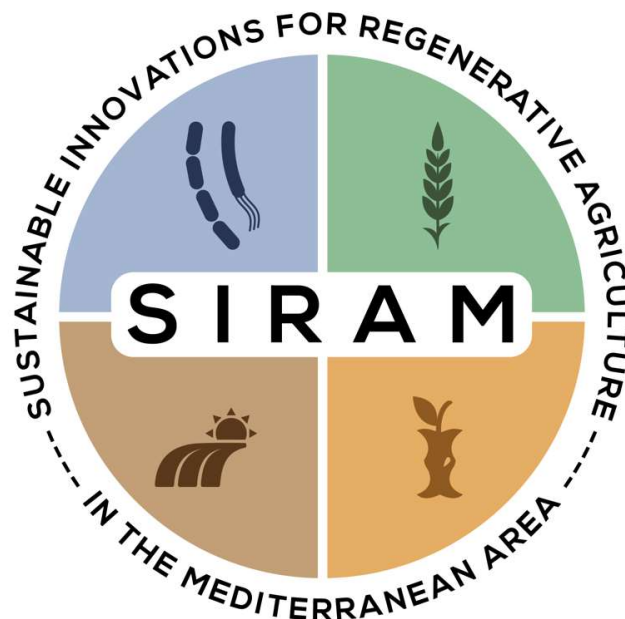




D1.4. REPORT ON STRATEGIES TO INCLUDE LOCAL RESISTANT VARIETIES IN COMBINATION WITH MICROBIAL ANTAGONISTS TO OVERCOME PLANT PESTS AND DISEASES

Lead Beneficiary: Regional Centre of Agricultural Research of Sidi Bouzid (CARRA)

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Date: 01/03/2023



DOCUMENT INFORMATION

Grant Agreement	
Project title	Sustainable innovations for Regenerative Agriculture in the Mediterranean area
Project acronym	SIRAM
Project coordinator	Edoardo Puglisi, UCSC
Project duration	1 June 2022 – 31 May 2025 (36 months)
Related work packages	WP 1 – Screening and selection of beneficial microorganisms, recyclable biomasses and resistant varieties
Related task(s)	Task 1.3 Resistant varieties .
Lead organisation	CRRA
Contributing partner(s)	UCSC, CRRA, ENAM, USMBA, IRTA, UC, ARC
Due date	28 February 2023
Submission date	01 March 2023
Dissemination level	Confidential, only for members of the Consortium including the Commission Services

EXECUTIVE SUMMARY

The present Deliverable (D1.4) contains the results obtained in the context of Task 1.3 (T1.3. resistant varieties). In particular, Table 1 lists the local resistant varieties for application in SIRAM case studies with their available characteristics such as crop, target pathogen or pest, resistance level and associated strategies. Moreover, details on methods (phenotypic and

molecular) to study the varietal resistance are given. Finally, some conclusions and recommendations for this task are detailed.

1. RESISTANT VARIETIES FOR APPLICATION IN THE PROJECT CASE STUDIES

Several regenerative agricultural practices have been adopted to decrease pests and pathogens impact. However, the application of environment-friendly methods individually failed to control it. Therefore, the practices of regenerative agriculture, including the screening of existing crop varieties, microbial strains and organic waste biomasses, will be screened to choose the best strategies for Mediterranean areas. These approaches are in agreement with UN Sustainable Development Goals.

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. Local cultivars from each country will be screened for tolerance/resilience to the most diffused pests and diseases on varying environmental conditions (water stress, salinity stress, heat stress, etc.). As described in the project proposal, Case Studies 3, 5, 6, 7, and 8/4 foresee the test of resistant varieties.

CSs 3, 5 and 7 will test fruit tree crops (olive and citrus) resistance, CS 6 will test wheat and barley and CSs 7 and 8 will use vegetable crops (tomato and potato). CSs 3 and 7 (ENAM and CRRRA) will focus both on the olive crop as a varietal resistance testing against two different pathogens. CSs 3, 5, 6, 7 and 8/4 will screen resistance towards oomycetes and fungal disease and CS8 will also test resistance of potato and tomato cultivars against nematodes. Table 1 reports the crop chosen for application in these Case Studies, detailing their resistance pathogens.

In particular:

- In **CS3**, it will be evaluated the resistance of olive collection varieties located at Agro-Pôle Olivier Meknès (ENAM) to *Phytophthora* root and crown rot. Interestingly, this collection contained Moroccan, Spanish and other Mediterranean olive varieties. The results would be very interesting as resistant/tolerant varieties can be combined with screened beneficial PGPR.

- In **CS5**, it will be tested, under greenhouse conditions, most effective bacterial isolates (T.1.1), citrus seedlings with/out the presence of each pathogen, and the proposed control strategies will be verified. For each disease, different concentrations of pathogens/antagonists and different citrus rootstocks with different disease susceptibility will be used. Then, recommendations and protection programs will be developed, taking into account alternative methods for citrus rootstocks with different resistance in different climatic conditions.
- In **CS6**, field experiments on rainfed winter arable crops fertilized with manure will use adapted selected varieties. On top, the development of a series of wheat and barley varieties will be followed to detect adaptation to most incident pests and pathogens and the local climate. Crop yield and quality and soil quality characteristics linked to water dynamics will be assessed.
- In **CS7**, varietal resistance experiments will be carried out on olives and tomatoes. For both crops, pot experiments will be realized to screen some local or introduced varieties for their resistance toward fungal disease (*Verticillium* and *Fusarium* wilt caused by *Verticillium dahliae* and *Fusarium f.sp. lycopersici*). In addition, the same varieties will be tested on different environmental stress (water stress for tomatoes and salinity for olive trees). Then, the recommendation on selected varieties and strategies under different environmental conditions will be developed.
- In **CS8+CS4**, the available partially resistant potato cultivars (Spunta/Lady Rosetta) only confer resistance to *G. rostochiensis* but some cultivars partially resistant to *G. pallida* can also be found. However, there are no potato cultivars resistant to *Meloidogyne* spp. Some previous studies indicate that there is a great variation in the same cultivar considering their behavior against *Meloidogyne* spp. In T1.3, two partially resistant potato cultivars will be used in pot assays to determine the effects on population densities of the two PCN species and of two or three *Meloidogyne* species that are a threat to potato crops like, for example, *M. chitwoodi*. The assays will compare the effects of the partially resistant cultivars; the bio-tools (bacteria and fungi) alone and the cultivars together with the bio-tools. It is expected that the effects of the partially resistant cultivars together with the bio-tools will become a

new methodology to control nematodes that is economically and environmentally friendly.

Table 1: Resistance varieties experiment proprieties in all cases studies

Part- ner	Case study	Crop	Target pathogen/pest	Level of resistance	Environmental conditions (abiotic stress)	Associated pillars/ strategies
ENAM	CS3	Olive	Oomycetes pathogens (<i>Phytophthora</i> / Pythium root and crown rot)	resistant/tolerant/susceptible		Microbial consortia (PGPR)
USM- BA	CS5	Citrus	Oomycetes pathogens (<i>Phytophthora</i> /Pythium root and crown rot)			
IRTA	CS6	Wheat/ barley	Different leaf diseases	Resistant/Tolerant/ Susceptible (more levels depending on the disease)	mild winter/ hot spring- time/water stress	
CRRRA	CS7	1. Tomato	Fusarium wilt (<i>Fusarium oxysporum</i> f.sp. <i>lycopersici</i>)	resistant/susceptible	water stress	Microorga- nisms and com- posts
		2. Olive	Verticillium wilt (<i>Verti- cillium dahliae</i>)	resistant /partially resistant	salinity	
UC	CS8	1. Potato	Nematodes (<i>Meloidog- yne</i> spp. and <i>Glo- boderia</i> spp.)	Partially resistant		Microbial consortia (PGPR/PG PF)
ARC	CS4	2. Tomato				

2.

Greenhouse experiments under controlled conditions will be performed to evaluate local lines referring to the background of cultivated crops. Various accessions will be used as a source for tolerance and resistance to a wide range of fungal pathogens. Biotic and combined stress experiments will be planned. Then, agronomic traits will be measured such as chlorophyll content, damage reduction of shoot and root dry weights and salt tolerance index (calculated as the ratio of (fresh or dry) shoot biomass under salt stress and biomass un-

der control conditions for each genotype). In addition, the disease incidence of the main pathogenic fungi of each crop study will be determined by means of disease severity (expressed as disease index (DI), assessed at several times dpi (days post inoculation) in different lines. The transcriptomic analysis could be also applied to discover genes responsible for salt and/or pathogens tolerance in crops (e.g. tomato). An available olive collection at ENAM (Agro-Pôle Olivier Meknès) will be tested for their tolerance to *Phytophthora* root and crown rot under greenhouse conditions in pot experiments. As olive trees are attacked by many *Phytophthora* species, this collection will be tested at least against three predominant *Phytophthora* species (CS3). The metagenomic approach would be used to investigate the resistant mechanisms. The screening will be performed also by PCR (Q-PCR) on relevant molecular genetic markers for resistance/tolerance of plant parasites and pathogens (Table 2).

At ARC, a greenhouse experiment will be conducted with the collaboration of UC team (CS4/CS8) to evaluate the efficacy of PGPR/PGPF isolates on two potato varieties with reference to tolerance and resistance to plant parasitic nematodes (PPNs). The agronomic traits such as plant growth parameters, enzyme activities, phenol content, etc., as well as the nematode population density will be evaluated. Transcriptomic (e.g. RNA-seq) analyses on roots of selected optimal potato/PGP combinations will be performed to identify the mechanisms involved in the improved tolerance/resistance and to highlight genes and pathways involved in plant response to PPNs (Table 2).

Table 2: ??????? in all cases studies

Case study	Experiments		Pathogenicity		
	Crop	(Pot/field)	Agronomic traits	parameters	Genomic study
CS3	Olive	Pot expe-	growth parameters and disease severity		gene expression/
		riment			microbial community
CS5	Citrus	Pot expe-	growth parameters and disease severity		Microbial community
		riment			
CS6	Wheat/ barley	Field expe-	Crop yield and quality	Severity in plant	-----
		riment			
CS7	1. To-	Pot expe-	growth parameters and	disease index, vas-	transcriptomic/gene de-

	mato	riment/ field	soil quality	cular wilt diameter	fense expression (q-PCR)
		Pot expe-			
	2. Olive	riment	Chlorophylle content		metabolomic
CS8	Potato		Growth parameters/	nematode	
CS4	Tomato		enzyme activities/ phenol content	population density	transcriptomic/gene defense expression (q-PCR)

3. CONCLUSIONS AND NEXT STEPS

In several Mediterranean pathosystems, the integration of resistant varieties and regenerative agricultural practices has been advocated as sustainable strategies for plant mitigation towards biotic and abiotic stress.

An increase in fruit yield and a decrease in stress severity were observed for several varietal resistance-regenerative agricultural practices combinations. Numerous assays under the greenhouse and open fields will be assessed in order to develop an efficient integrated approach to control crop diseases and pathogens.