UNIVERSITÀ DEGLI STUDI DELLA TUSCIA | DIPARTIMENTO DI SCIENZE AGRARIE E FORESTALI

Dottorato di Ricerca in Scienze delle Produzioni Vegetali e Animali
PhD Programme in Plant and Animal Science
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Coordinatore/Coordinator: Prof. Rosario Muleo

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PhD student: Maria Lia Di Romana

Posizione/Position
X With scholarship
☐ Without scholarship
☐ Reserved for research center employees
☐ Industrial PhD
☐ Other typology

Supervisor
Ljiljana Kuzmanovic
Affiliazione/Affiliation
Department of Agriculture and Forest Sciences (DAFNE) – University of Tuscia

Co-Tutor

Affiliation

Main place of research: Department of Agriculture and Forest Sciences (DAFNE) – University of Tuscia

Research title: "Genetic improvement of abiotic stress tolerance in durum wheat by exploiting root system plasticity and introgression of genetic variability from wheat wild relatives through chromosome engineering".

Short description of the research activity
(Max 10000 characters, included spaces)

1. Introduction and State of the Art

Climate change is posing increasingly severe challenges to global agriculture, especially in the Mediterranean basin, where rising temperatures, irregular precipitation and progressive land degradation are intensifying abiotic stresses such as drought, heat and salinity (del Pozo et al., 2019; Patterson et al., 2013). These stresses are worsened by anthropogenic factors, including unsustainable irrigation practices and the use of brackish water, which accelerate soil salinization and contribute to desertification processes (Abdennour et al., 2021). Among the crops most affected is durum wheat (DW, *Triticum turgidum* ssp. *durum*), a key component of the Mediterranean diet and a major agricultural commodity in wheat-producing countries such as Italy. Its adaptive capacity is limited by a narrow genetic base, the result of domestication and long-term selection focused on few agronomic traits, reducing resilience to environmental stressors. Projections suggest wheat yield stability may decline by ~8% for every 1 °C temperature increase (Xiong et al., 2024). In southern Italy, traditional DW-growing regions are already experiencing reduced productivity linked to heat, irregular rainfall and increasing soil salinity (Ceoloni et al., 2014).

Root traits play a central role in plant adaptation to abiotic stresses. Root architecture, comprising depth, angle, branching and biomass distribution, influence the plant's ability to access resources under stress. Although roots are not directly exposed to aerial temperatures, they remain highly sensitive to soil conditions, including salinity and moisture fluctuations. However, the genetic and physiological basis of root adaptation remains insufficiently explored, largely due to the technical difficulties of observing root systems in situ (Karlova et al., 2021; Yashavanthakumar et al., 2023). Recent phenotyping advancements, such as rhizotrons

and shovelomics, have enabled detailed analysis of root growth both in controlled environments and field conditions (Ober et al., 2021; York et al., 2018). When integrated with molecular "omics" approaches—transcriptomics, proteomics and metabolomics—these tools allow the identification of biological pathways and candidate genes involved in abiotic stress tolerance (Arbona et al., 2013; Templer et al., 2017; Srivastav et al., 2018). To overcome the limited genetic variability of DW, attention has increasingly shifted to wild wheat relatives, especially species of the Triticeae tertiary gene pool. These species naturally inhabit harsh environments and represent valuable reservoirs of genetic diversity for improving stress resilience (Ceoloni et al., 2014, 2015, 2017). In particular, *Thinopyrum ponticum* and *T. elongatum* are recognized for their natural tolerance to extreme environmental conditions, including high salinity and heat (Deal et al., 1999; Liu et al., 2015). A powerful approach to exploit these wild genetic resources is chromosome engineering (CE), a non-GMO cytogenetic strategy that facilitates the transfer of defined chromosomal segments from wild species into elite wheat cultivars. This approach allows the transfer of beneficial traits/genes such as those conferring disease resistance, salt and heat tolerance, and yield stability while minimizing disruption to the overall genome (Ceoloni et al., 2005, 2015; Tounsi et al., 2024). Over the past two decades, recombinant DW lines carrying *Thinopyrum* segments, especially on chromosome arm 7AL, have been developed and tested, showing improved performance under multiple abiotic stresses (Kuzmanovic et al., 2018; Giovenali et al., 2023, 2024). These benefits include increased root volume (Virili et al., 2015), better maintenance of root growth and plasticity under drought through favourable alien alleles of the *LATERAL ROOT DENSITY* (*LRD*) gene (Placido et al., 2020; Di Romana et al., 2024). Under salt stress, the presence of *Th. elongatum* introgressions in some of the DW-*Thinopyrum* recombinants have also shown to improve root growth, germination and antioxidant activity (Tounsi et al., 2024). Under heat stress at anthesis, *Th. ponticum* introgressions improve spike fertility, water retention and accumulation of protective osmolytes (Giovenali et al., 2023, 2024).
These findings emphasize the potential of targeting root traits to improve abiotic stress resilience in DW, by reaching to wild gene pool. A deeper understanding of the root system is critical for developing resilient DW varieties, yet the more extensive knowledge on the alien species' potential in improving abiotic stresses in cultivated wheat is still lacking. With new tools such as rhizotrons, shovelomics, and integrated omics approaches, it is now possible to identify key root traits and their genetic basis (York et al., 2018; Boudiar et al., 2021). Targeting the root system and harnessing the genetic potential of wild relatives, can redirect DW breeding toward sustainable productivity under salinity and heat stress, by ensuring resilience to climatic and agronomic pressures.

2. Work plan

Analyse response to heat, salt and heat+salt stresses in roots and shoots of the available chromosomally engineered, near-isogenic DW-*Thinopyrum* spp. recombinant lines. A systems biology approach will be applied, including morpho-physiological and "omics" (transcriptomic, metabolomic) analyses to identify key biological networks differentially regulated under stress. Primary recombinants carrying *Th. ponticum* segments (e.g., R5+, R112+) and secondary recombinants containing smaller *Th. elongatum* segments nested within *Th. ponticum* regions (e.g., R69-9/R5+, R69-9/R112+) will be compared to corresponding control lines lacking alien chromatin.
In the first year, phenotyping experiments will be conducted in controlled growth chambers using rhizotron platform. Root growth dynamics will be monitored by capturing sequential images before and after stress imposition. Root number, total and primary length, root angle, lateral root density and root biomass distribution will be quantified through dedicated software. Shoot traits, including height, biomass, chlorophyll content (SPAD), stomatal conductance, chlorophyll fluorescence, and relative water content, will also be measured. After that, root to shoot biomass ratio, root length to plant height ratio, specific root length and the dynamics of root development in time and space will be analysed. Multifactorial ANOVA will test the effect of the presence/absence of alien segments. At selected timepoints, root and shoot tissues will be sampled (five plants per treatment), frozen and stored at –80 °C for subsequent "omics" analyses.
In the second year, based on phenotypic differences and literature data, targeted "omics" analyses (e.g. transcriptomics, metabolomics), will be conducted. Shoot and root tissues sampled at the timepoints which

caused major differences in the plant response between introgression carrier- and non-carrier genotypes, will be analysed by the "omics". This will allow to investigate the adaptive molecular responses of the selected genotypes to salt and heat stress. Statistical comparison between segment carrier and non-carrier near isogenic lines will be conducted to identify gene and metabolite networks linked to the alien chromatin. Combination of the information from these extensive "omics" datasets with morpho-physiological data, will provide insights into key regulatory pathways and potential biomarkers involved in stress response. This will shed the light on specific functional roles of alien introgressions in shaping DW root traits and growth dynamics under abiotic stresses and therefore enhance the plant's resilience. Possibly, during the second and third year, a field trial in an environment with saline soil can be set up to evaluate the recombinants' productivity and correlate this data with those of controlled conditions.
In the third year, based on transcriptomic analyses results, a subset of key differentially expressed genes, potentially involved in the differential response to heat and salt stresses, will be selected for validation by targeted expression analysis. Particularly those mapped to chromosome group 7 and overlapping with *Thinopyrum* introgressions, will be validated by qRT-PCR in root and shoot tissues across multiple developmental stages. This integrative strategy will elucidate how alien chromatin contributes to improved root architecture and stress resilience in DW.

Training activities: Courses and seminars planned and organised by the Teachers' Council

Signature (Supervisor)



Signature (PhD student)

