ACCADEMIA NAZIONALE DEI LINCEI

MEETING

CROP PROTECTION: CURRENT STATE AND PROSPECTS FOR HUMAN HEALTH AND THE ENVIRONMENT

16TH NOVEMBER 2023

Scientific committee: Roberto BASSI (Linceo, Università di Verona), Paola BONFANTE (Lincea, Università di Torino), Michele MORGANTE (Linceo, Università di Udine), Andrea RINALDO (Linceo, Università di Padova), Bruno CARLI (Linceo, Università di Firenze), Enrico PORCEDDU (Linceo, Università della Tuscia), Cosimo PALAGIANO (Linceo, Sapienza Università di Roma), Corinna GUERRA (Università di Venezia).

PROGRAMME

European Food Production relies on crop chemical protection from pests and diseases. Unfortunately, the widespread, uncorrected use of these molecules is source of pollution: it contaminates water, soil, and air, causes biodiversity loss, and promotes pest resistance. Furthermore, the purchasing and application of these protectants are expensive, affect the competitiveness of the agricultural sector and can affect unwise users. Yet, the consequences of a 50% reduction in their use by 2030, a goal of the European Union, are not clear.
The meeting will address the following aspects.

- Socio-economic problems deriving from the use of agro chemicals.
- Mechanism in the public perception of pesticides.
- Focus on microorganisms: the effect of pesticides on the soil microbiota.
- Alternative strategies for disease pest control: genetics and introduction of resistance alleles in fruit plant cultivars.
- Alternative strategies for pest control: transgenesis and "genome editing" of resistance genes in cultivated species.
- Future perspectives.

Thursday 16 November

10.00 *Welcome addresses*

Chair: Roberto BASSI (Linceo, Università di Verona)

10.15 José Ramón BERTOMEU-SANCHEZ (Institut Interuniversitari López Piñero, Universitat de València): *Recent Historical Research on Pesticides: Agnotology, Environmental Justice and the Role of History*

10.45 Marco TREVISAN (Università Cattolica di Piacenza): *A world without pesticides - The consequences of giving up chemical crop protection*

11.15 Coffee break

11.30 Francesco PENNACCHIO (Università di Napoli): *Insect multitrophic interactions and sustainable plant bioprotection*

12.00 David TURRÀ (Università di Napoli): *Plant-pathogen interaction: signaling mechanisms and their manipulation*

12.30 Paola BONFANTE (Lincea, Università di Torino): *Beneficial microbes as biofertilisers and bioprotectants: Perspectives for their use in agricultural management*

Chair: Enrico PORCEDDU (Linceo, Università della Tuscia),

14.00 Silvia VEZZULLI (Fondazione Edmund Mach): *Genetic dissection of pathogen resistance and conventional breeding strategies in grapevine*

14.30 Aline KOCK (University Regensburg): *Potential and Challenges of RNA-Based resistance*

15.00 Marc F. SCHETELIG (Justus-Liebig-University, Giessen, Institute for Insect Biotechnology): *Beyond Chemicals: Genetic Pathways for Optimized SIT in Sustainable Agriculture*

15.30 Mario PEZZOTTI (Fondazione Edmund Mach, Università di Verona): *Expression of transgenes encoding pest control factors in grapevine and other species*

16.00 Coffee break

16.30 Michele MORGANTE (Linceo, Università di Udine): *Genome editing vs transgenesis vs classic genetics for pest control*

17.00 Giovanni CARRADA (Scrittore): *Paradoxes of the public perception of pesticides*

17.30 Roberto BASSI (Linceo, Università di Verona): *Conclusions*

ROMA - PALAZZO CORSINI - VIA DELLA LUNGARA, 10
Segreteria del convegno: convegni@lincei.it – http://www.lincei.it

Tutte le informazioni per partecipare al convegno sono disponibili su:
https://www.lincei.it/it/manifestazioni/crop-protection-convegno

Per partecipare al convegno è necessaria l'iscrizione online.
Fino alle ore 10 è possibile l'accesso anche da Lungotevere della Farnesina, 10.
I lavori potranno essere seguiti dal pubblico anche in streaming

L'attestato di partecipazione al convegno viene rilasciato esclusivamente a seguito di partecipazione in presenza fisica e deve essere richiesto al personale preposto in anticamera nello stesso giorno di svolgimento del convegno

Insect multitrophic interactions and sustainable plant bioprotection
Francesco PENNACCHIO (Università di Napoli)

Reduction of pesticide use in agriculture requires an increasing availability of sustainable tools and strategies of pest control. It is highly desirable that current research efforts aim at developing novel, highly specific biopesticides and bioprotection strategies based on knowledge obtained from the understanding of the molecular mechanisms underlying plant-insects multitrophic interactions, at metaorganism level.

This approach allows to use biocontrol agents as a source of virulence factors or of molecular information on which to base technologies reproducing their negative impact on pests and to develop bioinspired pest control tools for sustainable plant protection. Moreover, understanding the mechanisms underlying insect multitrophic interactions paves the way towards the definition of protection strategies for beneficial insects and the ecosystem services they provide.

Plant-pathogen interaction: signaling mechanisms and their manipulation
David TURRÀ (Università di Napoli)

Soil-borne fungal pathogens are responsible for a wide variety of plant diseases and have significant impacts on agriculture, horticulture, and natural ecosystems worldwide. The soil-borne fungal ascomycete *Fusarium oxysporum* provokes vascular wilt disease in more than a hundred different plant crop species, causing devastating economic losses both in field and greenhouse settings. Its infectious cycle starts when survival structures (i.e. conidia and chlamydospores) germinate in the soil; hyphae track shallow chemical gradients released from plant roots and penetrate host tissues via the formation of characteristic needle-shaped structures. An in-depth comprehension of both endogenous and exogenous signals regulating these processes and of how such signals are transduced in the cell is fundamental to designing low-impact strategies for pathogen control. Here, we used reverse genetics approaches, fluorescence microscopy, and microfabrication of biomimetic plant platforms to gain fundamental insights on the chemical, physical, and mechanical cues driving fungal germination, tropic behaviors, and pathogenicity. We further identified metabolites from rhizosphere-inhabiting microorganisms able to modulate and interfere with the colonization process of the soil-borne pathogenic fungus *F. oxysporum*. Overall, our research generates new insights on how plant microbiomes play crucial roles in disease suppression and represents a framework for the development of more sustainable and environmentally friendly approaches to disease management.

Beneficial microbes as biofertilisers and bioprotectants: Perspectives for their use in agricultural management
Paola BONFANTE (Lincea, Università di Torino)

Using less agrochemicals as fertilisers and pesticides is one of the main aims of European strategies in the context of the Farm to Fork Strategy. Among the eight Integrated Pest Management principles, a relevant one is the protection and enhancement of important beneficial organisms. Arbuscular mycorrhizal fungi (AMF), a group of obligate biotrophic soil fungi, are lead actors in this scenario, due to their capacities of biofertilisers (Salvioli and Bonfante, 2023) and bioprotectors (Pozo and Azcon-Aguilar, 2007, Jung et al., 2012). While a part of their hyphae proliferates in the soil taking up minerals, a conspicuous part of their body lives associated with the roots of around 70% of land plants, with whom they establish a nutritional balance: while AMF provide minerals to the plant, they receive reduced carbon from the photosynthetic host (Genre et al, 2020). During the interaction, AMF strongly impact on the main metabolic pathways of the host plant along a root-shoot axis (Chialva et al, 2023), often leading to an increased plant biomass. Another consistent result of the symbiosis is the elicitation of plant immune system. This priming effect leads to a Mycorrhiza Induced Resistance, which is induced when the plant is challenged by a plethora of pathogens, from fungi to viruses and herbivores (Zeng et al 2022; Papantoniou et al 2021; Fiorilli et al, 2018). These benefical features of AMF as biofertilisers and bioprotectors offer new perspectives for their use in agricultural mangement. However, due to their still enigmatic life cycle, these fungi are more difficult to formulate into an effective biostimulant than, for example, any nitrogen-fixing rhizobacterium. Standardised tests to ensure the quality of the products released on the market are strongly required.

Genetic dissection of pathogen resistance and conventional breeding strategies in grapevine

Silvia VEZZULLI (Fondazione Edmund Mach)

"*Vinum debet esse naturale ex genimine vitis et non corruptum*". The Eucharistic wine must be made with pure grapes that must not be contaminated in any way. This is how wine was born in the monastery of the Augustinians, and that is how the genetic improvement of grapevine implemented over the decades at the Agricultural Institute of San Michele all'Adige (since 1874; Trentino, Italy) has been oriented to make the cultivation of grapes always more sustainable. This concept is still current and meets the worldwide urgent need of reducing the use of chemicals, under a climate crisis scenario. Since the beginning of the twentieth century, the varieties introduced in Trentino and the new cultivars produced by pioneer breeders have already embraced the principle of sustainable viticulture. During this first phase it was mainly focused on the search for agronomical and qualitative improving characteristics, while a second phase concentrated on the crossbreeding activity for resistance to biotic stresses began in 2010 at the now-established Edmund Mach Foundation (FEM).

The technique of crossbreeding, free or controlled, has always been a source of variability and introduction of characters that are not present in the parents. The first established approach was based on i) "*vinifera* × *vinifera*", in particular Teroldego×Lagrein and Malvasia di Candia Aromatica×Muscat Ottonel populations created in the '90s. The F1 individuals obtained were selected on 1-25-100 plants for qualitative/quantitative parameters of grape and chemical/sensorial analysis of must/wine upon microvinification. The selected genotypes were characterized by DNA fingerprinting, ampelographic and ampelometric descriptors, phenology, and wine profiling. The overall evaluation of various parameters allowed for the identification of some genotypes with looser clusters than those of their parents, in association with a minor sensitivity to grey mould, and other ones with earlier ripening time or different anthocyanin/tannin content. The second strategy followed two objectives, both related to the implementation of multiple disease resistance in the breeding program. The plan ii) "*vinifera* × non-*vinifera*" aimed at obtaining resistant materials with the historical varieties of Trentino as a noble parent (e.g. Nosiola), using genotypes acquired from other breeding programs (e.g. Merzling) as disease resistance donors. For the plan iii) "non-*vinifera* × non-*vinifera*", upon an initial phase of scouting the FEM complex genetic pool of resistance traits to the main ampelopathies, a group of accessions were selected as resistance donors. They were used as parental lines in the process of introgression and stacking (or "pyramiding") of resistance loci (genomic regions) for downy and powdery mildew: the final goal was to obtain a durable resistance at leaf and bunch level. Thus, through Marker-Assisted Parental Selection (MAPS), various genotypes with stacked loci reached the open field and were then used for crossbreeding purposes. Subsequently, the optimization of phenotyping and genotyping protocols was conducted for a time-efficient and cost-effective Marker-Assisted Seedling Selection (MASS).

Recently, several genotypes were screened also for black rot resistance and a bi-parental segregating population study has been carried out to dissect the genetic basis of this "emerging" disease towards the development of a marker toolkit. The final goal is the identification of responsible genes underlying the loci of interest, by sequencing and phasing the donor genomes of disease resistance along with other relevant traits. This outcome will inform both conventional breeding approaches and new genomic techniques (i.e. cisgenesis and genome editing) applied to grapevines.

In conclusion – upon multi-year agronomic surveys, grape quality composition evaluation and wine tastings – in 2018 four new varieties were registered at the National Register of Grapevine Varieties, for their novel organoleptic characteristics and resilience to grey mould. Indeed, in 2020 additional four new (mid)-resistant varieties to downy and powdery mildew, as well as black rot, were patented. Being employable for various oenological goals, these new releases, along with several upcoming prototypes, pave the way towards a more sustainable viticulture.

Beyond Chemicals: Genetic Pathways for Optimized SIT in Sustainable Agriculture

Marc F. SCHETELIG (Justus-Liebig-University, Giessen, Institute for Insect Biotechnology)

In recent years, sustainable agricultural practices have gained significant attention as concerns about environmental degradation and chemical over-dependence continue to grow. The Sterile Insect Technique (SIT) stands out as a promising biotechnological approach to pest control, reducing the need for chemical pesticides. Traditionally, SIT involves the release of sterilized male-only populations of pest insects to mate with wild females, resulting in non-viable offspring and subsequent population decline. However, methodologies to separate males and females before sterilization and release have only been developed for a few species and rely primarily on uncharacterized genetic markers identified through classical genetics. This talk delves into cutting-edge research exploring novel genetic markers as alternatives to building sex separation strains. By leveraging modern genetics, we can fine-tune SIT, optimizing the fitness of released insects while ensuring effective population control. We will discuss the molecular mechanisms underpinning these genetic strategies, the potential benefits for crop yield and protection, and the implications for sustainable agriculture's future.

Expression of transgenes encoding pest control factors in grapevine and other species

Mario PEZZOTTI (Fondazione Edmund Mach, Università di Verona)

Genetically controlled plant resistance represents the most efficient and mature tool for a truly sustainable crop protection. Resistant varieties obtained by conventional breeding are widely adopted whenever available, but the present challenges that agriculture is facing need urgent development of new tailored genotypes, with improved durable resistance against old and emerging pathogens and pests.

Since the discovery and cloning of the first resistance genes, an impressive amount of literature has greatly improved our fundamental knowledge of pathogenicity and resistance mechanisms in plants. Hundreds of plant genomes have been sequenced. The development of -omics and bioinformatic tools together with transient or stable genetic engineering techniques have allowed the functional analysis of countless plant genes, and the definition of their roles in plant development or stress responses, among others.

This wealth of information has also shed light on the molecular bases of plant-microbe interactions and more specifically on the different steps of pathogen perception, subsequent signal transduction and expression of defense components deployed by plants, that could be exploited for the production of new resistant commercial varieties of many crops. Hundreds of plant genes have been functionally characterized for their actual role in disease resistance and can be introduced into susceptible varieties, with a fast and effective way, without compromising the quality traits of the original receiving genotype. This is especially interesting for perennial crops that are vegetatively propagated, the genetic asset of which is obviously modified by crossing.

One of the advantages of genetic engineering over classical breeding is the possibility to expand the mining of genetic resource beyond the boundaries of plant species or even of the plant kingdom, and in fact the most successful commercial applications of biotechnological disease resistance reside in the use of sequences and genes derived from the pathogens themselves, especially from viruses.

A comprehensive description of all successful examples of improved disease resistance obtained in the labs or in greenhouse by different types of genetic engineering would be impossible.

On the other hand, the investments of further development of this knowledge is still relatively limited.

Beside the very successful and widespread use of Bt crops, most pathogen- or pest-resistant genotypes have never reached the field trial phase and remained confined in laboratories and only a handful of pathogen-resistant varieties have reached the market. This is surely partly due to the discouraging low public acceptance of the biotechnological approach, but adverse perception of transgenics is not the only constrain to investments on development of resistant transgenic crops: 30 year of research have shown the intricate network governing different types of resistance to biotic and abiotic stresses and its interconnections with plant growth and productivity, so that some resistance phenotypes may come with pleiotropic adverse effects on development or with increased susceptibility to pathogens with a different life-style, or abnormal reactions to abiotic stress. Also, the large variety of pathogens affecting each crop and their fast evolution ability, hampers the efforts to identify durable and broad-spectrum resistant traits with a simple genetic control.

More research is needed to achieve a more comprehensive overview of the plant balance between stress response and growth, and to understand how the expression of interesting transgenes can be regulated to limit detrimental effects, e.g. by directing their expression under natural or synthetic pathogen-inducible promoters.

In conclusion modern transgenic approaches certainly have the potential to provide strong support to a more sustainable agriculture, the large body of knowledge produced in the last decades is there, together with many plant prototypes produced all around the world and represent a fundamental rich legacy for future generations, despite the present constrains to its application. Of course, genetic engineering is unlikely to provide all the solutions alone, and we should avoid overselling the concept: in a more realistic view, future pest management in agriculture should rely on a combination of genetic innovation, new antimicrobial strategies, plant health strengtheners and correct management of the soil microbiota.