

Tritordeum, barley landraces and ear photosynthesis are key players in cereal resilience under future extreme drought conditions

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ABSTRACT

Drought is the main factor limiting cereal production in the Mediterranean basin and Climate Change will exacerbate its effects. Among the strategies to mitigate Climate Change impact on cereal production, we highlight the development of drought-resilient crops better adapted to future extreme conditions, either by i) using heritage germplasm (e.g., landraces) or ii) developing novel species (e.g., crop hybrids). Our study aimed to identify key functional traits and stress-tolerant germplasm to contribute to designing drought-resilient crops under future Mediterranean climatic conditions. For that, we conducted an innovative approach combining a late-sowing field trial with two contrasting water regimes to simulate future extreme drought conditions, the use of high-throughput phenotyping devices and an infrared gas analyser to characterise leaf and ear photosynthesis, biochemistry, growth, and stress responses during the reproductive stage, and a novel linear mixed-effects model to integrate these results with final agronomical data. Modern durum wheat and barley, barley landraces and tritordeum varieties were grown and evaluated as individual plants. Our results identified barley landrace SBCC010 and tritordeum Coique as promising resilient germplasm. These genotypes showed a grain set maintenance and a higher allocation of resources to the ears compared to modern varieties, higher leaf and ear greenness, and ear photosynthesis and thermostability during the reproductive stage, particularly under stress conditions. We conclude the necessity of including ear photosynthesis in the breeding programs relying on adaptive germplasm as barley landraces and novel cereal hybrids as tritordeum to design drought-resilient cereals for future extreme Mediterranean environments.

1. Introduction

Agriculture, and in particular cereal cultivation, faces the challenge of having to increase yields at a rate of >2 % annually to meet the current and future demands from a growing population, with changes in the dietary habits and the adverse effects of climate change (Evans and Lawson, 2020; Ray et al., 2013). Despite the progress in current breeding programs, the development of novel genome-editing technologies, and

the optimization of crop management practises, crop improvement and food security are seriously threatened by high inter-annual climate variability and climate change predictions in many agroenvironments (FAO, 2018; Jagadish et al., 2014). One clear example is Mediterranean agriculture, where cereal production constitutes an invaluable nutritional and cultural richness, heritage, and a significant economic activity (PRIMA, 2022). Unfortunately, cereal production is constantly threatened by drought periods because of high temperatures and scarce

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water availability during the reproductive stages (Martínez-Peña et al., 2023). In fact, the frequency and intensity of drought periods have been especially increasing in areas with a Mediterranean climate during the last decade (EEA, 2019; FAO, 2018), whereas climate models point towards the prevalence and hardening of these conditions due to Climate Change (Bento et al., 2021).

Among the main cultivated cereals in temperate climate regions, barley (*Hordeum vulgare* L.) is one of the most drought-tolerant species because of its genetic richness inherited from its wild ancestor, *Hordeum spontaneum* (Kebede et al., 2019). Despite its natural drought tolerance and adaptability to grow in different climatic conditions, drought compromises barley grain yield and quality (Cammarano et al., 2019). Therefore, it is urgent to find solutions that contribute to mitigating the impact of climate change on cereal production, such as the development of climate-resilient crops, either by i) using heritage germplasm (e.g., wild relatives, landraces, etc.) to improve resilience in high-yielding modern varieties or ii) developing novel species adapted to future limiting growth conditions, such as crop hybrids (EEA, 2019; Katamadze et al., 2023).

Crop landraces are locally adapted crop varieties that usually present lower yields than modern genotypes, but are prized for their high diversity, grain quality and tolerance to stresses (Yahiaoui et al., 2014). For example, Cheng et al. (2024) showed that the A. E. Watkins landrace collection of bread wheat can still be used to confer superior traits in modern wheat to achieve sustainable food security. Therefore, cereal landraces are becoming increasingly employed as valuable genetic sources in crop breeding programs, particularly for crop improvement under low-productivity climatic conditions (Yahiaoui et al., 2014; Boudiar et al., 2020; Katamadze et al., 2023). One of the most promising novel climate-resilient crops is tritordeum [\times *Tritordeum* (Ascherson et Graebner)]. This species was obtained from a cross between durum wheat (*Triticum turgidum*) and wild barley (*Hordeum chilense*, Roem et Schultz), releasing the first commercial cultivar in 2010 (Papadopoulos et al., 2024). The interest in the use of tritordeum is increasing because of its nutritional quality, with higher fibre, unsaturated fatty acids, and antioxidants than commonly used wheat genotypes (Ballesteros et al. 2003; Vadim et al. 2020) and its versatility in producing end-food products such as bread, pasta, malta, and other baked goods (Mattera et al. 2017; Visioli et al. 2020). In addition, owing to the inherited genome of *Hordeum chilense*, it has been pointed out to its tolerance to dry and hot climatic conditions (Kakabouki et al. 2021; Villegas et al. 2010), granting tritordeum a high potential for its expansion in the Mediterranean agriculture and economy (Basso et al., 2013).

Given the need to test multiple species and varieties for crop improvement and climate change mitigation, plant phenomics have evolved significantly in recent decades to improve plant breeding efficiency. However, high-throughput plant phenotyping (HTPP) is still considered to be one of the main bottlenecks limiting genetic gains owing to the cost of the sensors, data management, and interpretation of the results by non-expert users (Araus et al., 2023). HTPP contributes to the study of the genotype-by-environment interactions and the identification of superior varieties and key phenotypic traits that define cereal ideotypes under Mediterranean climatic conditions, among other specific uses (Araus et al., 2022). For example, thermal sensors allow the prediction of the water status of plants, stress symptoms, and/or stomatal behaviour at the canopy and organ levels (Quemada et al., 2021). Spectroradiometers are useful for measuring a wide range of reflected radiation in the visible, near-infrared, and shortwave infrared regions (Vergara-Díaz et al., 2020a, 2020b). Data derived from the spectral reflectance of plant material are processed to non-invasively calculate many vegetation indices, which offer a comprehensive overview of plant functional traits such as leaf area index, water and nitrogen status, greenness, pigment content, photosynthetic light-use efficiency, and cell structure (Pauli et al., 2016; Vergara-Díaz et al., 2020a; Vicente et al., 2019).

One of the most relevant traits associated with plant growth and

grain yield and, at the same time, most affected by water scarcity conditions, is photosynthesis (Feller et al., 2016). Recently, some studies have proposed the use of leaf hyperspectral reflectance as a proxy for the estimation of photosynthetic capacity, although we are still far from obtaining precise results (Araus et al., 2022; Buchailot et al., 2022; Kumagai et al., 2022). Fluorometers and Infra-Red Gas Analysers (IRGAs) provide more accurate information than hyperspectral data (Fernández-Calleja et al., 2020; Kuhlger et al., 2016; Sanchez-Bragado et al., 2016). On one hand, fluorometers with quick pulse protocols at the ground level are rapidly expanding for the estimation of leaf light-harvesting capacity as an intermediate HTPP solution for small- to medium-sized field trials (Kuhlger et al., 2016). On the other hand, IRGAs cannot be considered HTPP devices because they require a long time to obtain steady-state results. Nevertheless, their use provides invaluable information that cannot be substituted as long as spectroscopic estimation models show precise and accurate. A potential key trait that has been highlighted in different studies on wheat under Mediterranean climatic conditions is ear photosynthesis. During most phases of the reproductive stage, wheat ears present better stay-green phenotype and stress resilience than flag leaves under limiting conditions, contributing significantly to final grain filling (Martínez-Peña et al., 2022; Molero and Reynolds, 2020; Sanchez-Bragado et al., 2020). In the pseudo-cereal quinoa, panicle photosynthesis also plays a significant role during reproductive stages and under salt stress (Vergara-Díaz et al., 2024). To our knowledge, the contribution of ear photosynthesis to grain filling under drought conditions has not been tested in barley and tritordeum, although photosynthetic (Hosseini et al., 2012) and molecular (Abebe et al., 2009) analyses of barley awns have indicated its potential photosynthetic capacity.

Therefore, it is essential to conduct experiments under future Mediterranean extreme drought conditions to evaluate the response of potential drought-resilient genetic resources in cereals during key developmental stages for grain yield. The use of state-of-the-art HTPP sensors and modern IRGA systems in such experiments can allow for the identification of key plant functional traits and strategies desired for future Mediterranean environmental conditions. Based on this, the present study aimed to i) identify drought tolerant physiological traits by relying in a panel of adaptive germplasm to future Mediterranean extreme conditions and integrating plant responses along the reproductive stage, and ii) investigate the role of leaf and ear photosynthesis in stress resilience and plant agronomic performance. We hypothesise that although barley landraces and tritordeum genotypes may achieve lower yields compared with modern varieties due to less breeding progress, they will deal better under limiting growing conditions owed to their inherent drought tolerant traits, showing an enhanced (less reduced) water status and photosynthetic capacity compared to modern genotypes. We also hypothesise that ear photosynthesis will play a more relevant role in canopy photosynthesis as stress and senescence progress, contributing significantly to agronomic performance.

2. Materials and methods

2.1. Plant material, experimental setup and environmental growth conditions

Modern malting barley (RGT Planet) and durum wheat (Euroduro) genotypes were grown together with four barley landraces (SBCC010, SBCC154, SBCC155 and SBCC156) and two commercial tritordeum genotypes (Aucan and Coique) in Lisbon (Instituto Superior de Agronomia, 38°7088.982' N, -9°1822.618 W), Portugal, under irrigated and rainfed conditions. The modern malting barley and durum wheat genotypes were used as control modern cereals, being widely used in the Iberian Peninsula. The barley landraces were selected from the Spanish Barley Core Collection for being spring landraces with a high yield tolerance under Mediterranean climatic conditions (Yahiaoui et al. 2014). Tritordeum genotypes were obtained from VIVAGRAN S.L. (Spain), being

included in the panel as potential new hybrid species adapted to Mediterranean conditions (Papadopoulos et al., 2024).

A split-plot individual-plant field experiment was conducted to study the genotype-by-environment interaction. The idea was to mimic a greenhouse experiment growing the plants individually to perform exhaustive phenotypic characterisation, but in the field to ensure that environmental conditions are as close as possible to real field conditions without limitations such as reduced root growth by using pots or reduced light intensity or quality. Thus, in our study, individual plants were defined as independently replicated experimental units (n) (Rogers et al., 2021). Concretely, plants were grown in 4 plots of 4 m × 1.05 m, two plots were set for irrigated conditions and two for rainfed conditions (Fig. 1A). In each plot, the genotypes were randomly distributed in rows (4 × 2 rows, 8 genotypes). Additionally, two rows of the genotype Planet were added as borders in each plot. In each row, 15 plants were grown per genotype, letting a space of 0.15 cm between each plant of the same species and 0.26 cm between each row and plant species, minimising resource competition between species and allowing to distinguish the tillers corresponding to each individual plant. Therefore, from the 30 plants per genotype and water regime (2 rows of 15 plants), various plants from the middle of each row per plot were selected depending on the type of measurement (as defined below) to reduce possible border effects. Overall, the whole experiment encompassed 33.5 m². The soil was characterised by a 3.56 % organic matter content, 5.2 g kg⁻¹ carbonate, 0.24 g kg⁻¹ phosphate, 0.71 g kg⁻¹ N and a pH of 7.39.

As a proof-of-concept to simulate future limiting climatic conditions

in the Mediterranean agroenvironment (EEA, 2019; Trambly et al., 2020; Bento et al., 2021), a late-sowing trial was carried out by sowing on 1st of April of 2022 (0 days after sowing, DAS), while harvest and the collection of final agronomic and physiological data took place on 11th of July of 2022 (101 DAS). The average temperature and relative humidity along the experiment was 19.2°C and 69.4 %, respectively. The precipitation during the whole experiment was extremely low, only reaching 47 mm. More information about the environmental growing conditions is detailed in Fig. 1B. This experimental design allowed us to expose plants to higher temperatures and lower relative humidity and precipitations compared to plants growing under normal spring conditions (Supplementary Fig. S1).

Seeds were stratified by germinating at 4 °C temperature on damp filter paper in a dark room for seven days to ensure a homogeneous germination. Afterwards, the seeds were moved to room temperature for 3 days to allow seedling establishment before being transplanted to the field. Field-grown plants were irrigated twice per week until 54 DAS, coinciding with the booting stage. For this purpose, tanks of 8 liters (2mm/plot) were employed, which allowed to irrigate homogeneously the different species within and between the plots and to have a control of the amount of the watering in every moment (Fig. 1B). In parallel, the soil water content (SWC) was monitored weekly with a Moisture Meter type HH2 with Theta Probe (Delta-T Devices, Cambridge, UK). During this period, the soil water content (SWC) was maintained around 18 % (from a maximum of 36 % in the sensor), which represented a 50 % of the field capacity (FC) (Fig. 1C). Then, the plots were divided in two

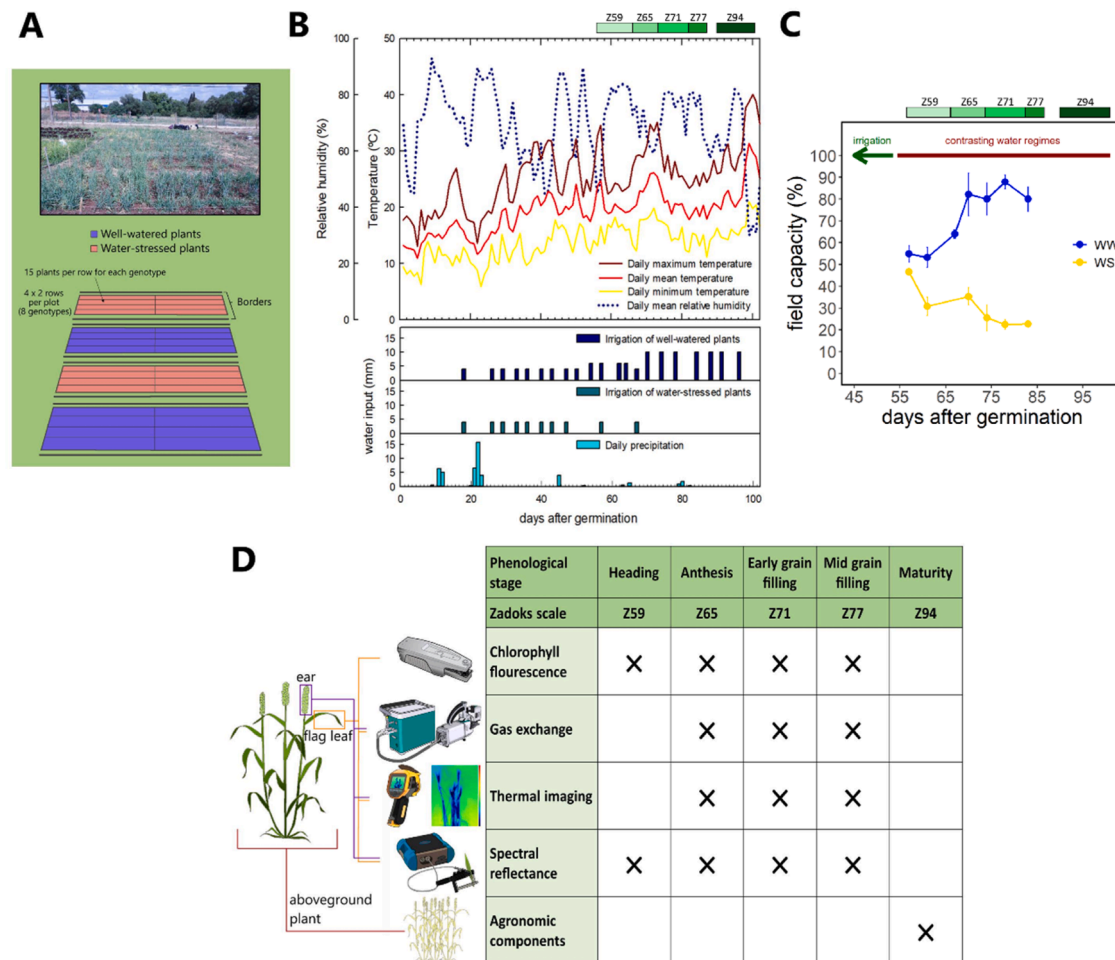


Fig. 1. (A) Experimental setup and (B) environmental growth conditions during the field trial correlated to the Zadocks scale. (C) The soil water content was monitored during the application of contrasting water regimes to determine the field capacity (FC) at the different key growth stages, correlating with the Zadocks scale. (D) Schematic representation of the measurements performed during the crop cycle.

water treatments: irrigated treatment (well-watered plants, WW), where plants were watered along the whole cycle and maintained at 60–80 % FC, and rainfed treatment (water-stressed plants, WS), watering with half of the irrigation than in WW till 70 DAS (Z51-60) and withholding the watering till the end of the experiment, reaching values of FC around 20–30 % (Fig. 1C). In each measuring day, 10 random measurements per water treatment were recorded by the Moisture Meter. By the end of the experiment, WW and WS plants received 133.5 and 44 mm of irrigation, respectively, together with the 47 mm of precipitation along the whole growing cycle (Fig. 1B).

2.2. Phenology and agronomic components

Phenological stages for heading, anthesis, early-grain filling (EGF), mid-grain filling (MGF), and maturity were monitored along the trial by visual inspection during periodical visits to the experiments. When plants reached the physiological maturity, Z94 (Alqudah and Schnurbusch, 2017; Zadoks et al., 1974), we collected randomly from the middle of the rows several plants per genotype and water regime ($n = 4-10$) due to interspecies plant growth variability. Different agronomic components at harvest were obtained: plant height, number of tillers and ears, plant dry weight, number of grains per ear, thousand grain weight (TGW), grain dry weight per plant, and harvest index (HI, calculated as the ratio of grain dry weight to total plant dry weight). To further study the effect of water limitation on agronomic traits at harvest in each genotype, we calculated the drought susceptibility index (DSI) expressed as a percentage. The index for each trait was obtained from the average values of WW and WS conditions, while the absolute values ($n = 4-10$) were employed for the ANOVA analysis to assess the significance between group means per trait and genotype. The following formula was used for the index calculation:

$$DSI (\%) = \frac{(Trait_{ws} - Trait_{ww})}{Trait_{ww}} \times 100$$

2.3. High-Throughput phenotypic characterisation of flag leaves and ear organs

We determined different phenotypic traits under both irrigated and rainfed conditions, including plant growth, chlorophyll-fluorescence traits, pigment content, water and nitrogen status, stress responses, organ area and temperature (Fig. 1D). Firstly, we used a MultispeQ V2.0 (Photosynq Inc., USA) and an ASD Fieldspec4 spectroradiometer (FS4, ASD Inc. PANalytical Company, USA). Due to some differences in the phenology between genotypes and treatments, we clustered these measurements at four key growth stages, whenever each plant reached each stage, including heading (Z59), anthesis (Z65), early-grain filling (Z71) and mid-grain filling (Z77) (Fig. 1D). The fully developed mature flag leaf per plant was measured with the MultispeQ V2.0 device avoiding shadows. All measurements across growth stages were consistently taken from the same plant within each row as a biological replicate ($n = 3-5$ per genotype and water regime). Different parameters were obtained from the MultispeQ data: relative chlorophyll content (SPAD), maximum Photosystem II (PSII) quantum efficiency (F_v'/F_m'), non-photochemical quenching (NPQ), fraction of open PSII centres (qL), quantum yield of PSII (Φ_{PSII}), the light that is used in non-photochemical processes (Φ_{NPQ}) and the light that is lost via non-regulated processes (Φ_{NO}) (Kuhlgert et al., 2016). FS4 was used to acquire leaf and ear spectra in the 350–2500 nm range using the ASD Plant Probe and Leaf Clip Version 2 ($n = 4$ per genotype and water regime). Spectra was processed using the RS3 and ViewSpec Pro softwares (ASD Inc. PANalytical Company, USA), and various spectral vegetation indices were calculated based on Vergara-Díaz et al. (2018) with some modifications (Supplementary Table S1). Thermal photos of flag leaves and ears were taken using a Ti480 PRO Infrared Camera (Fluke, USA). Measurements were taken at three growth stages (Z65, Z71 and Z77;

Fig. 1D) for each genotype and treatment combination ($n = 3-5$). Each thermal image of a plant (biological replicate) was used to extract the temperature of flag leaves and ears using the SmartView software (Fluke, USA). Three leaves and ears were measured for each plant as technical replicates, obtaining the mean temperature of the middle part of the organ from the apical to the basal part. Leaf and ear temperature depression (LTD and ETD, respectively) were then calculated by subtracting organ temperature from the ambient temperature.

2.4. Gas exchange measurements in flag leaves and ears

An infrared gas analyser Li-6800 system (Li-Cor Inc., USA) was used to determine gas exchange traits of flag leaves and ears at three growth stages: Z65, Z71 and Z77 (Fig. 1D). Three to four biological replicates were obtained per organ, genotype and treatment combination at each growth stage in different plants. Measurements were performed between 2–6 h after dawn in the fully expanded flag leaf and ear of the principal tiller. For flag leaves, gas exchange was determined in 2 cm² leaf sections using the 6800-01A Multiphase Flash Fluorometer chamber coupled to the LI-6800 Portable Photosynthesis System (Li-Cor Inc., USA). For the ears, we used the 36 cm² 6800-13 chamber together with the 6800-03 Large Light Source, coupled to the LI-6800 system, as we previously used for quinoa panicles (Vergara-Díaz et al., 2024). The conditions in the cuvettes during the measurements were 60 % relative humidity, a saturating photosynthetic light flux density of 1500 $\mu\text{mol}/\text{m}^2 \text{ s}$, provided by a red/blue (90/10) LED light source, 25°C and 400 ppm of CO₂. The following parameters were calculated in both flag leaves and ears: net photosynthetic rate (A_n), stomatal conductance (g_s), and intrinsic water use efficiency (WUE, calculated as A_n in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ divided by g_s in $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$). In addition, considering the area of each organ calculated with the RGB images, we further calculated total A_n , g_s and WUE per organ by multiplying the net values by total organ area. For that, on each day of measurement, RGB pictures of the measured organs were taken with an A6000 camera (Sony, Japan). RGB images were later analysed using the open-source ImageJ software (National Institutes for Health, USA) to calculate organ area in a two-dimensional plane. Lastly, the electron transport rate (ETR) and the electrons needed to fix a molecule of CO₂ (calculated as photosynthetic electron transport rate divided by A_n , ETR/A_n), were also considered in flag leaves. Photosynthetic parameters with subscripts “leaf” and “ear” refer to flag leaves and ears on an area basis, respectively, while “LEAF” and “EAR” refer to total organ.

2.5. Statistical analysis

The figures were generated in SigmaPlot v12.0 (Systat Software Inc., USA), the R environment v4.3.1 (R Core team, 2021) using the interface from RStudio v2022.12.0 (www.rstudio.com), and the ggplot2 package, the raster graphics editor Krita v5.0.6 (Krita Foundation, The Netherlands), and Microsoft Excel (Office 365, Microsoft Corporation, USA). The effect of water regime per genotype or the interactive effect of water regime and genotype were studied with one- and two-way analysis of variance (ANOVA) using agricolae package in R (de Mendiburu and Yaseen, 2020). Prior to the ANOVA analysis, the assumptions of normality and homogeneity by means of the Kolmogorov-Smirnov and Levene tests, respectively, were checked. Further post-hoc tests were carried out to assess differences between groups when $P < 0.05$ using Tukey’s Honest Significant Difference (HSD). Furthermore, linear mixed-effects models (LMMs) were used to analyse the phenotypic traits (chlorophyll fluorescence, gas exchange and organ temperature) per genotype and water regime integrating the results from the different measuring times (growth stages). The best linear unbiased estimator (based on the Akaike information criterion (AIC) and Bayesian information criterion (BIC) values) was calculated with the R package lme4 (Bates et al., 2015) using the following linear model as the basis, assuming a Gaussian error distribution:

$$Y_{ijkl} = \mu + G_i + W_j + (G \times W)_{ij} + S_k + R_l + e_{ijkl}$$

where Y_{ijkl} is the phenotypic value, μ is the overall mean, G_i is the fixed effect of the i^{th} genotype, W_j is the fixed effect of the j^{th} water regime, $(G \times W)_{ij}$ is the fixed effect of the ij^{th} genotype \times water regime interaction, S_k is the random effect of the k^{th} growth stage, R_l is the random effect of the l^{th} replication, and e_{ijkl} is the residual random error term with null mean and common error variance. We further used the R packages *lmerTest* (Kuznetsova et al., 2017), *emmeans* (Lenth, 2023) and *multcomp* (Hothorn et al., 2008) to estimate the significance of the fixed main factors and their interactions (according to type III analyses of variance with Satterthwaite's method and degrees of freedom approximation with Kenward-Roger's method), the means and the *post-hoc* comparisons between groups (i.e., genotype, water regime, and genotype \times water regime) with the Tukey HSD test. LMMs were also used to analyse organ-specific differences for photosynthesis-related traits and organ temperature depression at well-watered and water-stressed conditions, using the same models as described before but substituting the factor genotype by type of organ.

An exploratory multivariate analysis was performed for the 109 spectral indices measured in flag leaves and ears with a clustered heatmap using the package *pheatmap* (Kolde, 2019). To get an overview of the results from these indices, the means per genotype and water regime were shown for each organ (flag leaves and ears) after averaging the three growth stages obtained during the reproductive period when water stress was imposed (anthesis, early-grain filling and mid-grain filling). The data was scaled per trait in row direction. Moreover, using these data, we ran principal component analyses (PCAs) to select the top three spectral vegetation indices per water regime and organ contributing to the variability of dimension 1 and 2, with the aim to reduce the complexity and simplify data interpretation. Two final PCAs with all the traits measured during the reproductive period, averaged per genotype for the different growth stages, as well as the agronomic components, were generated for each water condition. For these analyses, the packages *FactoMineR* (Lê et al., 2008) and *factoextra* (Kassambara and Mundt, 2020) were used after scaling and centering the data. A second clustered heatmap was built with all the data (agronomic components and phenotypic attributes) using again the package *pheatmap*, scaling the data per trait in row direction. In this case, we studied the stress tolerance using a symmetric scale around 0 by calculating the \log_2 ratio of water-stressed over well-watered plants for each genotype to study their stress tolerance:

$$\text{stress response} = \log_2(\text{Trait}_{\text{WS}} / \text{Trait}_{\text{WW}})$$

3. Results

We conducted a field experiment with different water availabilities and late spring sowing, simulating extreme Mediterranean conditions of high temperatures, low humidity, and low rainfall (Fig. 1, Supplementary Fig. S1) to identify key physiological traits and drought-resilient crops. A comparison of the main climatic factors of our experiment (April–July 2022) with the conditions of the last three years in the area, considering the usual growing season for spring cereals (January–May 2020–2022), allowed us to evaluate the severity of the growth conditions. Thus, our trial was characterised by a shortening of the life cycle, an increase of 4.6 °C in daily mean temperature and decreases of 7.6 % and 79 % in daily mean relative humidity and precipitation, respectively. Because 2022 was an extremely dry and hot year during spring, survival irrigation was supplied during the early stages of cultivation (Figs. 1B–C).

3.1. The effect of genotypic variability and water availability on agronomic components under extreme Mediterranean conditions

Planet and Euroduro varieties were used as controls for widely used

spring/facultative varieties of barley and durum wheat, respectively, in the Iberian Peninsula, in addition to constituting the species that gave rise to the cereal hybrid tritordeum. Planet also constituted a control as a modern variety for comparison with barley landraces. Phenotyping was carried out according to the developmental stage of each variety at different moments of the reproductive stage (Fig. 1D) to avoid differences due to the phenology of each genotype (Supplementary Fig. S2A). It is worth to highlight the faster phenology and reduced grain filling period (time difference between anthesis and physiological maturity) of barley landraces SBCC155 and SBCC156 compared to Planet, and the higher grain filling period of tritordeums compared to control species (Supplementary Figs. S2A and S2B). Both genotypic variability and water regime had a significant effect on all agronomic and physiological traits studied at harvest (except for water regime on HI; Fig. 2A), being the interaction only significant for the number of ears.

Water stress clearly reduced all agronomic traits at harvest, with a large effect on grain number, plant dry weight, and grain dry weight, whereas DSI for these traits showed large genotypic variability (Figs. 2B–C). Regardless of the water regime, grain dry weight was higher in the modern control varieties Planet and Euroduro, followed by SBCC010 and SBCC156, and was lowest in Aucan. However, the differences between genotypes were much smaller or nonexistent under WS conditions (Fig. 2B) due to the lower drought susceptibility of tritordeums and barley landraces (except SBCC154) than that of the control varieties (Fig. 2C). For the rest of the agronomic components, DSI showed more significant declines due to WS conditions in control varieties than in barley landraces and tritordeums, with some exceptions. In terms of absolute values, the tritordeum Aucan showed the lowest values for all the agronomic components irrespective of the water treatment. However, in general the tritordeum Coique only presented lower values for the agronomic components compared to Planet and Euroduro under WW conditions (Fig. 2B). The barley landrace most affected by water stress, SBCC154, showed high values for the number of tillers and ears among the landraces, but lower grain number, plant height, and harvest index (Fig. 2B). The opposite occurred in SBCC010, with a lower number of tillers and ears, as well as a lower TGW, but higher grain number.

3.2. The effect of genotypic variability and water availability on chlorophyll fluorescence-related traits in flag leaves

Once plant survival was no longer at risk due to extreme weather conditions in the year 2022, two levels of irrigation were applied from the heading stage. Phenotypic results are presented as time courses to study the genotype \times water regime interaction at each growth stage and with LMMs to integrate the results of the different growth stages during the reproductive period. Overall, there were clear genotypic differences in SPAD and chlorophyll fluorescence traits in flag leaves at most growth stages, while water stress and genotype \times water regime effects were particularly affected at the latest stages (Fig. 3). SPAD decreased with leaf senescence and to a greater extent in water stress at mid-grain filling, with higher absolute values in tritordeum varieties, followed by Euroduro. F_v'/F_m' was again higher in tritordeum varieties, followed by SBCC010, with clear WS effects at mid-grain filling, particularly in landraces SBCC155 and SBCC156 due to advanced senescence and yellowing. Because of this, NPQt and Φ_{NPQ} reached significant high values in early- and mid-grain filling in SBCC155 and SBCC156, with lower values in tritordeums and SBCC010, as also shown by LMMs. As Φ_{PSII} , Φ_{NPQ} , and Φ_{NO} add up to 1, the extremely high values of Φ_{NPQ} in SBCC155 and SBCC156 at late stages led to these varieties having the lowest Φ_{PSII} and Φ_{NO} values. Conversely, Φ_{PSII} was higher in Euroduro, Planet, and Aucan. Φ_{NO} was significantly reduced by water stress, while Coique, SBCC010 and Aucan had the highest values. Finally, the interaction genotype \times water in the LMM showed that qL increased under WS conditions in barleys (Planet and landraces), with negligible differences in wheat and tritordeum.

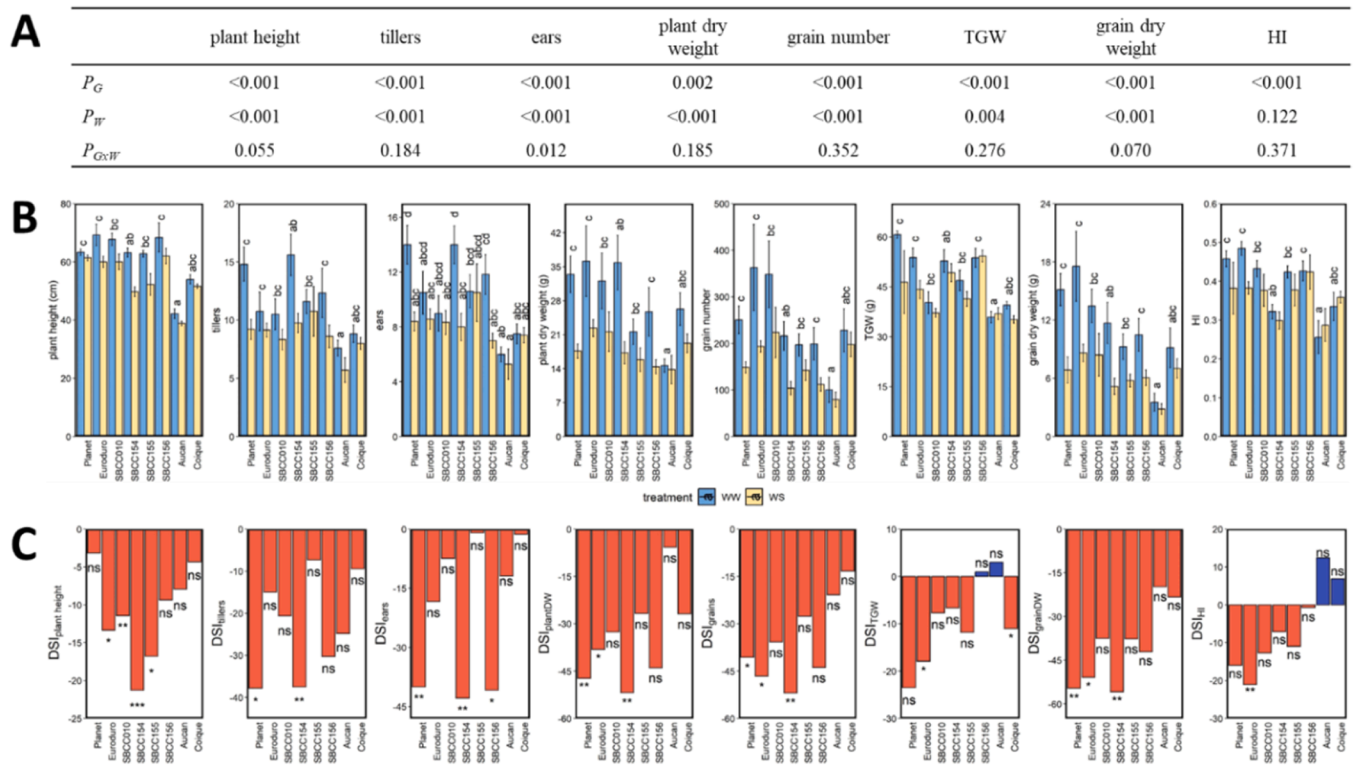


Fig. 2. Effects of water regime and genotypic variability on physiological and agronomic traits at harvest. In (A), the P values for the two-way ANOVAs are shown ($n = 4-10$), while the mean values \pm SE for each trait are visualised in (B). As the genotype-by-environment interaction was not significant for most traits, the different letters in (B) represent significant differences between genotypes irrespective of water regime ($P < 0.05$), except for the number of ears, where the letters refer to the genotype-by-environment interaction ($P = 0.012$). Drought susceptibility index (DSI) for each trait is shown in (C), representing the reduction/increase (%) in water-stressed compared to well-watered plants. Asterisks indicate a significant change between water regimes using the absolute values of each trait (ns, non-significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Abbreviations: HI, harvest index; P_G , P_W and $P_{G \times W}$, P values for genotype, water regime and their interaction, respectively; TGW, thousand grain weight; WS, water-stressed plants; WW, well-watered plants.

3.3. Characterisation of the photosynthetic capacity in flag leaf and ear organs in response to the genotype \times water regime interaction

There was a large genotypic variability in leaf and ear photosynthetic capacity and plasticity in response to water availability during the reproductive period (Fig. 4). After analysing the time courses and integrating three growth stages through the LMM analyses (Fig. 4A), a quantitative decline of the flag leaf photosynthesis (A_{leaf} and A_{LEAF}) and stomatal conductance ($g_{s,\text{leaf}}$ and $g_{s,\text{LEAF}}$) was observed as senescence and water stress progress, being genotype-dependent. SBCC156 irrespective of water regime, and SBCC155 under WS, showed advanced leaf senescence and no gas exchange values were obtained at mid-grain filling. SBCC155 presented the lowest values for A_{leaf} , A_{LEAF} , $g_{s,\text{leaf}}$, and $g_{s,\text{LEAF}}$, while they were not so low in SBCC156 due to its high values at earlier reproductive stages. In contrast, Euroduro and Coique showed the highest values for these traits under WW conditions, followed by Aucan and SBCC010. Under WS conditions, Euroduro and Coique again stood out for A_{leaf} , $g_{s,\text{leaf}}$, and $g_{s,\text{LEAF}}$, and Euroduro, SBCC156 and Planet for A_{LEAF} .

As for the leaves, barley landraces SBCC155 and SBCC156 showed advanced ear senescence, including also SBCC154 (Fig. 4B). Among them, SBCC156 showed the lowest values for all gas exchange parameters in ears, irrespective of the water regime. Interestingly, A_{ear} was higher in tritordeums, followed by SBCC010 and Euroduro, similarly as it happened for $g_{s,\text{ear}}$. When considering the whole organ, A_{EAR} was higher in SBCC010 and Coique, followed by Euroduro and Aucan, with a significant decrease by water stress. $g_{s,\text{EAR}}$ was higher in Euroduro and Coique, followed by Aucan and SBCC010, with also a significant decrease by water stress.

Regarding other interesting photosynthesis-related traits, WS

reduced ETR_{leaf} and increased $\text{ETR}_{\text{leaf}}/A_{\text{leaf}}$ ratio, WUE_{leaf} and WUE_{ear} (Supplementary Table S2). ETR_{leaf} was particularly higher in tritordeums and Euroduro irrespective of water regime, and lower in SBCC155 and SBCC156. $\text{ETR}_{\text{leaf}}/A_{\text{leaf}}$ ratio was higher in SBCC010, SBCC154, SBCC155, and Aucan, mainly under WS conditions. WUE_{leaf} and WUE_{ear} were higher in barley and its landraces, irrespective of water regime and when the leaves were alive, as well as in Aucan under WS conditions.

Additionally, we plotted together flag leaf and ear photosynthesis to compare statistically their contribution to grain filling during reproductive period by using LMMs (Fig. 5). The results showed that leaf photosynthesis is higher than that of the ear, but these differences are attenuated under WS conditions or when calculated per organ. Leaf photosynthesis decreased strongly under WS conditions, although Planet showed a high resilience. Interestingly, ear photosynthesis showed higher stability in all genotypes at different water regimes, with A_{EAR} being even higher than A_{LEAF} in both tritordeum varieties and SBCC010 under WS conditions. Linear regression models did not show a significant correlation between leaf and ear photosynthesis at any growth stage, except for one case at mid-grain filling conditioned by some absolute values of 0 at this stage (Supplementary Fig. S3).

3.4. Thermal imaging to evaluate flag leaf and ear temperature between genotypes and water regimes

Using thermal images, we analysed the flag leaf and ear temperatures to study the genotype \times water regime (Fig. 6A) and organ \times water regime (Fig. 6B) interactions by integrating different growth stages through LMMs since the absolute values can vary significantly between dates. WS significantly increased LTD and ETD, although these increases were

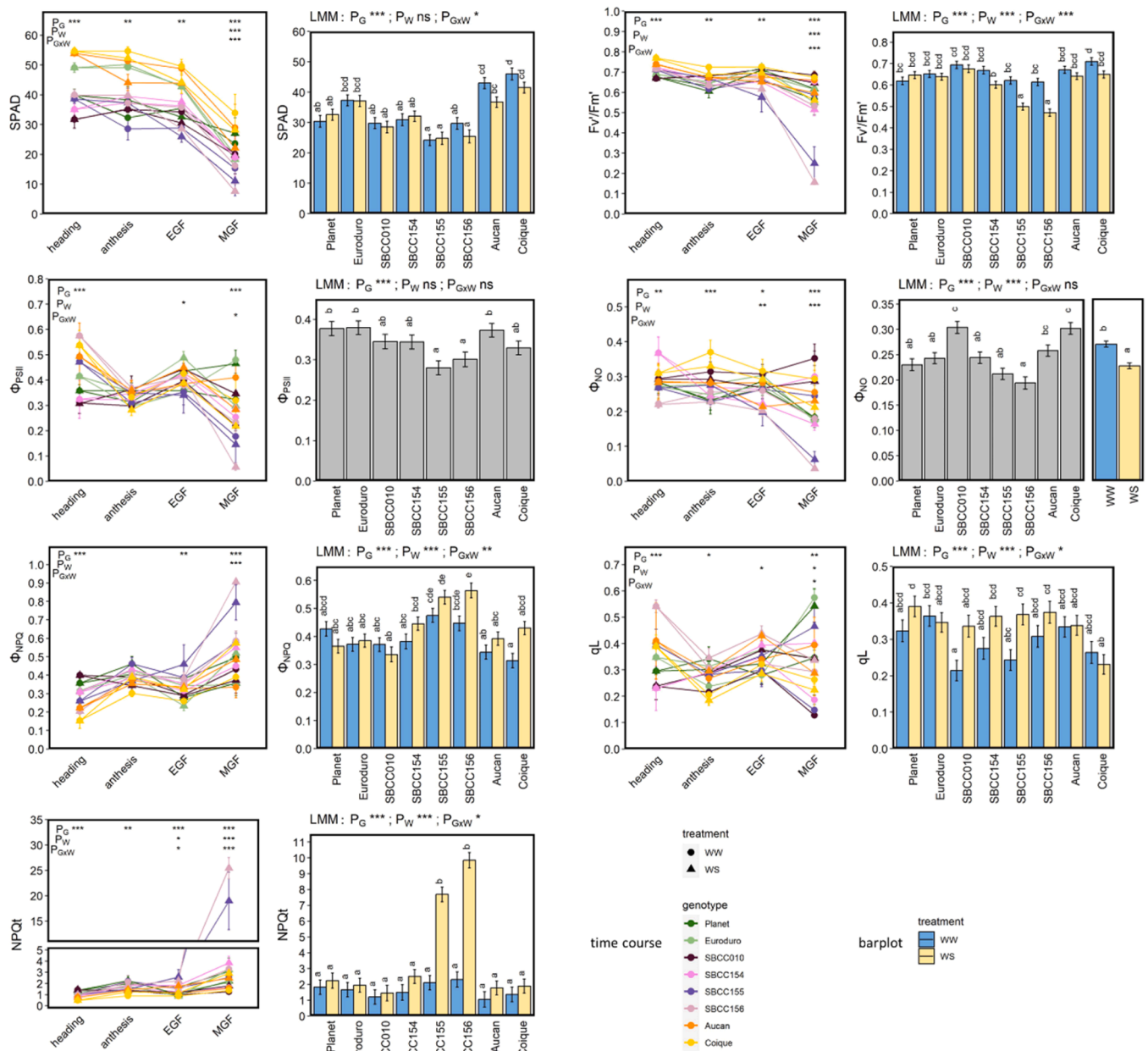


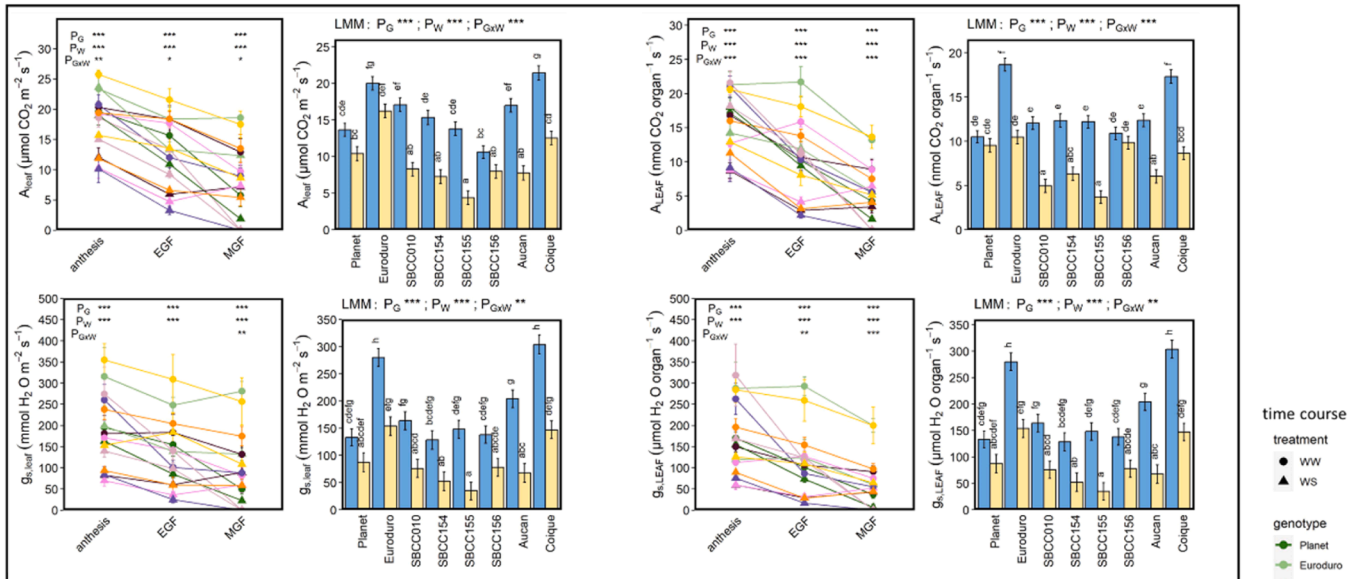
Fig. 3. Relative chlorophyll content (SPAD) and chlorophyll fluorescence-related traits in flag leaves of eight cereals under two water regimes. A two-way ANOVA was used in the time courses to study the main factors and the interactive effects of genotype × water regime at each growth stage ($n = 3-5$). Then, linear mixed-effects models (LMMs) were performed to study the interaction genotype × water regime during the whole reproductive period (considering anthesis, EGF, and MGF, excluding heading where there was no difference in water inputs). For LMMs, the significant interaction effects were plotted or, alternatively, the main effects. For all plots, mean values ± SE are shown for each group, while asterisks indicate significant differences between groups (ns, non-significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Abbreviations: EGF, early-grain filling; F_v'/F_m' , maximum Photosystem II quantum efficiency; MGF, mid-grain filling; NPQI, non-photochemical quenching; P_G , P_W and $P_{G \times W}$, P values for genotype, water regime and their interaction, respectively; qL, fraction of open Photosystem II centres; SPAD, relative chlorophyll content; WS, water-stressed plants; WW, well-watered plants; Φ_{NO} , the light that is lost via non-regulated processes; Φ_{NPQ} , the light that is used in non-photochemical processes; Φ_{PSII} , quantum yield of Photosystem II.

genotype dependent (Fig. 6A). The genotypes with the lowest LTD and ETD in WW conditions were the tritordeums, while in WS conditions they were also the lowest together with barley landraces, such as SBCC156. Control varieties Planet and Euroduro showed the highest (least negative) values for both LTD and ETD. Flag leaves warmed up to a greater extent than the ears under WS conditions (Fig. 6B), with the ears showing again greater stability among the different genotypes.

3.5. Organ-specific spectral vegetation indices in response to the genotype × water regime interaction

We calculated 109 spectral vegetation indices using the reflectance of leaves and ears in the range of 350 to 2500 nm (Supplementary Table S1). These indices were subdivided into 8 major functions, although some indices may have more than one. Overall, organ is the most clearly distinguishable category in the heatmap, compared to genotypes and water regimes (Fig. 7). Flag leaves are greener and with higher chlorophyll content than ears, as indicated by most indices for

A Flag leaves



B Ears

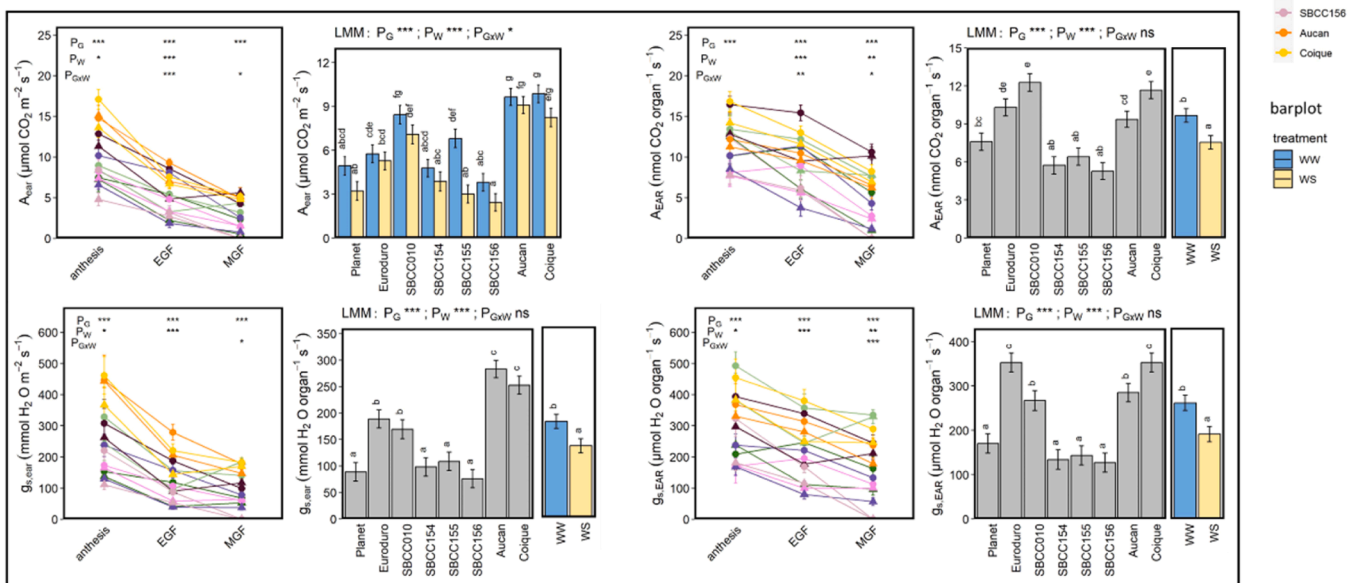


Fig. 4. Photosynthesis and stomatal conductance in (A) flag leaves and (B) ears of eight cereals under two water regimes. A two-way ANOVA was used in the time courses to study the main factors and the interactive effects of genotype \times water regime at each growth stage ($n = 3-4$). Then, linear mixed-effects models (LMMs) were performed to study the interaction genotype \times water regime during the whole reproductive period. For LMMs, the significant interaction effects were plotted or, alternatively, the main effects. For all plots, mean values \pm SE are shown for each group, while asterisks indicate significant differences between groups (ns, non-significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Abbreviations: A_{ear} and A_{EAR} , net photosynthetic rate in the ear expressed on an area basis or by total organ, respectively; A_{leaf} and A_{LEAF} , net photosynthetic rate in the flag leaf expressed on an area basis or by total organ, respectively; EGF, early-grain filling; $g_{s,ear}$ and $g_{s,EAR}$, stomatal conductance in the ear expressed on an area basis or by total organ, respectively; $g_{s,leaf}$ and $g_{s,LEAF}$, stomatal conductance in the flag leaf expressed on an area basis or by total organ, respectively; MGF, mid-grain filling; P_G , P_W and $P_{G \times W}$, P values for genotype, water regime and their interaction, respectively; WS, water-stressed plants; WW, well-watered plants.

these functions. The flag leaves of the two tritordeums and Euroduro showed the highest greenness and chlorophyll values regardless of water regime, while SBCC155 and SBCC156 were the lowest. In the ear, again the tritordeums and Euroduro, as well as SBCC010, excelled in greenness and chlorophyll, particularly under WS conditions. Spectral indices related to water status showed a greater variability between them, although most of them pointed to a higher water content in flag leaves than in ears. An opposite trend to the described above for greenness and chlorophyll was observed for the water status indices. Moreover, the relative content of carotenoids seemed to be higher in leaves, whereas

anthocyanins in ears. Carotenoids followed a similar pattern to chlorophyll content in terms of genotypic variability. The only specific N index (NDNI) showed higher values in Coique and Euroduro in flag leaves, and in the tritordeums, Euroduro and SBCC010 in ears, with a general decline under water stress. Structural C-related indices (cellulose and lignin) were higher in ears than in flag leaves. The stress-related indices showed more erratic pattern between organs, genotypes and water regime, although tritordeums, Euroduro and SBCC010 leaves and ears achieved lower values compared to the rest.

To reduce the number of variables and highlight the most relevant,

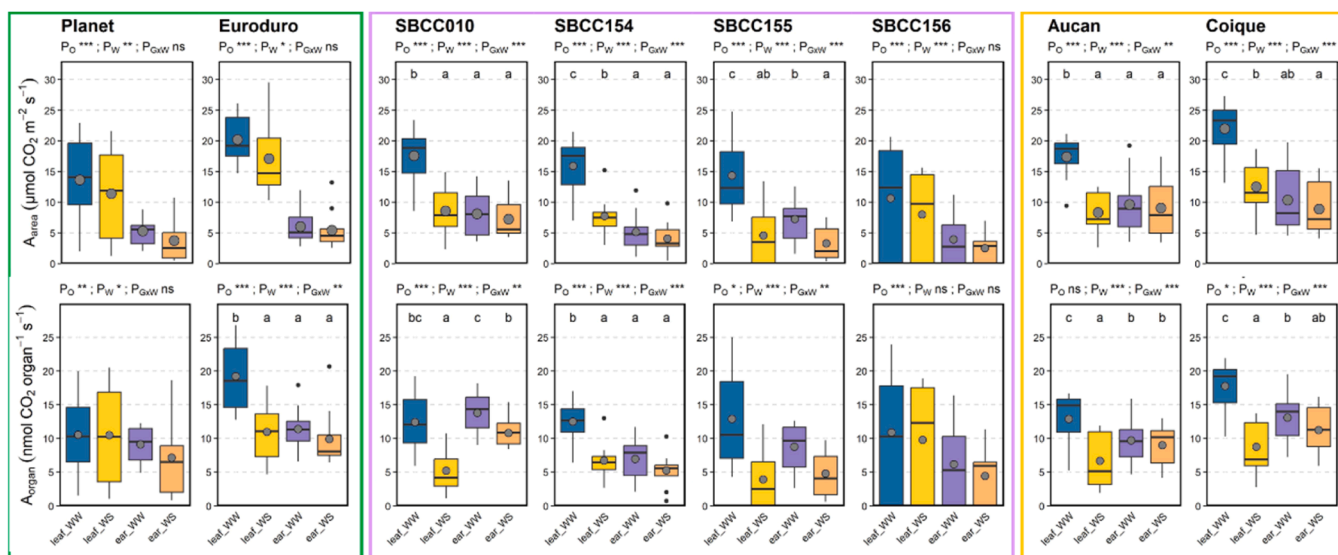


Fig. 5. Photosynthesis on an area basis (A_{area}) or per total organ (A_{organ}) in flag leaves and ears of eight cereals under two water regimes. All the values for each organ, irrespective of the growth stage, were used to build the boxplots per genotype ($n = 10-12$), with grey dots indicating the average value. Linear mixed-effects models (LMMs) were used to compare organs, water regimes, and their interactions, with letters indicating significant differences between groups for the interaction organ \times water regime (ns, non-significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Abbreviations: P_O , P_W and $P_{O \times W}$, P values for organ, water regime and their interaction, respectively; WS, water-stressed plants; WW, well-watered plants.

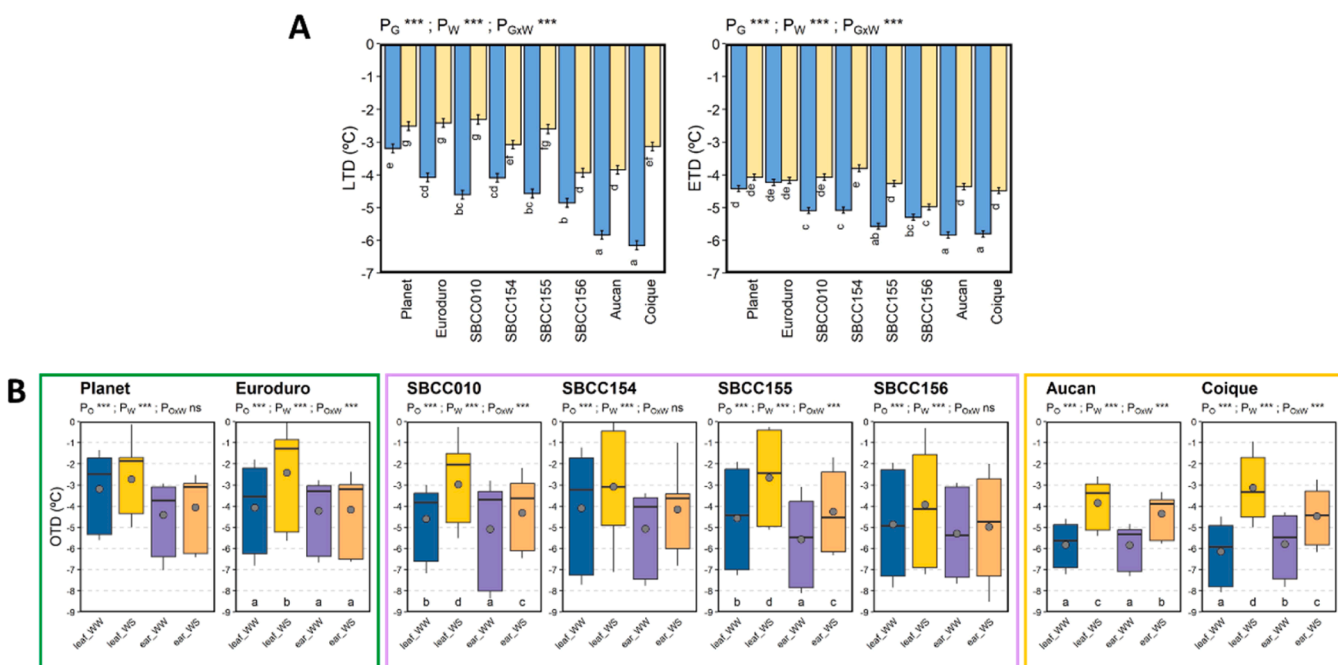


Fig. 6. (A) Genotype \times water regime or (B) organ \times water regime effects on leaf (LTD) and ear (ETD) temperature depression. In (A), mean values \pm SE are shown for each genotype and water regime, while in (B) all the values for each organ were used to build the boxplots per genotype, with grey dots indicating the average value ($n = 13-15$). In both cases, linear mixed-effects models (LMMs) were used to study the interactive effects of the factors during the whole reproductive period (anthesis, early-grain filling and mid-grain filling). Different letters indicate significant changes between groups for the interactions (ns, non-significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Abbreviations: OTD, organ temperature depression; P_G , P_O , P_W , $P_{G \times W}$, and $P_{O \times W}$, P values for genotype, organ, water regime, genotype \times water regime and genotype \times water regime interactions, respectively; WS, water-stressed plants; WW, well-watered plants.

we selected the top three indices that contributed most to the variability based on PCA dimensions 1 and 2, using average data of leaf and ear indices under WW and WS conditions separately (Supplementary Figs. S4A-B). This allowed us to identify indices that explain genotypic variability and not merely the effect of water stress. We highlighted six indices for the ears (SR3, GCTbc, GRVI, ARI, TCARI, and MCARI), and four for the leaves (ChINDI, NDRYMATTER, MCARI, and MRENDVI), as

two of them were repeated in both water regimes (Supplementary Figs. S4C-D). These indices were mainly associated with chlorophyll content and greenness, being higher in both tritordeums and Euroduro in flag leaves and ears, as well as SBCC010 in ears, compared with the other genotypes. Leaf water status was also highlighted through the NDRYMATTER index, being higher in the tritordeums and Euroduro (Supplementary Fig. S4C). Interestingly, the anthocyanin-related index

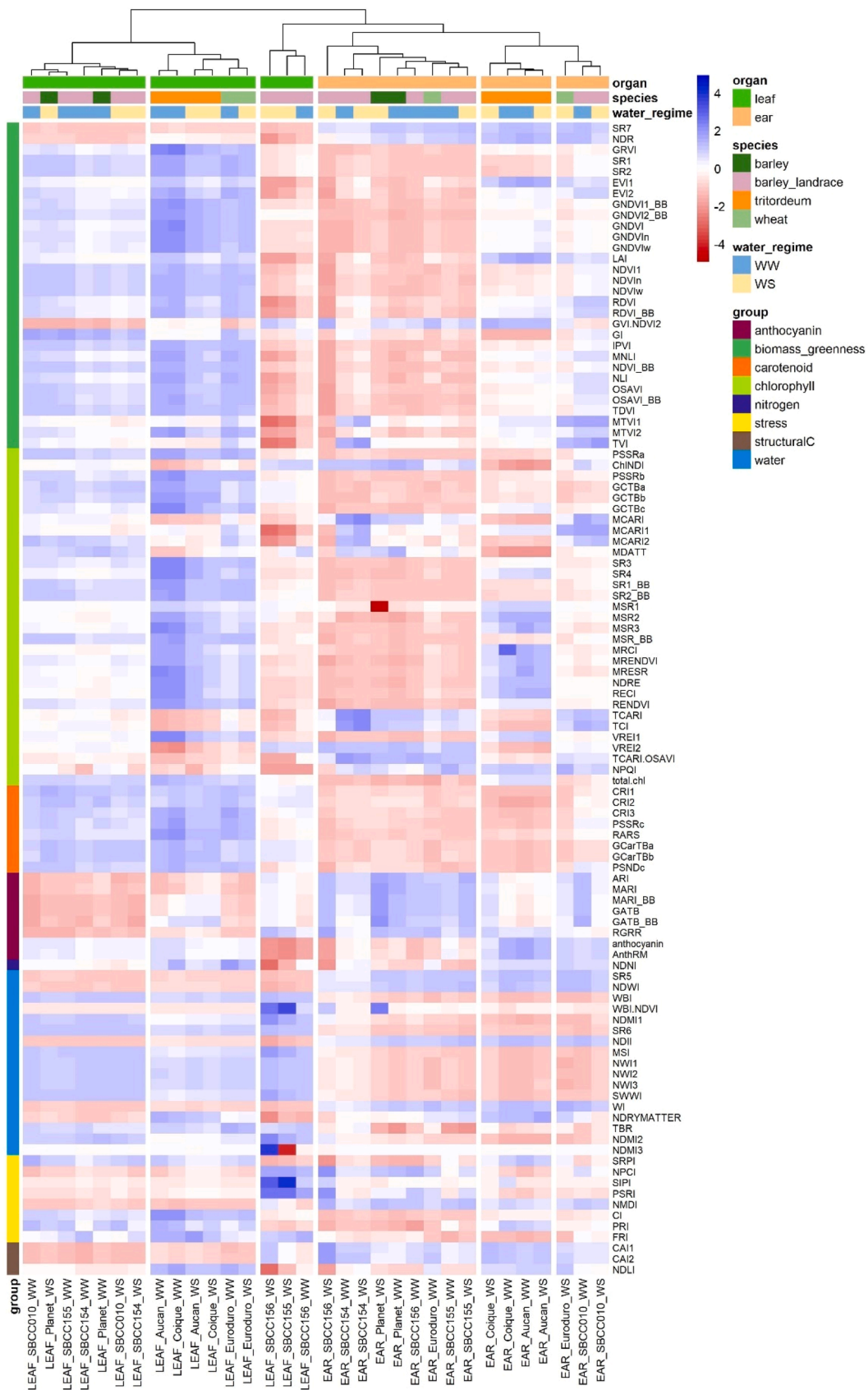


Fig. 7. Heatmap of spectral vegetation indices depending on organ, water treatment and genotype. Data were scaled by trait in row direction using mean values per group combination (organ × genotype × water regime). Abbreviations of the vegetation indices are explained in Supplementary Table S1. WS, water-stressed plants; WW, well-watered plants.

ARI was the most relevant under WS conditions in ears, being lowest in tritordeums, SBCC010 and Euroduro, particularly under WS conditions (Supplementary Fig. S4D).

3.6. Multivariate analysis to identify key traits contributing to yield-related traits and for water stress resilience

We built a PCA with all variables (in the case of spectral indices, only the top ones described above) that explained 43.4 % and 26.1 % of the variability by dimensions 1 and 2, respectively (Supplementary Fig. S5). The grouping according to cereal species (barley, wheat and tritordeum) was mainly emphasised. To better compare between genotypes and their tolerance to water stress, we generated a heatmap with \log_2 values

representing WS/WW ratios (Fig. 8) and PCAs for each water regime (Fig. 9). We observed that tritordeums showed the highest overall resilience for agronomic components, ear photosynthetic parameters, and organ temperatures (Fig. 8). Planet showed high stability of its photosynthetic and chlorophyll fluorescence traits in flag leaves, although it was among the least tolerant at agronomic level. Among the barley landraces, SBCC154 and SBCC156 were the most affected agronomically. Meanwhile, SBCC010 did not stand out in the resilience of specific traits, except LTD and Φ_{PSII} , but it did show average stability values for many agronomic and phenotypic traits. The PCAs in Fig. 9 summarise the results described throughout the text. Under WW conditions, we observed that among the barley landraces and tritordeums, SBCC010 and Coique stood out, being closest to the controls, mainly

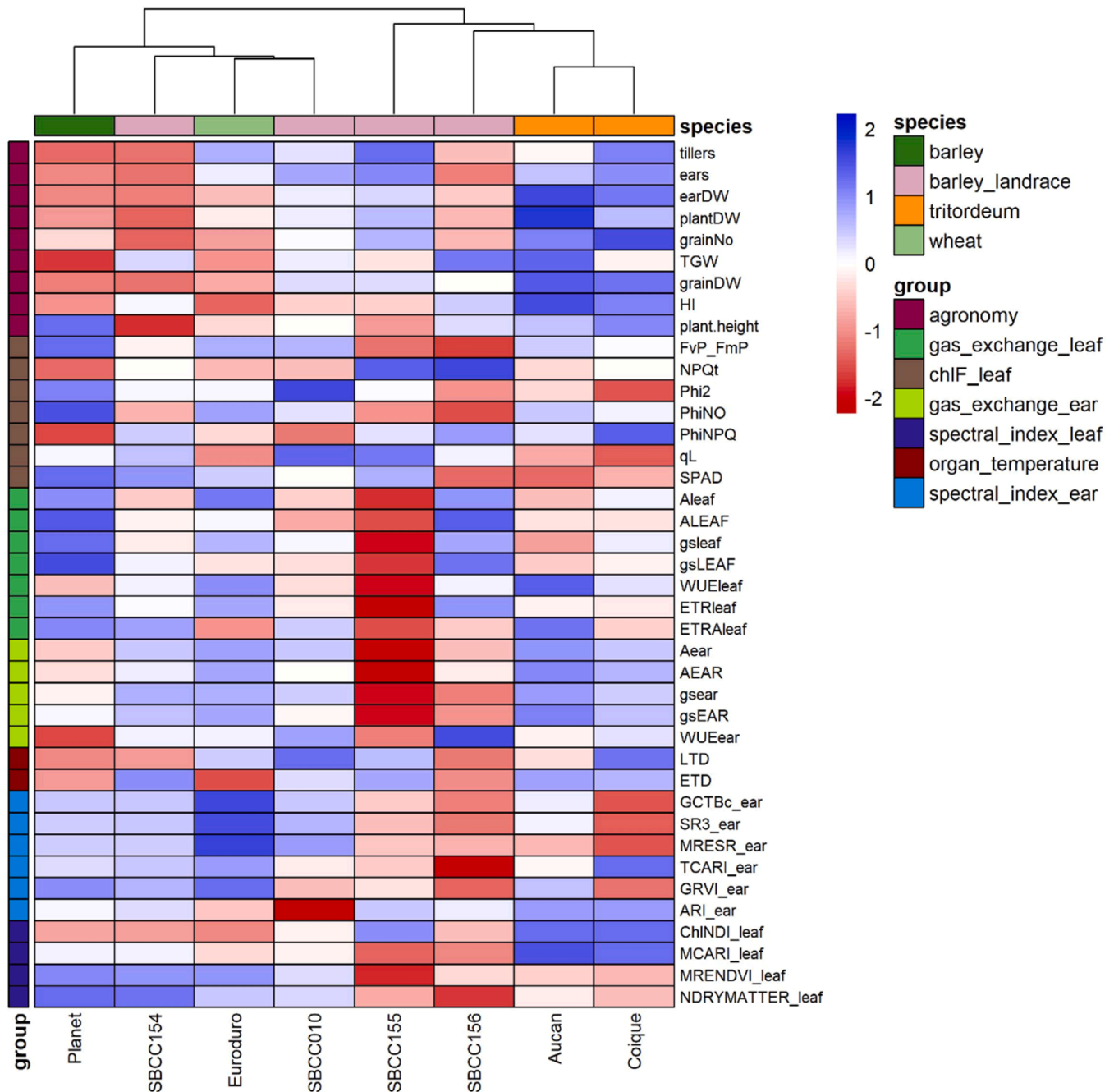


Fig. 8. Effect of water stress on agronomic components and phenotypic attributes during the reproductive period in eight cereal genotypes. Each trait was expressed as \log_2 ratio of water-stressed over well-watered plants. Data were scaled by trait in row direction per group combination (genotype \times water regime). Abbreviations of the traits are the same as in Figs. 2-7.

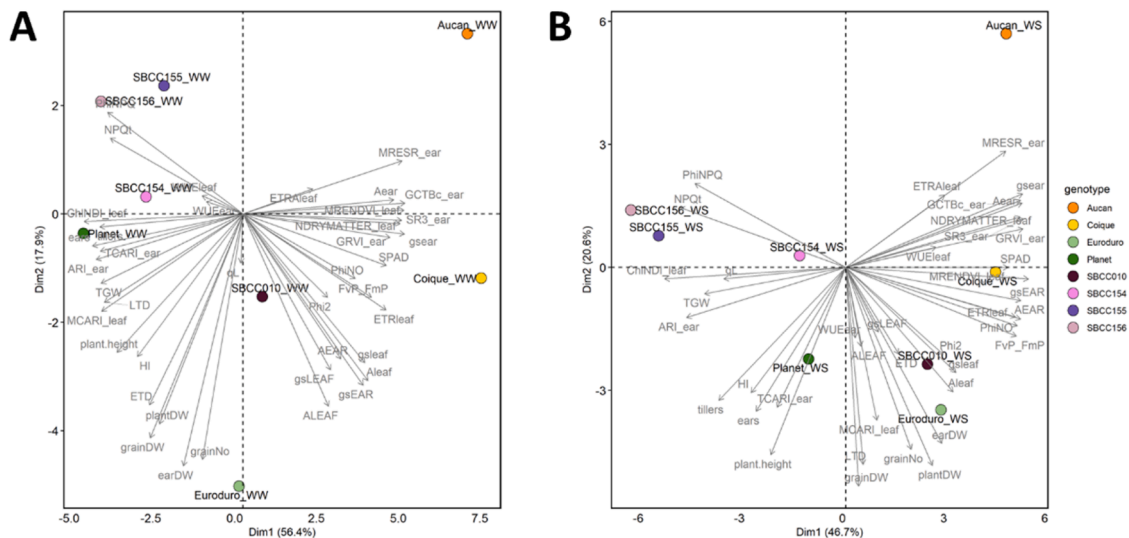


Fig. 9. Principal component analyses of agronomic components at harvest and physiological traits during reproductive period under (A) well-watered (WW) and (B) water stress (WS) conditions. Each physiological trait was averaged using the measurements taken at three growth stages (anthesis, early-grain filling and mid-grain filling). Abbreviations of the traits are the same as in Figs. 2-7.

with Euroduro that outperformed the rest in terms of agronomic components (Fig. 9A). In SBCC010 and Coique, the photosynthetic parameters of the leaf and ear, expressed both per area and total organ, as well as leaf and ear greenness and chlorophyll indices, were highest. Under WS conditions, a similar pattern was observed, but in this case SBCC010 and Coique were closer to Euroduro and Planet (Fig. 9B). The different photosynthetic and spectral parameters of the ear were outstanding in Coique, while the leaf photosynthetic and agronomic parameters were highest in Euroduro followed by SBCC010, Planet and Coique. Barley landraces SBCC155 and SBCC156 showed opposite values, with high results for NPQ and Φ_{NPQ} .

4. Discussion

4.1. Grain weight resilience to water stress by some barley landraces and tritordeums under extreme Mediterranean conditions is associated to grain number stability and harvest index

Under future climate conditions, the growth habit of spring cereal varieties will become even more important in the Iberian Peninsula compared to winter growth habits, as in many regions it may be difficult to achieve long periods of cold for vernalisation (Tun et al., 2021). For this reason, our study focused only on spring or facultative varieties. As a proof-of-concept design to achieve extreme Mediterranean drought conditions to identify key physiological traits for plant performance and resilience, we carried out a late-spring sowing under field conditions, where the plants grew under > 4.5 °C and < 80 % precipitation conditions compared to usual cereal growing season climatic conditions (Supplementary Fig. S1).

Attending to our agronomic results, a heterogeneous response was observed within barley landraces. Among them, we highlight the genotype SBCC010, a landrace with origin in the south of Iberian Peninsula, which presented the same grain dry weight per plant as modern varieties (Fig. 2A), but higher stability in response to contrasting water regimes (Fig. 2B). Yahiaoui et al. (2014) showed that barley landraces from the south of the Iberian Peninsula and the Ebro valley had high productivity under low production conditions and a delay in reaching heading stage, in line with our results for SBCC010 (Fig. 2 and Supplementary Fig. S2). This suggests that SBCC010 is a resilient variety reaching high yields under extreme hot and dry environments during the grain filling period by maintaining a high grain number per ear (Fig. 2), rather than high TGW as was observed in Yahiaoui et al. (2014). It is also

worth noting that SBCC010 is a 6-rowed cultivar, while the modern variety Planet and the other landraces are 2-rowed cultivars, affecting therefore TGW when we compare between them.

Different results were observed between the two tritordeums studied. Aucan presented a high drought tolerance but lower grain yield than the commercial wheat and barley varieties, in agreement with Kaka-bouki et al. (2020). It was one of the first commercial tritordeum varieties and, therefore, a breeding gap is expected when comparing with modern varieties. However, a second generation of tritordeums (Coique) already showed slightly lower grain dry weight values than Euroduro and Planet, differences that were not statistically significant and inexistent under WS conditions. This was due to a lower DSI in Coique compared with commercial barley and wheat, being our study the first report comparing these species under (future) extreme Mediterranean conditions. Although we must be cautious about generalising our agronomic results from only two environments and with a design focused on individual plants, these results suggest the high potential of tritordeum as adaptive cereal to Mediterranean conditions (Papadopoulos et al., 2024), showing that further breeding progress of this hybrid could close the existing yield gap compared to commercial varieties. The high grain filling period of tritordeums, also reported by Gallardo and Fereres (1993), and their high DSI for grain dry weight highlight their resilience during late growth stages. The maintenance of the number of grains per ear (as observed above for SBCC010) and the higher translocation of resources to the ear (lower DSI_{HI}) under WS conditions contributed to the grain weight stability in Coique.

Grain yield is a complex trait defined by the interaction of the environment with plant growth and development processes that occur throughout the life span of cereals (Carrera et al., 2023). The two main agronomical components that define grain yield are grain set and grain weight, where photosynthesis is the main physiological process involved (Reynolds et al., 2022). Based on our results, the grain number and, in the case of tritordeum, HI, are suggested as the main agronomic traits contributing to the stability of grain dry weight in SBCC010 and Coique compared to barley and wheat controls. Recent reviews carried out in wheat (Slafer et al., 2022) and for different cereals (Carrera et al., 2023) support our results, stating that the grain number per ear is a key trait determining grain yield in harsh environments. This trait is affected by flower production and flower survival, the latter mostly controlled by photosynthetic capacity during grain filling stages (Carrera et al., 2023 and references therein), motivating our further analyses of photosynthesis in green organs.

4.2. The photosynthetic capacity and greenness during the reproductive stage of leaves, but mainly ears, contribute the most to grain dry weight under extreme Mediterranean conditions

Leaf photosynthesis through gas-exchange measurements has been extensively studied in cereals, but not so much in other non-foliar photosynthetic organs. Previous research in barley awns (Hosseini et al., 2012), wheat awns and whole ears (Gómez et al., 2020; Maydup et al., 2014; Molero and Reynolds, 2020), rice panicles (Chang et al., 2020; Dharni et al., 2022) and even the panicles of the pseudo-cereal quinoa (Vergara-Díaz et al., 2024), leads us to suggest that photosynthesis of reproductive organs in cereals has a significant role in grain filling, particularly under abiotic stress conditions. However, among other issues, two main problem arises when ear photosynthesis contribution on grain yield is targeted. Firstly, the ear is a three-dimensional organ, which makes it difficult to measure with existing devices adapted to a laminar morphology, as well as the difficulty of expressing its contribution per area or per total organ (Dharni et al., 2022; Sanchez Bragado et al., 2016, 2020; Tambussi et al., 2021; Wu et al., 2022). Secondly, the complexity and time consuming of measuring plant gas-exchange at field conditions makes difficult to analyse ear gas-exchange along cereals reproductive stage.

In our study, we have carried out a pioneering characterisation of leaf and ear photosynthesis at three reproductive stages in a collection of cereals, including for the first time to our knowledge the whole ears of barley and tritordeum (Figs. 4 and 5). Although there is still a long way to improve the methodologies for ear photosynthetic characterisation, for instance by robust and high-throughput calculation of its three-dimensional area and the design of special chambers adapted to this organ (Molero and Reynolds, 2020; Wu et al., 2022; Dharni et al., 2022), our study on different cereals and growth stages sheds light on its relevance. Considering the genotypes studied and the three growth stages with our statistical model (LMM), our data indicated that A_{LEAF} was 13.4 and 7.5 $\mu\text{mol CO}_2 \text{ organ}^{-1} \text{ s}^{-1}$ during the reproductive period under WW and WS conditions, respectively, while A_{EAR} was 9.7 and 7.7 $\mu\text{mol CO}_2 \text{ organ}^{-1} \text{ s}^{-1}$. These results pointed that photosynthesis of the flag leaf had a higher drought susceptibility than that of the ear, demonstrating the ear resilience and high contribution to support grain filling (Figs. 8B and 9B).

The use of fluorimeters with fast measurement protocols has become popular on the market and in the scientific community, being a low-cost solution to characterize chlorophyll fluorescence parameters and chlorophyll content in a rapid manner (Fernández-Calleja et al., 2020). However, they have certain limitations that need to be taken into account. Due to their design, they are limited to laminar organs, mainly leaves, while the use of chlorophyll fluorescence imaging systems for other organs in the field is far from being implemented or still not considered a priority. The light-harvesting (related to chlorophyll fluorescence parameters) and CO_2 assimilation (gas exchange) capacities are generally coupled, but not as a rule, as they can respond at different speeds to stress events and other photosynthetic limitations may appear (Deans et al., 2019; Flexas et al., 2004). In our case, A_{leaf} was reduced under WS conditions, accompanied by a reduction in F_v'/F_m' and an increase in Φ_{NPQ} and NPQ_t , but Φ_{PSII} was not significantly modified. As g_s was severely affected by WS conditions, particularly in flag leaves (Fig. 4), this may indicate that the reduction of photosynthetic capacity was associated with the restriction of CO_2 supply to the site of carboxylation by diffusional effects, more than a limitation by the provision of energy for Calvin-Benson cycle. In our study, the fluorimeter demonstrated its usefulness in capturing genotypic variability during the reproductive period and the effects of water stress in late stages of this period. The values for the electron transport rate through PSII, measured with the IRGA's fluorimeter, and the maximum PSII quantum efficiency and chlorophyll content, measured with MultispeQ, showed the better photosynthetic capacity of tritordeums, Euroduro and SBCC010 flag leaves compared to the rest (Fig. 3 and Supplementary Table S2).

Time-course measurements highlighted its usefulness in identifying developmental stages of interest. For instance, as the developmental stage advanced, overall, WS plants presented higher Φ_{NPQ} rather than Φ_{NO} values, indicating that the photoprotection was carried out via dissipating excess energy as heat instead of other non-regulated processes (Khalil et al., 2021). Nevertheless, time-courses complicate the interpretation of the results, for which we suggest the integration of the results in models that facilitate their interpretation (e.g., LMMs).

Our previous studies together with that of other authors have shown that reproductive organs exhibit metabolic mechanisms of tolerance to abiotic stresses, including a particular pattern of the transcriptome, metabolome and proteome that differs from that of flag leaves in durum wheat, barley and rice (Hein et al., 2016; Jia et al., 2015; Lawas et al., 2019; Martínez-Peña et al., 2023; Sanchez-Bragado et al., 2020; Vicente et al., 2018, 2024; Vergara-Díaz et al., 2020a). The tolerance mechanisms include a greater stability in the expression of photosynthetic genes and the antioxidant system, and nitrogen and water status, that could contribute to the stabilisation of photosynthetic capacity as it was observed in our study, although future metabolic studies are required in this cereal collection. The observed higher ear photosynthetic capacity and stomatal conductance under WS of SBCC010 and Coique (Figs. 4B and 8B) could explain the maintenance of the grain set by providing greater photoassimilates to the developing grains and, in that way, avoid grain abortion caused by a source limitation due to drought impairments in sucrose production (Barnabas et al., 2008). In the end, the proximity of ear tissues such as awns, glumes and lemmas facilitate the transport of photoassimilates to the grains during late stages (Sanchez-Bragado et al., 2020). Additionally, ear photosynthesis in our study is underestimated due to the high respiration of developing grains that greatly influence gross photosynthesis (Chang et al., 2020; Hosseini et al., 2012; Gamez et al., 2020; Molero and Reynolds, 2020), although ear bracts and green pericarps may reflex part of the CO_2 released by grain respiration (Bort et al., 1996; Sanchez-Bragado et al., 2020; Tambussi et al., 2007). Further studies to deepen canopy photosynthesis at late growth stages should cover in detail the CO_2 fluxes during daily cycles in the different green organs, including net photosynthesis, respiration during the day and the night, and CO_2 refixation capacity, although this last process is methodologically a challenge.

It is interesting to note that leaf photosynthesis was not correlated with ear photosynthesis on an area basis or per organ in any water treatment in cereals (Supplementary Fig. S3). The leaf photosynthesis decrease does not stimulate that of the ear, or vice versa, at least not significantly. Molero and Reynolds (2020) also showed an independent response of leaf and ear photosynthesis for a panel of 45 wheat genotypes. This may indicate that there are no compensatory effects of photosynthesis between organs under water stress conditions. To date, breeding programs have mainly focused on leaf photosynthesis improvement, what is clearly observed in our study when data for modern varieties is compared with barley landraces and tritordeum (Fig. 4), but not on ear photosynthesis improvement (Reynolds et al., 2022; Tambussi et al., 2021). Although this fact needs to be observed in more studies with different growth conditions, our results suggest that (i) photosynthesis taking place in each organ operates independently, (ii) ear photosynthesis is relevant during late stages in drought-resilient crops, and (iii) ears are more adapted to harsh conditions than leaves. We therefore consider that both leaf and ear photosynthesis should be included in the breeding agenda to improve canopy photosynthesis and grain yield. The lower agronomic and physiological performance of genotypes with early senescence of green photosynthetic organs, such as SBCC155 and SBCC156, demonstrate the relevance of stay-green phenotypes for stress resilience.

4.3. High-throughput phenotyping for in vivo, non-destructive and rapid characterisation of green photosynthetic organs highlights the resilience of ears and SBCC010 and tritordeum varieties under limiting growth conditions

Novel HTPP approaches based on hyperspectral data linked to plant metabolism (Vergara-Díaz et al., 2020a, 2020b; Vicente et al., 2024) or thermal profiles (Mertens et al., 2023; Vergara-Díaz et al., 2020c; Vicente et al., 2018) are being used to understand the differential behaviour and resilience that may experience the various plant photosynthetic organs during cereal growth cycle, being a complement to gas exchange measurements. There was a large genotypic variability in organ temperature (Fig. 6). This measurement is dependent on the stage of development, the interplant variability (different tillers) and it is also susceptible to daily environmental conditions. Therefore, the measurements must be done in the shortest possible time on sunny days, use a control to standardise the results, measure the main stems on each plant and, in our case, integrate the measurements at different stages of development to get an overview of each species behaviour. In our experiment, ears were generally cooler than flag leaves, on average 0.5 °C under WW and 1.3 °C under WS conditions. This contrasts with previous experiments for field-grown wheat, where ears were at higher temperatures than leaves (Maydup et al., 2014; Vicente et al., 2018), and to the fact that the ears are at the top of the canopy, exposed to higher radiation. Our results therefore indicated that (i) ears were able to dissipate heat better than leaves, and/or (ii) leaves had an accelerated senescence compared to ears, particularly under stress conditions. Although one or both hypotheses may operate and are complementary, it is clear that ears had high thermal stability in contrasting water regimes as the reproductive stage progresses and avoiding the massive oxidative damage that is produced by heat stress in chloroplasts (Sadok et al., 2020). These data support the lower loss of photosynthetic capacity observed in ears compared to leaves under WS conditions. Particularly, tritordeum varieties showed the lowest (more negative) organ temperature depression (OTD) values for flag leaves and ears, suggesting their better resilience to extreme Mediterranean conditions compared with barley and wheat controls.

The integration of three-point measurements during the reproductive stage showed that flag leaves are generally greener and with higher water content than ears, as it was expected, matching with the higher photosynthesis per area observed in leaves (Fig. 4). However, as mentioned above, ears have an intricate three-dimensional structure, with several tissues overlapping, and high respiration rates that make difficult to calculate ear photosynthesis (and likely underestimate it). As a proxy for estimation of photosynthetic capacity and stress resilience, we observed that several of the genotypes with better leaf and ear photosynthetic capacity were associated with better vegetative indices for greenness, chlorophyll content, and nitrogen and water status, as was the case of Euroduro, tritordeums and SBCC010. It has been shown that water content of ears was more stable than in flag leaves under water stress in durum wheat (Martínez-Peña et al., 2023). Better water status in ears may be related to improved osmotic adjustment, avoiding massive cell dehydration (Tambussi et al., 2005). The worst performance of SBCC155 and SBCC156 (early senescence) could also be detected with spectral measurements, as well as with the pulse-amplitude modulated chlorophyll fluorometer. Interestingly, estimation of anthocyanins with ARI index showed that these pigments were more accumulated in ears compared to leaves, but lower in tritordeums, SBCC010 and Euroduro, the genotypes with better performance or tolerance under stress conditions (Figs. 8 and 9). Since the role of anthocyanins in delaying plant senescence has been proposed (Landi et al., 2015), our results may suggest that (i) ears constitutively have higher anthocyanin levels to act as “sunscreens” and protect them for the higher radiation exposure, being one of the tolerance mechanisms involved in ear resilience, and that (ii) genotypes with lower anthocyanin accumulation could be related to a lower need for stress protection.

Our data demonstrated the capacity of thermal sensors and spectroradiometers to obtain in vivo, non-destructive and rapid information of plant performance under field conditions (Araus et al., 2022), whose future development can most likely incorporate more detailed metabolic measurements that save time and costs in large-scale breeding programs. Further study of the biochemical and molecular mechanisms involved will contribute to the design of drought-resilient crops coping with climate change conditions.

5. Conclusions

Overall, our pioneering phenotypic characterisation of different green organs for various cereal species along the whole reproductive stage highlighted the importance of organ-specific stay-green phenotypes and the role of the ear as a drought-tolerant and thermostable organ, being urgent to include ear photosynthesis in breeding programs for Mediterranean agroenvironments. Furthermore, our results pointed out barley landrace SBCC010 and tritordeum variety Coique as promising germplasm for coping with future Mediterranean climatic constraints. We conclude that the study and selection of phenotypic traits in different plant organs, relying on old germplasm as barley landraces and new cereal hybrids as tritordeum, will be of great value for the development of drought-resilient cereals under future extreme environments. Further research is needed under multiple field-limiting growing conditions with a plot design to validate the results obtained at the agronomic level.

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CRedit authorship contribution statement

Ander Yoldi-Achalandabaso: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Aitor Agirresarobe:** Investigation, Formal analysis. **Arturs Katamadze:** Writing – review & editing, Investigation, Formal analysis. **Giulia Burini:** Investigation, Conceptualization. **Omar Vergara-Díaz:** Writing – review & editing, Investigation. **Mariana Mota:** Writing – review & editing, Resources, Methodology. **Cristina Oliveira:** Writing – review & editing, Resources. **Usue Pérez-López:** Supervision, Funding acquisition, Conceptualization. **Rubén Vicente:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

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Supplementary materials

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Data availability

Raw data, including biological replicates, are available from the corresponding author on reasonable request.

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