PhD School on Agriculture, Environment and Bioenergy

(http://sites.unimi.it/dottorato_aab/)

(XL cycle, 2024-27)

Project draft

1.Field of interest: *AGR/07*

2.Project title: Physiological and genetic determinants of water use efficiency in barley

3.Tutor: Dr. Alessandro Tondelli; **co-tutor:** Dr. Davide Guerra

4.Relevance of the topic and state of the art:

Barley is the most resilient among cereal crops, still its growth and yield can be significantly impacted by drought conditions (Dawson et al. 2015). Barley water use efficiency (WUE) measures how barley plants use water to produce biomass or yield, and it is an important trait for improving crop performance, especially in regions with limited water availability. The response of barley to water scarcity involves a combination of morphological/developmental, physiological, and molecular changes aimed at conserving water and maintaining essential biological functions (Cattivelli et al. 2008; Sallam et al. 2019). Early flowering is a stress escape strategy for accessions growing in environments characterized by terminal drought. Closing the stomata can help in minimizing water loss, even if this also limits carbon dioxide intake and reduce photosynthesis. In addition, the cell turgor can be maintained through the accumulation of osmolytes such as proline, glycine betaine, and sugars. At the molecular level, drought stress triggers the expression of specific genes encoding for proteins that protect cellular structures, facilitate osmotic adjustment, and repair damage caused by stress. Differences in the response to water deprivation have been observed in the barley germplasm, with accessions showing high transpiration and maximum productivity under well-watered conditions but rapid transpiration decrease under drought, in contrast with accessions characterized by water-conserving behavior (Appiah et al. 2023). Understanding and harnessing these responses may be useful for improving barley water use efficiency and ensuring stable production under challenging environmental conditions.

5.Layout of the project (draft)

5.1. Materials & Methods:

Different barley genetic resources are available at CREA - Research centre for Genomics and Bioinformatics, such as germplasm collections extensively characterized at the molecular level (Bustos-Kort et al. 2019), a doubled haploid mapping population developed from cultivars with contrasting responses to water deprovation, BC2F4 introgression lines of *Hordeum spontaneum* into the barley cultivar Nure, barley mutants and others. Such genetic materials will be screened according to phenotyping methods preliminary tested at the host centre and related to barley transpiration and water use efficiency, for example leaf stomatal count with handheld digital microscopes, followed by genetic analyses based on association or QTL mapping. Candidate genes will be identified taking advantage of state-of-the-art genomic resources available for barley. Genotypes with contrasting phenotypes and carrying allelic variants at candidate genes will be further tested through measurement of daily transpiration under contrasting conditions in growth chambers, coupled with direct

measurement of leaf transpiration with LI-600 Porometer/ Fluorometer, or through continuous measurement of plant water balance and biomass gain through the PlantArray physiological phenotyping gravimetric platform installed in the CREA-GB greenhouse (Dalal et al. 2020). Gene expression analyses based on RNAseq data will support the characterization of candidate genes, that will be validated through genome editing and/or TILLING at the host centre or in collaboration with national and international partners.

5.2. Schedule and major steps (3 years):

WP1: testing phenotyping protocols on parents of mapping population and selected accessions from diversity panels. Screening of populations and germplasm collections. Years 1-2

WP2: identification of candidate genes through genetic and transcriptome analysis. Year 2.

WP3: validation of candidate genes. Years 2-3

WP4: stage at external laboratory and writing of articles and PhD thesis. Years 2-3

6. Available funds (to support research):

Approximately 30,000 euros from the FACCE-JPI RecoBar project

7. Co-Financing (to support the bourse):

Approximately 76,000 euros from CREA-Research centre for Genomics and Bioinformatics

8. Literature:

Appiah M, Abdulai I, Schulman AH, Moshelion M, Dewi ES, Daszkowska-Golec A, Bracho-Mujica G and Rötter RP (2023) Drought response of water-conserving and non-conserving spring barley cultivars. Front. Plant Sci. 14:1247853. doi: 10.3389/fpls.2023.1247853

Bustos-Korts D, Dawson IK, Russell J, Tondelli A, Guerra, D, et al. (2019) Exome sequences and multi-environment field trials elucidate the genetic basis of adaptation in barley. The Plant Journal 99: 1172-1191. DOI: 10.1111/tpj.14414

Cattivelli L; Rizza F; Badeck, FW; Mazzucotelli E.; Mastrangelo AM; Francia E; Marè C; Tondelli A; Stanca AM (2008) Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. Field Crop Res., 105, 1–14

Dalal A, Shenhar I, Bourstein R, et. al. (2020) A Telemetric, Gravimetric Platform for Real-Time Physiological Phenotyping of Plant–Environment Interactions. JoVE DOI: 10.3791/61280

Dawson IK, Russell J, Powell W, Steffenson B, Thomas WT, Waugh R (2015) Barley: a translational model for adaptation to climate change. New Phytol 206:913–931

Sallam A, Alqudah AM, Dawood MFA, Baenziger PS, Börner A. Drought Stress Tolerance in Wheat and Barley: Advances in Physiology, Breeding and Genetics Research. Int J Mol Sci. 2019 Jun 27;20(13):3137. doi: 10.3390/ijms20133137