JOURNAL OF WATER AND LAND DEVELOPMENT
e-ISSN 2083-4535
Polish Academy of Sciences (PAN) Institute of Technology and Life Sciences – National Research Institute (ITP – PIB)

JOURNAL OF WATER AND LAND DEVELOPMENT
DOI: 10.24425/jwld.2025.156056
2025, No. 67 (X–XII): 240–252

Photosynthetic efficiency and gene expression responses of maize (*Zea mays* L.) seedlings to diverse abiotic stresses

Hazem M. Kalaji[1)], Piotr Dąbrowski[2)], Justyna Nowakowska[3)], Piotr Kiełtyk[3)], Jacek Wróbel[4)], Anna M. Linkiewicz*[3)]

[1)] Warsaw University of Life Sciences, Institute of Biology, Department of Botany and Plant Physiology, 159 Nowoursynowska St, 02-776, Warsaw, Poland

[2)] Warsaw University of Life Sciences, Institute of Environmental Engineering, 159 Nowoursynowska St, 02-776, Warsaw, Poland

[3)] Cardinal Stefan Wyszynski University in Warsaw, Faculty of Biology and Environmental Sciences, Institute of Biological Sciences, 1/3 Wóycickiego St, 01-938, Warsaw, Poland

[4)] West Pomeranian University of Technology in Szczecin, Department of Bioengineering, 17 Słowackiego St, 71-434, Szczecin, Poland

* Corresponding author

RECEIVED 16.06.2025 ACCEPTED 19.11.2025 AVAILABLE ONLINE 30.12.2025

Abstract: Abiotic stress can cause long-term disruptions in the function of the photosynthetic apparatus, while also modulating the expression of defence-related genes like dehydrin 1 (*dhn*1) and heat shock protein 70 (*hsp*70). This study assessed the effects of prolonged drought, flooding, salinity, heavy metals, heat, low temperatures, and frost on photosynthetic efficiency in maize by combining chlorophyll fluorescence analysis with the *dhn*1 and *hsp*70 expression. All stress conditions resulted in significant reductions in photosynthetic performance, particularly in the maximum quantum yield for primary photochemistry (F_V/F_M), total complementary area between the fluorescence induction curve (Area) and time to reach the maximal fluorescence (t_{FM}), indicating impairment of photosystem II (PSII). The stress and time-dependent variability in chlorophyll fluorescence parameters underscores their value as biomarkers of photosynthetic stress. The high sensitivity of performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors (PI_{ABS}) and total performance index for energy conservation from photons absorbed by PSII until the reduction of photosystem I terminal electron acceptors (PI_{total}) confirms their relevance in eco-functional assessments. Gene expression analysis revealed downregulation of *dhn*1 under drought, flood, and salinity, while *hsp*70 remained stable, suggesting either stress exhaustion or activation of alternative protective mechanisms. The transient nature of *dhn*1 expression may reflect an early and short-lived response to stress.

This study underscores the value of integrating chlorophyll fluorescence with gene expression analysis to elucidate crop physiological and molecular responses to prolonged abiotic stress, providing essential insights for safeguarding photosynthesis and improving maize stress tolerance.

Keywords: abiotic stresses, bioindicator, chlorophyll *a* fluorescence, dehydrin 1 (*dhn*1), heat shock protein 70 (*hsp*70), long-term stress response, non-invasive biomarkers, relative gene expression

INTRODUCTION

Plants continuously face various biotic and abiotic stressors (Mantri *et al.*, 2012), with drought, flood, salinity, heavy metals, heat, and cold being most critical. These disrupt physiological and biochemical functions, reducing growth and yield, and require genetic and agronomic interventions. Drought limits shoot or root elongation, induces stomatal closure, and impairs photosynthesis via reduced CO_2 uptake and ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCo) activity (Gu *et al.*, 2019; Ahluwalia, Singh and Bhatia, 2021; Mubeen *et al.*, 2021; Yang *et al.*, 2021). It lowers leaf water content and damages

photosynthetic organs (Song, Zhou and He, 2021), reduces transpiration (Klimešová *et al*., 2021), and alters water use efficiency (*WUE*) depending on the severity of the stress (Farooq *et al*., 2009). Photosynthetic efficiency declines (Chen *et al*., 2021) due to less CO_2 assimilation (Helm *et al*., 2020).

Flooding, intensified by climate change, causes hypoxia or anoxia, impairing root respiration and nutrient uptake, leading to chlorophyll degradation, stomatal closure, and biomass loss (Luan *et al*., 2018; Pan *et al*., 2021; Qi *et al*., 2021). Prolonged submergence causes cell death and yield loss; flooding also promotes the spread of soil-borne diseases and toxin accumulation.

Salinity induces osmotic stress, nutrient imbalance, and reduced biomass and stomatal conductance, with effects varying depending on the genotype and growth stage (Iqbal, S. *et al*., 2020). High salinity reduces chlorophyll fluorescence (Shin, Bhandari and Lee, 2021), triggers antioxidant responses (Zheng *et al*., 2019), and lowers chlorophyll content (Tobiasz-Salach, Mazurek and Jacek, 2023). Plants respond to environmental stress through ion regulation and osmolyte synthesis (Sevgi and Leblebici, 2023).

Heavy metals (Cd, Pb) are highly toxic, affecting morphology, metabolism, and yield (Kohli *et al*., 2017; Chtouki *et al*., 2021; Baruah *et al*., 2023). Even at low concentrations, Cd causes chlorosis, necrosis, and embryonic damage in maize (Ruiz-Huerta *et al*., 2022).

Heat stress impairs photosynthesis, affecting photophosphorylation, the Calvin cycle, and thylakoid integrity (Mihaljević *et al*., 2020; Lakshmi *et al*., 2023). It deactivates Rubisco activase (RCA) (Scafaro *et al*., 2016), downregulates chloroplast proteins (Wang *et al*., 2019), and induces reactive oxygen species (ROS), damaging photosystem II / photosystem I (PSII/PSI) (Distéfano *et al*., 2017; Iqbal, H. *et al*., 2020; Mustafa *et al*., 2021).

Cold stress inhibits photosynthesis and enzyme activity (Zhang *et al*., 2020), damages chloroplasts (Lamers, Meer van der and Testerink, 2020), reduces stomatal conductance and metabolite transport (Pandey *et al*., 2018; Ramazan *et al*., 2021), disrupts glycolysis and the Tricarboxylic acid cycle (TCA) (Li *et al*., 2020). It lowers electron transport efficiency (Bhattacharya, 2022), and frost causes dehydration and membrane damage (Chen *et al*., 2017; Wang, Head and Hauser, 2023). Tolerance involves complex molecular responses (Kosová, Vítámvás and Prášil, 2014; Dąbrowski *et al*., 2024a; Kosová *et al*., 2025).

Physiological responses (e.g., photosynthetic efficiency) are closely linked to molecular mechanisms (e.g., gene expression). This study integrates chlorophyll fluorescence (ChFl) analysis and real-time polymerase chain reaction (RT-qPCR)-based gene expression of dehydrin 1 (*dhn*1) and heat shock protein 70 (*hsp*70) to correlate PSII performance with transcriptional activity (Kalaji and Guo, 2008). This dual approach reveals a nuanced framework for studying physiological and molecular responses, indicating metabolic prioritisation or adaptive exhaustion. It may also provide robust biomarkers for stress tolerance and inform about strategies to enhance crop resilience.

Physiological changes, such as alterations in photosynthetic performance, occur before visible morphological modifications in plants exposed to stress (Kalaji and Guo, 2008). The analysis of ChFl kinetics has proven to be a practical approach for evaluating the impact of environmental stresses on photosynthesis. This method provides valuable insights into the electron transport process, focusing on PSII – the most stress-sensitive component of the photosynthetic apparatus, which plays a critical role in a plant's response to adverse conditions (Baker and Rosenqvist, 2004). Among the analytical approaches, the JIP-test is particularly significant, as it evaluates PSII functionality and its response to environmental stressors (Dąbrowski *et al*., 2024b; Dąbrowski *et al*., 2024c). This comprehensive analysis enhances the understanding of PSII dynamics under different unfavourable environmental conditions (Strasser, Tsimilli-Michael and Srivastava, 2004; Zivčák *et al*., 2014).

Several external stimuli trigger oxidative stress in the plant cells, and chaperone proteins can alleviate damage caused by ROS. Stressors that disrupt photosynthesis also trigger signalling cascades involving ROS, abscisic acid (ABA), and other pathways, which lead to the activation of stress-protective genes, including: *dhn*1 – involved in cold/drought tolerance, stabilisation of proteins and membranes, *hsp*70 – helps refold misfolded proteins and prevents aggregation under heat or oxidative stress. To investigate the molecular response of the *Zea mays* (L.) leaves, which were put under three prolonged stress factors (drought, flood and salinity), the expression of the *dhn*1 and *hsp*70 was investigated.

Dehydrins (*dhn*1) belong to numerous classes of chaperone proteins, which are involved in the protection of plasma membranes and prevent destabilisation of deoxyribonucleic acid (DNA) conformations in the nucleus (Sun *et al*., 2021). The cytoplasmic dehydrin 1 encoded by the *dhn*1 gene plays an important role in drought and osmotic stress tolerance in *Arabidopsis thaliana* (L.) Heynh. and many crops (Timperio, Egidi and Zolla, 2008; Sena *et al*., 2018; Sun *et al*., 2021). Findings in maize suggest that *dhn*1 messenger ribonucleic acid (mRNA) expression is an early stress response (Jiao *et al*., 2022), with the peak in roots around 72 h after the stress application. However, dehydrin proteins are highly stable and can persist in cells long after mRNA levels decline, ensuring prolonged protection during stress. The other studied gene, *hsp*70, encodes a member of *hsp*70 chaperones mainly located in the plant cytoplasm and plastids. In plants, their expression often varies in response to environmental biotic and abiotic stresses (Timperio, Egidi and Zolla, 2008; Rana *et al*., 2018) mainly through modification of protein transport and plant development (Seguí-Simarro, Testillano and Risueño, 2003; Su and Li, 2010; Sarkar, Kundnani and Grover, 2013; Bionda *et al*., 2016), to ensure an important physiological role assigned to the protection against drought (Aghaie and Tafreshi, 2020), salinity (Rana *et al*., 2018), flood (Saleem *et al*., 2024), and heavy-metal exposure (Moreira-de-Sousa, Souza de and Fontanetti, 2018).

This study aimed to precisely determine the changes caused by prolonged abiotic stresses on photosynthetic efficiency through the analysis of chlorophyll fluorescence and the expression of *dhn*1 and *hsp*70 genes in maize leaves. Moreover, to understand how stress affects the function of PSII and whether maize, in response to prolonged stress, employs defence mechanisms based on the expression of the selected genes.

MATERIALS AND METHODS

EXPERIMENT DESIGN AND PLANT GROWTH CONDITIONS

The experiment was conducted in the greenhouse of Warsaw University of Life Sciences (Pol.: Szkoła Główna Gospodarstwa Wiejskiego – SGGW) between March 13th and May 3rd, 2024. The

Zea mays (L.) cv. 'SM Perseus' from Hodowla Roślin Smolice Sp. z o.o. the IHAR group was used. Under greenhouse conditions, plants were illuminated only by sunlight. The microclimate parameters in the greenhouse were as follows: temperature ~23°C, light intensity 200 µmol (photon)·m^{-2}·s^{-1} with 16/8 day/night ratio, CO_2 level ~710 ppm, and relative humidity (RH) ~65%. Seeds sprouted in dark conditions 10 days after germination (March 12th). The experiment began on March 22nd, 2024, with five seedlings placed in ten single pots (50 plants in total) of 700 cm^3 capacity each, filled with standard podzolic soil (soil specific gravity ~1.7 g·cm^{-3}) from Prof. Marian Górski Experimental Station in Skierniewice (Poland), fertilised with manure and legumes. Soil moisture was maintained at 60% of field capacity. The seedlings were grown under the same conditions (no stress applied) until April 5th, when eight different stresses (drought, flood, salinity, heavy metals (cadmium (Cd) and lead (Pb)), heat, low temperature, and frost) were applied, except for the two control pots.

To induce drought stress, watering was withheld for the last 14 days of the experiment for half of the plants being tested, causing the soil moisture to decrease gradually to approximately 10%. To simulate flooding, during the last 14 days of the experiment, half of the plants were submerged in water sourced from three quaternary wells, each with a depth of about 30 m and a capacity of 40 m^3·h^{-1}. The water level was maintained approximately 10 mm above the soil surface. To induce salinity stress, half of the tested plants were irrigated with saline water (NaCl) during the last 14 days of the experiment, maintaining a salinity level of 120 mmol NaCl. Heavy metal stresses were applied by 0.134 g·dm^{-3} of $PbCl_2$ or 0.163 g·dm^{-3} of $CdCl_2$. The temperature was lowered to –16°C for the application of frost stress and to 7°C for the low-temperature treatment.

From each pot, three out of five plants were chosen for measurement. The study was conducted on the second, third and fourth fully developed leaves of each plant, taking three measurements on each leaf, resulting in 15 measurements from each environment. Samples were taken from the top, middle and bottom of the leaf blade at three-weekly intervals.

CHLOROPHYLL FLUORESCENCE AND CHLOROPHYLL CONTENT MEASUREMENTS

One leaf from each plant in each pot was randomly selected for the measurement of chlorophyll *a* fluorescence. This was assessed using a HandyPEA fluorimeter (Hansatech Instruments Ltd., UK), and all chlorophyll fluorescence (ChFl) parameters measured after dark adaptation for at least 25 min (JIP-test) are presented in Figure S1. Each leaf sample was illuminated with continuous saturating actinic light (3.500 µmol (photon)·m^{-2}·s^{-1}) for 1 second. Measurements were performed on April 12th, 15th and 24th of 2024, 8, 11 and 20 days after the first stress application, respectively.

Chlorophyll content, flavonols and nitrogen balance index were measured using a Dualex device (Force-A, France) on 8th, 15th, 19th and 24th April, corresponding to 4th, 11th, 15th, and 20th days after stress application, respectively. Measurements were taken on the same leaves as those used for chlorophyll fluorescence.

GENE EXPRESSION STUDY – EXPRESSION OF TWO SELECTED GENES IN MAIZE LEAVES UNDER ABIOTIC STRESS

The relative gene expression was analysed in 16 plants subjected to drought, flood, and salinity treatments, compared to non-treated plants as a control (3–5 plants per treatment). On the 21st day after the initial stress application, samples of fresh maize leaves were frozen in liquid nitrogen and stored at –80°C. For the ribonucleic acid (RNA) isolation, 100 mg of frozen material per plant was ground in liquid nitrogen and subjected to Fenozol treatment following the manufacturer's instructions (A&A Biotechnology, Gdańsk, Poland). Obtained RNAs were immediately cleaned from deoxyribonucleic acids (DNAs) with Tornado™ DNAse (2 U/µl), following the manufacturer's instructions (A&A Biotechnology Gdańsk, Poland). The RNA quantity and quality were determined using the DS-11 FX spectrophotometer (TK Biotech, DeNovix, USA).

For each reaction of reverse transcription, 1 µg of cleaned RNA was used and proceeded with High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) according to the manufacturer's instructions. The reverse transcription (RT) reaction was programmed in C1000™ Thermal Cycler (Bio-Rad CA, USA), following three steps: 1) 10 min at 25°C, 2) 120 min at 37°C, 3) 5 min at 85°C, then held at 4°C.

The quantitative polymerase chain reactions (qPCRs) were performed using 1–10 ng of complementary deoxyribonucleic acid (cDNA) with primers designed for two stress-responding genes encoding dehydrin 1 (*dhn*1) and heat shock protein 70 (*hsp*70), and two genes encoding housekeeping proteins – actin 1 (*act*1) and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) (Tab. 1), frequently used for the relative expression analyses in many crops, including maize (Hu *et al*., 2010; Manoli

Table 1. Primer sequences for stress-responding and reference genes in maize

Gene	Forward primer 5'–3'	Reverse primer 5'–3'
*dhn*1[1)]	CATCATGGAGTACGGTCAGC	ACTAGGTTGCCGTACTGGTC
*hsp*70[1)]	ACCACTTCGTCCAGGAGTTCAA	GCCCTCATACAGCGAGTCAATCT
*act*1[2)]	ACCAAGCAGCATGAAGATCAAG	ACCCTCCAATCCAGACACTGTAC
GAPDH[2)]	ACTGTGGATGTCTCGGTTGTTG	CCTCGGAAGCAGCCTTAATAGC

[1)] stress-responding gene.
[2)] housekeeping gene.
Explanations: *dhn*1 = dehydrin 1, *hsp*70 = heat shock protein 70, *act*1 = actin 1, *GAPDH* = glyceraldehyde 3-phosphate dehydrogenase.
Source: own study.

et al., 2012; Jin *et al*., 2019; Li *et al*., 2023). All primer sequences were designed using the NCBI Nucleotide database (https://www.ncbi.nlm.nih.gov/) for exon-exon translated regions, 22–25 nucleotide long, melting temperature – Tm < 60°C, product size < 100 bp, and narrowed to *Zea mays* (L.) (NM_001111949.1). The specificity of the newly designed primer sequences was verified using NCBI Blastn (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) against the *Zea mays* (L.) (taxid: 4577) transcript database.

For the expression analysis, three to five plants that had been subjected to a given stress, as well as three control plants, were used. Three qPCR analyses were performed, using cDNA from a minimum of three plants. Two analytical replicates were performed for each gene, using the Thermo ABI QuantStudio 7 Flex real-time PCR instrument (Thermo Fisher Scientific Inc, Waltham, MA, USA) and the PowerUp™ SYBR™ Green Master Mix Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA), with the thermal profile: deactivation for 2 min 50°C, initial denaturation at 95°C for 10 min, then 40 cycles of denaturation at 95°C for 15 sec, annealing at 60°C for 15 sec, extension for 1 min at 72°C. The presence of the single amplicon was confirmed as a single peak of the melting curve. Two negative controls (NC) were included for the qPCR: one, "NC-RT", consisted of all compounds except MultiScribe™ RT; the second, "H_2O", consisted of diethyl pyrocarbonate (DEPC) treated, nuclease-free water (A&A Biotechnology, Gdansk, Poland) added instead of cDNA.

The gene expression data were analysed using Applied Biosystems' DataAssist 3.01 software (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Normalisation and visualisation of the expression of the *dhn*1 and *hsp*70 genes relative to the expression levels of the reference genes and the control plants were performed using a relative quantification strategy based on the ΔCT and log2-ΔΔCT methods (Schmittgen and Livak, 2008), employing the RQdeltaCT package (Zalewski, 2024) within the R statistical programming environment, R version 4.4.2 (R Core Team, 2024).

STATISTICS

Induction curves of chlorophyll *a* fluorescence and differential ΔVt curves were obtained by subtracting the control curve from the first sample for maize under various abiotic stress treatments, control, drought, flood, salinity, Cd, Pb, heat, low temperature, and frost. In these plots, the black curve represents chlorophyll *a* fluorescence of control plants and is included in each stress treatment panel as a reference. Changes in JIP-test chlorophyll *a* fluorescence parameters under different abiotic stress treatments on three dates (April 12th, 15th and 24th, 2024) are presented as radar plots and as boxplots. Chlorophyll *a* fluorescence analysis was performed on 30 control plants (not subjected to stress treatments), with 15 plants per stress treatment on each observation date. The fluorescence parameter values presented on radar plots were averaged for each treatment, normalised to the control group (set as 100%), and expressed as percentage changes (either increases above or decreases below 100%) relative to the control. Chlorophyll content (Chl), flavonoids (Flav), and the nitrogen balance index (NBI) of maize under different stress treatments were measured on four dates: April 8th, 15th, 19th, and 24th, 2024, and are displayed as boxplots. Measurements for Chl, Flav, and NBI were taken from 30 control plants and 15 plants per stress treatment on each observation date.

To compare chlorophyll *a* fluorescence parameters, as well as Chl, Flav, and NBI values, across the treatments, a non-parametric Kruskal–Wallis test was used, followed by post hoc pairwise Wilcoxon rank-sum tests (Logan, 2010) with the Holm correction for multiple comparisons (Holm, 1979). Non-parametric tests were chosen due to deviations from normality, as assessed with the Shapiro–Wilk test. All statistical analyses were performed in R version 4.4.2 (R Core Team, 2024).

RESULTS AND DISCUSSION

CHLOROPHYLL A FLUORESCENCE AND CHLOROPHYLL CONTENT

None of the applied stress factors altered the OJIP chlorophyll fluorescence curves measured on the 8th day (Fig. 1), indicating that there was no early impairment of photosynthesis. By the 11th day, frost stress caused severe flattening of the OJIP curve, which may indicate severe impairment of photosystem II (PSII) and downstream electron transport. Salinity and Cd stress reduced all points except O, reflecting disruptions in energy trapping and electron transport. Heat and low temperature lowered I and P phases, indicating impaired electron flow beyond Q_A and possible photosystem I (PSI) related dysfunction. Drought, flood, and Pb showed no early deviations. On the 20th day, drought increased O with reduced I and P, suggesting over-reduction and lowered photochemical efficiency. Heat curves resembled frost damage. The Cd stress intensified; low temperature effects plateaued, and salinity showed a lower P point. Flood and Pb slightly increased P, which may signal partial recovery or adaptation. Fluorescence changes reflected primary quinone acceptor of PSII (Q_A) redox status (Tsimilli-Michael, 2020), with frost and heat showing the strongest O–P alterations, salinity and Cd affecting J–I (Schansker, Tóth and Strasser, 2005), and low I–P indicating chloroplast structural damage (Strasser *et al*., 2010).

The stress factors altered certain JIP test parameters (Fig. 2). Drought and salinity increased fluorescence when all photosystem II reaction centres (PSII RCs) are open (F_0) and absorption flux per reaction centre (ABS/RC), but decreased maximal fluorescence, when all PSII RCs are closed (F_M), maximal variable fluorescence at time t (F_V), maximum quantum yield for primary photochemistry (F_V/F_M), maximum quantum yield for primary photochemistry at any time t (φ_{Po}), performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors (PI_{ABS}), total performance index for energy conservation from photons absorbed by PSII until the reduction of PSI end electron acceptors (PI_{total}) and efficiency/probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side RE (δ_{Ro}). Flood reduced F_M, F_V/F_M, energy flux not intercepted by a reaction centre (DI_0/RC), electron flux reducing end electron acceptors at the PSI acceptor side, per RC (RE_0/RC), φ_{Po}, and PI_{ABS} only on the 20th day. The Cd and Pb lowered F_0 early, and later reduced F_M, F_V, φ_{Po}, efficiency/probability that an electron moves further than Q_A^- (the reduced form of Q_A) (ψ_{Eo}), quantum yield for electron transport (φ_{Eo}), PI_{ABS}, PI_{total}, δ_{Ro}, while increasing ABS/RC, DI_0/RC. Heat increased ABS/RC, normalised the total area above the OJIP curve (S_m), electron transport flux

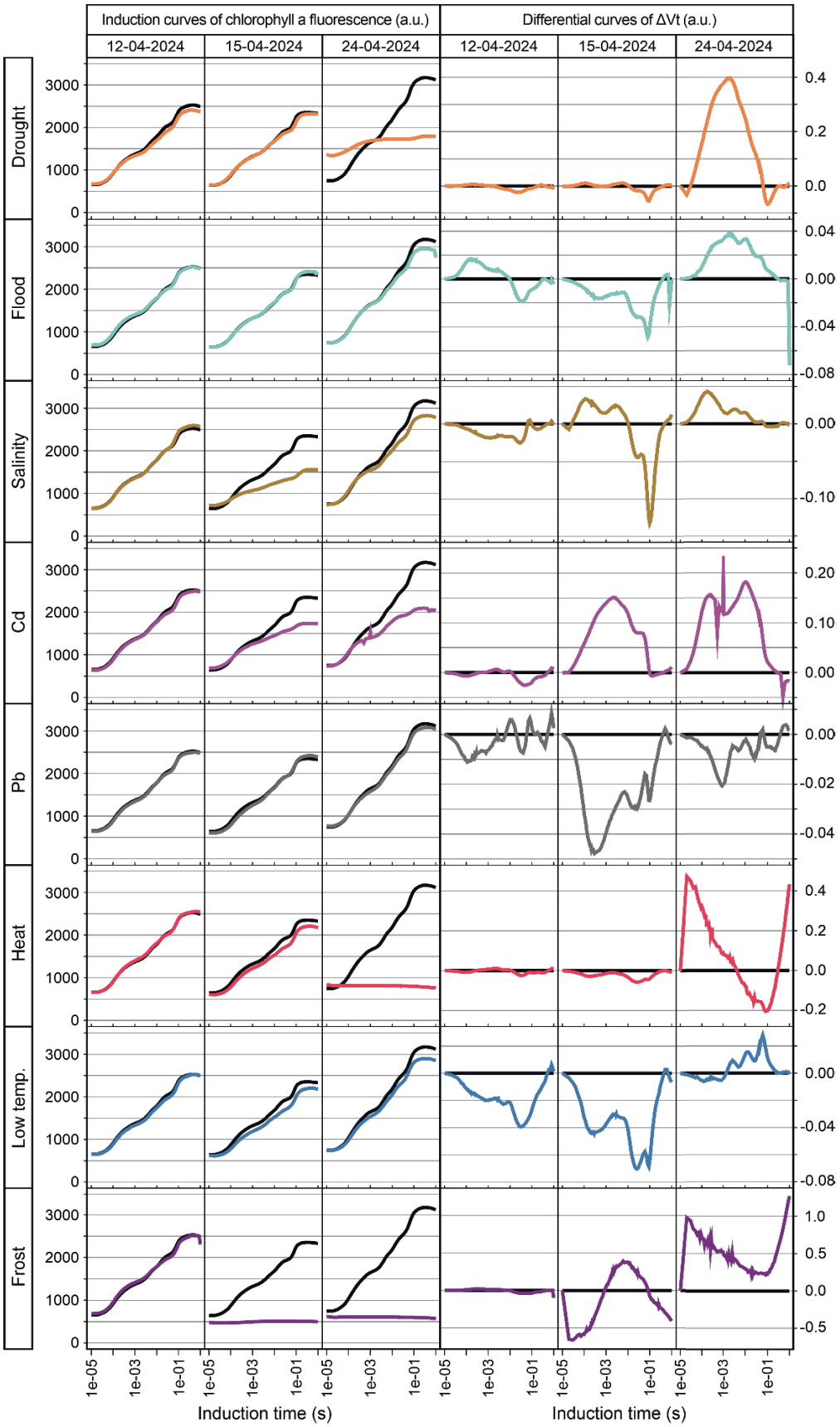


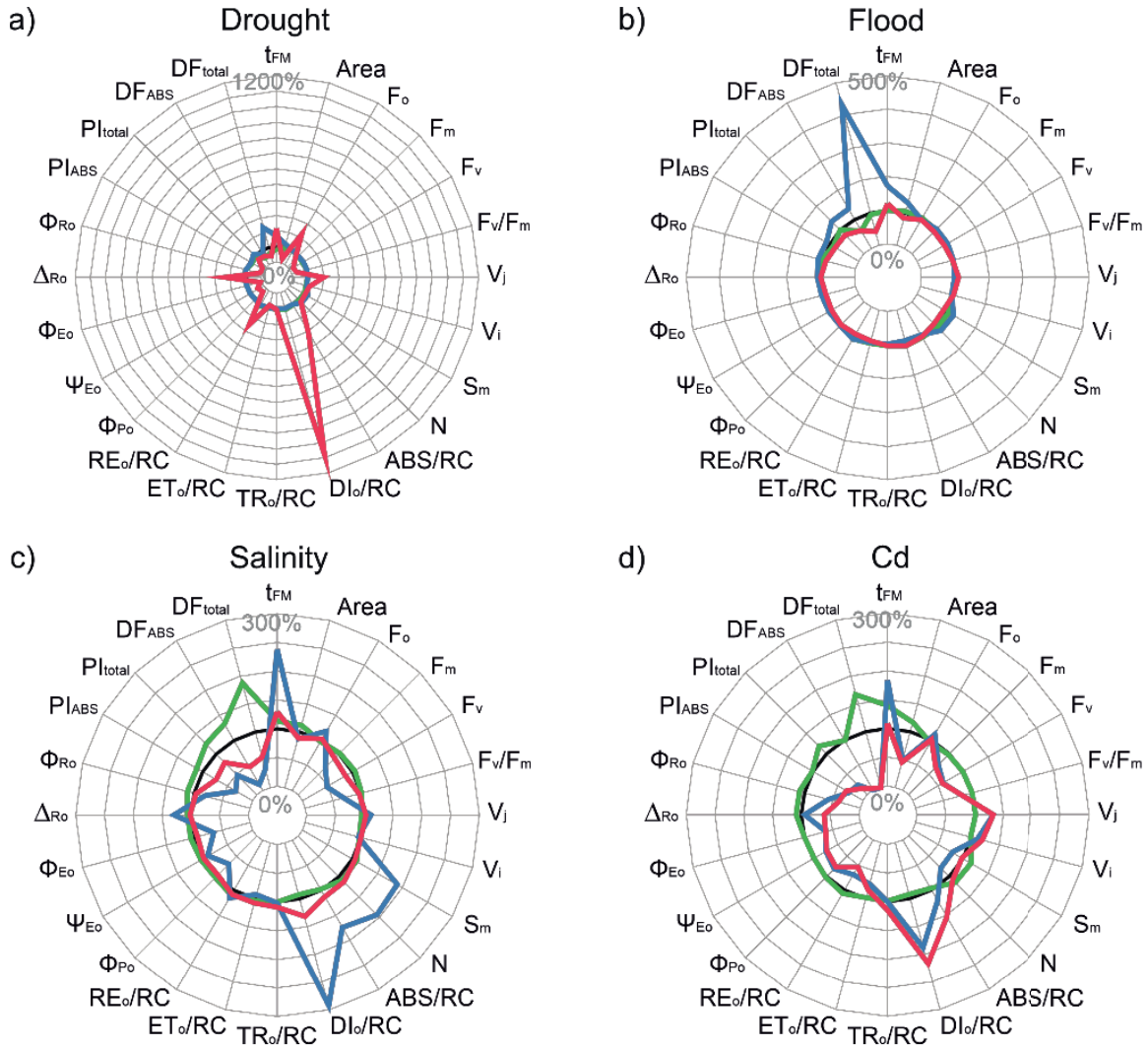
Fig. 1. Induction curves of chlorophyll *a* fluorescence and differential curves of ΔVt of maize under various abiotic stress treatments: control, drought, flood, salinity, Cd, Pb, heat, low temperature, and frost; the black curve in each plot represents the chlorophyll *a* fluorescence of the control plants; measurements were taken 8, 11 and 20 days from the stress application (April 12th, 15th and 24th, 2024); source: own study

(further than Q_A^-) per RC (ET_0/RC), RE_0/RC, but reduced time (in ms) to reach the maximal fluorescence (t_{FM}), total complementary area above the fluorescence induction curve (Area) and later F_M, F_V, F_V/F_M, φ_{Po}, φ_{Eo}, PI_{ABS}, PI_{total}. Low temperature and frost reduced F_M, F_V, and F_V/F_M. Area, increased energy flux not intercepted by a reaction centre (DI_0/RC). Frost further reduced φ_{Po}, φ_{Eo}, and quantum yield for reduction of end electron acceptors at the PSI acceptor side (φ_{Ro}). Heat, low temperature and frost reduced Area and t_{FM}, indicating smaller electron acceptor pools (Schreiber, Bilger, and Neubauer, 1995) or slowed plastoquinone reduction (Reigosa and Weiss, 2001). Drought raised F_0, suggesting PSII antenna impairment (Dinç *et al.*, 2012; Brestič *et al.*, 2015). Under most stressors, F_M, F_V, and F_V/F_M decreased, indicating photoinhibition (Björkman and Demmig, 1978; Tikkanen, Mekala and Aro, 2014; Dąbrowski *et al.*, 2024a; Dąbrowski *et al.*, 2024b). The ET_0/RC and RE_0/RC declined under heat, low temperature, and frost, reflecting reduced energy conversion efficiency (Strasser, Tsimilli-Michael and Srivastava, 2004; Strasser *et al.*, 2010). The ψ_{Eo} and ϕ_{Ro} vary with stress type, affecting electron transfer efficiency (Tsimilli-Michael, 2020). The PI_{ABS} and PI_{total} decreased under most stresses, confirming sensitivity to early damage of the photosynthetic apparatus, particularly the reaction centres and electron transport chain (Strasser, Tsimilli-Michael and Srivastava, 2000; Rodríguez *et al.*, 2023).

There were no differences in chlorophyll (Chl) content among the treatments 8 days after the stress application (Fig. 3). By the 11th day, Cd and frost had reduced the Chl content. By the 15th day, heat, low temperature and frost had also lowered Chl. By the 20th day, all stressors except low temperature had reduced Chl. Our results suggest that Cd and frost significantly reduce Chl content, indicating that these stressors impair chlorophyll biosynthesis or promote pigment degradation.

Cadmium is known to disrupt the chloroplast structure and inhibit key enzymatic activities involved in chlorophyll synthesis (Ghosh and Singh, 2005), while frost damages both cell membranes and photosystem II, resulting in pigment loss (Theocharis, Clement, and Barka, 2012). Reduced Chl levels have also been observed in response to heat, low temperature and frost, highlighting the cumulative negative impact of abiotic stress on photosynthetic pigments. In particular, heat stress compromises thylakoid membrane integrity and accelerates the breakdown of chlorophyll (Wahid *et al.*, 2007).

There was a slight change in flavonoid content at the start of the study, but drought (on the 15th day) and flooding (on the 20th day) increased their levels. Frost, salinity and drought all reduced flavonoid levels on the 20th day. Changes in flavonoid content reflected the plant's antioxidant response. The accumulation of these compounds is a well-documented response to cold



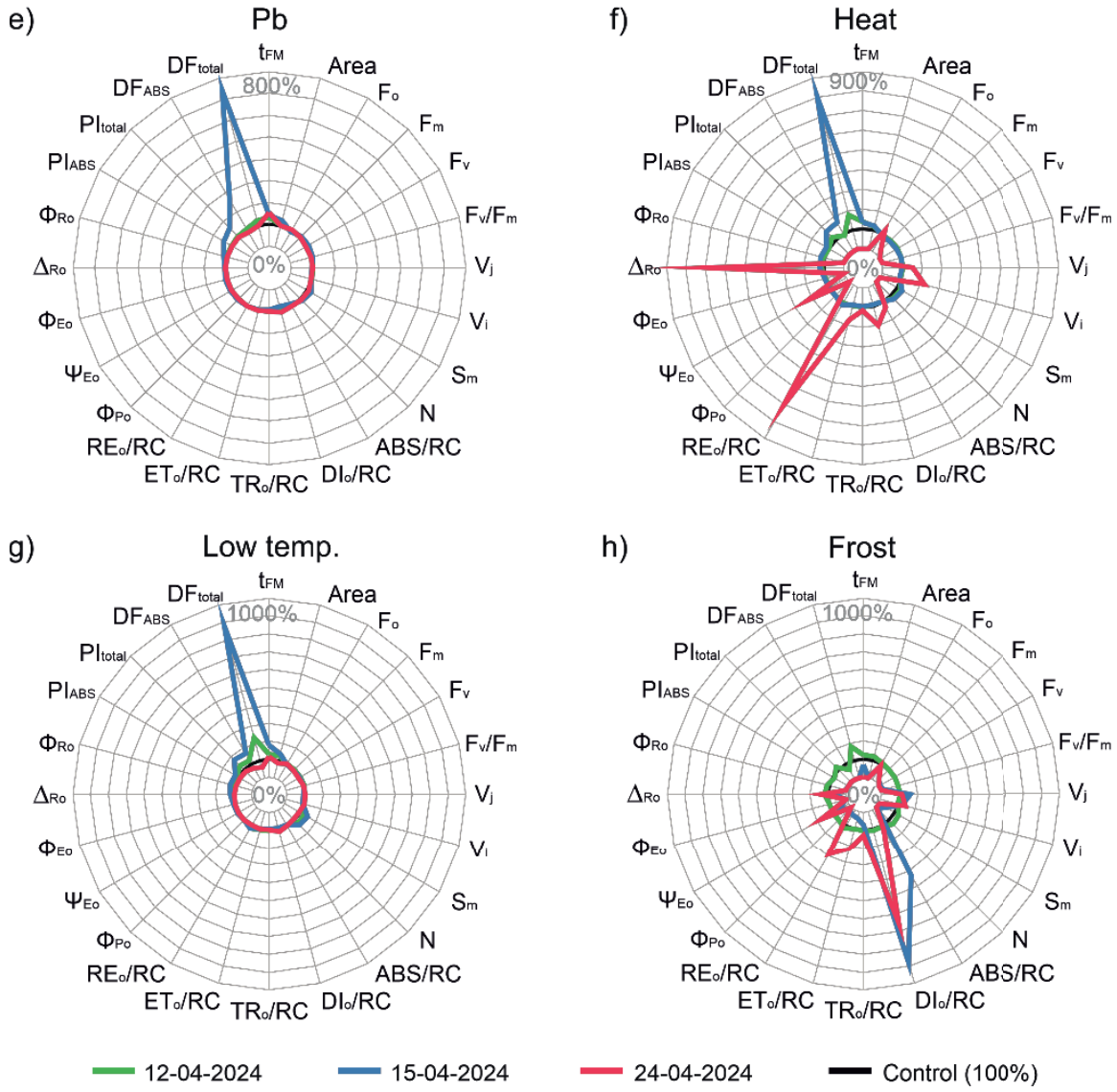


Fig. 2. Radar plots of JIP-test parameters normalised to control values in maize under various abiotic stress treatments: a) drought, b) flood, c) salinity, d) Cd, e) Pb, f) heat, g) low temperature, h) frost; measurements were taken 8, 11 and 20 days from the stress application (April 12th, 15th and 24th, 2024); t_{FM} = time (in ms) to reach the maximal fluorescence (F_M), Area = total complementary area above the fluorescence induction curve, F_0 = fluorescence when all PSII RCs are open, F_M = maximal fluorescence, when all PSII RCs are closed, F_V = maximal variable fluorescence at time t, F_V/F_M = maximum quantum yield for primary photochemistry, ABS/RC = absorption flux per RC, TR_0/RC = trapped energy flux per RC (reaction center), RE_0/RC = electron flux reducing end electron acceptors at the PSI acceptor side, per RC, ET_0/RC = electron transport flux (further than Q_A^-), per RC, DI_0/RC = energy flux not intercepted by an RC, dissipated in the form of heat, fluorescence, or transfer to other systems, at time t = 0, φ_{Po} = maximum quantum yield for primary photochemistry at any time t, φ_{Eo} = quantum yield for electron transport (ET), φ_{Ro} = quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE), ψ_{Eo} = efficiency/probability that an electron moves further than Q_A^-, δ_{Ro} = efficiency/probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side (RE), N = turnover number (expresses how many times Q_A is reduced in the time interval from 0 to t_{FM}), S_m = normalised the total area above the OJIP curve, PI_{ABS} = performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors, PI_{total} = total performance index for energy conservation from photons absorbed by PSII until the reduction of PSI end electron acceptors, DF_{ABS} = driving force (potential) for energy conservation from photons absorbed by PSII until the decrease in intersystem electron acceptors, DF_{total} = driving force (potential) for energy conservation from photons absorbed by PSII until the reduction of PSI end electron acceptors; source: own study

conditions, where they function as protective antioxidants (Chalker-Scott, 1999; Banti *et al.*, 2010; Sharma *et al.*, 2019). The nitrogen balance index (NBI) reflects nitrogen status and overall metabolic activity (Janská *et al.*, 2010). This parameter declined under frost in the early stages, increased under drought and Pb on the 11th day, decreased under drought on the 15th day, and fell under flooding, Cd and heat by the 20th day. This decreased possibly indicates temporary overcompensation in nitrogen assimilation or reduced flavonoid biosynthesis, which affects NBI values inversely. A transient increase in NBI during early drought stress has also been reported prior to prolonged water deficit, leading to metabolic decline (Netto *et al.*, 2005). On the 20th day, flood, Cd, and heat stress caused a consistent reduction in NBI, underscoring the cumulative metabolic burden

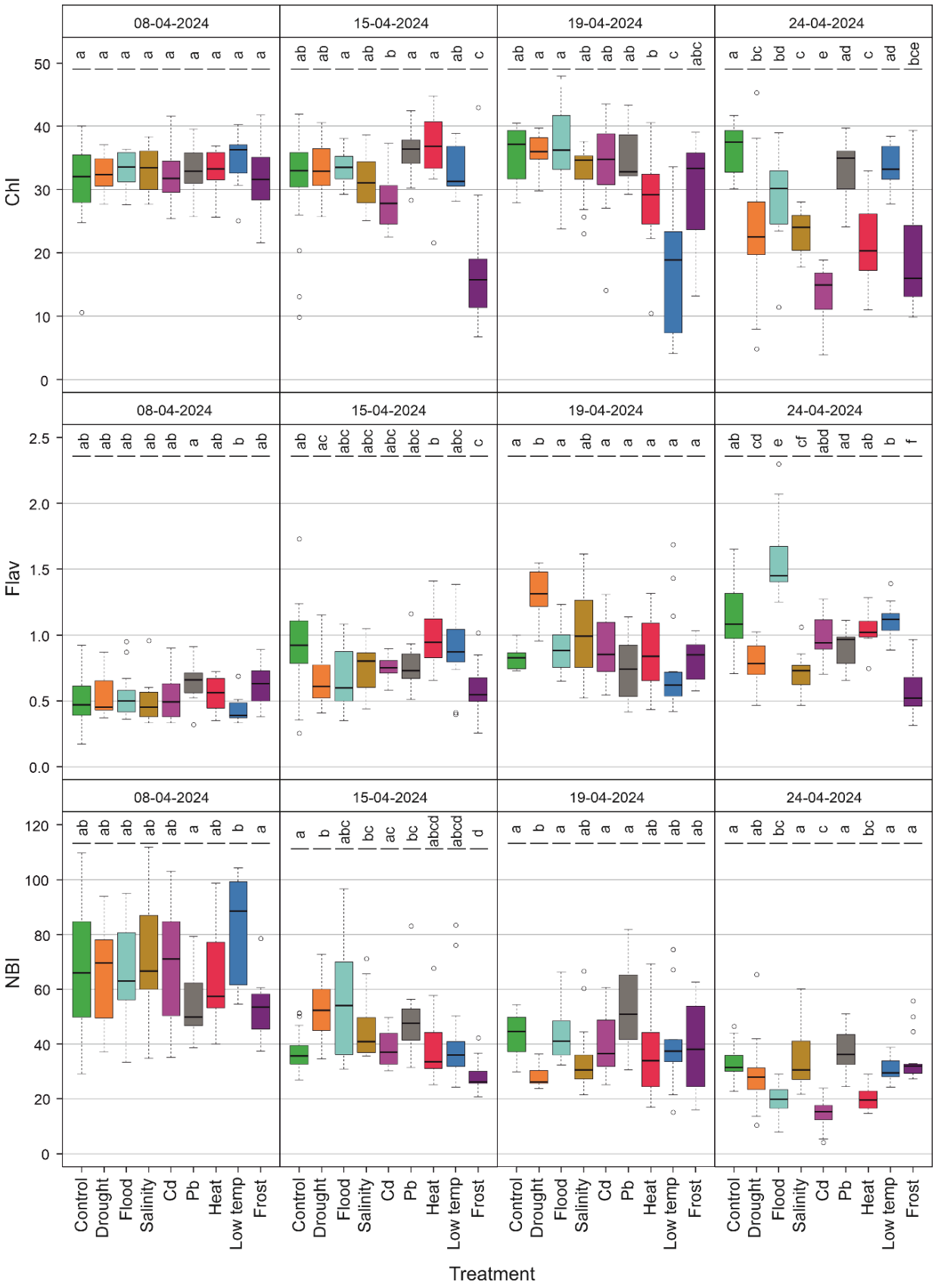


Fig. 3. Chlorophyll content (Chl), flavonoids (Flav), and nitrogen balance index (NBI) of maize under various abiotic stress treatments: control, drought, flood, salinity, Cd, Pb, heat, low temperature, and frost; measurements were taken 4, 11, 15 and 20 days from the stress application (April 8th, 15th, 19th, and 24th, 2024); letters above the boxes indicate results of a post hoc Wilcoxon rank-sum test; treatments not sharing a letter differ significantly at the 0.05 level; horizontal lines within boxes represent medians, boxes indicate the interquartile range (IQR; 25–75%), and whiskers extend to 1.5 IQR, with data points beyond this range considered potential outliers; source: own study

imposed by these stressors, which are known to disrupt nitrogen uptake, assimilation, and internal balance (Piršelová, Boleček and Gálusová, 2016; Chen *et al*., 2021).

GENE EXPRESSION LEVELS

After 21 days of the stress, a statistically significant decrease in normalised relative expressions was observed in maize leaves for the dehydrin 1 (*dhn*1) gene in all treatments (Fig. 4a and Fig. S2a). The heat shock protein 70 (*hsp*70) gene was slightly, but not significantly, down-regulated by drought and flood (Fig. 4b and Fig. S2b). Only the salinity treatment caused a slightly (not significantly) higher level of the *hsp*70 expression compared to the control plants.

Abiotic stress factors, such as drought, flood, and salinity, impair photosynthesis by disrupting the functions of PSII and PSI. We observe that long-term stress leads to decreased expression of *dhn*1 and has no statistically significant impact on *hsp*70 expression levels in maize leaves subjected to 3 weeks of stress. This may indicate alternative adaptive mechanisms in maize cv. 'SM Perseus' that may reflect rapid early gene induction followed by protein-level stabilisation, as seen in *dhn*1 root expression. Water-related stresses limit root uptake either via water scarcity (drought/salinity) or hypoxia-induced "physiological drought" (flood). These usually trigger osmo-protective gene overexpression, including dehydrins and late embryogenesis abundant (LEA) proteins, maintaining membrane integrity (Farooq *et al*., 2024). Stress-responsive gene regulation varies significantly across species and genotypes. This regulatory response can be highly dynamic, changing over time as the plant continues to experience various forms of abiotic stress. Our data show that prolonged stress diminished *dhn*1 activity, weakening its protective role. Genes like ZmDHN1 (abscisic acid (ABA) dependent) are key in early stress defence but may downregulate as energy-conservation mechanisms prevail. The observed down-regulation of *dhn*1 in response to salt and drought stress may suggest a stress-specific regulatory mechanism of dehydrin genes in maize. This is consistent with findings in wheat and barley. In these species, specific dehydrin isoforms are also either up- or downregulated depending on the type and duration of stress (Kosová, Vítámvás and Prášil, 2014; Kosová *et al*., 2025). The lack of *dhn*1 upregulation may reflect a transition from acute to chronic stress responses, where other protective pathways (such as osmolyte accumulation, antioxidant response) dominate.

The other gene, *hsp*70, interacts with key enzymes across organelles, protecting against degradation. Its expression rises under early stress in maize, grapevine, soybean, and sugarcane, with enhanced tolerance (Klimešová, Holková and Středa, 2017; Kozeko, 2021). In our case, only salinity triggered modest *hsp*70 upregulation at day 21, correlating with partial recovery in F_V/F_M values, suggesting a late-phase stress adaptation. These results indicate a stress-duration-dependent shift from gene activation to energy-saving responses in maize leaves, aligning with fluorescence and molecular data. Similarly, while *hsp*70 expression appears stable in some spinach varieties under heat and cold, proteomic data reveal upregulation of multiple HSP chaperones, indicating overlooked post-transcriptional regulation (Li *et al*., 2019). To address these limitations, future studies should integrate RNA-seq and proteomics to uncover gene-protein networks. For instance, a meta-analysis of maize transcriptomes under abiotic stress identified over 2000 differentially expressed genes (Forestan *et al*., 2016), highlighting the complexity of stress responses. Such multi-omics approaches could better elucidate the interactions between *dhn*1, *hsp*70, and key regulators, such as ZmDREB2A, under flooding and cold conditions (Zhou *et al*., 2022), thereby enhancing our understanding of maize adaptation and aiding in the identification of biomarkers for breeding stress-resilient varieties.

Focusing on *dhn*1 and *hsp*70 in gene expression analysis provides only a partial view of the transcriptomic response of maize to abiotic stress. These genes represent only a small part of the broader stress response network, which includes transcription factors such as MYB and WRKY, as demonstrated in transcriptomic studies of *Zea mays* (L.) under drought and salinity conditions (Jiao *et al*., 2022). This narrow scope limits the

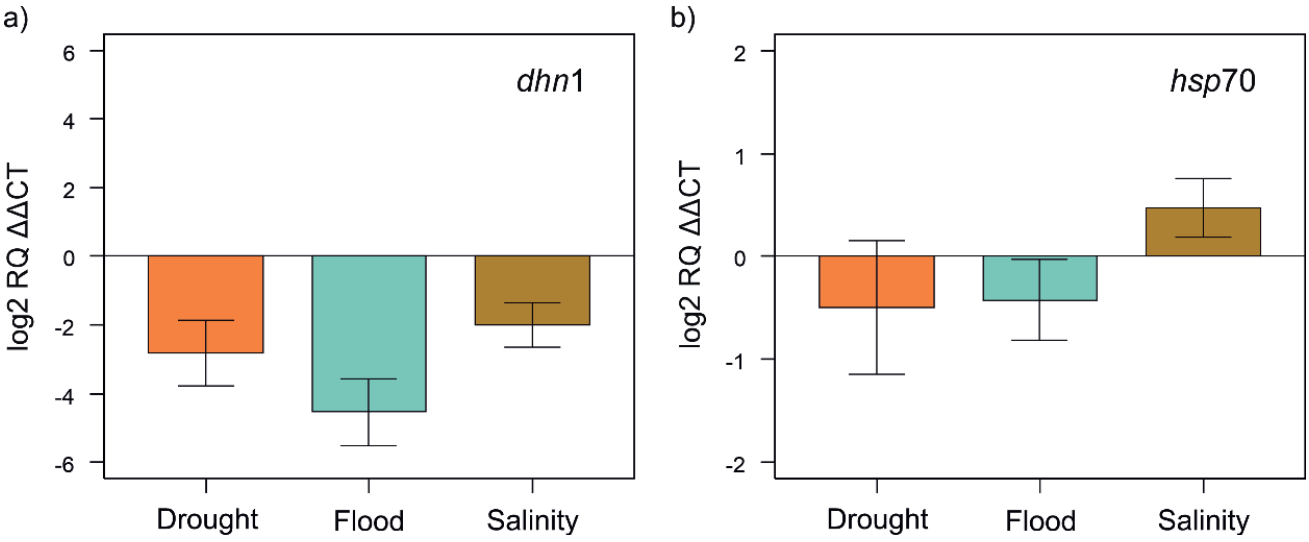


Fig. 4. Normalised relative expression of a) dehydrin 1 (*dhn*1) and b) heat shock protein 70 (*hsp*70) genes in *Zea mays* (L.) leaves under drought, flood, and salinity conditions; the bars represent mean relative gene fold change in a target sample expression vs reference genes actin 1 (*act*1) and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), normalised by an expression in treatment-relevant control plants; whiskers represent standard error of mean (SE); source: own study

generalisation of conclusions, as *dhn*1 downregulation under drought, flooding, and salinity may obscure the activation of alternative pathways, such as osmoprotectant biosynthesis genes identified in proteomic analyses under heavy metal stress (Baruah *et al.*, 2023).

Understanding such gene-specific responses can inform breeding programs to enhance early-stage stress detection and long-term resilience. In practical terms, combining chlorophyll fluorescence parameters and early stress-responsive gene expression provides a useful screening tool for selecting maize genotypes with superior stress tolerance. This approach can be integrated into breeding pipelines focused on improving crop performance under increasing abiotic stress conditions due to climate change.

CONCLUSIONS

This study demonstrates that diverse abiotic stress factors significantly impair photosynthetic efficiency in maize, as reflected by alterations in chlorophyll fluorescence parameters and gene expression profiles. The most pronounced disruptions in the chlorophyll fluorescence induction curves, particularly in the O–P phase, were observed under frost and heat stress, indicating reduced Q_A reduction efficiency and compromised activity of the oxygen-evolving complex (OEC). In contrast, salinity and cadmium stress predominantly affected the J–I phase, suggesting limitations in electron transport between photosystem II (PSII) and photosystem I (PSI).

Core fluorescence parameters such as maximum quantum yield for primary photochemistry (F_V/F_M), total complementary area above the fluorescence induction curve (Area), and time to reach the maximal fluorescence (t_{FM}) exhibited significant declines under all analysed stress conditions, confirming their strong diagnostic value in assessing PSII functionality and electron transport capacity. Additionally, efficiency/probability that an electron moves further than Q_A^- (ψ_{Eo}) and quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE) (ϕ_{Ro}) values varied depending on the type and timing of stress, highlighting their usefulness in evaluating electron transfer efficiency on both PSII and PSI sides. The photosynthetic performance indices and total performance index for energy conservation from photons absorbed by PSII until the reduction of PSI end electron acceptors (PI_{total}) proved particularly sensitive to stress, validating their application in performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors (PI_{ABS}) ecophysiological screening and environmental monitoring.

At the molecular level, prolonged exposure to drought, flood, and salinity led to a marked downregulation of the dehydrin 1 (*dhn*1) gene, suggesting early activation followed by depletion of protective responses. In contrast, heat shock protein 70 (*hsp*70) expression remained stable, indicating either post-transcriptional regulation or the involvement of alternative protective pathways. These findings suggest that chronic stress conditions may exhaust the plant's defence systems, shifting metabolic priorities and potentially invoking gene-specific protective mechanisms that warrant further investigation.

Overall, our results emphasise that the selection of appropriate physiological and molecular markers should be tailored to the type and duration of stress. Chlorophyll fluorescence analysis remains a universally applicable, non-invasive tool for assessing plant responses across a range of abiotic stress conditions. When integrated with gene expression profiling, it provides a comprehensive framework for evaluating stress tolerance and photosynthetic performance in maize. Nevertheless, integrating transcriptomic and proteomic analyses is crucial to fully elucidate the complex regulatory networks underlying maize responses to abiotic stress, thereby enabling a more accurate identification of key genes and pathways for breeding resilient varieties.

ABBREVIATIONS

Abbreviations can be found in the Supplementary Materials Table S1.

SUPPLEMENTARY MATERIALS

Supplementary material to this article can be found online at: https://www.jwld.pl/files/Supplementary_material_67_Kalaji.pdf.

AUTHOR CONTRIBUTIONS

1st Author (contribution – 25%): study design, data collection, data interpretation, manuscript preparation, literature search. 2nd Author (contribution – 25%): study design, data collection, data interpretation, manuscript preparation, literature search. 3rd Author (contribution – 15%): study design, data collection, data interpretation, manuscript preparation, literature search. 4th Author (contribution – 15%): statistical analysis, data interpretation, manuscript preparation. 5th Author (contribution – 5%): data interpretation, manuscript preparation. 6th Author (contribution – 15%): study design, data collection, data interpretation, manuscript preparation, literature search.

FUNDING

This research was funded by the Polish Ministry of Science and Higher Education, grant number INB-13-2024 and DEC-INB-2/24.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

REFERENCES

Aghaie, P. and Tafreshi, S.A.H. (2020) "Central role of 70-kDa heat shock protein in adaptation of plants to drought stress," *Cell Stress Chaperones*, 25, pp. 1071–1081. Available at: https://doi.org/10.1007/s12192-020-01144-7.

Ahluwalia, O., Singh, P.C. and Bhatia, R. (2021) "A review on drought stress in plants: Implications, mitigation and the role of plant growth promoting rhizobacteria," *Resources, Environment and*

Sustainability, 5, 100032. Available at: https://doi.org/10.1016/j.resenv.2021.100032.

Baker, N.R. and Rosenqvist, E. (2004) "Applications of chlorophyll fluorescence can improve crop production strategies: An examination of future possibilities," *Journal of Experimental Botany*, 55, pp. 1607–1621. Available at: https://doi.org/10.1093/jxb/erh196.

Banti, V. *et al.* (2010) "The heat-inducible transcription factor *HsfA2* enhances anoxia tolerance in *Arabidopsis*," *Plant Physiology*, 152, pp. 1471–1483. Available at: https://doi.org/10.1104/pp.109.149815.

Baruah, N. *et al.* (2023) "Phytotoxic responses and plant tolerance mechanisms to cadmium toxicity," *Journal of Soil Science and Plant Nutrition*, 23, pp. 4805–4826. Available at: https://doi.org/10.1007/s42729-023-01525-8.

Bhattacharya, A. (2022) "Effect of low temperature stress on photosynthesis and allied traits: A review," in Bhattacharya, A. (ed.) *Physiological processes in plants under low temperature stress*. Singapore: Springer, pp. 199–297. Available at: https://doi.org/10.1007/978-981-16-9037-2_3.

Bionda, T. *et al.* (2016) "Eukaryotic Hsp70 chaperones in the intermembrane space of chloroplasts," *Planta*, 243, pp. 733–747. Available at: https://doi.org/10.1007/s00425-015-2440-z.

Björkman, O. and Demmig, B. (1987) "Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins," *Planta*, 170, pp. 489–504. Available at: https://doi.org/10.1007/BF00402983.

Brestič, M. *et al.* (2015) "Low PSI content limits the photoprotection of PSI and PSII in early growth stages of chlorophyll *b*-deficient wheat mutant lines," *Photosynthetic Research*, 125, pp. 151–166. Available at: https://doi.org/10.1007/s11120-015-0093-1.

Chalker-Scott, L. (1999) "Environmental significance of anthocyanins in plant stress responses," *Photochemistry and Photobiology*, 70, pp. 1–9. Available at: https://doi.org/10.1111/j.1751-1097.1999.tb01944.x.

Chen, J. *et al.* (2017) "*Arabidopsis* WRKY46, WRKY54, and WRKY70 transcription factors are involved in brassinosteroid-regulated plant growth and drought responses," *Plant Cell*, 29, pp. 1425–1439. Available at: https://doi.org/10.1105/tpc.17.00364.

Chen, J. *et al.* (2021) "Effects of drought on the relationship between photosynthesis and chlorophyll fluorescence for maize," *IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing*, 14, pp. 11148–11161. Available at: https://doi.org/10.1109/JSTARS.2021.3123111.

Chtouki, M. *et al.* (2021) "Effect of cadmium and phosphorus interaction on tomato: Chlorophyll *a* fluorescence, plant growth, and cadmium translocation," *Water Air Soil Pollution*, 232, 84. Available at: https://doi.org/10.1007/s11270-021-05038-x.

Dąbrowski, P. *et al.* (2024a) "Photosynthetic efficiency of plants as an indicator of tolerance to petroleum-contaminated soils," *Sustainability*, 16, 10811. Available at: https://doi.org/10.3390/su162410811.

Dąbrowski, P. *et al.* (2024b) "Relationship between photosynthetic performance and yield loss in winter oilseed rape (*Brassica napus* L.) under frost conditions," *Photosynthetica*, 62, pp. 240–251. Available at: https://doi.org/10.32615/ps.2024.025.

Dąbrowski, P. *et al.* (2024c) "Photosynthetic performance and yield losses of winter rapeseed (*Brassica napus* L. var. *napus*) caused by simulated hail," *Plants*, 13, 1785. Available at: https://doi.org/10.3390/plants13131785.

Dinç, E. *et al.* (2012) "The Chl *a* fluorescence intensity is remarkably insensitive to changes in the chlorophyll content of the leaf as long as the Chl *a/b* ratio remains unaffected," *Biochimica et Biophysica Acta (BBA) – Bioenergetics*, 1817, pp. 770–779. Available at: https://doi.org/10.1016/j.bbabio.2012.02.003.

Distéfano, A.M. *et al.* (2017) "Heat stress induces ferroptosis-like cell death in plants," *Journal of Cell Biology*, 216, pp. 463–476. Available at: https://doi.org/10.1083/jcb.201605110.

Farooq, M. *et al.* (2009) "Plant drought stress: Effects, mechanisms and management," *Agronomy for Sustainable Development*, 29, pp. 185–212. Available at: https://doi.org/10.1051/agro:2008021.

Farooq, M. *et al.* (2024) "Real-time expression and in silico characterization of pea genes involved in salt and water-deficit stress," *Molecular Biology Reports*, 51, 18. Available at: https://doi.org/10.1007/s11033-023-09064-2.

Forestan, C. *et al.* (2016) "Stress-induced and epigenetic-mediated maize transcriptome regulation study by means of transcriptome reannotation and differential expression analysis," *Scientific Reports* 6, 30446. Available at: https://doi.org/10.1038/srep30446.

Ghosh, M. and Singh, S.P. (2005) "A review on phytoremediation of heavy metals and utilization of its byproducts," *Applied Ecology and Environmental Research*, 3, pp. 1–18. Available at: https://doi.org/10.15666/aeer/0301_001018.

Gu, L. *et al.* (2019) "Sun-induced chlorophyll fluorescence and its importance for biophysical modeling of photosynthesis based on light reactions," *New Phytologist*, 223, pp. 1179–1191. Available at: https://doi.org/10.1111/nph.15796.

Helm, L.T. *et al.* (2020) "Solar-induced chlorophyll fluorescence and short-term photosynthetic response to drought," *Ecological Applications*, 30, e02101. Available at: https://doi.org/10.1002/eap.2101.

Holm, S.A. (1979) "Simple sequentially rejective multiple test procedure," *Scandinavian Journal of Statistics*, 6, pp. 65–70. Available at: https://www.jstor.org/stable/4615733 (Accessed: April 02, 2025).

Hu, X. *et al.* (2010) "Heat shock protein 70 regulates the abscisic acid-induced antioxidant response of maize to combined drought and heat stress," *Plant Growth Regulators*, 60, pp. 225–235. Available at: https://doi.org/10.1007/s10725-009-9436-2.

Iqbal, H. *et al.* (2020) "Improving heat stress tolerance in late planted spring maize by using different exogenous elicitors," *Chilean Journal of Agricultural Research*, 80, pp. 30–40. Available at: https://doi.org/10.4067/S0718-58392020000100030.

Iqbal, S. *et al.* (2020) "The response of maize physiology under salinity stress and its coping strategies," in A. Hossain (ed.) *Plant stress physiology*. London: IntechOpen Limited, pp. 1–25. Available at: https://doi.org/10.5772/intechopen.92213.

Janská, A. *et al.* (2010) "Cold stress and acclimation – what is important for metabolic adjustment?," *Plant Biology*, 12, pp. 395–405. Available at: https://doi.org/10.1111/j.1438-8677.2009.00299.x.

Jiao, P. *et al.* (2022) "Integration of mRNA and microRNA analysis reveals the molecular mechanisms underlying drought stress tolerance in maize (*Zea mays* L.)," *Frontiers in Plant Science*, 13, 932667. Available at: https://doi.org/10.3389/fpls.2022.932667.

Jin, H. *et al.* (2019) "Maize leaves drought-responsive genes revealed by comparative transcriptome of two cultivars during the filling stage," *PLOS ONE*, 14, e0223786. Available at: https://doi.org/10.1371/journal.pone.0223786.

Kalaji, M.H. and Guo, P. (2008) "Chlorophyll fluorescence: A useful tool in barley plant breeding programs," in A. Sanchez, S.J. Gutierrez (eds.) *Photochemistry research progress*. New York: Nova Publishers, pp. 439–463.

Klimešová, J. *et al.* (2021) "Transpiration and water use efficiency of maize in different soil moisture conditions," *Acta Horticulturae et Regiotecturae*, 24, pp. 105–109. Available at: https://doi.org/10.2478/ahr-2021-0031.

Klimešová, J., Holková, L. and Středa, T. (2017) "The expression of dehydrin genes and the intensity of transpiration in drought-stressed maize plants," *Cereal Research Communications*, 45, pp. 355–368. Available at: https://doi.org/10.1556/0806.45.2017.017.

Kohli, S.K. *et al.* (2017) "Role of salicylic acid in heavy metal stress tolerance: Insight into underlying mechanism," in R. Nazar, N. Iqbal and N. Khan (eds.) *Salicylic acid: A multifaceted hormone*. Singapore: Springer, pp. 123–144. Available at: https://doi.org/10.1007/978-981-10-6068-7_7.

Kosová, K. *et al.* (2025) "How to survive mild winters: Cold acclimation, deacclimation, and reacclimation in winter wheat and barley," *Plant Physiology and Biochemistry*, 220, 109541. Available at: https://doi.org/10.1016/j.plaphy.2025.109541.

Kosová, K. Vítámvás, P. and Prášil, I.T (2014) "Proteomics of stress responses in wheat and barley – search for potential protein markers of stress tolerance," *Frontiers in Plant Science*, 5, 711. Available at: https://doi.org/10.3389/fpls.2014.00711.

Kozeko, L. (2021) "Different roles of inducible and constitutive HSP70 and HSP90 in tolerance of *Arabidopsis thaliana* to high temperature and water deficit," *Acta Physiologiae Plantarum*, 43, 58. Available at: https://doi.org/10.1007/s11738-021-03229-x.

Lakshmi, G. *et al.* (2023) "Exogenously applied plant growth regulator protects rice from heat-induced damage by modulating plant defense mechanisms," *Journal of Crop Science and Biotechnology*, 26, pp. 63–75. Available at: https://doi.org/10.1007/s12892-022-00162-4.

Lamers, J., Meer van der, T. and Testerink, C. (2020) "How plants sense and respond to stressful environments," *Plant Physiology*, 182, pp. 1624–1635. Available at: https://doi.org/10.1104/pp.19.01464.

Li, G. *et al.* (2023) "Selection and validation of reference genes in virus-infected sweet potato plants," *Genes*, 14, 1477. Available at: https://doi.org/10.3390/genes14071477.

Li, S. *et al.* (2019) "Heat-responsive proteomics of a heat-sensitive spinach variety," *International Journal of Molecular Sciences*, 20 (16), 3872. Available at: https://doi.org/10.3390/ijms20163872.

Li, S. *et al.* (2020) "Changes in photosynthesis and respiratory metabolism of maize seedlings growing under low-temperature stress may be regulated by arbuscular mycorrhizal fungi," *Plant Physiology and Biochemistry*, 154, pp. 1–10. Available at: https://doi.org/10.1016/j.plaphy.2020.05.025.

Logan, M. (2010) *Biostatistical design and analysis using R: A practical guide*. Chichester: Wiley-Blackwell.

Luan, H. *et al.* (2018) "Elucidating the hypoxic stress response in barley (*Hordeum vulgare* L.) during waterlogging: A proteomics approach," *Scientific Reports*, 8, 9655. Available at: https://doi.org/10.1038/s41598-018-27726-1.

Manoli, A. *et al.* (2012) "Evaluation of candidate reference genes for qPCR in maize," *Journal of Plant Physiology*, 169, pp. 807–815. Available at: https://doi.org/10.1016/j.jplph.2012.01.019.

Mantri, N. *et al.* (2012) "Abiotic stress responses in plants: Present and future," in P. Ahmad and M.N.V. Prasad (eds.) *Abiotic stress responses in plants: Metabolism, productivity and sustainability*. New York: Springer. Available at: https://doi.org/10.1007/978-1-4614-0634-1_1.

Mihaljević, I. *et al.* (2020) "Photochemical efficiency of photosystem II in two apple cultivars affected by elevated temperature and excess light in vivo," *South African Journal of Botany*, 130, pp. 316–326. Available at: https://doi.org/10.1016/j.sajb.2020.01.017.

Moreira-de-Sousa, C., Souza de, R.B. and Fontanetti, C.S. (2018) "HSP70 as a biomarker: An excellent tool in environmental contamination analysis – A review," *Water Air Soil Pollution*, 229, 264. Available at: https://doi.org/10.1007/s11270-018-3920-0.

Mubeen, M. *et al.* (2021) "Effect of plant growth promoting bacteria and drought on spring maize (*Zea mays* L.)," *Pakistan Journal of Botany*, 53, pp. 731–739. Available at: https://dx.doi.org/10.30848/PJB2021-2(38).

Mustafa, T. *et al.* (2021) "Exogenous application of silicon improves the performance of wheat under terminal heat stress by triggering physio-biochemical mechanisms," *Scientific Reports*, 11, 23170. Available at: https://doi.org/10.1038/s41598-021-02594-4.

Netto, A.T. *et al.* (2005) "Photosynthetic pigments, nitrogen, chlorophyll *a* fluorescence and SPAD-502 readings in coffee leaves," *Scientia Horticulture*, 104, pp. 199–209. Available at: https://doi.org/10.1016/j.scienta.2004.08.013.

Pan, J. *et al.* (2021) "Mechanisms of waterlogging tolerance in plants: Research progress and prospects," *Frontiers in Plant Science*, 11, 627331. Available at: https://doi.org/10.3389/fpls.2020.627331.

Pandey, S.N. *et al.* (2018) "Diversity, functions, and stress responses of soil microorganisms," in D. Egamberdieva, P. Ahmad and Q.S. Wu (eds.) *Plant microbiome: Stress response*. Cham: Springer, pp. 1–19.

Piršelová, B., Boleček, P. and Gálusová, T. (2016) "Effect of cadmium and arsenic on chlorophyll fluorescence of selected soybean cultivars," *Russian Journal of Plant Physiology*, 63, pp. 469–473. Available at: https://doi.org/10.1134/S1021443716040129.

Qi, M. *et al.* (2021) "Photosynthetic resistance and resilience under drought, flooding, and rewatering in maize plants," *Photosynthetic Research*, 148, pp. 1–15. Available at: https://doi.org/10.1007/s11120-021-00825-3.

R Core Team (2024) *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: https://www.r-project.org (Accessed: June 05, 2025).

Ramazan, S. *et al.* (2021) "Combined gas exchange characteristics, chlorophyll fluorescence, and response curves as selection traits for temperature tolerance in maize genotypes," *Photosynthetic Research*, 150, pp. 213–225. Available at: https://doi.org/10.1007/s11120-021-00829-z.

Rana, R.M. *et al.* (2018) "HSP70 mediated stress modulation in plants," in A.A. Asea and P. Kaur (eds.) *Heat shock proteins and stress*. Cham: Springer, pp. 281–290. Available at: https://doi.org/10.1007/978-3-319-90725-3_13.

Reigosa, R.M. and Weiss, O. (2001) "Fluorescence techniques," in R.M. Reigosa (ed.) *Handbook of plant ecophysiology techniques*. Dordrecht: Academic Publishers, pp. 155–171.

Rodríguez, A.A. *et al.* (2023) "Field and genetic evidence support the photosynthetic performance index (PI_{ABS}) as an indicator of rice grain yield," *Plant Physiology and Biochemistry*, 201, 107897. Available at: https://doi.org/10.1016/j.plaphy.2023.107897.

Ruiz-Huerta, E.A. *et al.* (2022) "Bioaccumulation of heavy metals and As in maize (*Zea mays* L.) grown close to mine tailings strongly impacts plant development," *Ecotoxicology*, 31, pp. 447–467. Available at: https://doi.org/10.1007/s10646-022-02522-w.

Saleem, M.H. *et al.* (2024) "Advances in physiochemical and molecular mechanisms of abiotic stress tolerance in plants," *Journal of Crop Health*, 76, pp. 753–767. Available at: https://doi.org/10.1007/s10343-024-00993-x.

Sarkar, N.K., Kundnani, P. and Grover, A. (2013) "Functional analysis of Hsp70 superfamily proteins of rice (*Oryza sativa*)," *Cell Stress Chaperones*, 18, pp. 427–437. Available at: https://doi.org/10.1007/s12192-012-0395-6.

Scafaro, A.P. *et al.* (2016) "Heat tolerance in a wild *Oryza* species is attributed to maintenance of Rubisco activation by a thermally stable Rubisco activase ortholog," *New Phytologist*, 211, pp. 899–911. Available at: https://doi.org/10.1111/nph.13963.

Schansker, G., Tóth, S.Z. and Strasser, R.J. (2005) "Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl *a* fluorescence rise OJIP," *Biochimica et Biophysica Acta (BBA) – Bioenergetics*, 1706(3), pp. 250–261. Available at: https://doi.org/10.1016/j.bbabio.2004.11.006.

Schmittgen, T.D and Livak, K.J. (2008) "Analyzing real-time PCR data by the comparative C_T method," *Nature Protocols*, 3, pp. 1101–1108. Available at: https://doi.org/10.1038/nprot.2008.73.

Schreiber, U., Bilger, W. and Neubauer, C. (1995) "Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis," in E.D. Schulze and M.M. Caldwell (eds.) *Ecophysiology of photosynthesis*, Vol. 100. Berlin: Springer, pp. 49–70. Available at: https://doi.org/10.1007/978-3-642-79354-7_3.

Seguí-Simarro, J.M., Testillano, P.S. and Risueño, M.C. (2003) "Hsp70 and Hsp90 change their expression and subcellular localization after microspore embryogenesis induction in *Brassica napus* L.," *Journal of Structural Biology*, 142, pp. 379–391. Available at: https://doi.org/10.1016/S1047-8477(03)00067-4.

Sena, S.J. *et al.* (2018) "Expansion of the dehydrin gene family in the Pinaceae is associated with considerable structural diversity and drought-responsive expression," *Tree Physiology*, 38, pp. 442–456. Available at: https://doi.org/10.1093/treephys/tpx125.

Sevgi, B. and Leblebici, S. (2023) "Tuz Stresinin Bitkiler Üzerindeki Etkileri ve Geliştirilen Tolerans Mekanizmaları [Effects of salt stress on plants and developed tolerance mechanisms]," *Duzce University Journal of Science and Technology*, 11, pp. 1498–1516. Available at: https://doi.org/10.29130/dubited.1171221.

Sharma, A. *et al.* (2019) "Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress," *Molecules*, 24, 2452. Available at: https://doi.org/10.3390/molecules24132452.

Shin, K.Y., Bhandari, R.S. and Lee, G.J. (2021) "Monitoring of salinity, temperature, and drought stress in grafted watermelon seedlings using chlorophyll fluorescence," *Frontiers in Plant Science*, 12, 786309. Available at: https://doi.org/10.3389/fpls.2021.786309.

Song, X., Zhou, G. and He, Q. (2021) "Critical leaf water content for maize photosynthesis under drought stress and its response to rewatering," *Sustainability*, 13, 7218. Available at: https://doi.org/10.3390/su13137218.

Strasser, R.J. *et al.* (2010) "Simultaneous *in vivo* recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*," *Biochimica et Biophysica Acta (BBA) – Bioenergetics*, 1797(6–7), pp. 1313–1326. Available at: https://doi.org/10.1016/j.bbabio.2010.03.008.

Strasser, R.J., Tsimilli-Michael, M. and Srivastava, A. (2000) "The fluorescence transient as a tool to characterize and screen photosynthetic samples," in M. Yunus, U. Pathre and P. Mohanty (eds.) *Probing photosynthesis: Mechanisms, regulation, and adaptation*. London: Taylor & Francis, pp. 445–483.

Strasser, R.J., Tsimilli-Michael, M. and Srivastava, A. (2004) "Analysis of the chlorophyll *a* fluorescence transient," in G.C. Papageorgiou, Govindjee (eds.) *Chlorophyll a fluorescence: A signature of photosynthesis*. Dordrecht: Springer, pp. 321–362. Available at: https://doi.org/10.1007/978-1-4020-3218-9_12.

Su, P.H. and Li, H.M. (2010) "Stromal Hsp70 is important for protein translocation into pea and *Arabidopsis* chloroplasts," *Plant Cell*, 22, pp. 1516–1531. Available at: https://doi.org/10.1105/tpc.109.071415.

Sun, Z. *et al.* (2021) "Plant dehydrins: Expression, regulatory networks, and protective roles in plants challenged by abiotic stress," *International Journal of Molecular Science*, 22, 12619. Available at: https://doi.org/10.3390/ijms222312619.

Theocharis, A., Clement, C. and Barka, E.A (2012) "Physiological and molecular changes in plants grown at low temperatures," *Planta*, 235, pp. 1091–1105. Available at: https://doi.org/10.1007/s00425-012-1641-y.

Tikkanen, M., Mekala, N.R. and Aro, E.M. (2014) "Photosystem II photoinhibition-repair cycle protects Photosystem I from irreversible damage," *Biochimica et Biophysica Acta (BBA) – Bioenergetics*, 1837, pp. 210–215. Available at: https://doi.org/10.1016/j.bbabio.2013.10.001.

Timperio, A.M., Egidi, M.G. and Zolla, L. (2008) "Proteomics applied on plant abiotic stresses: Role of heat shock proteins (HSP)," *Journal of Proteomics*, 71, pp. 391–411. Available at: https://doi.org/10.1016/j.jprot.2008.07.005.

Tobiasz-Salach, R., Mazurek, M. and Jacek, B. (2023) "Physiological, biochemical, and epigenetic reaction of maize (*Zea mays* L.) to cultivation in conditions of varying soil salinity and foliar application of silicon," *International Journal of Molecular Sciences*, 24, 1141. Available at: https://doi.org/10.3390/ijms24021141.

Tsimilli-Michael, M. (2020) "Revisiting JIP-test: An educative review on concepts, assumptions, approximations, definitions and terminology," *Photosynthetica*, 58, pp. 275–292. Available at: https://doi.org/10.32615/ps.2019.150.

Wahid, A. *et al.* (2007) "Heat tolerance in plants: An overview," *Environmental and Experimental Botany*, 61, pp. 199–223. Available at: https://doi.org/10.1016/j.envexpbot.2007.05.011.

Wang, K. *et al.* (2019) "The role of C2H2 zinc finger proteins in plant responses to abiotic stresses," *Physiologia Plantarum*, 165, pp. 690–700. Available at: https://doi.org/10.1111/ppl.12728.

Wang, Y.-Y., Head, D.J. and Hauser, B.A. (2023) "During water stress, fertility modulated by ROS scavengers abundant in *Arabidopsis* pistils," *Plants*, 12, 2182. Available at: https://doi.org/10.3390/plants12112182.

Yang, X. *et al.* (2021) "Response mechanism of plants to drought stress," *Horticulturae*, 7, 50. Available at: https://doi.org/10.3390/horticulturae7030050.

Zalewski, D. (2024) *RQdeltaCT: Relative quantification of gene expression using delta Ct methods. R package version 1.3.0.* Available at: https://doi.org/10.32614/CRAN.package.RQdeltaCT (Accessed: April 01, 2025).

Zhang, Z. *et al.* (2020) "Calcium is involved in exogenous NO-induced enhancement of photosynthesis in cucumber (*Cucumis sativus* L.) seedlings under low temperature," *Scientia Horticulturae*, 261, 108953. Available at: https://doi.org/10.1016/j.scienta.2019.108953.

Zhao, H. *et al.* (2019) "Effects of salt stress on chlorophyll fluorescence and the antioxidant system in *Ginkgo biloba* L. seedlings," *HortScience*, 54(12), pp. 2125–2133. Available at: https://doi.org/10.21273/HORTSCI14432-19.

Zhou, S. *et al.* (2022) "Small heat shock proteins and dehydrins synergistically stabilize chloroplast proteins during salt stress," *Plant Cell Environment*, 45, pp. 847–864. Available at: https://doi.org/10.1111/pce.14256.

Zivčák, M. *et al.* (2014) "Photosynthetic responses of sun- and shade-grown barley leaves to high light: Is the lower PSII photochemical efficiency in shade leaves associated with decreased PSII antenna size?," *Photosynthetic Research*, 119, pp. 339–354. Available at: https://doi.org/10.1007/s11120-014-9969-8.