*Project Proponent: Prof. Nunzio D'Agostino, Dept. of Agricultural Sciences*

**Title: Polyphenol oxidases in wheat: expression atlas construction and allelic variant discovery under drought stress and microbial interactions.**

**Objectives of the research project**: The primary objective of this project is to investigate the expression dynamics and regulatory mechanisms of polyphenol oxidase (PPO) genes in wheat under drought stress, with a particular focus on interactions with plant growth-promoting bacteria (PGPB). By examining tissues from both hexaploid and tetraploid wheat (flag leaves, roots, and immature grains), the study aims to construct a comprehensive PPO expression atlas under abiotic stress conditions. A key goal is to determine how beneficial microbial consortia modulate PPO gene expression and contribute to enhanced drought tolerance, particularly during critical developmental stages. To this end, the project integrates targeted RNA sequencing using Ion AmpliSeq™ to capture and quantify PPO transcript levels with high sensitivity. In parallel, variant discovery will be conducted through alignment of RNA-seq reads to reference wheat genomes to identify single nucleotide polymorphisms (SNPs). Functional predictions of these variants, followed by validation via Sanger sequencing, will uncover novel allelic variations potentially linked to adaptive stress responses. This project is inherently interdisciplinary, bridging plant genomics, microbiology, bioinformatics, and crop physiology. Collaborations between molecular biologists, computational scientists, and agronomists are central to achieving an integrated understanding of PPO function in both quality and stress-resilience contexts. The integration of high-throughput sequencing, microbial ecology, and functional genomics offers a systems-level perspective critical for the development of climate-resilient wheat varieties.

**Innovation and originality of the project:** This project introduces a novel and integrative approach to understanding the dual role of polyphenol oxidases (PPOs) in wheat—traditionally studied for their impact on product quality—by repositioning them as critical components of the plant’s response to environmental stress. While past breeding efforts have primarily focused on reducing PPO activity to enhance the visual appeal of wheat-based products, this project breaks new ground by investigating the regulatory and functional significance of PPOs in stress tolerance, particularly under drought conditions. The originality of the project lies in its comprehensive examination of PPO gene expression across different wheat ploidy levels (hexaploid and tetraploid), multiple tissue types, and in response to both abiotic stress and microbial interactions. By incorporating plant growth-promoting bacteria (PGPB) into the experimental framework, the project explores an under investigated dimension—how beneficial microbiota modulate PPO expression and contribute to drought resilience through systemic signaling mechanisms. Technologically, the project stands out through its use of targeted RNA sequencing via Ion AmpliSeq™, a highly efficient method for transcriptome enrichment and quantification, enabling precise monitoring of PPO gene expression dynamics. Furthermore, the integration of variant discovery through high-resolution SNP calling and validation via Sanger sequencing adds a genomic layer to the analysis, facilitating the identification of functional allelic variants that may confer adaptive advantages. The combination of expression atlas construction, allelic variation mapping, and microbial interaction studies in the context of drought stress represents a significant advancement in both the functional genomics of wheat and the broader field of climate-resilient agriculture. This integrative and multidisciplinary strategy not only enhances the scientific understanding of PPOs but also opens new pathways for breeding programs that balance quality improvement with environmental adaptability.