGPR - *Chryseobacterium humi* ECP37  
 Endophyte - *Ochrobacterium haematophilum* ZR3-5  
 AMF - *Rhizophagus irregularis*  
 Mix - PGPR + Endophyte + AMF

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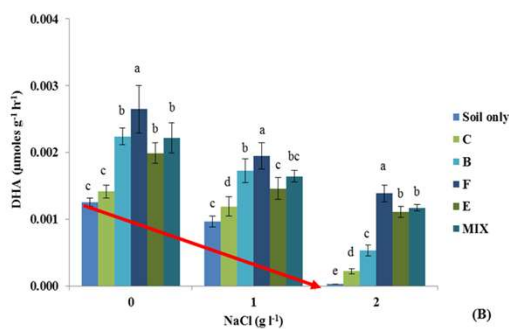
## Soil enzymes



(A)



(C)



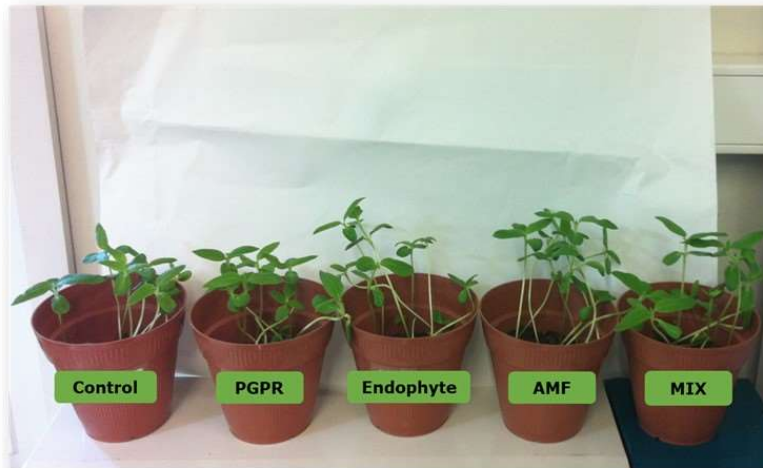
(B)

- The activity of soil enzymes was severely affected by salinity
- Bioinoculants increased the activity of all enzymes

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## Plant Biomass

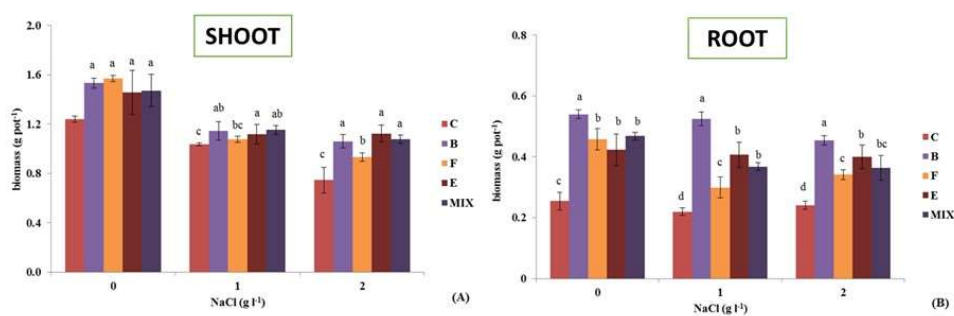
After 4 weeks.....



Inoculated plants showed higher shoot biomass than non-inoculated

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## Plant Biomass

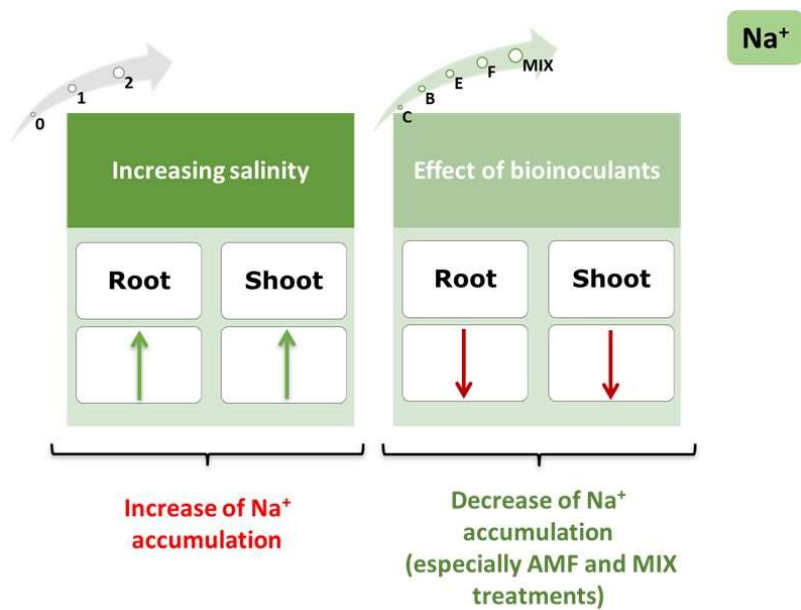


- Saline water decreased plant biomass (roots and shoots)
- Bioinoculants mitigated the adverse effects of salt by increasing biomass production

↑ 62-85% (ROOT)

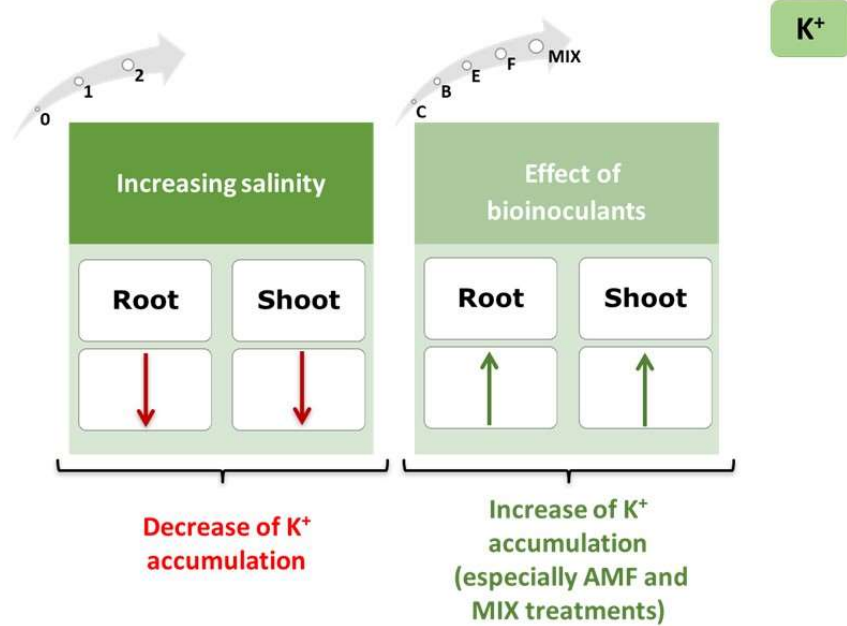
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## Ion accumulation



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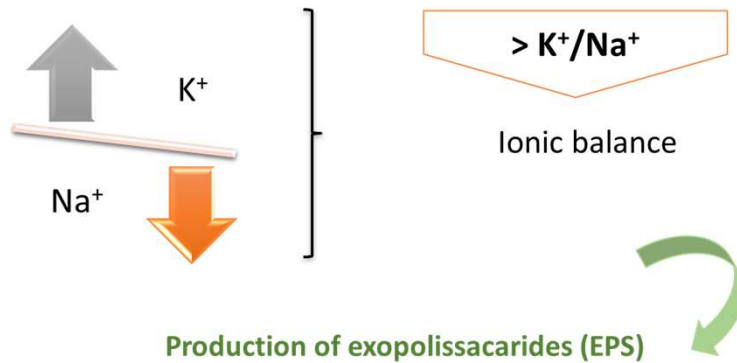
## Ion accumulation



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## Ion accumulation

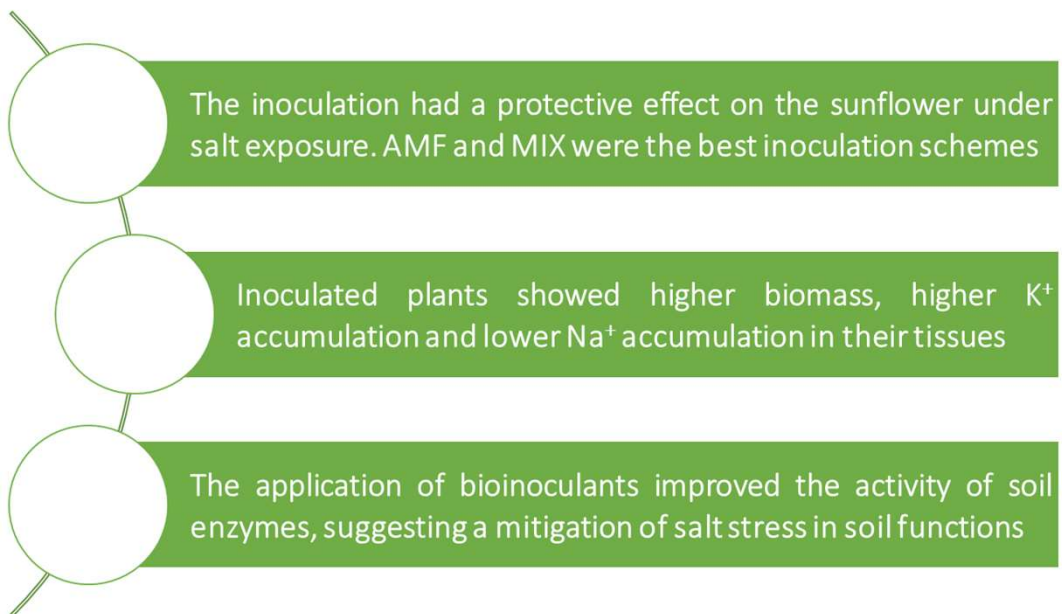
### Effects of bioinoculants on ionic balance



- Binding of Na<sup>+</sup> preventing its absorption by plants
- Beneficial for the structure and aggregation of soil

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## Conclusions



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## Project ReCROP - Bioinocula and CROPping systems: a biotechnological approach for improving crop yield, biodiversity and REsilience of Mediterranean agro-ecosystems

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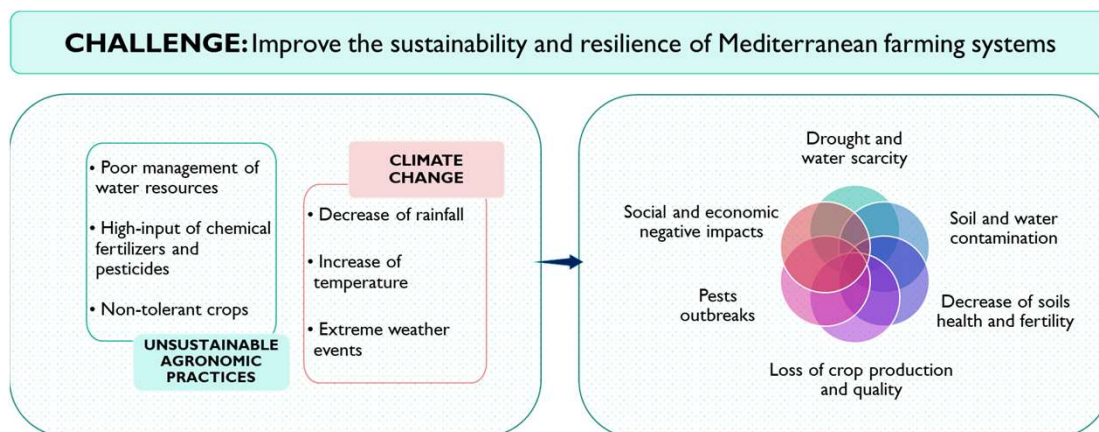
PRIMA  
Section 2 Call 2020 - Multi-topic  
Topic 2.2.1 (RIA) Re-design the agro-livelihood systems to ensure resilience

Co-funded by:



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### The basis for ReCROP project



...fostering sustainability and resilience of agricultural production systems (...) combined use of biotechnological tools, such as bioinoculants (mycorrhiza-MF and plant growth-promoting bacteria-PGPB), and environmentally friendly agronomic practices.

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## ReCROP consortium



### 7 countries:

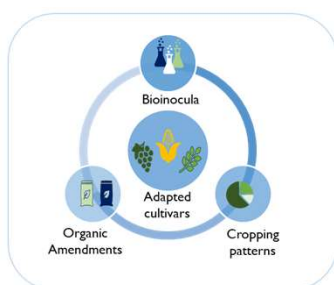
Portugal, Spain, France,  
Italy, Morocco, Egypt,  
Tunisia

### 11 Partners:

- ESB-UCP, Portugal
- IDARN, Portugal
- ADVID, Portugal
- NEIKER, Spain
- CSIC, Spain
- UPVM-CEFE, France
- UM-CEE-M, France
- IRET-CNR, Italy
- FSTG, Morocco
- FOAFU, Egypt
- INAT, Tunisia

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## ReCROP Strategy



- **The established and newly set-up field sites** in each country are being used to study the beneficial effects of cropping systems, bioinoculation, and amendments in 3 important Mediterranean crops - **vineyards, maize and aromatic/medicinal plants**



A **multi-actor approach** is being implemented to:

- identify barriers and opportunities related to technical and socioeconomic aspects (close proximity to farmers to incorporate local knowledge).
- identify solutions to increase crop production in a sustainable way while ensuring farmer's incomes

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## Expected impacts

### IMPACTS: Redesigned farming systems with improved resilience capacity

#### Provision of ecosystems services

- Increase of soils' health, fertility and functions

#### Sustainable and resilient farming

- Climate change adaptation
- Resilient agroecosystems

#### Preservation of natural resources

- Water conservation
- Increase of biodiversity

#### Social and Economic development

- Increase farmers' income and satisfaction
- Stakeholders' Networks

- characterize from a socioeconomic point of view the drivers that can encourage farmers to switch towards greener practices and promote their acceptability
- comprehensive characterization of soil biodiversity through the combination of classical taxonomy and NGS technologies to promote "productivity-biodiversity" binomials; the data collected in all established field trials will help farmers in decision making via a comprehensive assessment of soil biodiversity profiles
- Implementation of the innovative sustainable solutions that contribute to a more resilient and sustainable agriculture
- **Guidelines for farmers** concerning **widely adaptable agricultural practices** for soil health and sustainable agricultural productivity in the Mediterranean region

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