ORPRAMed

Risk assessment of introduction of *Xanthomonas citri* subsp. *citri* (*Xcc*) through commercial trade of ornamental rutaceous plants in the Mediterranean basin

Coordinator Paola CARUSO,
CREA-OFA, Italy
paola.caruso@crea.gov.it
CONSORTIUM & OTHER STAKEHOLDERS

Project start date: 01/03/2016 - Project end date: 28/02/2019
Overall budget: 1,328.000 € - ARIMNet2 grant: 474,000.00 €

Coordinator: Germplasm collections of Citrus and Citrus Rutaceae relatives. Genomics and transcriptomics expertise. Sequencing of Murraya paniculata and transcriptomic analysis to identify the main genes involved in the disease.


Specialized in species of Xanthomonas. Expertise on molecular mechanisms involved in the early steps of the infection process. Evaluation of Xcc ability to survive on surface of the different species tested.

Population biology of genetic variants of the pathogen, host specialization and fitness-associated traits, and molecular diagnostics. Xcc ability to in planta multiplication and exudation from lesions in relation with its genetic diversity and Validation of the EMA/PMA-PCR method as the new tool for Xcc analysis.

Laboratory tuning in Turkey based on EPPO procedures. Citrus canker surveys in Turkey.
Protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community, regulates the introduction to all Member States of plants or parts of Citrus, Fortunella, Poncirus, and their hybrids.

EFSA opinion (EFSA-PLH, 2014) on the risk to plant health of Xcc and Xca for the EU territory identified seven entry pathways of citrus xanthomonads. Among them there are ornamental rutaceous plants: commercial trade; and import by passenger traffic. The risk of introduction by these 2 pathways is considered “likely”.

Forbids the introduction within the Community of some rutaceos genera from countries where Huanglongbing disease of citrus is present.

Assess the introduction of Xanthomonas citri subsp. citri in the Mediterranean basin through Ornamental Citrus Rutaceous Relatives.

BACKGROUND
PROJECT OBJECTIVES

Goal:

- ORPRAMed (Ornamental Rutaceous Plants Xcc Risk Assessment in Mediterranean) aims to assess the risk of the introduction of Xcc in the Mediterranean region by means of ornamental Citrus relatives, generating and improving our understanding of the interactions between the bacterium and ornamentals citrus and related genera.

Objective 1
- To enquire about the actual flows of the most economically important citrus ornamental species, as well as of ornamental rutaceous relatives, whose distribution and trade are actually unknown.

Objective 2
- To provide new data on the behavior of many ornamental Rutaceae in relationship to Xcc.

Objective 3
- To enlarge the knowledge about the epiphytic and endophytic colonization of the pathogen and its ability to survive within studied plants.

Objective 4
- Sequencing genome of ornamental Citrus relatives not yet available and performing transcriptomic analysis to identify candidate genes involved in the plant response to the disease.

Objective 5
- To acquire methods, protocols to work with quarantine bacterial diseases as Xcc, to face possible pathogenic threats.

Objective 6
- To provide useful information for the definition and/or update of the European directive on plant protection.
ORPRAMed promotes a multidisciplinary approach involving the use of diverse scientific areas and methods including:

- Raising the awareness of the scientific community as well as general public about this issue
- Establishing a network of researchers with different expertise and local plant protection services and nurseries.
ACTIVITIES / WORK PACKAGES

WP 0 - Coordination

- Task 1.1 Trade analysis on ornamental Rutaceae plants flows (Di3A)
- Task 1.2 Pest risk assessment (Di3A)
- Task 1.3 Economic impact assessment (Di3A)

WP 1 - Economics and trade of ornamental Rutaceae plants in the Mediterranean region

WP 2 - Host status of ornamental Rutaceae species and mechanisms of Xcc survival and colonization in ornamental plants

Task 2.1 Host status of ornamental Rutaceae species (Di3A, CREA-OFA)

Task 2.2 Testing of Xcc ability to survive on surface of the different species (INIA)

Task 2.3 In planta multiplication of Xcc and exudation from lesions in relation with its genetic diversity (CIRAD)

Task 2.4 Xcc survey in Turkey and application of EPPO protocol PM7/44 (BCRI, CU)

Task 2.5 Ring test to validate de EMA/PMA-PCR method (All Partners / CIRAD leading)

Task 3.1 Ornamental rutaceous relatives genome sequencing (CREA-OFA)

Task 3.2 Transcriptomic analysis (CREA-OFA)

WP 3 - Genomic and transcriptomic analysis of the resistant/susceptible genotypes

WP 4 – Dissemination Activities
STUDY AREA / SITES
RESULTS SO FAR

The sequencing of *M. paniculata* was accomplished using Illumina HiSeq 2500 platform. Statistics of assembly are very good: Genome prediction and functional annotation are in progress.

- **N₉₀** scaffold size in kb, with gaps = 309.6 (it means that the 90% of the assembly is included in scaffold longer than 300 kbp).
- **N₅₀** scaffold size in kb, with gaps = 1385 (it means that N₅₀ is over one megabase).

Surveys were conducted in Turkey (Adana, Mersin and Hatay provinces) for verifying the presence of symptoms of CBC in a sample of citrus orchards and in all ornamental nurseries. From the survey, no suspect symptoms of the diseases were observed.

<table>
<thead>
<tr>
<th>Provinces</th>
<th>Surveyed areas (ha)</th>
<th>N. of total nurseries</th>
<th>N. of surveyed nurseries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adana</td>
<td>~4050</td>
<td>28</td>
<td>22</td>
</tr>
<tr>
<td>Mersin</td>
<td>~2600</td>
<td>63</td>
<td>26</td>
</tr>
<tr>
<td>Hatay</td>
<td>~1900</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>~8550</td>
<td>109</td>
<td>61</td>
</tr>
</tbody>
</table>
PUBLICATIONS & DISSEMINATION ACTIVITIES

ARIMNet2 Mid-Term Evaluation Meeting, 12 October 2017, Montpellier

Date | Journal/Congress/Workshop/Conference | Article/Oral presentation/Poster/Brochure | Title
---|---|---|---
19-23 June 2017 | 15th Congress of the MPU - Mediterranean Phytopathological Union – Spain | Oral presentation and Poster | Is *Xanthomonas citri* subsp. *citri* (*Xcc*) knocking at the doors of the Mediterranean basin?
19-23 June 2017 | 15th Congress of the MPU - Mediterranean Phytopathological Union – Spain | Oral presentation | Assessment of the host status of ornamental rutaceous species to *Xanthomonas citri* pathovars causing Citrus Bacterial Canker.
12-14 December 2016 | Joint EFSA - EPPO Workshop: Modelling in Plant Health – Italy | Poster | The risk assessment of introduction of citrus canker in the Mediterranean basin through Citrus rutaceous relatives: ORPRAMed project
18-23 September 2016 | XVIII Congreso de la Sociedad Española de Fitopatología (SEF 2016) - Spain | Poster | ORPRAMed: un progetto transnazionale per valutare il rischio d’introduzione del Cancro batterico degli agrum (CBC) nel bacino del Mediterraneo
18-23 September 2016 | International Citrus Congress 2016 | Poster | The risk assessment of introduction of citrus canker in the Mediterranean basin through citrus rutaceous relatives: ORPRAMED project
18-23 September 2016 | International Citrus Congress 2016 | Poster | Ornamental rutaceous species potential asymptomatic vectors for citrus diseases
19-22 September 2016 | XXII SIPaV Congress - Società Italiana di Patologia Vegetale - Italy | Oral presentation | An international multidisciplinary project to assess the risk of introduction of *Xanthomonas citri* subsp. *citri*.
13-16 September 2016 | LX Annual Congress of Italian Society of Agricultural Genetics | Poster | ORPRAMed project: risk assessment of introduction of *Xanthomonas citri* subsp. *citri* through commercial trade of ornamental rutaceous plants in the Mediterranean Basin

Interviews to different national/local broadcasting channels and online newspapers
Project Partner exchange visits

- A CREA-OFA research fellow will visit INIA for a 5 weeks exchange. Accordingly, a request of grant was submitted and accepted in the framework of EuroXanth (Cost Action n. 16107) first call for Short Term Scientific Missions.
NEXT STEPS / PERSPECTIVES

Other related current projects
- EuroXanth (COST Action n. 16107)

Future research projects
- Full proposal submitted to H2020-SFS-2017-2 call
- Full proposal submitted to H2020-SFS-2017-2 call
- Other projects to be submitted exploiting ORPRAMed results
PROJECT FUNDERS

[Logos of project funders: MIPAAF, INIA, ANR, GDAR]

- Italy MIPAAF
- Spain INIA
- France ANR
- Turkey (General Directorate of Agricultural Research and Policy) GDAR
Thank you for your attention!