



Med-Berry

DEVELOPING NEW STRATEGIES
TO PROTECT STRAWBERRY CROP
IN MEDITERRANEAN COUNTRIES



Med-Berry 2nd Newsletter - October 2022



Horizon 2020
European Union

Med-Berry is a PRIMA (Partnership for Research and Innovation in the Mediterranean Area) financed project started the 1st of September 2019.



Editorial

Med-Berry is a PRIMA (Partnership for Research and Innovation in the Mediterranean Area) research project, funded by the European Union started in September 2019. The project is coordinated by the University of Bologna, and it involves seven Academic Institutions and two companies from five Mediterranean Countries (Italy, Spain, France, Morocco, and Turkey).

Med-Berry project will develop a range of alternative and competitive solutions to reduce drastically the use of pesticides in strawberry farming and to manage phytosanitary emergencies and diffusion of new diseases caused by temperature in the Mediterranean area. To this end the project will adopt conventional and genomic strategies aiming to potentiate natural resistance and will integrate them with modern transgene-free technologies to create new sustainable and environment-friendly integrated pest management solutions. Med-Berry will contribute to improve the economic situation of the Mediterranean countries: the results will not only allow to decrease the use of conventional Plant Protection Products (PPP) in strawberry but will also provide important inputs for the protection of other Mediterranean crops.

The main Med-Berry objectives are:

- to provide new solutions to improve control management of strawberry crop in Mediterranean region through valorization of local germplasm to be used in conventional breeding programmes;
- to provide innovative tools to potentiate natural strawberry immunity mechanisms against fungal pathogens through development of New Breeding Technologies protocols (cis-genesis and intra-genesis).
- to develop new agrochemicals solutions based on RNAi technology
- to tackle the social consequences by involving strawberry growers and consumers to integrate the economic factors with a broad overview of the territorial and agricultural context in which the end users operate.

You can find more information about the project on our website (<https://medberry-prima.eu/>)





Med-Berry Work Progresses

New Breeding Technologies such as cis-genesis and intra-genesis allow to exploit the natural resistance of crops through potentiating of immunity mechanism.

Cis/intragenesis technology offers great advantages over conventional breeding to develop pathogen-resistant strawberry crops, allowing the development of transgene-free plants as well as overcoming difficulties such as handling complex genetic material, long-term breeding protocols and introduction of unwanted traits.

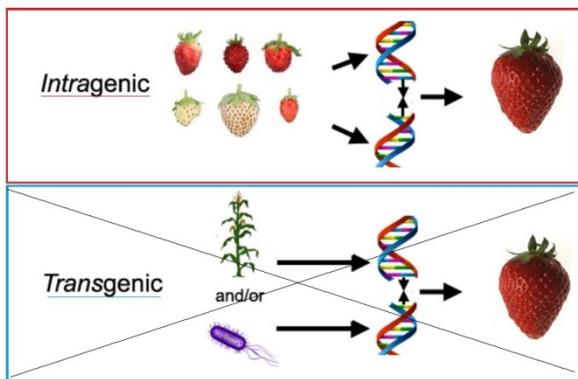


Figure 1. New Breeding Technologies provide important advantages in developing resistant varieties with respect to conventional breeding, since they avoid managing complex genetic material, introducing undesired traits and allow the development of GMO-free plants.

We have constructed four RNAi intragenic DNA cassettes to target key strawberry genes involved in fruit resistance to pathogens. Strawberry lines carrying these intragenic cassettes together with the *Malus domestica MdMyb10* gene as a natural selectable marker have been obtained. Great difficulties were encountered in selecting intragenic lines using this marker, so we have constructed new RNAi-silencing plasmids containing the same four intragenic cassettes and the kanamycin resistance gene for selection. The *kan^r* gene will subsequently be removed from the genome of the selected strawberry lines using

CRISPR genome editing technology. Currently, strawberry Kan^R lines have been obtained and acclimatized and infection tests are being carried out against different fungal pathogens to check increased resistance in fruits.



Figure 2. *In vitro* cis/intragenic plants expressing MdMYB reporter gene cassette.

Different lines of the Italian cultivar Sveva (*Fragaria x ananassa*) have been obtained expressing four different gene constructs for pathogens resistance and *MdMYB* reporter gene have been obtained (Figures 2, 3). The plants will be soon acclimatized for inoculation tests.

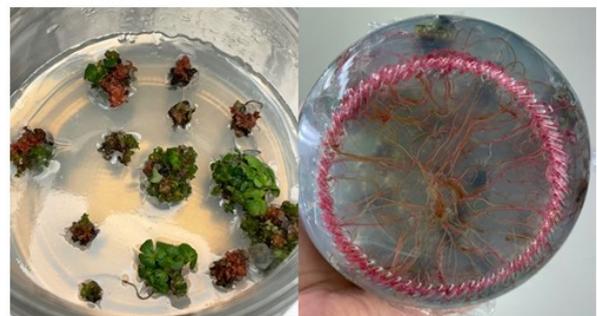


Figure 3. *In vitro* plants expressing MdMYB reporter gene cassette. Transformation process.



Nowadays, it has become urgent to identify new molecules harmless to human and environment to replace pesticides.

Recent techniques using RNA interference (RNAi), which define the ability of double-stranded RNA (dsRNA) to inhibit the expression of target pathogen genes by silencing, have been suggested for crop protection in an environmental-friendly way. Host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS) are strategies based on the use of dsRNAs directly *in planta* or on plant surfaces. The use of new small natural molecule in developing plant defense strategies is considered more efficient and safer for environment and consumers. More tools and more knowledge are needed to assess its efficiency in fruit crops, e.g. strawberry, and against different fungal pathogens.



Figure 2. *C. gloeosporioides* HIGS plants at University of Bologna (Italy). Plants have been multiplied and acclimatized by VICA nursery (Spain) and delivered to University of Bologna (Italy) to be transplanted and characterized.

To date, chemicals and fungicides are being used to control strawberry infection by many fungal pathogens (e.g. *Colletotrichum* spp. *Botrytis cinerea*, *Podosphaera aphanis*). Med-Berry project aims to develop RNAi based technologies against the main strawberry pathogens infections. Using the *Colletotrichum*, *Botrytis* and *P. aphanis* databases currently available, have been used to design several hairpin-based gene cassettes which target important pathogen genes.

Several transformed lines of Italian and Spanish strawberry cultivars (*Fragaria* x *ananassa*)

expressing RNAi constructs for the resistance against different pathogens (HIGS) have been obtained. Currently, to verify the increase in resistance, susceptibility tests to this pathogen are being carried out by inoculating leaves detached from elongated shoots of *in vitro* plants of these strawberry HIGS lines.



Figure 3. Spray Induced Gene Silencing (SIGS) experiments at Polytechnic University of Marche (Italy).

Furthermore, Spray-induced gene silencing method has been validated as useful tool to reduce gray mold disease on strawberry plants cultivated in the greenhouse (Figures 3, 4).



Figure 4. Spray Induced Gene Silencing (SIGS) experiments in greenhouses at Polytechnic University of Marche (Italy). dsRNA treated plants have been compared with negative control (without any molecule) and a commercial fungicide.

Spray Induced Gene Silencing approach (SIGS) has also been used as control strategy against powdery mildew in greenhouses at Invenio under natural inoculation conditions (Figures 5, 6). Overall, 3 trials have been carried out, applying 3 different concentrations of naked dsRNA molecules (chemically synthesised) on susceptible plants cultivated in the greenhouse. SIGS treatments seemed equivalent to the organic one. None off-targets effects on fruits were observed. This study was the first experiment of SIGS strategy in strawberry production conditions. Further trials are required to evaluate the SIGS efficiency. Various factors could be tested to increase the SIGS effects such as other dsRNA concentrations, several successive treatments for a same trial, application of treatments earlier in the plant stage, another cultivation system and period and the use of tolerant strawberry cultivar.



Figure 5. Spray Induced Gene Silencing (SIGS) experiments at INVENIO (France). High pressure devices have been used to spray strawberry plants.



Figure 6. Third independent SIGS trials in Spring 2022 in France. Assays have been performed in greenhouses under natural inoculum condition in Powdery mildew susceptible strawberry variety.



International Horticultural Congress

International Symposium on Advances in Berry Crops 14-22 August 2022, Angers, France

The International Horticultural Congress (IHC) is the most important scientific event organized every four years since 1959 in the fields related to Horticultural crops, raw or processed (fruit, vegetables, aromatic and medicinal plants, seeds and roots, ornamental plants, landscaping, vineyard. under the auspices of ISHS (International Society for Horticultural Science). ISHS is a truly global network comprising over 6,000 members. Its aim is to promote and foster research and education in horticultural science and to facilitate cooperation and knowledge transfer on a global scale through events and publications.

Med-Berry consortium joined the International Horticultural Congress presenting several works related to project's activities. The IHC was also the occasion to finally meet in presence for the annual project meeting and the General Assembly after a long 'on-line only' time (Fig. 7).



Figure 7. Some of the Med-Berry partners which met in presence during the Symposium on 'Advancement on Berry Crops'. From left to right: Dr. *Mustapha Arbaoui* (Institut Agronomique et Vétérinaire Hassan, Morocco), Dr. *José-Javier Higuera-Sobrino* and Prof. *José L Caballero* (University of Córdoba, Spain), Prof. *Elena Baraldi* (University of Bologna, Italy), Prof. *Nesibe Ebru Kafkas* (University of Cukurova, Turkey), Dr. *Francesca Negrini* and Dr. *Alice Ciofini* (University of Bologna). Seated: Dr. *Angela Ricci*, Dr. *Silvia Sabbadini* and Dr. *Luca Capriotti* (Marche Polytechnic University, Italy).



The following works have been presented during the International Strawberry Symposium:

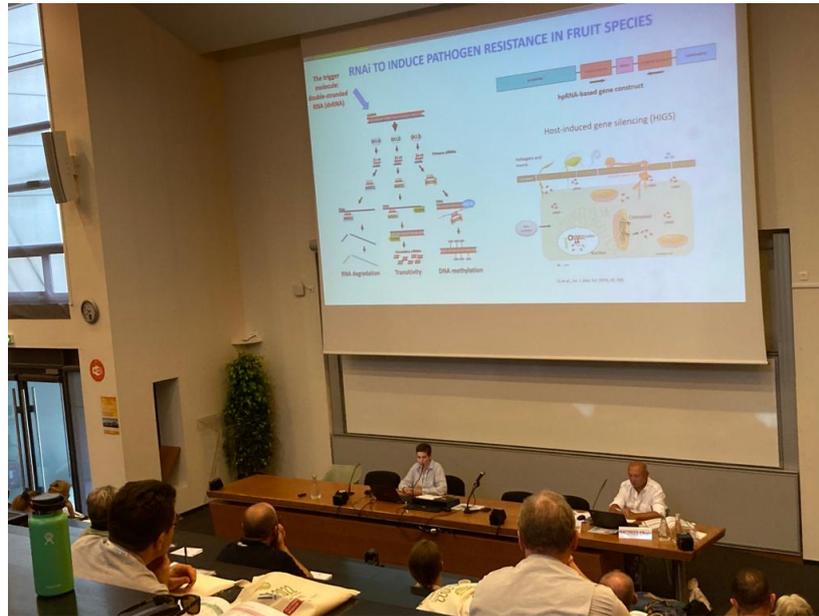


Fig. 8. Dr. Silvia Sabbadini (Marche Polytechnic University) Oral presentation during the Symposium on 'Advancement on Berry Crops'.

Marche Polytechnic University_Oral Presentation: Sabbadini S., Negrini F., Capriotti L., Ciofini A., Ricci A., Gebremichael D. E., Baraldi E., Mezzetti B. **HIGS and SIGS approaches targeting *Cyp51* and *SdhD* of *B. cinerea* to control grey mould disease in strawberry and grapevine**

University of Córdoba_Oral Presentation: José-Javier Higuera-Sobrino, Victoria Súnico, Lucía Cervantes-Cárdenas, Rosario Blanco-Portales, Enriqueta Moyano, Juan Muñoz-Blanco, José L Caballero. **A HIGS approach targeting the *DCL1*, *Cyp51* and *CHS* genes of the pathogen to control *Colletotrichum acutatum* infection of strawberry**

INVENIO_Poster Presentation: Christophe Carmagnat, Eliot Wilmotte, Raphaël Maître, Francesca Negrini, Silvia Sabbadini, Marion Turquet, Philippe Chartier, Bruno Mezzetti, Elena Baraldi, Aurélie Petit. **SIGS, an innovative strategy for powdery mildew resistance in strawberry crop?**

University of Cukurova_Poster Presentation: Nesibe Ebru Kafkas, Duygu Ayvaz Sönmez, Salih Kafkas, Paizilia Aibiubula, Hale Esen , Omer Faruk Bilgin, Seral Yucel. **Breeding strategies on gray mould disease in Turkey**



Publications

J.J. Higuera-Sobrino, R. Blanco-Portales, E. Moyano, A. Rodríguez-Franco, J. Muñoz-Blanco, J.L. Caballero (2021) Silencing of strawberry pathogen defence related candidate genes by using specific strawberry fruit ripening-related promoters: an intragenic approach to improve fruit quality and resistance. *ActaHortic* DOI:10.17660/ActaHortic.2021.1309.14.

Victoria Súnico, José Javier Higuera, Francisco J Molina-Hidalgo, Rosario Blanco-Portales, Enriqueta Moyano, Antonio Rodríguez-Franco, Juan Muñoz-Blanco, José L Caballero (2021) The Intragensis and Synthetic Biology Approach towards Accelerating Genetic Gains on Strawberry: Development of New Tools to Improve Fruit Quality and Resistance to Pathogens. *Plants (Basel)* Dec 25;11(1):57. doi: 10.3390/plants11010057.

José-Javier Higuera-Sobrino, Victoria Súnico, Lucía Cervantes-Cárdenas, Rosario Blanco-Portales, Enriqueta Moyano, Juan Muñoz-Blanco, José L Caballero (2022) A HIGS approach targeting the *DCL1*, *Cyp51* and *CHS* genes of the pathogen to control *Colletotrichum acutatum* infection of strawberry. International Horticultural Congress 2022. S19 - Session O3 - Biotic and abiotic stresses. <https://app.ihc2022.org>.



Events

European Researcher Night



University of Córdoba (Spain) UCO group has participated actively in the Spanish “XI edition of European Night of Research and researchers” (XI edición de la Noche Europea de los Investigadores). Members of the UCO team have had an informal meeting with ordinary citizens who have come to learn about science and the main activities of the Med-Berry project.

This meeting took place in traditional courtyards (“patios”) of the city of Córdoba (World Heritage Site) on the night of September 30th, 2022. You can have more information on the following links:

[https://lanochedelosinvestigadores.fundaciondescubre.es/;](https://lanochedelosinvestigadores.fundaciondescubre.es/)

<http://www.uco.es/investigacion/ucci/es/noticias-gen/item/3934-mas-de-veinte-patios-cordobeses-abren-sus-puertas-a-la-ciencia-en-la-noche-europea-de-los-investigadores>

<https://cadenaser.com/andalucia/2022/07/06/todas-las-claves-para-disfrutar-de-la-noche-europea-de-los-investigadores-en-cordoba-radio-cordoba>

<https://www.cordobahoy.es/articulo/cultura/cordoba-celebra-xi-edicion-noche-europea-investigadores/20221001095034123945.html>



Fig.9 University of Cordoba group at the European Researcher Night.



International Strawberry Congress



Fig. 10. From left to right: Béatrice Denoyes (NRAE, France), Nesibe Ebru Kafkas (University of Cukurova, Turkey), Bruno Mezzetti (Marche Polytechnic University).

Upcoming Events

Med-Berry Winter School

14-15 November 2022, Bordeaux (France)

In the framework of the Med-Berry project, INRAE partner holds the Med-Berry School in Bordeaux (France) on 14-15 Nov. 2022.

This training course offers 9 talks about resistance to biotic and abiotic stresses, genetics and breeding (conventional and NBT).

The classroom school is favoured to facilitate discussions (Fig. 11) but online courses are allowed.

Free registration fees. Only travel, meals and accommodation costs.

For further information (program, accommodation and travelling details, online option), please contact: amelia.gaston@inrae.fr



Fig. 11. Location of the Med-Berry Berry School.



Winter School Program

DAY 1 - Monday 14th, 2022

14:00 - 14:30	Welcome and introduction to INRAE, Med-Berry project and Berry School program Amélia GASTON, Aurélie Petit
14:30 - 15:00	Introduction of Berry School participants
15:00 - 16:00	<i>title pending (GWAS genomic selection powdery mildew)</i> Alexandre Prohaska and Pol Rey Serra
16:00 - 16:30	<i>Coffee break</i>
16:30 - 17:15	Strawberry cultivation and breeding technologies in Turkey Ebru Kafkas
17:15 - 18:00	Plant architecture: a tool for fine phenotyping of strawberry Amélia GASTON
18:00	<i>end of Day 1</i>

DAY 2 - Tuesday 15th, 2022

9:30 - 10:15	Living in harmony with your enemy : What CWR (crop wild relatives) populations can teach us. Véronique Decrooq
10:15 - 11:00	<i>title pending (disease resistance - breeding)</i> Valérie Schrudi-Levraud
11:00 - 11:30	<i>Coffee break</i>
11:30 - 12:15	HIGS and SIGS approaches targeting different <i>B. cinerea</i> genes to control grey mould disease in strawberry Silvia Sabbadini
12:30 - 14:00	<i>Lunch</i>
14:00 - 14:45	Advances in New Breeding Technologies Michel Hernould
14:45 - 15:30	Genetic determinism of an important abiotic stress in sweet cherry : rain-induced fruit cracking José Quero-Garcia
15:00 - 15:30	<i>Coffee break</i>
15:30 - 16:30	<i>title pending (INVENIO, strawberry breeding program)</i> Aurélie Petit and Justine Perrotte
16:30 - 17:00	conclusion - feedback

For more information visit med-berry website

<https://medberry.mycity.it/eventi/1451435/berry-winter-school>

or contact amelia.gaston@inrae.fr



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