ZeroParasitic

Newsletter #2,

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NEWSLETTER *ZeroParasitic*

Word from the Coordinator

ZeroParasitic is a three-year project, aiming to deliver innovative sustainable solutions to overcome broomrape plant parasitism in key Mediterranean cropping systems. The main goal of ZeroParasitic is to integrate innovative solutions into a realistic framework through a trans-disciplinary, multi-actor effort targeting broomrapes, which is one of the most critical disease/weed in Mediterranean countries, posing a significant threat to various key cropping systems in the region. Until now, the project is being implemented successfully, with some changes due to the Covid 19 pandemic, but the objectives have been achieved. The communication with the consortium was sustained. Despite our continuous contact, the Executive Board & General Assembly meetings have been held for the sharing of all the progress and decision making, as well as some internal meeting among WP leaders for exchange of knowledge and sharing protocols of practices. Both the Kick-Off meeting and the first annual meeting were organized, performing efficiently day-to-day management ensuring that the project stays always focused on its objectives. From my side, as the coordinator, I feel completely pleased from the progress of the project which couldn't be achieved without the partners valuable contribution.

INSIDE THIS NEWSLETER:

Pattern recognition receptors (PRRs) in host plants

Plants can sense microbial attack by detection systems for "pathogen-associated molecular patterns" (PAMPs). They are inducing defense responses such as ethylene production and a burst of reactive oxygen species (ROS). We addressed the question whether tomato might detect and respond to molecular signals associated with the parasitic plant in a manner comparable to the plant immune responses to microbe-associated molecular patterns (MAMPs) or are they just perceived indirectly, for example through DAMPs (damage associated molecular patterns). We exploited the natural variation between susceptible S. lycopersicum and resistant S. pennellii to identify the receptor for the P. ramosa factor. Preliminary results showed that pre-purified extracts of broomrapes such as *P. ramosa, P. aegyptiaca* induce ethylene production in *S. lycopersicum* cv. M82/*S. pennellii* and that ethylene response depends on different doses of pre-purified broomrape extracts. Moreover, we found that the broomrape PAMPs appeared to be present not in all parts of broomrapes including shoot tips, stems and flowers, but especially seeds or premature seeds indicating that this factor could be produced only at certain developmental (e.g. germination) or infectious stages. To purify and identify the P. ramosa factor, we established a purification scheme involving sequential separation steps that will be further improved and scaled-up in the next months.

Detecting virulent broomrapes and identifying host resistance

Understanding the molecular basis of broomrape virulence and plant resistance are key for the development for biocontrol solutions and tolerant crop varieties. Analysis of root exudates as belowground defense substances, will generate unique phytohormone and metabolite profiles to determine how roots sense and interact with broomrape germination, development and infection in tomato and faba bean. In parallel, natural tomato variants/introgression lines and transgenic plants with altered pattern recognition receptors (PRR's) will be infected with different Orobanche/Phelipanche and checked for successful infection events and the parasite growth utilizing well-established bio-assays. Regarding the genetic diversity of broomrapes, new DNA polymorphism and existing or newly identified from the hormonal and metabolite profiles candidate genes will be studied and proposed as genetic markers to pinpoint pathogenicity of broomrape biodiversity in seed lots and extrapolated in the soil. Additionally, the degree of resistance of tomato and faba bean genotypes will be associated with the expression level of host genes related to resistance/tolerance to parasitism.

Boomrape parasitism in tomato

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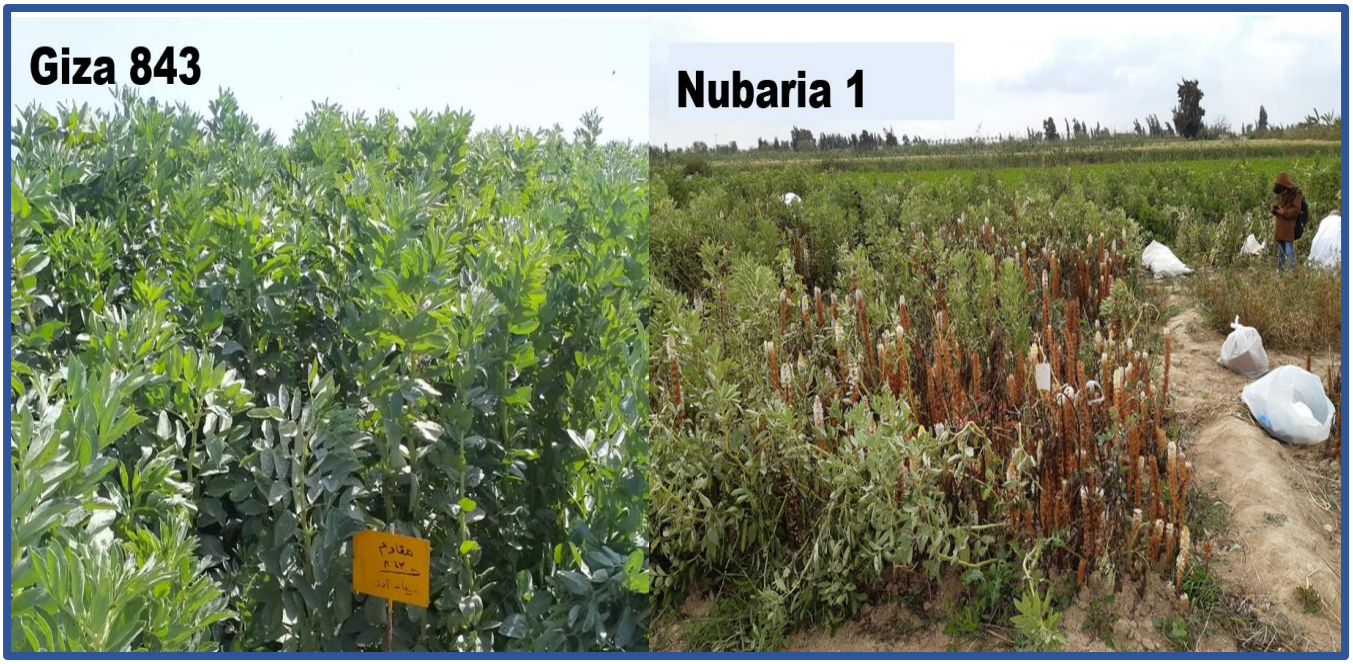
Faba bean cultivars susceptibility to parasitic weed infestation



Faba bean cultivars Resistant (Giza 843) and susceptible Nubaria 1 field (Nubaria Farm,April, 2021)

Field experiment were performed in both Abis and Nubaria farms. Four faba bean cultivars were investigated for their susceptibility to infestation by *Orobanche. Crenata*, in naturally *Orobanche* infested soil. The experimental data showed that, both susceptible (Nubaria 1 and Giza 714) and resistant cultivars (Giza843 and Misr 3) were infected by broomrape. Giza 843 was the most resistant followed by Misr 3 and Giza 714. While Nubaria 1 was very susceptible. Survived broomrape spikes among Nubaria 1 cultivar produced the highest broomrape seeds compared to other cultivars.

Effect of intercropping on the emergence of the parasite spikes

Evaluation of intercropping of fenugreek, radish and flax on the emergence of broomrape spikes and seed bank was conducted using faba bean resistant and susceptible cultivars for two successive seasons. Intercropping flax or fenugreek significantly reduced the infestation levels by *O.crenata*. Flax was more effective in reducing infestation rate by *O.crenata* than fenugreek. In contrast radish was in-effective in reducing infestation. Moreover, the flax or Fenugreek intercropping significantly increased the pod yield of faba bean.

Fenugreek intercropped within faba bean

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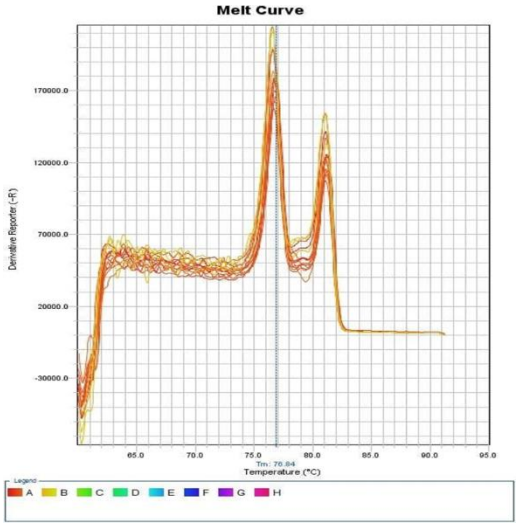
Analysis of the expression of host genes associated to resistance

Three introgression lines: LA4060, LA4062 & LA4062, as well as three commercial tomato hybrids of *S.lycopersicum* were sowed and inoculated with *O.ramosa* seeds. Untreated plants were used also as controls. Photosynthetic rate and morphological characteristics are recorded after inoculation. The aim is to characterize the plant material as broomrape resistant/tolerant or sensitive after the host plant gets parasitized by the parasite. In this perspective, host-plant material from roots & leaves will be collected for further molecular analysis of gene expression of specific host genes associated to resistance.

Commercial hybrids & ILs seedlings 2 weeks after broomrape inoculation

Exploiting the genetic diversity of broomrapes



HRM melt curve of broomrape DNA material

Appropriate Primer probes have been ordered and HRM experiments are carried out to identify genetic differences among different broomrape species as well as the identification of intraspecific genetic differences. DNA sequencing of PCR products from some species will follow and these samples are going to be used as positive controls.

Target	Name	Sequence (5'-3')	Source	Purpose
trnL	trnLC(F)	CGAAATCGGTAGACGCTACG	Taberletetal., 1991	PCR and sequencing
	trnLHRMR	GGGGATAGAGGGACTTGAACC		
rbcL	1F	ATGTCACCACAAACAGAAAC	Manen etal., 2004	PCR and sequencing
	1352R	CAGCAACTAGTTCAGGRCTCC		
trnL	trnL-Z1-F	CGGTAGACGCTACGGACTTA	Rolland et.al., 2016	HRM
	rnL-Lg-2R	ATGGGACTCTATCTTTATTCTC		
rbcL	rbcL-lg-1-F	AACCTGAAGTTCCGCCTGAA		
	rbcL-Z2-R	AGTACATCCCAACAGGGGAC		
its	ITS-F	TTTCACTCCCTCACATGCCCAC	this study	HRM
	ITS-R	TCTGCAATTCACACCAAGTATCGC		
	ITS-R2	CACCCCACCAGCAACACGTC		
rps2	RPS2-F1	ATCCTAAAATGGCACCTTATATCTC	this study	HRM
	PRS2-F2	AAACAAYTCTTGATTGTTGGTACAAA		
	RPS2-R	CATATATTTAATCCCGCCCAGAT		

Primers for HRM and PCR sequencing

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Economic effectiveness as one of the key factors affecting the decision to adopt a novel practice

Since economic effectiveness is one of the key factors affecting farmers' decision to adopt a novel practice, an economic feasibility analysis, aiming to provide with a socioeconomic assessment in an integrated manner that will accommodate the diversity of social and economic conditions of targeted production systems, is always required. An integrated economic assessment framework, which uses established analytical tools in order to demonstrate how the new solutions will specifically affect the economic performance of farms, has to be developed. Technical and economic data indicators of farm management have to be collected, while specific questions should record in detail management practices related to broomrape (preventive or curative, biological or chemical etc) and the resource requirements of each practice (capital and labor).

Farmers' mindset towards the adoption of new methods

"Further research is required and has to focus specifically on how farmers conceive and evaluate a potential shift from chemical herbicides to new weed control strategies."

However, the introduction, adoption and implementation of new weed management methods is a challenging process and does not only depend on economic results. Implementing new approaches for weed management will require participatory approaches and multidisciplinary groups. Further research is required and has to focus specifically on how farmers conceive and evaluate a potential shift from chemical herbicides to new weed control strategies. The examination of farmers' perspective towards the adoption of new weed management methods is crucial towards this change. Factors either enabling adoption or considered as barriers to the adoption of new practices by farmers need to be investigated and solutions to overcome those barriers should be proposed.

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Inducers: Testing their effect on faba beans resistance

Assays in petri dishes

The assay was placed in petri dish co-culture in greenhouse in order to understand the behavior and the interaction between faba bean and *O. crenata* or *O. foetida* in response to seed coating. Preliminary results showed that all treatments decreased the orobanche germination percentage compared to check. Reduction varied from 14.28 % to 57.35% for O. crenata and from 14.60% to 41.89% for *O. foetida*. The highest percentage of reduction in seed germination of orobanche was observed in faba bean coated by Trianum-P. This reduction reached 57.35 % and 41.89% for *O. crenata* and *O. foetida*, respectively. This experiment was conducted again to confirm the results. Orobanche attachments and some biochemical parameters will be also determined in the future.

Mapping the parasitism in tomato

Satellite data acquisition and production of the initial NDVI maps took place in order to map the parasitism in tomato. Specifically, the ESA Sentinel 2 (S-2) platform was selected to be employed for the purposes of this study. To produce the initial NDVI (Normalized Difference Vegetation Index) maps, satellite images were acquired from the Copernicus Scihub portal for the Sentinel-2 mission for dates matching (± 3 days) the dates of the UAV flights. The Sentinel Application Platform (SNAP) software was employed for image processing and the QGIS software for the final map productions (colour palettes and illustrations). In the next steps of the task, these initial NDVI maps will be calibrated and optimized, utilizing the UAV images and other data, to achieve the final maps that will present the illustrate parasitism.

Satellite data acquisition and production of the initial NDVI maps

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