SCIENZE A SISTEMA PER LA SOSTENIBILITÀ
La ricerca al Dipartimento di Biologia Ambientale
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Metabolomics insights for the evaluation of safety, quality, and origin of wheat and selection of bioactive endophytes

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agritech National Center for Technology in Agriculture
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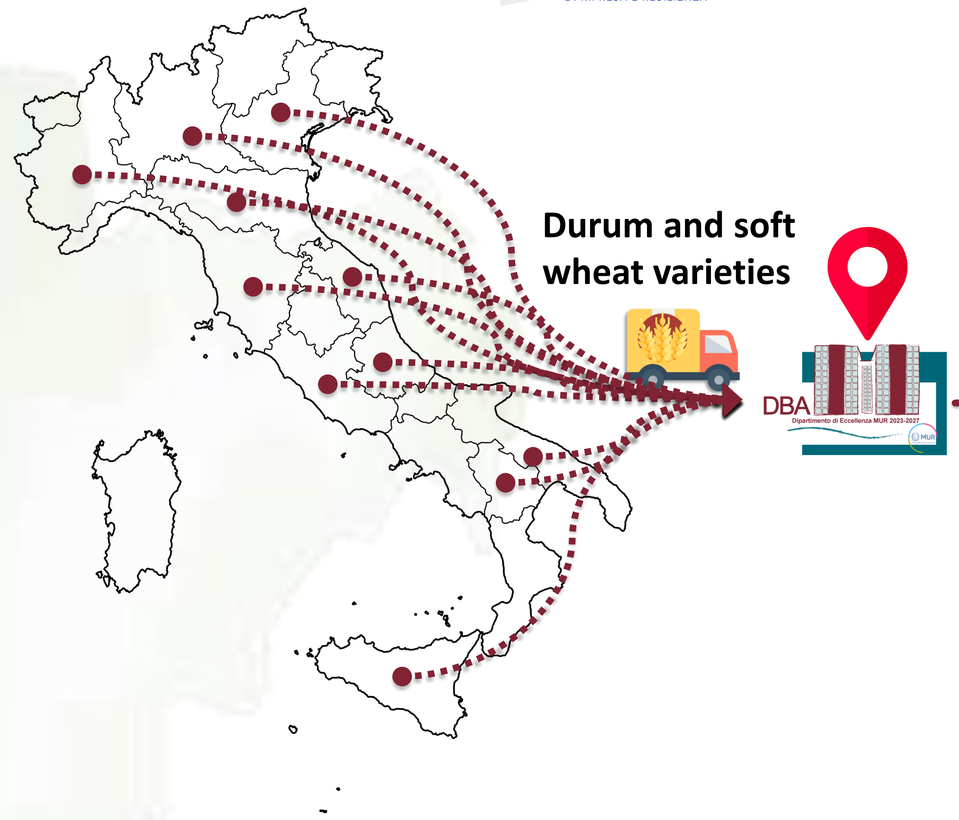
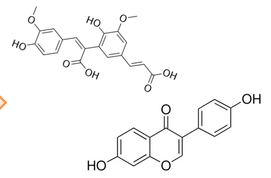


Untargeted analysis

HRAM Orbitrap

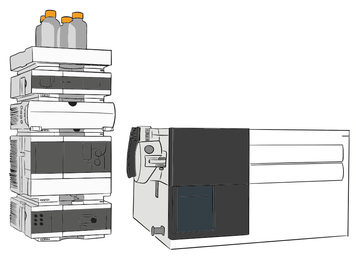
METRO FOOD-IT

Multivariate data analysis

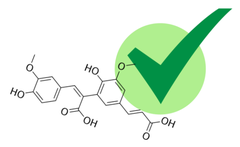


Markers identification

Targeted analysis



HPLC-MS/MS



Markers validation

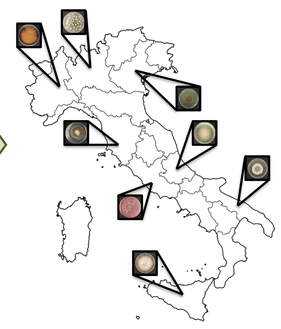
Volatilome analysis

INTEGRATED SMART PHYTOTRON

VOCs identification

Selection of bioactive endophytes

In vitro growth, DNA sequencing and double culture assay



Introduction

This research project, funded by Agritech and supported by Metrofood facilities, focuses on developing technologies to ensure the safety and quality of agri-food products. Specifically, the metabolomic and ecological profile of twenty-four Italian and two commercial varieties of durum and soft wheat will be studied in detail. Wheat varieties will be investigated in order to identify endogenous molecules that can be used as potential biomarkers to define wheat origin, quality, and safety; in addition, wheat endophytic fungi will be isolated and studied, with a focus on pathogenic fungi and potentially bioactive fungi.

Methodologies

Wheat varieties will initially be analyzed with an "**untargeted**" approach using liquid chromatography coupled with high-resolution mass spectrometry (HRAM Orbitrap) to find potential biomarkers. Subsequently, "**targeted**" approaches with high-performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS) will be used to validate biomarkers. As future prospects, wheat cultivars will be studied in **smart integrated phytotrons** equipped with tools for real-time analysis of volatile organic compounds produced by plants. Isolation of wheat endophytic fungal colonies will be conducted on PDA medium and will be performed on wheat seeds whose surface has been sterilized with water:ethanol (90:10 v/v) and water-sodium hypochlorite (92:8 v/v). An initial screening of the isolated strains will be conducted by phenotypic observations of the mycelium and spores. DNA extraction and sequencing will subsequently allow the identification of isolated and grown *in vitro* species. Double culture assays will also be performed to study the ability of potential biocontrol agents against wheat pathogens. Briefly, both candidate as biocontrol agents and wheat pathogens will be spotted on solid or liquid medium and their growth-inibiting activity will be evaluated.

Preliminary results

Different extraction methods of polar (e.g., polyphenols, alkaloids, polypeptides, etc.) and apolar (e.g., fatty acids, oxylipins, etc.) compounds have been studied and refined for wheat samples. Data obtained with Compound Discoverer (*Fig. 1*) from wheat polar extracts of *Colombo*, *Odisseo*, *Shrekan* and *Core* varieties reveal that the metabolomic profile obtained through these extraction methods allows us to separate different wheat varieties according to their metabolomic profile. A remarkable fungal biodiversity has been successfully isolated from the twenty-four Italian and two commercial durum and soft wheat samples (*Fig. 2 a and b*).

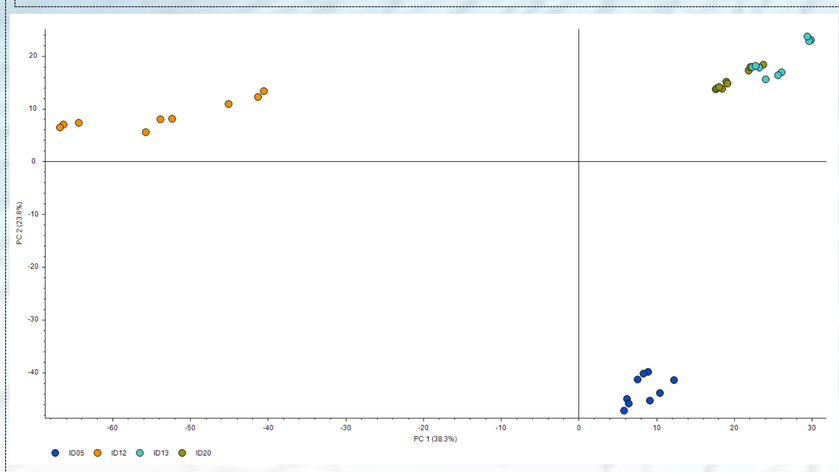


Fig. 1 - Principal Components Analysis *of all identified compounds in a polar extract of four wheat varieties (ID05 - Colombo; ID12 - Odisseo; ID13 - Shrekan; ID20 - Core).*

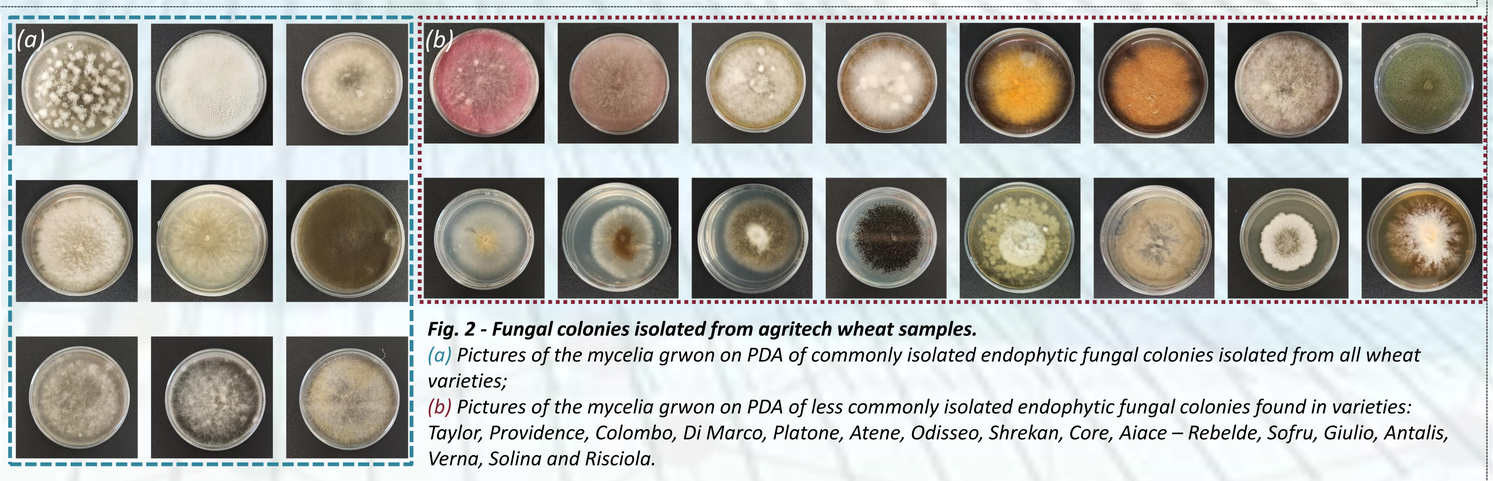


Fig. 2 - Fungal colonies isolated from agritech wheat samples.
(a) Pictures of the mycelia grwon on PDA of commonly isolated endophytic fungal colonies isolated from all wheat varieties;
(b) Pictures of the mycelia grwon on PDA of less commonly isolated endophytic fungal colonies found in varieties: Taylor, Providence, Colombo, Di Marco, Platone, Atene, Odisseo, Shrekan, Core, Aiace – Rebelde, Sofru, Giulio, Antalis, Verna, Solina and Risciola.

Future prospects

The results of this research project will allow us to delineate in more detail the metabolomic profile of durum and soft wheat as well as its differences in Italian varieties. Molecular markers that will emerge from multivariate statistical and machine learning analysis will be used as analytical tools to trace origin, quality and safety of durum and soft Italian wheat, as well as to implement innovative sensors useful for companies to monitor the entire production process. Study of endophytic fungal colonies of wheat varieties obtained from different regions and cultivated in distinct ways will enable us to delineate more clearly the effects of certain agronomic practices, as well the climate and also the metabolomic profile of wheat itself on the composition of the endophytic fungal microbiome.

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