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Photosynthetic health of winter wheat following overwintering stresses in controlled conditions

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Abstract

Spring plant regrowth can be impacted by overwintering stresses such as ice encasement and winter desiccation. The photosynthetic ramifications of these two stresses and whether chlorophyll fluorescence-based parameters during spring recovery may be efficient at differentiating winter survival of winter wheat (*Triticum aestivum*) is not well known. A panel of 10 winter wheat genotypes from various origins were exposed to surface ice encasement (2.54 cm deep) or winter desiccation in a low-temperature growth chamber and were transferred to a growth chamber containing high throughput photosynthetic imagers, which measured photosynthetic efficiency and non-photochemical quenching (NPQ) associated parameters. Antioxidant enzyme activity and lipid peroxidation were also measured but were only significant in response to treatment duration. Low-temperature dormancy or prolonged effects of each winter stress caused a decline in $F_v/F_{m,}$ but F_v/F_m was not different in response to genotype. The NPQ parameters revealed dynamic stress responses and were better at distinguishing responses to each stress and between genotypes compared to F_v/F_m. Most NPQ parameters were recovered to control levels after 24 to 50 h of recovery. Measurements of qI and qE were effective for screening winter wheat genotypes for surface ice encasement. The Φ_{II} values were slower to recover for winter desiccated plants compared to the surface ice-treated plants, indicating that this parameter may be a good indicator of soil moisture-associated stress during winter. Detailed photosynthetic health assessments including NPQ parameters are valuable for detecting overwintering stresses to overwintering crop species such as winter wheat.

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Introduction

Overwintering crops such as winter wheat (*Triticum aestivum*) often suffer from winterkill damage, particularly in northern regions of the world. Winter wheat crops are sensitive to winter damage and significant crop loss can occur annually due to various winter stresses[1]. Winterkill is an important economic issue for winter wheat farmers with little advances in breeding for specific winter stresses other than low temperature or freezing tolerance[2]. Winterkill damage refers to one or more abiotic stresses that prevent spring regrowth due to the necrosis of overwintering structures, such as crown tissues[3]. The abiotic stresses most associated with winterkill are temperatures below hardiness levels, freeze/thaw cycles, ice encasement, and winter desiccation[4]. With climate change threatening more severe weather events and fluctuating temperatures during cold acclimation and de-acclimation, and lack of persistent snow cover[5], it is important to understand the physiology of overwintering crops during severe conditions such as ice encasement and winter desiccation on spring recovery.

Winter wheat is susceptible to various winterkill-related stresses with precipitation and topography playing a major role in winter weather exposure. Low-lying, poorly drained areas, or areas where soil freezes to preclude drainage, ice encasement is problematic. On exposed slopes or areas lacking snow cover, winter desiccation is possible[6,7]. Ice encasement can cause hypoxia or anoxia, the accumulation of toxic byproducts in soils and plant tissue due to fermentation pathways, and low temperature stress[8,9]. Winter wheat and other cereal grasses can be killed in less than 1 week of ice encasement and exposure to ice encasement can highly limit subsequent freeze tolerance and spring recovery[10]. Interspecies comparisons have indicated that winter wheat is more sensitive than other grass species to winter stresses such as ice encasement[9], but intraspecies evaluations of recovery from extreme winter conditions are lacking. Ice encasement for some short-statured or mown grass species is a complete encasement. For winter wheat plants after fall growth, complete ice encasement is less likely since some leaves would be long enough to protrude above the ice layer. Therefore, the condition used in this study will be termed surface ice, which primarily would influence the stem, crown, and root structures. The soil would be less prone to drying underneath the ice and is used here to provide a comparison of no ice cover and drying soil to surface ice with adequate soil moisture.

While drought of winter wheat during the summer growing season is highly investigated and it is generally accepted that dry conditions during grain filling of winter wheat reduces yield traits to a greater extent than in other periods of the growing season[11], information on the ramifications of plant productivity and health following winter desiccation during dormancy for winter wheat is limited. A desiccation period prior to cold and freezing conditions was able to be a sufficient substitute for a cold acclimation period for winter rye (*Secale cereale*) and

winter wheat allowing for sufficient freezing tolerance and survival compared to plants that went through several weeks of cold acclimation[12]. The mechanism is thought to be related to not only the water content of the plants but also changes in protein and lipid content and characteristics[13]. This clearly shows the profound effect of timing of dry conditions on plant responses and defenses during winter. As protective snow cover may become variable or reduced due to climate change, the physiological effects of winter desiccation on dormant, overwintering structures and plant regrowth need to be more clearly understood.

Vigor during spring recovery has been directly associated with plant productivity and yield, with the earliest spring recovery contributing to high grain yields[14], and early spring frost influenced tillering behavior, and yield traits[1]. While stand counts indicating the emergence of recovered vs non-recovered plants are often recorded to document spring vegetation, these measures do not account for the physiological or photosynthetic health of recovered plants. The health of photosynthetic apparati, which are very sensitive to damage[15], is critical during plants' stress recovery for adequate carbohydrate creation for growth. The plant photosynthetic apparatus is where the primary reactions of photosynthesis are mediated by protein complexes that are embedded in the thylakoid membranes of chloroplasts[16]. Stress conditions negatively affect photosynthesis in most plants by altering the ultrastructure of the organelles and the concentration of various pigments and metabolites involved in this process and energy absorption can exceed the amount capable of being utilized by the plant causing reactive oxygen species generation[17,18]. Non-photochemical quenching (NPQ) traits of plants aim to reduce oxidative damage to photosynthetic apparati, particularly photosystem II (PSII), and dissipate excess energy[19]. Energy dependent quenching (qE) and photoinhibition quenching (qI) are NPQ-associated traits and include heat and energy release mechanisms such as the production of pigments and other protective changes[20]. $NPQ_{(T)}$ is a methodology to measure NPQ without the requirements of a dark adaptation period[21] and Φ_{NPQ} is a parameter that estimates the flux of excitation energy into NPQ pathways or the yield induced by downregulatory processes[22]. Evaluating whether chlorophyll fluorescence-based parameters are useful for evaluating spring health and recovery from winter in overwintering crop species will increase our understanding of plant physiological responses of this time in the growing cycle.

Photosystem damage is highly connected to oxidative damage in plants during stress. Antioxidant activity and lipid peroxidation may be good indicators of winter recovery damage. During plant stress, reactive oxygen species (ROS) are formed and can accumulate to high amounts, overwhelming the antioxidant system and causing damage to plant structures[23]. Cold stress or cold acclimation can upregulate the activity of various antioxidant enzymes in winter wheat plants[24]. Whether antioxidant activities or lipid peroxidation are influenced by surface ice or winter desiccation in various winter wheat genotypes is unclear.

Thus, the objectives of this research were to determine if there are intraspecies variations in chlorophyll fluorescence parameters during recovery from winter desiccation or a surface layer of ice among a small set of winter wheat genotypes and to evaluate whether these conditions alter the health of cold-hardened photosynthetic apparati. It was hypothesized that winter tolerance may be associated with genotypic origin of the winter wheat plants and NPQ-associated parameters may be good indicators of stress level. Winter desiccation may influence photosynthetic efficiency during spring to a greater extent than plants having an ice layer that held moisture in the soil profile. Phenotyping using these parameters may be a viable method for indicating the recovery potential for screening breeding populations to identify winterkill-tolerant winter wheat genotypes.

Materials and methods

Plant material and growth conditions

A set of 10 winter wheat genotypes from various winter wheat breeding programs were used in the study (Table 1). Seeds were placed in pots (6.35 cm wide and 8.89 cm deep) containing sandy loam (65.9% sand, 14.9% silt, 19.2% clay, Typic Hapludault) soil on 17 March 2021 for year 1 and 16 July 2021 for year 2. All pots were treated with a preventative drench fungicide (Subdue Maxx, Corteva Agriscience, Indianapolis, IN, USA) 2 d before vernalization on 17 Mar 2021 and 16 July 2021 (same day as planting). Pots were put in a vernalization room for 8 weeks (about 2 months) at 10 °C. After the vernalization period, on 14 May 2021 (experiment 1) and 10 Sept 2021 (experiment 2), the plants were placed in greenhouse for 2 d before being moved to a growth chamber that has low-temperature capabilities (LTCB-19; Biochambers, Winnipeg, Manitoba, Canada) to begin simulated fall acclimation. Simulated fall acclimation occurred on 16 May 2021 for experiment 1, and 12 September 2021 for experiment 2. The growth chamber conditions were stepped down from 10 °C for 2 weeks with a 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light level and 10/14 h photoperiod, then 4 °C for two weeks, 2 °C for two weeks, and then −1 °C/2 °C day/night for the remaining simulated winter period with the latter three temperature conditions at a light level of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 10/14 h photoperiod, 0% relative humidity. The de-acclimation period occurred between the low-temperature chamber and the DEPI chamber and included 3 d at 10 °C to allow for ice layer melting and defrosting.

Stress treatments and duration

Winter treatments included plants that were exposed to: 1) an ice layer on the soil surface; or 2) winter desiccation imposed as no ice cover and no supplemental watering at low temperature freezing conditions (no ice layer). Ice layer treatment was imposed by misting plants at −1 °C with deionized water for

Table 1. Soft red winter wheat genotypes, abbreviation, and university of origin.

Genotype	Abbreviation	Origin
05222A1-1-2-7-1	PU	Purdue University
IL07-19334	UI	University of Illinois
MI16R0898	MSU	Michigan State University
OH15-131-31	OSU1	Ohio State University
OH15-165-51	OSU2	Ohio State University
OH15-89-68	OSU3	Ohio State University
X11-0010-10-9-5	UK1	University of Kentucky
X11-0081-8-10-3	UK2	University of Kentucky
X11-0120-13-4-5	UK3	University of Kentucky
X11-0249-17-17-3	UK4	University of Kentucky

5 min every 30 min for 2 d until an ice layer formed (approximately 2.54 cm on each soil surface). Ice layers were checked daily throughout the study and misted as needed to maintain a consistent ice layer. While crown tissue was submerged under ice, leaf tissues were not completely submerged under the ice layer and were exposed to growth chamber conditions. Plants that were not covered with an ice layer stopped receiving water at 4 °C temperature and therefore had a total drying period of four weeks before treatment began. A total of 210 pots were used for each experiment in year 1 and year 2. On day 0, a total of 30 pots were used. For all other sampling days (days 4, 10, and 20), 60 pots were used on each day (30 for no ice and 30 ice-treated pots). On 0, 4, 10, or 20 d of treatment, winter wheat plants from both ice-encased and winter desiccation plants were transferred to a dynamic environmental photosynthetic imaging (DEPI) growth chamber. Plants from each treatment were removed from these conditions after a recovery period of four days. In experiment 1, day 0 plants were placed in the DEPI chamber on 14 June 2021 and were taken out on 19 June 2021. Day 4 plants were placed in the DEPI chamber on 19 June 2021 and were taken out on 23 June 2021. Day 10 was on June 25, 2021, and out 29 June 2021. Day 20 in on 5 July 2021, and out 9 July 2021. In experiment 2, day 0 plants were placed in the DEPI chamber on 29 Sept 2021, and were taken out 3 Oct 2021. Day 4 plants were placed in DEPI chamber on 3 Oct 2021 and removed 7 Oct 2021. Day 10 plants were placed 9 Oct 2021 and taken out 13 Oct 2021. Day 20 was on 19 October 2021 and taken out of the DEPI chamber on October 23, 2021.

Photosynthetic imaging

The DEPI chamber lighting system was programmed to measure and calculate parameters including the maximum quantum efficiency of photosystem II (PSII) in the dark-adapted state (F_v/F_m), nonphotochemical quenching (NPQ), quantum efficiency of PSII under steady-state actinic light (Φ_{II}), photosynthetic efficiency energy quenching (qE), and photoinhibition (qI)[25]. All parameters were measured for 12 h every 1 h except F_v/F_m which was measured every 24 h during the experiment, due to dark adaptation requirements. Conditions in the DEPI chamber included 400 μmol·m^{-2}·s^{-1} light with a 12 h photoperiod and a day/night temperature regime of 22/16 °C.

Antioxidant activity

Fresh tissues were collected directly following recovery in the DEPI chamber. For extraction, 0.2 g of fresh tissue was added into 2 mL of extraction solution containing 50 mM phosphate buffer solution (PBS) and 1% polyvinylpolypyrrolidone (PVPP), homogenized on ice, and centrifuged for 20 min at 11,000 rpm.

Malondialdehyde (MDA) content was measured to indicate levels of lipid peroxidation according to Dhindsa et al.[26] and Zhang & Kirkham[27] with some modifications. An extraction solution of 0.8 mL was mixed with 0.4 mL of MDA reaction solution, containing 20% w/v trichloroacetic acid and 0.5% w/v tert-butyl-alcohol, and heated in a water bath at 95 °C for 30 min. Samples were cooled quickly on ice and the absorbance of the mixture was measured at 520 and 600 nm.

Ascorbate peroxidase (APX) activity was determined by monitoring the initial ascorbate oxidation by hydrogen peroxide at 290 nm with modifications[28]. A 3 mL reaction solution was used containing 2.75 mL of 100 mM sodium acetate (pH 5.8), 0.05 mL of 0.003 mM ethylenediaminetetraacetic acid, 0.05 mL of 5 mM hydrogen peroxide (H_2O_2), and 0.05 mL of 10 mM ascorbate acid, and 0.100 mL of enzyme. The absorbance at 290 nm was recorded once every 10 s for 60–80 s. Peroxidase (POD) activity was measured by monitoring the increase in absorbency at 460 nm as guaiacol was oxidized, according to the method of Chance & Maehly[29]. A 25 μL volume of enzyme extract was added to 3 mL of reaction mixture consisted of 33.3 mM phosphate buffered saline at a pH of 6.00.083% guaiacol, and 0.025% H_2O_2[30].

Statistical analysis

The experimental design was a complete randomized block design with three replicate pots. Winter treatment type was the main block and plant genotype, and winter treatment duration were randomized within each block. Lme4 and emmeans packages were used in R Studio (Version 4.2.1, Boston, MA, USA). There were three ice encasement durations (Day 0, 4, 10, and 20). Pots were randomly placed in a growth chamber and the experiment was repeated within the same growth chamber for two years. There was no statistical interaction between experiments 1 and 2 so data are pooled together. Normality was assessed using normal quantile plots, histograms, and residual plots. All data measured and calculated were analyzed using analysis of variance (ANOVA) in R Studio (Version 4.2.1, Boston, MA, USA). A principal component analysis (PCA) was conducted on photosynthetic traits using the FactoMinR package in R[31]. Mean separation was carried out using Fisher's protected least significant difference ($\alpha = 0.05$).

Results

Chlorophyll fluorescence parameters during recovery

Plants that were exposed to cold acclimation only and then went straight into a brief de-acclimation period at 10 °C for 2 d and then to recovery (day 0 plants) had consistent average Φ_{II} and other values for NPQ parameters over the entire recovery time and therefore are presented as a threshold values for each parameter. For instance, day 0 plants had an average of approximately 0.4 for Φ_{II}.

For plants exposed to 4, 10, or 20 d of overwintering and comparing fluorescence parameters that were measured, F_v/F_m was the least impacted by experimental factors and their interactions compared to other parameters (Table 2). Main and interacting effects of ice duration and ice treatment were significant for F_v/F_m but the main or interacting effects for genotype was not significant. The F_v/F_m value decreased significantly with the duration of stress, with day 10 displaying the lowest value. On day 0 the average F_v/F_m was 0.750, 0.729 on day 4, 0.668 on day 10, and marginally higher on day 20 at 0.692. Lower F_v/F_m values were detected for winter desiccation compared to plants with surface ice (Fig. 1).

The Φ_{II} responses and other NPQ parameters were significant for most main and interacting effects based on ANOVA analysis (Table 2). Plants within either surface ice or winter desiccation had low initial Φ_{II} values at the start of recovery (time 0), which ranged from 0.1–0.3 (Fig. 2). For day 10 plants the Φ_{II} values took longer and never reached the day 0 plant threshold values for the winter desiccation treatment compared to the surface ice treatment. On days 4 and 20, Φ_{II}

Table 2. Analysis of variance for main treatment factors and interactions of the maximum quantum efficiency of photosystem II in the dark-adapted state (F_v/F_m), quantum efficiency of PSII under steady-state actinic light (Φ_{II}), nonphotochemical quenching (NPQ), photoinhibition (qI), photosynthetic efficiency energy quenching (qE), ascorbate peroxidase activity (APX), peroxidase activity (POD), and malondialdehyde content (MDA) of wheat plants exposed to simulated winter conditions in two experiments in growth chambers in 2021.

Effect	F_v/F_m	Φ_{II}	Φ_{NPQ}	$NPQ_{(T)}$	qI	qE	APX		POD		MDA	
							Leaf	Crown	Leaf	Crown	Leaf	Crown
Genotype (G)	NS	***	***	***	***	***	NS	*	NS	*	***	***
Duration of stress (D)	***	***	***	***	***	***	***	***	***	NS	***	***
Stress treatment (S)	*	***	NS	NS	***	***	***	NS	***	*	NS	***
G × D	NS	***	***	***	***	***	NS	NS	NS	NS	NS	**
G × S	NS	**	***	***	***	***	NS	NS	NS	NS	NS	NS
D × S	*	***	***	**	***	***	***	NS	NS	NS	NS	**
G × D × S	NS	***	***	***	***	***	NS	NS	NS	NS	NS	NS

Stress treatments included low temperature conditions combined with either surface ice or winter desiccation for a given duration. * p values ≤ 0.05, ** p value ≤ 0.01, *** p value ≤ 0.001, NS = not significant $p > 0.05$.

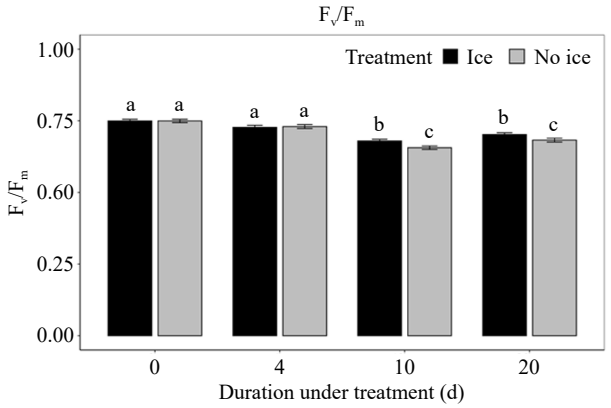

Fig. 1 Maximum quantum efficiency of photosystem II (F_v/F_m) of winter wheat during a recovery period at 22 °C following a winter period of 0, 4, 10, or 20 d with surface ice (black), or winter desiccation treatment (no ice, grey) in growth chambers at −1 °C. Letters indicate differences between treatment and duration under treatment using Fisher's least significant difference (LSD) at $p \leq 0.05$.

value recovery rates were similar for surface ice and winter desiccation-treated plants. Along with duration under treatments impacting Φ_{II} responses, the winter wheat genotypes varied significantly in their responses. Under both treatments, MSU exhibited consistently high Φ_{II} values in both treatments, except for day 20 plants with surface ice treatment, which UK3 and UK2 had the highest Φ_{II} values. Genotypes OSU2, UK4, and PU had the lowest values for both treatments.

For $NPQ_{(T)}$ responses, the main effects of treatment were not significant, whereas interactions between genotype/day x treatment were significant indicating a cross-over interaction (Table 2). On day 0, $NPQ_{(T)}$ responses remained relatively consistent throughout the recovery period with an average of 5.28 (Fig. 3). Both surface ice and winter desiccation treatments initially caused very high $NPQ_{(T)}$ values compared to day 0 plants within the first 12 h, which then began to stabilize around 24 h. From 24 to 100 h of recovery the $NPQ_{(T)}$ values remain consistent across genotype and stress treatment at an average of approximately 5 (data not shown). Within the first 24 h is when primary genotype variation in $NPQ_{(T)}$ responses were found. PU and UK4 generally exhibited high $NPQ_{(T)}$ values during ice encasement and winter desiccation on day 20 whereas MSU consistently showed the lowest $NPQ_{(T)}$ values. On sampling days 4 and 10, plants exposed to surface ice had higher $NPQ_{(T)}$ responses in the first 12 h compared to those that experienced winter desiccation. However, by day 20, winter desiccation plants showed higher $NPQ_{(T)}$ values.

Trends in Φ_{NPQ} responses were similar in response to different durations of stress and stress treatment with trends for day 4 and 10 being consistent with day 20 (all data not shown, Fig. 4a, b). The primary genotypic differences responses for Φ_{NPQ} occurred during the first 12 h of recovery and were most differential between genotypes for day 20 plants. PU sustained a higher value of Φ_{NPQ} compared to most other genotypes.

Just like the fluorescence parameters $NPQ_{(T)}$ and Φ_{NPQ}, qE initially started with high response values within the first 12 h (Fig. 5). With ice cover treatment, the plants displayed on average higher initial values for days 4, 10, and 20 compared to winter desiccation plants. Day 10 for both surface ice and winter desiccation treatments had higher qE responses compared to days 4 and 20. The qE response trends additionally differ across genotypes under both ice encasement and winter desiccation. UK4 initially shows high qE values under ice encasement but experiences a significant decline by day 20. UI consistently exhibits low qE values across all days and treatments. MSU similarly maintains low qE values, particularly with winter desiccation. UK3 and OSU3 show consistently high qE values under winter desiccation.

Photoinhibition (qI) was a good indicator of stress condition since there was a notable difference in the response in qI for surface ice compared to winter desiccated plants (Table 2, Fig. 6). Plants subjected to winter desiccation consistently showed qI values below the control value of 4.68 on all days. In contrast, surface ice-treated plants had high qI values within the first 12 h, with days 10 and 20 showing the largest response compared to day 4 plants. While winter desiccation did not exhibit the same high values as observed in plants with surface ice, genotype differences were observed on day 10 within the first 12 h. The qI values were generally highest for UK4 and OSU3, while UI and MSU had the lowest. On day 4 and 20, PU and OSU2 had the highest qI values, while UK2 and MSU were the lowest on day 4, and UK3 and UI on day 20. For winter desiccation, OSU3 and PU had the highest qI on day 4, and UK3 and UK1 on day 10. For both days 4 and 10, OSU1 and UI recorded the lowest values. By day 20, UK4 and UK2 showed the highest qI values, with PU and UI at the lowest.

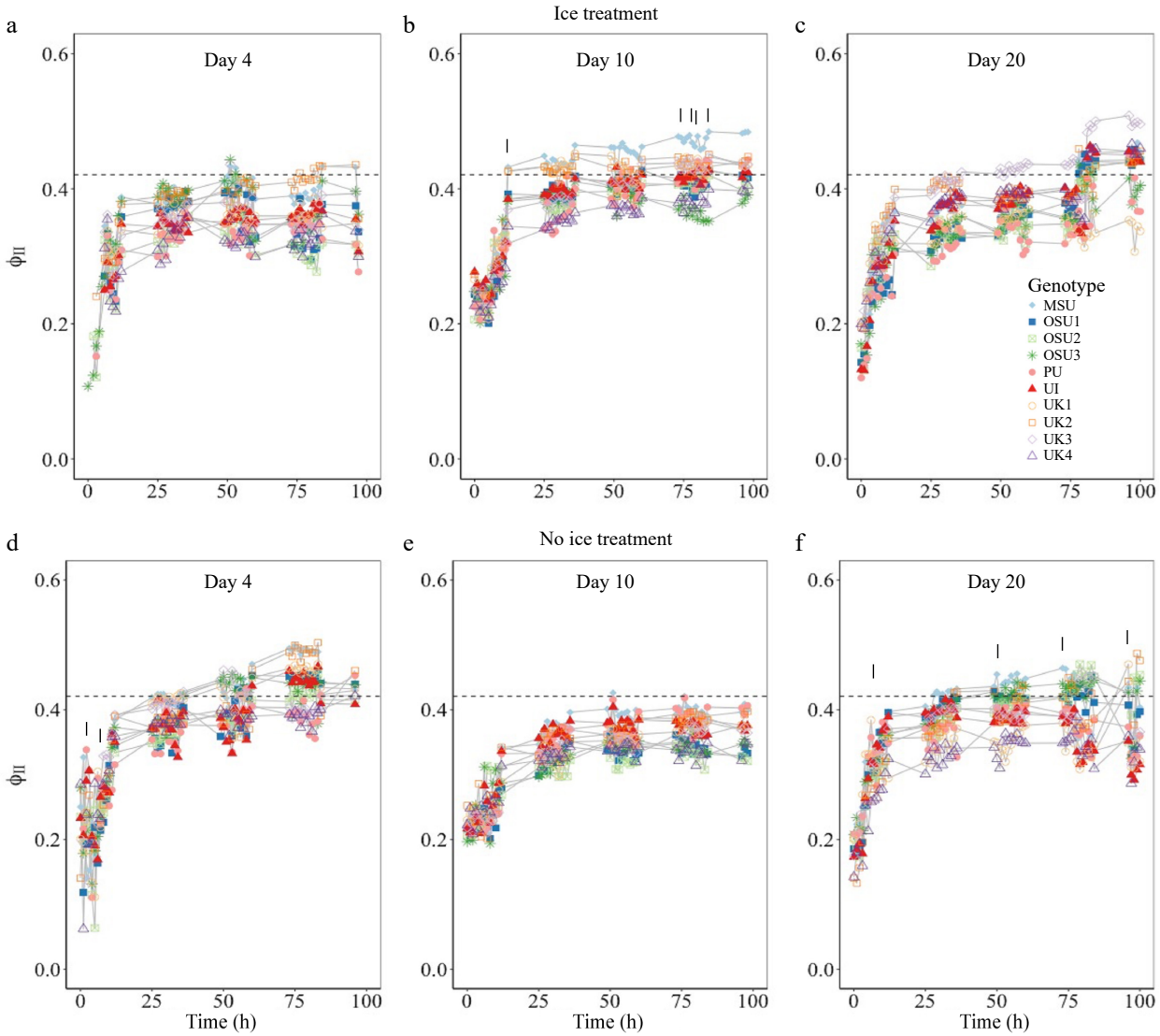

Fig. 2 Quantum yield of photosystem II (Φ_{II}) for winter wheat plants during a recovery period at 22 °C following a winter period in growth chambers at −1 °C. The winter period was for either (a) 4, (b) 10, or (c) 20 d under surface ice (ice treatment), or winter desiccation (no ice treatment) for (d) 4, (e) 10, or (f) 20 d. Dashed lines indicate the average control group values of day 0 plants i.e. those that were cold acclimated only. Vertical lines indicate Fisher's least significant difference (LSD) values between genotypes on respective hours and days ($p \leq 0.05$).

Malondialdehyde content and antioxidant enzyme activity

Lipid peroxidation, measured by MDA content, was assessed in both the leaf and crown tissue of the winter wheat plants. Both leaf and crown tissues exhibited a significant increase in MDA throughout the treatments. In leaf tissues, significant damage was observed on days 10 and 20 compared to day 0 (Fig. 7a). For crown tissue, significant damage was evident on days 4, 10, and 20 (Fig. 7b), and surface ice resulted in significantly higher MDA content compared to the winter desiccation treatment. Additionally, the main effect of genotype was significant in leaf and crown tissues. Specifically, in leaf tissues, genotype UI exhibited higher MDA content compared to most other genotypes. In crown tissues, genotype PU demonstrated the highest MDA content. In contrast, genotype UK2 consistently showed the lowest MDA content in both leaf and crown tissues.

Ascorbate peroxidase (APX) in leaf tissue showed a significant interaction between the duration under ice and the type of treatment (Table 2). Relative to day 0, both ice encasement and winter desiccation treatments resulted in increased APX activity on days 4, 10, and 20. The surface ice treated plants higher APX activity compared to winter desiccation-treated plants (Fig. 8a). In crown tissue, APX activity was influenced by both genotype and duration of treatment (Fig. 8b). Genotypes UK4 exhibited higher APX activity levels than some genotypes such as OSU1, UI, UK2 and UK3. In crown tissue, APX activity was highest on day 20 (Fig. 8c). The POD activity was overall higher than APX activity in both leaf and crown tissues and were elevated due to the duration of stress treatment (Fig. 8d, e). POD activity was overall higher in plants exposed to surface ice compared to no ice (Fig. 8f). In crown tissue, there were minimal significant differences in POD activity among genotypes. Genotype UI exhibited higher POD activity at 175 units $g^{-1}{\cdot}min^{-1}$ DW than MSU, which had an average activity of 95 units $g^{-1}{\cdot}min^{-1}$ DW (data not shown). POD activity in crown tissues was significant due to the main effect of stress

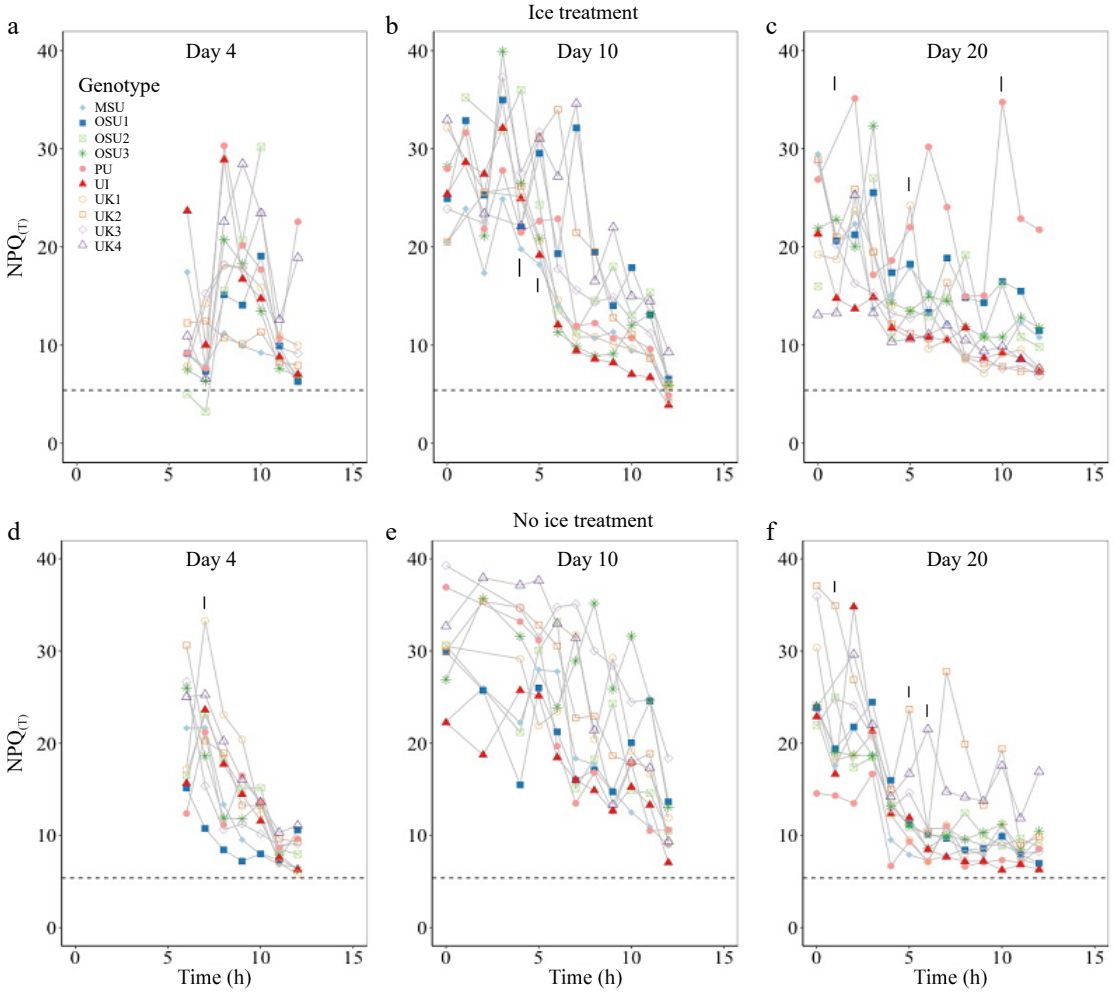

Fig. 3 Non-photochemical quenching ($NPQ_{(T)}$) of winter wheat plants for the first 12-h recovery period (22 °C) after (a) 4, (b) 10, or (c) 20 d of surface ice (ice treatment), or (d) 4, (e) 10, and (f) 20 d of winter desiccation (no ice treatment) in growth chambers at −1 °C. Dashed lines indicate the average control group values of day 0 plants (i.e. those that were cold acclimated only). Vertical lines indicate Fisher's least significant difference (LSD) among genotypes on a given hour during recovery of each respective duration under treatment ($p \leq 0.05$).

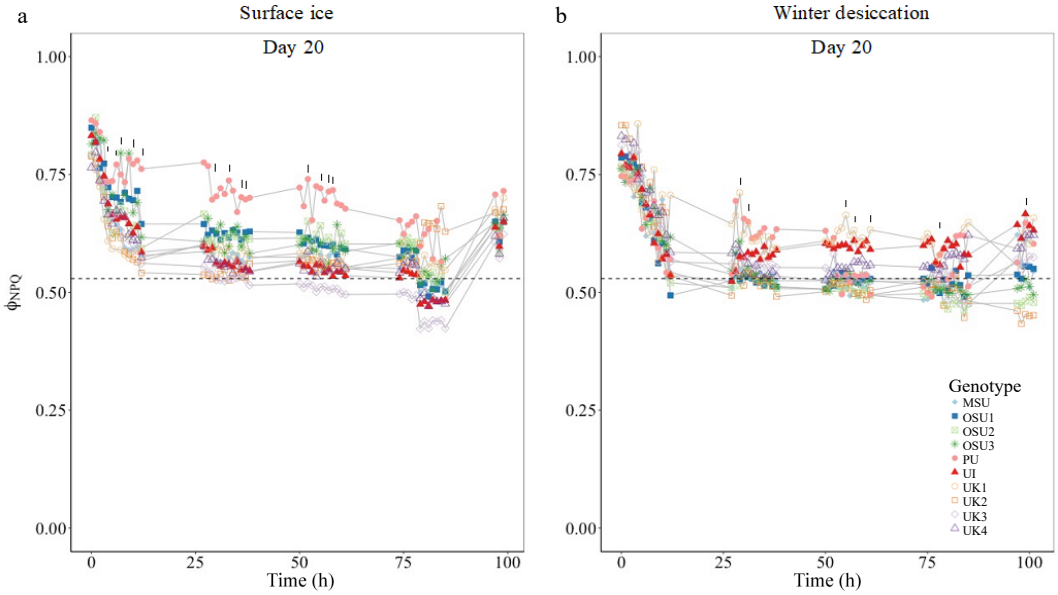

Fig. 4 Recovery of Φ_{NPQ} values at 22 °C of winter wheat plants following winter dormancy (−1 °C), and (a) surface ice, or (b) winter desiccation for 20 d of treatment in growth chambers. Dashed lines indicate the average control group values of day 0 plants i.e. those that were cold acclimated only. Vertical lines indicate Fisher's least significant difference (LSD) values between genotypes on respective hours ($p \leq 0.05$).

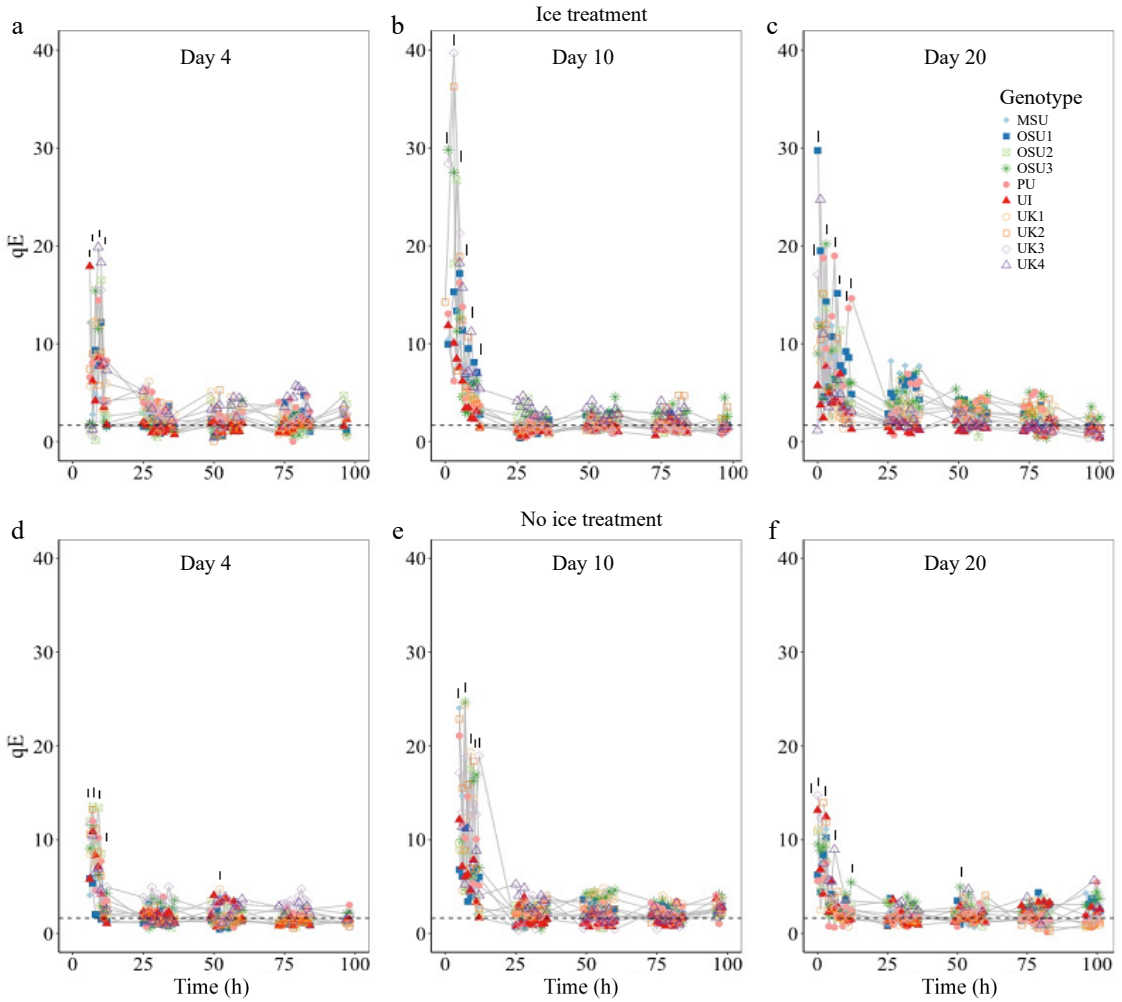

Fig. 5 Energy-dependent quenching (qE) of winter wheat plants during a recovery period (22 °C) following winter dormancy (−1 °C), and (a) 4, (b) 10, or (c) 20 d of surface ice, or (d) 4, (e)10, and (f) 20 d of winter desiccation treatment in growth chambers. Dashed lines indicate the mean of the control group (i.e. those that were cold acclimated). Vertical lines indicate Fisher's least significant difference (LSD) values between genotypes on a given hour of recovery ($p \leq 0.05$).

treatment, with surface ice having a higher average activity of POD compared to the no-ice treatment.

Principal component analysis

To assess the impact of stress treatment on photosynthetic traits, principal component analysis (PCA) revealed that for surface ice-treated plants on day 4 and day 20, Φ_{NPQ} contributed significantly to the variation (Fig. 9a, c). For day 10 Dim1 and Dim2 contributed 84.2% and 8.5% to the variation among genotypes, respectively, with the significant contributors from Φ_{II}, Φ_{NPQ}, and qI (Fig. 9b). For the plants following winter desiccation on day 4, Dim1 and Dim2 accounted for 51% and 28.5% of the variation, respectively, with Φ_{II} as the primary contributor (Fig. 9d). On day 10, where Dim1 and Dim2 contributed 45.1% and 24.5%, respectively. qI and qE are identified as the predominant contributors (Fig. 9e). On day 20, Dim1 and Dim2 contributed 52.4%, and 32%, with $NPQ_{(T)}$ and the main contributor (Fig. 9f).

Discussion

The maximal quantum efficiency of photosystem II (F_v/F_m) declined due to prolonged surface ice treatment or winter desiccation compared to cold acclimated only plants but to a greater extent for the winter desiccation treatment. The decline for both treatments is similar to the effect of cold hardening on photosynthetic apparati. Oat (A*vena sativa*) plants exhibited a significant and reversible decrease in F_v/F_m during cold hardening[32]. Hurry & Huner[33] found that high light and cold grown spring and winter genotypes of wheat each exhibited a decline in F_v/F_m. Low temperature dormancy or prolonged effects of each winter stress implemented in the current study also caused a decline in F_v/F_m. No significant differences were detected between genotypes for F_v/F_m, which may indicate that for these stresses it is not the most effective parameter to detect photosynthetic changes due to these stresses.

The quantum yield (Φ_{II}) was effective at differentiating among stress treatments and genotypes. The Φ_{II} values were generally slower to recover for winter desiccated plants compared to the surface ice-treated plants and therefore may be a good indicator of soil moisture-associated stress during winter. Regarding genotype, UK4 and UK2 took longer to reach control Φ_{II} levels; however, these differences were not always statistically significant and the short time of 12 h to reach control levels indicates low practical significance.

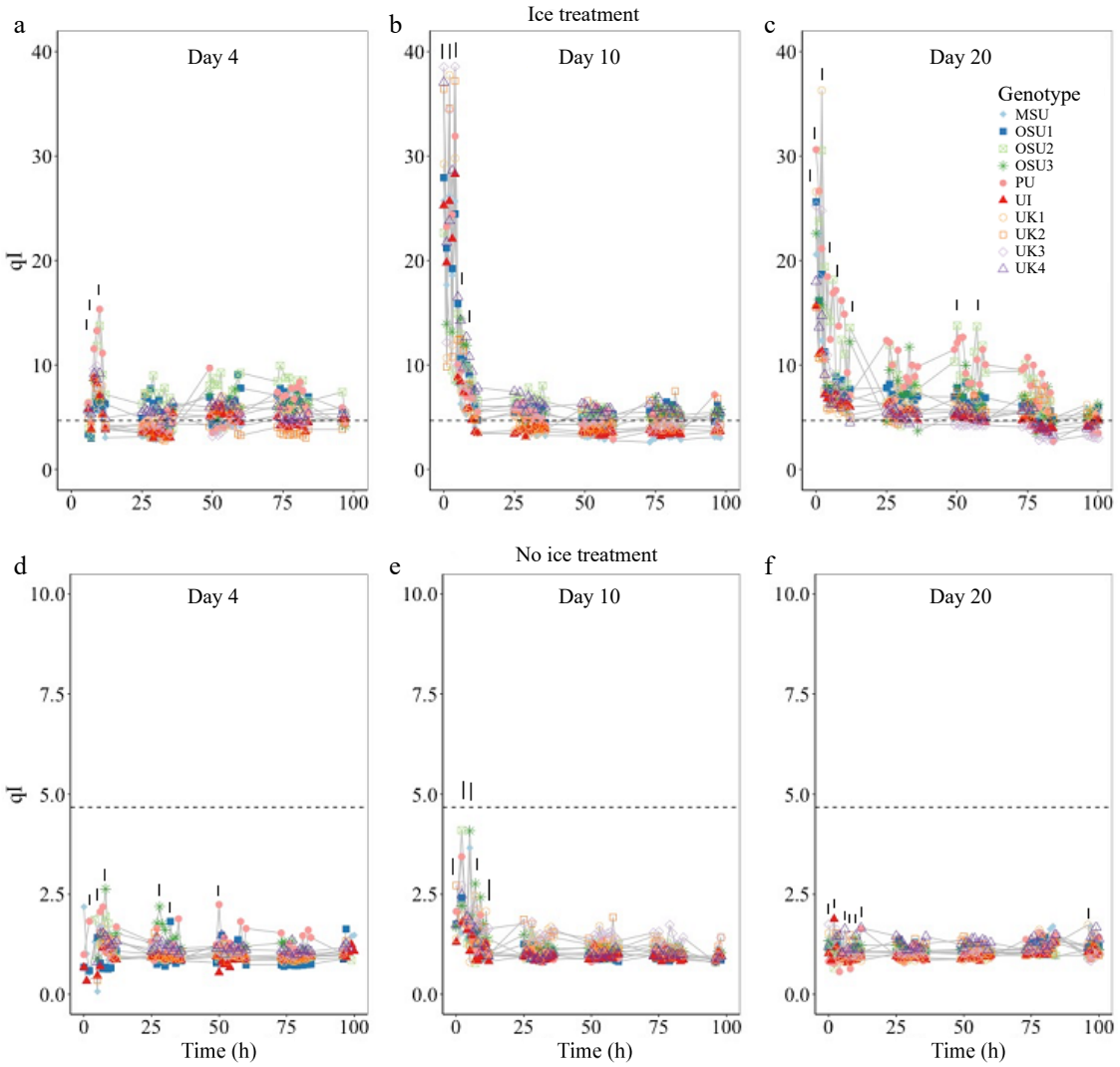

Fig. 6 Photoinhibition (qI) responses during a recovery period (22 °C) of winter wheat plants following a winter period (−1 °C) with a surface ice treatment for (a) 4, (b) 10, or (c) 20 d or winter desiccation for (d) 4, (e) 10, or (f) 20 d of treatment in growth chambers. Dashed lines indicate the mean of day 0 (i.e. those plants that were only cold acclimated). Vertical lines indicate Fisher's least significant difference (LSD) values between genotypes on a given hour of recovery ($p \leq 0.05$).

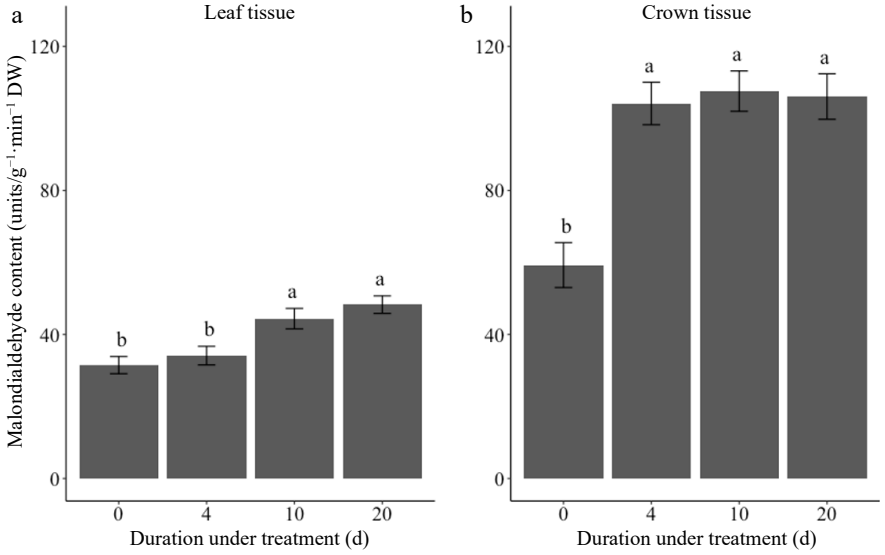

Fig. 7 Malondialdehyde content in (a) leaves, and (b) crown tissues of winter wheat in growth chamber conditions. Letters indicate significant difference between duration of treatment ($p \leq 0.05$).

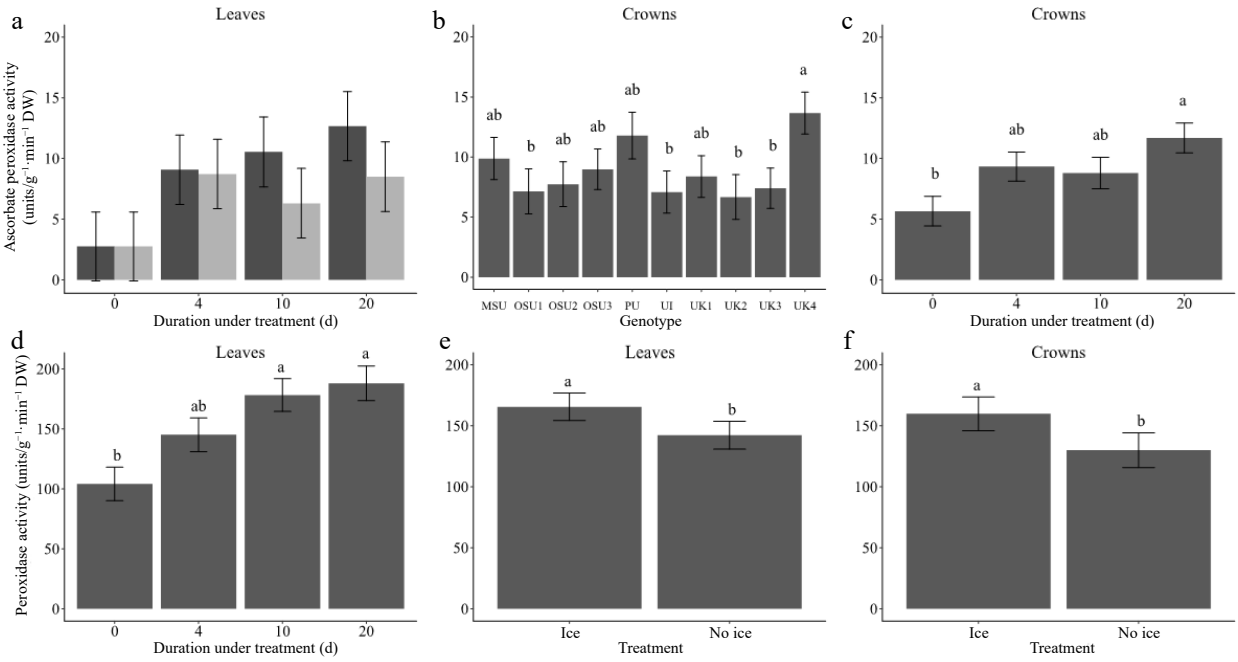

Fig. 8 Ascorbate peroxidase activity (APX) in (a) leaves as influenced by duration, (b) crowns in response to genotype, and (c) crown tissues in response to duration. Peroxidase activity (POD) in (d) leaves in response to duration and (e) leaves and (f) crowns in response to stress treatment of winter wheat in growth chamber conditions. Letters indicate significant differences based on Fisher's protected least significant difference values ($p \leq 0.05$).

The Φ_{NPQ} values decreased to control plant (only cold acclimated) levels throughout the recovery period for plants exposed to either stress treatment. The Φ_{NPQ} measurements indicated genotypic differences in recovery, particularly for day 20 of surface ice with PU likely having the highest damage or highest activation of Φ_{NPQ} even after 12 h of recovery. The $NPQ_{(T)}$ values were very high following winter dormancy for plants of all treatments compared to plants that were only cold acclimated. This could be due to: 1) an experimental artifact in that plants did not have much time to go through an extended de-acclimation period. Plants were moved to the DEPI chamber following a short duration to melt at 10 °C to allow for chlorophyll fluorescence measurements to commence; or 2) both stresses caused significant damage to winter wheat photosystems and caused the need for high levels of energy quenching and the need for thermal dissipation as plants adjusted to different light and temperature conditions. The $NPQ_{(T)}$ measurements did not resolve among winter wheat genotypes for potential stress tolerance levels for most dates; however, not having to dark adapt plants while measuring $NPQ_{(T)}$ allowed for more frequent assessment of this parameter and the measurement was well suited to this low temperature experiment since immediately following low-temperature conditions there is little active growth during the 0 to 12 h of the recovery period. Any growth or changes after measurements of Fm values can render NPQ, qE, and qI useless[21].

The photoinhibition (qI) component of NPQ is primarily associated with dissipation of excess absorbed light energy as heat and or mechanisms without thermal dissipation. The photoinactivation is thought to be directly linked to the damage of the D1 protein within the PSII complex[34]. The qI results were the most differential when comparing the surface ice treatment to the winter desiccation treatment. Winter desiccated plants had minimal to no response of qI whereas qI was very high for many genotypes immediately following surface ice. Surface ice could have caused mild hypoxia that resulted in a sudden re-exposure to oxygen when the ice was melted, particularly for any crown or leaf structures that persisted below the ice. Sudden re-exposure to oxygen is known to cause physiological stress, primarily from oxidative stress[35]. The major environmental change in light and temperatures used in this study could have caused damage to the D1 protein or cold acclimation and low temperatures could have resulted in cold-hardened downregulation of the function of D1 complexes. The interaction of cold hardening and photoinhibition has been evaluated in winter and spring wheat[33]. Cold-hardened leaves recovered photosynthetic efficiency faster than non-hardened leaves. Cold hardening-associated changes in susceptibility to photoinhibition were likely related to cold-hardened photosynthetic apparati and less associated with the capacity for repair of photosynthetic apparati. Winter wheat genotypes that had elevated levels of photoinhibition included PU and OSU2. Measurements of qI may be an effective method to screen winter wheat genotypes for winter stresses such as ice encasement that may cause significant levels of photoinhibition. Our results indicate that the timing of measurement is important since most differences were found within the first 12-to-24-h period of recovery. The winter desiccation plants did not have major changes in photoinhibition. Dry conditions could have been a source of preconditioning for oxidative stress to prevent the accumulation of photoinhibition characteristic of qI measurements; however, additional work would be needed to determine this.

The qE parameter, which is a measure of a rapid process of energy-dependent quenching that occurs in the light harvesting complexes of PSII and indicates a major pH-dependent response associated with activating changes in membranes and accessory pigment production[18], was activated by

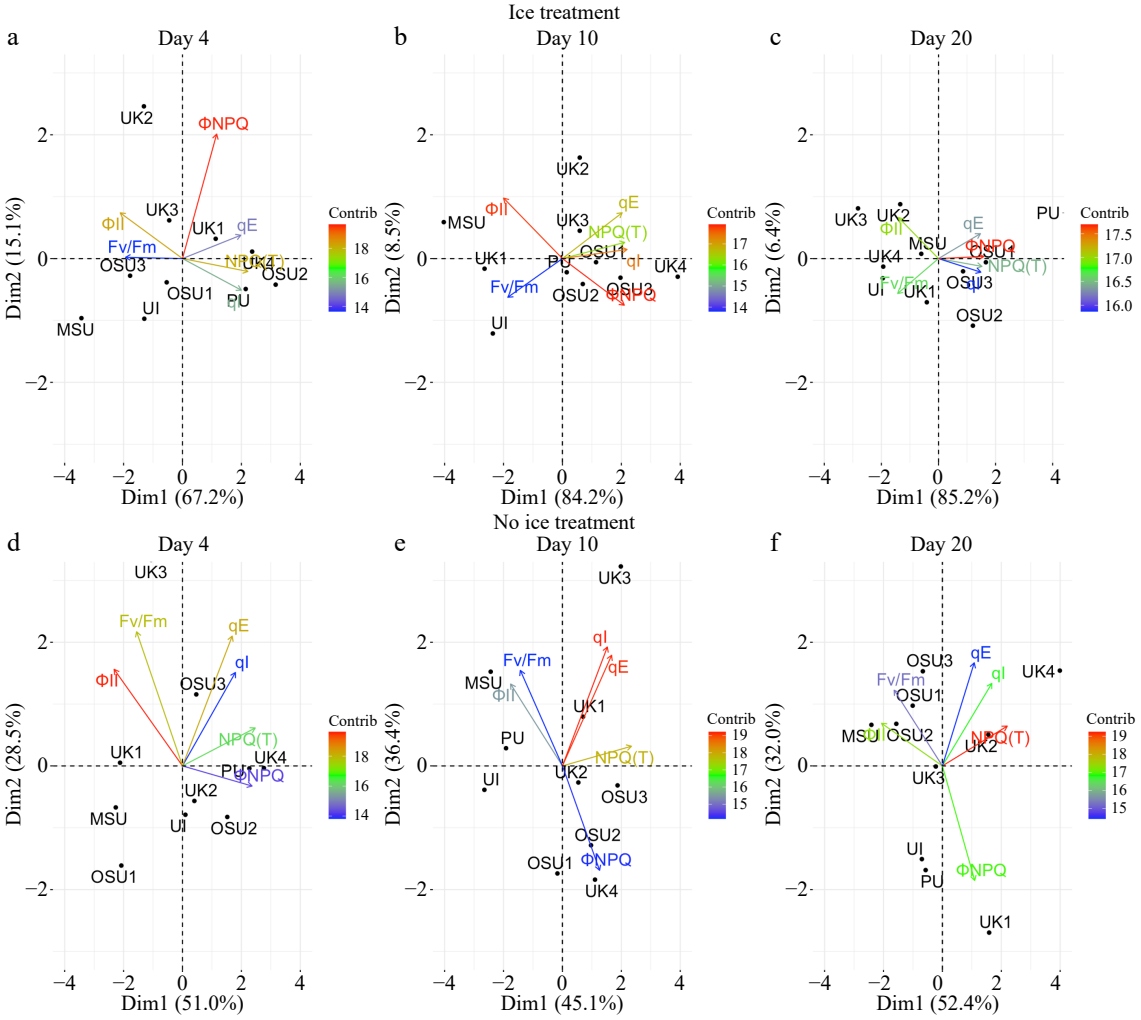

Fig. 9 Principal component analysis (PCA) of winter wheat genotypes following growth chamber winter conditions and surface ice treatment for (a) 4 d, (b) 10 d, and (c) 20 d or winter desiccation treatment for (d) 4 d, (e) 10 d, or (f) 20 d. The highest contributors to the variation are shown in red, and the lowest shown in blue. These groupings are based on maximum efficiency of photosystem II (F_v/F_m), non-photochemical quenching ($NPQ_{(T)}$), Φ_{NPQ}, quantum yield of photosystem II (Φ_{II}), and photoinhibition (qI).

exposure to low-temperature conditions, surface ice and the winter desiccation treatment. The values also fluctuated greatly in the first few hours during recovery, which could be indicative of a large physiological response of these mechanisms. Cold acclimated only plants had very low levels of qE. Surface ice treatment for 10 and 20 d caused the greatest activation of qE. A similar response occurred for the no-ice treatment plants but to a lesser extent than in plants exposed to surface ice. It is possible that the presence of surface ice required plants to have additional requirements for light acclimation, likely moderated by pH and pigment formation[36]. A need for rapid acclimation to light and temperature adjustment could have triggered this response. In tobacco plants (*Nicotiana tabacum*), rapid adjustment to light conditions promoted plant biomass and productivity and was intimately tied to qE responses[37]. Additional work is needed to specifically measure the mechanisms behind qE following winter conditions for grass species.

Lipid peroxidation and activation of antioxidant enzymes (APX and POD) occurred following prolonged winter stress in leaf tissue and crown tissue; however, few responses in these measurements occurred due to genotype or treatment type. A major limitation was that these samples were evaluated following a recovery period of 4 d. The winter wheat plants were likely to experience more oxidative stress on days 0 through 4. An earlier sampling time point would have provided more interpretive power in relation to the photosynthetic health measurements. During photosynthetic imaging in the DEPI chambers it is important not to disturb the plants to get accurate photosynthetic health readings.

Conclusions

Winter wheat plants were photosynthetically resilient to the surface ice and soil drying conditions implemented in controlled conditions in this study. Photosynthetic efficiency and health were regained for most measured parameters after one to two days. Some genotypes of southern origin took longer to regain photosynthetic health, such as for Φ_{II}, compared to northern genotypes; however, this was not always clear and consistent for each stress or duration. Additional knowledge of whether activating these defenses rapidly or not requiring the need for these defenses plays a greater role in winter stress

survival in plants is needed to better interpret the results found here. Regardless, this is the first report of major changes and results showing the importance of non-photochemical quenching parameters following winter stresses such as surface ice encasement and winter desiccation. Measurements of qI and qE were effective for screening winter wheat genotypes for surface ice encasement, which may have caused significant levels of photoinhibition. The Φ_{II} values were generally slower to recover for winter desiccated plants compared to the surface ice-treated plants and therefore may be a good indicator of soil moisture-associated stress during winter. The $\Phi_{NPQ,}$ qI, and qE measurements indicated potential physiological mechanisms that could be occurring following winter stress conditions. Mechanisms associated with NPQ following cold and winter stress conditions are not well-investigated and may be important for future breeding studies to better understand the winter resilience of winter wheat and increase crop productivity.

Author contributions

The authors confirm contribution to the paper as follows: funding acquisition: Merewitz E, Kramer D; experimental design and methodology conceptualization: Olson E, Merewitz E, Kramer D; experiment execution, data collection, and analysis: Miller K, Hall D; genotype physiological analysis and maintenance: Merewitz E, Miller K; draft and final manuscript preparation: Merewitz E, Miller K, Kramer D, Hall D, Olson E. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest

The authors declare that they have no conflict of interest.

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