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RESEARCH ARTICLE

Higher tolerance to freezing stress may accelerate the distribution and invasion of wild barley (*Hordeum spontaneum* Koch) and feral rye (*Secale cereale* L.)

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Abstract Background: Rising winter temperatures and increasing seasonal variability particularly during autumn and spring have contributed to the enhanced survival and proliferation of cold-tolerant weeds such as wild barley and feral rye in wheat fields. These resilient weeds compete aggressively with winter wheat and exhibit high adaptability to fluctuating environmental conditions. **Objective**: This study aimed to evaluate the freezing tolerance of wheat, wild barley, and feral rye by assessing survival rates, electrolyte leakage, regrowth characteristics, and the maximum photochemical efficiency of photosystem II (Fv'/Fm'). **Methods**: Plants were exposed to freezing temperatures (0, -3, -6, -9, -12, -15 and -18°C) in tillering stage and their tolerance was measured by determining the temperature at which 50% electrolyte leakage occurred in leaves (T_{EL50}), the lethal temperature for 50% survival (LT_{50su}), seedlings dry matter and Fv'/Fm' at -18°C **Results**: Feral rye exhibited the lowest T_{EL50} (–16°C), while wild barley showed the highest (–12°C). Wild barley also had the highest LT_{50su} (–12.6°C), whereas feral rye had the lowest (–15.4°C). At –15°C, the reduction in dry matter for feral rye and wild barley was 33% and 84%, respectively. At –18°C, Fv'/Fm' decreased by 65%, 70%, 76%, and 39% for wheat cultivars (Pishgam, Ghezel Khoushe), wild barley, and feral rye, respectively. **Conclusions**: Rising winter temperatures may diminish wheat's capacity to withstand freezing stress, while more resilient species, such as wild barley and feral rye, may thrive. This shift could result in heightened competition and potential invasions in wheat fields due their natural adaptation to milder freezing conditions.

Keywords: Electrolyte Leakage; Feral rye; Fv'/Fm'; Photosystem II; Survival.

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1. Introduction

Wild barley (*Hordeum spontaneum* Koch) and feral rye (*Secale cereale* L.) are among the most troublesome autumn weeds commonly infesting winter wheat (*Triticum aestivum* L.) fields, leading to notable yield reductions (Hosseini et al., 2022; Kumar et al., 2021). Their ability to tolerate various abiotic stresses, including cold temperatures, along with high growth plasticity and prolific seed production, has enabled their widespread establishment in autumn cereal crops across many cold and temperate regions (Izadi-Darbandi et al., 2020). As members of the Poaceae family, wild barley and feral rye share physiological and ecological traits with wheat. Characteristics such as prolific tillering, dense leaf coverage, considerable height, and allelopathic effects confer a competitive advantage to these weeds over wheat (Burger, Ellstrand, 2014).

Fluctuations in seasonal temperatures, particularly during autumn and spring, can influence frost tolerance in crops and affect the dynamics of weed populations (Vyse et al., 2019). These temperature variations may alter the distribution patterns of invasive and weedy species. The recent increase in the prevalence of certain weed species in autumn cropping systems reflects their resilience to temperature variability and their adaptive potential to diverse environmental conditions. Even moderate frost events can damage crops that have limited cold tolerance, whereas frost-resistant weeds such as wild barley and feral rye are able to survive, regenerate, and expand within these agroecosystems (Cici, Van Acker, 2009; 2011).

The population structure of plant species, especially invasive and weedy taxa, is largely shaped by their relative fitness under prevailing environmental conditions (Henckes et al., 2019). Fitness parameters for weeds include traits such as vigor, seed production, germination behavior, dormancy, and competitive ability (Li et al., 2021). Therefore, investigating cold tolerance and relative fitness of both crops and associated weeds under freezing stress can provide critical insights into their competitive interactions and inform management strategies.

Cold stress is a major abiotic constraint affecting the growth and productivity of winter cereals and other plants in many regions worldwide (Liu et al., 2019). Beyond directly impacting plant growth, cold stress influences the competitive balance between crops and weeds and plays a key role in the dispersal and geographic distribution of invasive species. For instance, Cici and Van Acker (2011) documented significant variation in cold

tolerance among facultative winter annual weeds collected across Canada, demonstrating that cold tolerance is a critical factor in successful establishment and spread.

Key physiological indicators used to assess cold tolerance include survival percentage (SU%), regrowth capacity, cell membrane stability, and the performance index of photosystem II (PSII), commonly expressed as Fv'/Fm', following exposure to freezing temperatures (Hoermiller et al., 2018; Hasanfard et al., 2023). The Fv'/Fm' ratio measures the maximum quantum efficiency of PSII under light-adapted conditions and serves as a sensitive indicator of photosynthetic performance. Reductions in Fv'/Fm' after freezing stress reflect damage to PSII and consequent declines in photosynthetic efficiency (Rapacz, 2007). Nabati et al. (2021) emphasized that the survival rate post-freezing stress is directly linked to plant recovery potential and is essential for predicting yield losses in frost-prone areas, where even moderate freezing can significantly reduce crop productivity.

Understanding plant responses to freezing stress is fundamental to elucidating the mechanisms of cold acclimation, which in turn influence plant survival, adaptation, and distribution. Previous studies have demonstrated that wild species and their weedy relatives often retain—or even enhance—their cold tolerance over time, due to long-term exposure to natural selection in harsh and variable environments, including colder climates (Cici, Van Acker, 2011; Hasanfard et al. 2021). In contrast, cultivated crops such as wheat has undergone intensive breeding and genetic selection, primarily aimed at improving yield and agronomic performance under controlled conditions. This targeted selection may have inadvertently reduced its resilience to abiotic stresses such as freezing. This biological and ecological divergence between domesticated crops and their wild or weedy counterparts forms the rationale for our hypothesis. Despite the significant agricultural impact of feral rye and wild barley as autumn-emerging weeds in wheat-based systems, their cold tolerance and adaptive capacity under freezing stress remain insufficiently characterized. Therefore, this study aims to address this gap by evaluating and comparing the freezing responses of wheat, feral rye, and wild barley under controlled experimental conditions.

2. Materials and Methods

2.1 Plant materials and growth conditions

In this study, wheat cultivars (cv. Pishgam and Ghezel Khoushe) and two common annual winter weeds in wheat fields, including wild barley and feral rye, were used. These wheat cultivars were selected for their high cold tolerance and widespread cultivation in the region. Wheat seeds were obtained from the Khorasan Razavi Agricultural and Natural Resources Research Center, while weed seeds were collected from infested farms around Mashhad, Iran. To break the dormancy of wild barley seeds, they were treated with a 0.2% potassium nitrate (KNO_3) solution for three days at 5°C in the dark (Hasanfard et al., 2021). Plastic pots (12 cm in diameter and 20 cm deep) were filled with a mixture of sand, field soil, and peat moss (1:1:1, w/w). Seeds were sown at a depth of 4–6 cm, with a density of eight seeds per pot, on November 4, 2018. Irrigation was applied immediately after planting and continued throughout the growing season based on the water requirements of the pots. To induce cold acclimation in seedlings, they were grown outdoors under natural conditions (Figure 1) at the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran (36°15' N, 59°28' E; 985 m. s. l.), until the tillering stage on January 20, 2019.

2.2 Experimental treatments

The plants were subjected to seven freezing temperatures (-3°C, -6°C, -9°C, -12°C, -15°C, and -18°C), based on the typical temperature patterns observed in the region during the growing season, with 0°C used as the control. At the end of the tillering stage, the plants were transferred to a thermogradient freezer (Weiss Technik). The initial temperature of the freezer was set at 5°C and then gradually decreased at a rate of 2°C per hour. Upon reaching -3°C, the seedlings were sprayed with a solution containing Ice Nucleation Active Bacteria (INAB), specifically Pseudomonas syringae, to induce ice nucleation within the plant tissues and prevent supercooling (Lindow, 1983). The plants were maintained at each target temperature for one hour. Immediately after the freezing treatment, the pots were moved to a growth chamber maintained at 5 ± 1°C for approximately 24 hours to slow the thawing process. They were then transferred to a greenhouse set at 20 ± 1°C with a relative humidity of 40–50%, where they remained for 21 days under natural photoperiod conditions. Plant survival and regrowth were subsequently assessed.

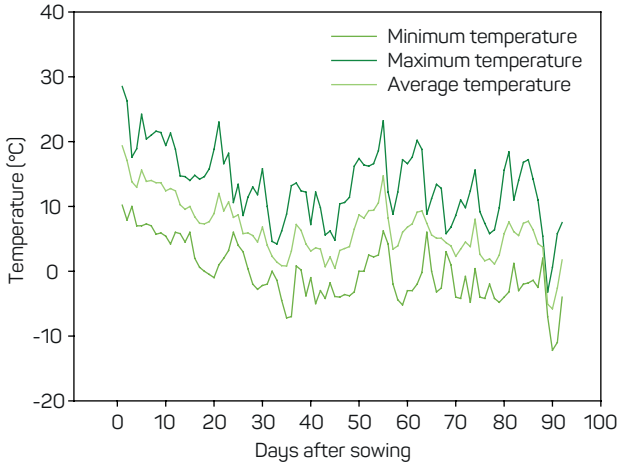

Figure 1 - Minimum, maximum and their average temperatures in November and December of 2018 and January 2019 in Mashhad, Iran

2.3 Electrolyte leakage

Cell membrane injury caused by freezing stress was evaluated by measuring electrolyte leakage (EL). Twenty-four hours after exposure to freezing temperatures, one plant with two fully developed true leaves and an intact crown was removed from each pot. The leaves and crown were separated and placed into individual vials containing 50 mL of deionized water, following the method described by Nezami et al. (2012). The samples were then placed on a shaker at 100 rpm for six hours, after which the initial electrical conductivity (EC_1) was measured using a conductivity meter (Jenway Model 4510, UK). To determine the total electrical conductivity (EC_2), which represents complete cell membrane damage, the samples were autoclaved at 110°C and 1.2 atm for 30 minutes. After cooling, the samples were again shaken for six hours, and EC_2 was recorded. EL was calculated as a percentage using the following formula:

$$EL\% = (EC1 / EC2) \times 100$$

Where EL% is the percentage of electrolyte leakage, EC_1 is the initial EL measured at a specific freezing temperature, and EC_2 is the total EL measured after autoclaving the samples. The temperature at which 50% EL occurred (TEL_{50}) was estimated by plotting the average EL% values against the corresponding freezing temperatures. A sigmoid regression model was fitted to the data using the following equation:

$$Elp = Eli + (Elm - Eli) / [1 + e^{(-B (T - Tm))}]$$

Where ELp is the predicted electrolyte leakage (%), ELi is the minimum EL (baseline leakage at the highest temperature), ELm is the maximum EL (typically close to 100%), e is the base of the natural logarithm (approximately 2.718), B represents the slope of the curve (indicating the rate of increase in leakage with decreasing temperature), T is the absolute value of the applied freezing temperature, and Tm is the temperature corresponding to 50% EL(TEL_{50}), representing the inflection point of the curve.

2.4 Survival and plant regrowth

After 21 days of recovery following the freezing treatment, several regrowth characteristics were evaluated. These included plant height, number of leaves, leaf area (measured using a leaf area meter, Delta-T Devices Ltd.), leaf chlorophyll content (measured using a chlorophyll content meter, Opti-Sciences CCM-200), and plant dry matter (both shoot and root). At the end of the regrowth period, the plant SU% for each replicate was calculated using the following formula:

$$SU\% = (\text{Number of surviving plants} / \text{Total number of plants}) \times 100$$

All surviving plants were used to evaluate the regrowth characteristics, and the average values were calculated for each pot. Ultimately, each pot was considered as a single replicate in the analysis.

2.5 Chlorophyll fluorescence

The maximum photochemical efficiency of PSII under light conditions (Fv'/Fm') was measured in the youngest fully expanded leaves, both before and seven days after exposure to freezing stress. This measurement was performed using a pulse-modulated fluorometer (Model OS1-FL, Opti-Sciences, Hudson, NH, USA).

2.6 Statistical analysis

A completely randomized design (CRD) was employed in a 4 × 7 factorial arrangement with four replications. The first factor was plant species, and the second factor was freezing temperatures. For traits such as the lethal temperature for survival (LT_{50}su) and the temperature at which TDM was reduced by 50% ($RDMT_{50}$), data were analyzed using a CRD with plant species as the sole factor. Prior to conducting the analysis of variance (ANOVA), the normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. Data transformation was not required.

Each experiment was conducted in two independent runs with a one-week interval. Since no significant differences were observed between the runs ($p > 0.05$), data from both runs were pooled. ANOVA was performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA), and mean comparisons were conducted using the Least Significant Difference (LSD) test at a significance level of $p \leq 0.05$. Graphs were generated using SigmaPlot software (version 11; Systat Software Inc., San Jose, CA, USA).

3. Results

3.1 Electrolyte leakage percentage (EL%) and T_{EL50}

As temperature decreased, the percentage of EL% in both the leaf and crown tissues of all tested plants increased (Figure 2). The highest EL% was observed in wild barley, while the lowest was recorded in feral rye. In the wheat cultivars and feral rye, EL% in both leaf and crown tissues did not change significantly at -9°C. However, in wild barley, EL% began to increase at -6°C, indicating its greater sensitivity to freezing stress.

The lowest and highest temperatures causing 50% EL in the leaf ($T_{EL_{50}}$) were observed in feral rye (-16°C) and wild barley (-12°C), respectively (Figure 3). Although no significant differences were found between the wheat cultivars, the $T_{EL_{50}}$ of feral rye was approximately 1.5°C lower than that of wheat cv. Pishgam. In crown tissues, the lowest and highest $T_{EL_{50}}$ values were also

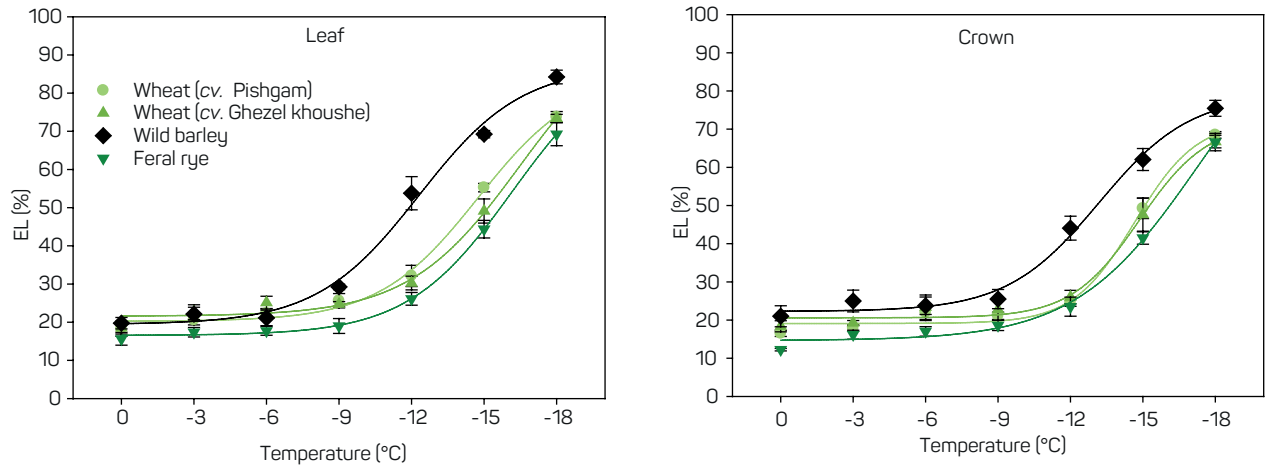

Figure 2 - Effect of freezing temperatures on electrolytes leakage (EL%) of leaf and crown in wheat cultivars (*cvs.* Pishgam and Ghezel khoushe), wild barley and feral rye. Bars represent the standard error around each mean

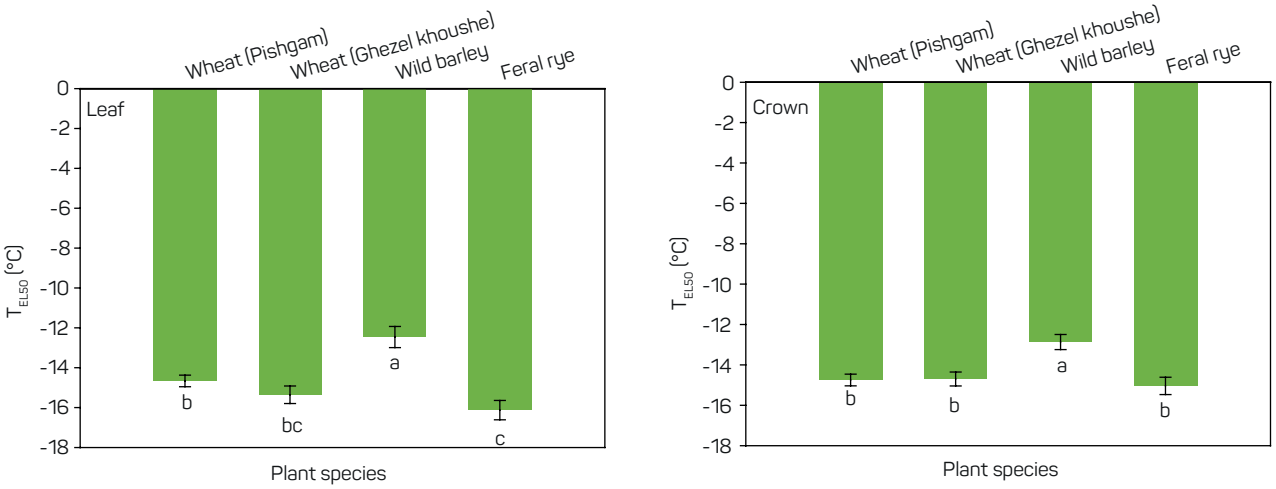

Figure 3 - Temperature causing 50% electrolyte leakage (T_{EL50}) of wheat cultivars (*cvs.* Pishgam and Ghezel khoushe), wild barleyand feral rye. Means with same letters do not have any significant differences according to LSD ($p \leq 0.05$). Bars represent the standard error around each mean

recorded in feral rye (-15°C) and wild barley (-13°C), respectively (Figure 3), with no significant differences in crown $T_{EL_{50}}$ between feral rye and the wheat cultivars. Interestingly, the crown $T_{EL_{50}}$ in feral rye was higher (i.e., less negative) than its leaf $T_{EL_{50}}$, indicating that the crown tissue may be more vulnerable to freezing injury in this species.

3.2 Survival percentage (SU%) and LT_{50su}

The SU% of wheat cultivars did not change significantly at -9°C (Figure 4). However, a sharp decline in SU% was observed beginning at -12°C, reaching 0% at -18°C. In contrast, feral rye maintained 100% survival up to -12°C, after which SU% gradually declined, reaching a minimum of 10% at -18°C. For wild barley, SU% remained at 100% up to -6°C but declined steadily thereafter, reaching 0% at -18°C (Figure 4).

Significant differences were observed in the lethal temperature at which 50% of plants survived (LT_{50}su). Wild barley exhibited the highest LT_{50}su at -12.6°C, indicating lower cold tolerance, while feral rye showed the lowest LT_{50}su at -15.4°C (Table 1). The LT_{50}su values for feral rye and the wheat cultivars were not statistically different.

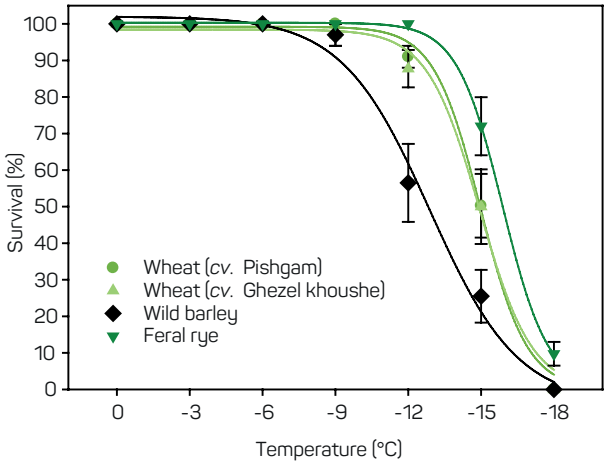

Figure 4 - Effect of freezing temperatures on survival% in wheat cultivars (*cvs.* Pishgam and Ghezel khoushe), wild barley and feral rye as measured 21 days after the application of freezing stress. Bars represent the standard error around each mean

Table 1 - LT_{50su} and $RDMT_{50}$ of two cultivars of wheat (*cvs.* Pishgam and Ghezel khoushe), wild barley and feral rye

Plant species Traits	Wheat (cv. Pishgam)	Wheat (cv. Ghezel khoushe)	Wild barley	Feral rye
LT_{50su} (°C)	-15.0±0.2^{b}	-14.8±0.4^{b}	-12.6±0.8^{a}	-15.4±0.4^{b}
$RDMT_{50}$ (°C)	-14.0±0.5^{b}	-14.5±0.4^{bc}	-11.9±0.3^{a}	-15.4±0.2^{c}

Means with same letters do not have significant difference according to LSD ($p \leq 0.05$). Numerical values are mean ± SE
LT_{50su}: Lethal temperature for 50% survival; $RDMT_{50}$: Reduced Total Dry Matter Temperature 50%

3.3 Plant height

The interaction between plant species and temperature on plant height was not significant (Table 1S). Therefore, only the main effects of plant species and temperature on plant height are presented. As temperature decreased from 0°C to -12°C, no significant changes in plant height were observed (Figure 5a). However, at -15°C and -18°C, plant height decreased by 17% and 92%, respectively, compared to the control (0°C). Among the surviving plants, the highest average plant height (12 cm) was recorded at 0°C, while the lowest (1 cm) was recorded at -18°C, indicating a 92% reduction.

In terms of plant species, feral rye produced the tallest plants (13 cm on average), while wild barley had the shortest (7 cm) (Figure 5b). No significant differences in plant height were observed between the two wheat cultivars.

3.4 Leaf area (LA)

Among the surviving plants, the highest LA was observed in feral rye at -3°C, reaching 117 cm^2 per plant, while the lowest LA was recorded in wheat (cv. Ghezel Khoushe) at -15°C, with 14 cm^2 per plant (Table 2). In wild barley, LA decreased by 49%, 68%, and 100% at -12°C, -15°C, and -18°C, respectively, compared to the control. In feral rye, reductions in LA were more moderate, with a 22% decrease at -15°C and an 81% decrease at -18°C relative to the control.

3.5 Dry matter (DM) and $RDMT_{50}$

Feral rye, shoot dry matter (SDM) was 38% lower at -15°C compared to the control (Table 2). Similarly, in wheat cultivars Pishgam and Ghezel Khoushe, as well as wild barley, SDM decreased by 73%, 58%, and 86%, respectively, at -15°C relative to the control. Root dry matter (RDM) in feral rye plants was 21% lower at -15°C than the control. In contrast, the RDM of wheat cultivars Pishgam and Ghezel Khoushe and wild barley decreased by 54%, 55%, and 82%, respectively, under the same conditions (Table 2).

Total dry matter (TDM) in feral rye, wheat cultivars Pishgam and Ghezel Khoushe, and wild barley was reduced by 33%, 65%, 57%, and 84%, respectively, at -15°C compared to the control (Table 2). The temperature causing a 50% reduction in total dry matter ($RDMT_{50}$) was -15.4°C for feral rye, while for wheat cultivars Pishgam and Ghezel Khoushe and wild barley, were -14.0°C, -14.5°C, and -11.9°C, respectively (Table 2S, Table 1).

3.6 Chlorophyll content (SPAD) and Fv'/Fm'

The highest chlorophyll content among the treatments was observed in feral rye at temperatures ranging from 0°C to -12°C, with values between 34 and 36, while the lowest chlorophyll content (ranging from 0 to 2) was recorded across all species at -18°C (Table 2). Compared to the control, chlorophyll content at -18°C decreased by 100% in wheat cultivars and wild barley, and by 95% in feral rye (Table 2). The reduction in maximum photochemical efficiency of PSII under light conditions (Fv'/Fm') at -18°C relative to 0°C was 65% in wheat cv. Pishgam, 70% in cv. Ghezel Khoushe, 76% in wild barley, and 39% in feral rye (Figure 6).

3.7 Relationship between SU%, EL%, and Fv'/Fm'

A strong negative relationship was observed between SU% and EL% in both leaf and crown tissues, with a high regression coefficient (R^2 = 0.99) (Figure 7), indicating that as EL% increased, SU% decreased across the plant species. Similarly, the regression analysis between SU% and the maximum photochemical efficiency of PSII under light conditions (Fv'/Fm') showed a high positive correlation (R^2 = 0.81), where SU% decreased as Fv'/Fm' declined (Figure 7).

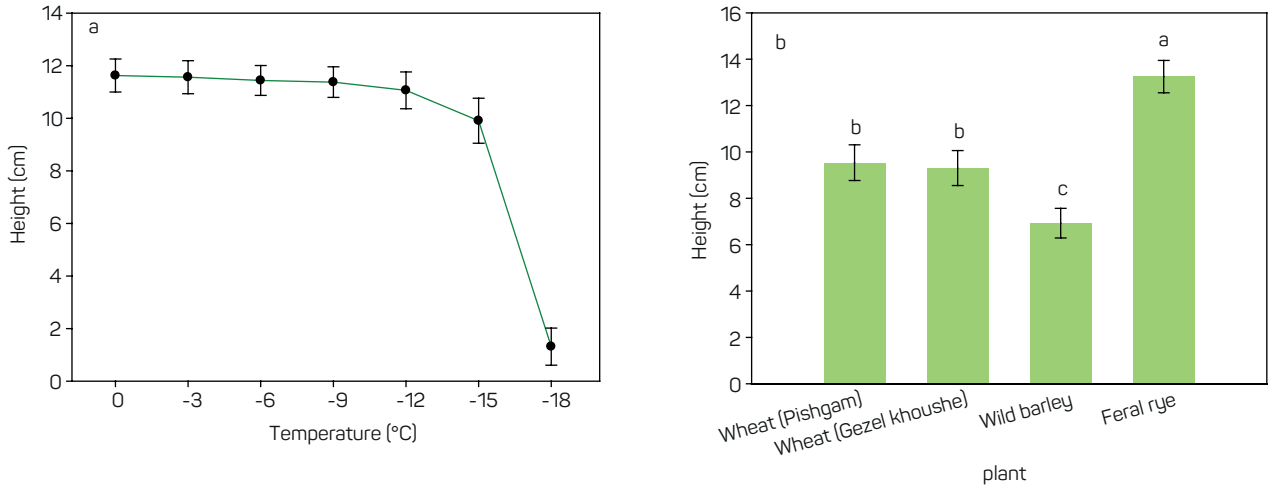

Figure 5 - Effect of freezing temperatures on height (a) of wheat cultivars (*cvs.* Pishgam and Ghezel khoushe), wild barley and feral rye (b) 21 days after freezing stress. Means with same letters do not have significant difference according to LSD ($p \leq 0.05$). Bars represent the standard error around each mean

Table 2 - Leaf area, shoot, root and total dry matter and chlorophyll content (SPAD) in wheat cultivars (*cvs.* Pishgam and Ghezel khoushe), wild barley and feral rye 21 days after freezing stress

Treatment		Traits				
Plant species	Temperature (°C)	Leaf area ($cm^2 plant^{-1}$)	Dry matter ($mg\ plant^{-1}$)			Chlorophyll content
			Shoot	Root	Total	
Wheat (*cv.* Pishgam)	0	22(100*) ±3.5^{e}	646(100)±9.2^{c}	536(100)±20.8^{c}	1181(100)±27.5^{c}	23(100)±0.8^{b}
	-3	21(95)±3.4^{e}	650(100.6)±9.0^{c}	549(102)±24.7^{c}	1198(101)±21.5^{c}	24(104)±1.3^{b}
	-6	21(95)±3.7^{e}	646(100)±8.1^{c}	536(100)±28.8^{c}	1182(100)±33.6^{c}	23(100)±0.9^{b}
	-9	21(95)±3.5^{e}	619(96)±34.2^{cd}	552(103)±28.6^{c}	1170(99)±29.2^{cd}	23(100)±0.8^{bc}
	-12	20(90)±3.4^{e}	410(63)±67.2^{de}	512(95)±22.8^{cd}	922(78)±48.6^{d}	18(78)±2.3^{de}
	-15	15(68)±4.3^{ef}	172(26)±62.2^{fg}	246(46)±48.8^{f}	418(35)±104.7^{ef}	7(30)±1.0^{g}
	-18	0(0)±0.0^{f}	0(0)±0.0^{g}	0(0)±0.0^{i}	0(0)±0.0^{g}	0(0)±0.0^{h}
Wheat (*cv.* Ghezel khoushe)	0	27(100)±1.0^{de}	668(100)±14.3^{c}	500(100)±27.7^{cd}	1168(100)±31.5^{cd}	22(100)±0.6^{bc}
	-3	29(101)±1.3^{de}	671(100.)±17.1^{c}	490(98)±27.3^{cd}	1161(99)±38.4^{cd}	24(109)±0.6^{b}
	-6	27(50)±1.1^{de}	644(96)±10.7^{cd}	512(102)±24.9^{cd}	1156(98)±27.6^{cd}	24(109)±0.9^{b}
	-9	27(50)±1.7^{de}	626(93)±15.4^{cd}	499(99)±27.0^{cd}	1125(96)±37.9^{cd}	25(104)±1.3^{b}
	-12	24(47)±2.2^{e}	465(69)±51.7^{cd}	488(97)±35.5^{cd}	953(81)±47.9^{cd}	20(83)±0.7^{cd}
	-15	14(24)±4.1^{ef}	280(30)±75.5^{ef}	226(45)±25.1^{f}	506(43)±94.9^{e}	12(50)±0.8^{f}
	-18	0(0)±0.0^{f}	0(0)±0.0^{g}	0(0)±0.0^{i}	0(0)±0.0^{g}	0(0)±0.0^{h}
Wild barley	0	53(100)±2.6^{c}	563(100)±25.5^{cd}	452(100)±9.4^{de}	1016(100)±18.1cd	17(100)±0.4^{e}
	-3	54(101)±4.1^{c}	576(102)±28.2^{cd}	454(100.)±9.2^{de}	1030(101)±23.8^{cd}	17(100)±0.5^{e}
	-6	53(100)±3.8^{c}	563(100)±25.5^{cd}	441(97)±7.7^{de}	1003(98)±21.3^{cd}	18(105)±0.8^{de}
	-9	44(83)±8.1^{cd}	556(98)±18.8^{cd}	404(89)±28.6^{e}	960(94)±42.9^{cd}	16(94)±0.6^{e}
	-12	27(50)±7.9^{de}	309(54)±51.9^{ef}	123(27)±31.0^{g}	432(42)±82.7^{e}	12(70)±2.3^{f}
	-15	17(32)±6.0^{ef}	81(14)±15.6^{fg}	83(18)±25.8^{gh}	164(16)±28.0^{fg}	2(11)±0.7^{h}
	-18	0(0)±0.0^{f}	0(0)±0.0^{g}	0(0)±0.0^{i}	0(0)±0.0^{g}	0(0)±0.0^{h}
Feral rye	0	115(100)±9.5^{a}	2004(100)±94.2^{a}	899(100)±29.3^{a}	2903(100)±85.0^{a}	36(100)±1.4^{a}
	-3	117(101)±8.6^{a}	2082(103)±108.8^{a}	910(101)±29.3^{a}	2992(103)±86.6^{a}	35(97)±0.3^{a}
	-6	113(98)±8.0^{a}	1987(99)±126.8^{a}	924(102)±33.1^{a}	2911(100.)±111.1^{a}	34(94)±1.1^{a}
	-9	107(93)±10.1^{ab}	1901(94)±118.2^{a}	850(94)±75.2^{a}	2751(94)±169.0^{a}	34(94)±1.1^{a}
	-12	109(94)±11.4^{a}	1927(96)±142.2^{a}	903(100.)±28.4^{a}	2831(97)±132.1^{a}	34(94)±0.9^{a}
	-15	90(78)±13.7^{b}	1246(62)±316.0^{b}	707(78)±28.5^{b}	1952(67)±33.92^{b}	22(61)±1.2^{bc}
	-18	22(19)±10.3^{e}	30(1.5)±11.6^{g}	31(3)±10.9^{hi}	61(2)±22.4^{g}	2(5)±0.6^{h}

Means with same letters do not have significant difference according to LSD ($p \leq 0.05$)
Numerical values are mean ± SE
*The numbers in parentheses indicate the changes in means to the control.

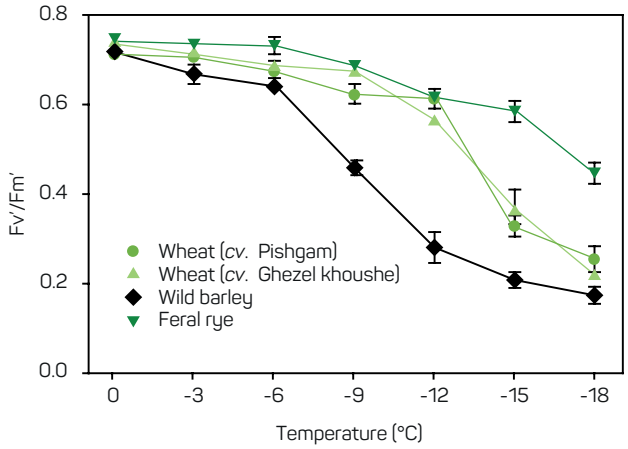

Figure 6 - Changes in the value of maximum photochemical efficiency of photosystem II (Fv'/Fm') in the studied plant species at freezing temperatures. The error bars indicate standard error (SE)

4. Discussion

The recent widespread invasion of wild barley and feral rye underscores their remarkable tolerance to temperature fluctuations and their robust adaptive capacity under changing climatic conditions. Increasing autumn and winter temperatures associated with climate change have impaired the ability of many crops to undergo sufficient cold acclimation, thereby diminishing their tolerance to low temperatures (Hekneby et al., 2006). In contrast, wild barley and feral rye have reportedly expanded their range into new habitats, including semi-arid regions, temperate highlands, and higher latitudes areas that were previously considered marginal or unsuitable for their establishment (Hosseini et al., 2022; Kumar et al., 2021). Anecdotal evidence suggests that this expansion may be facilitated by milder winters and a reduced incidence of severe frost events, highlighting a possible link between climate change and the spread of these cold-resilient species. While systematic studies are still needed to fully quantify these shifts, initial observations point to the growing potential of these species to exploit new ecological niches.

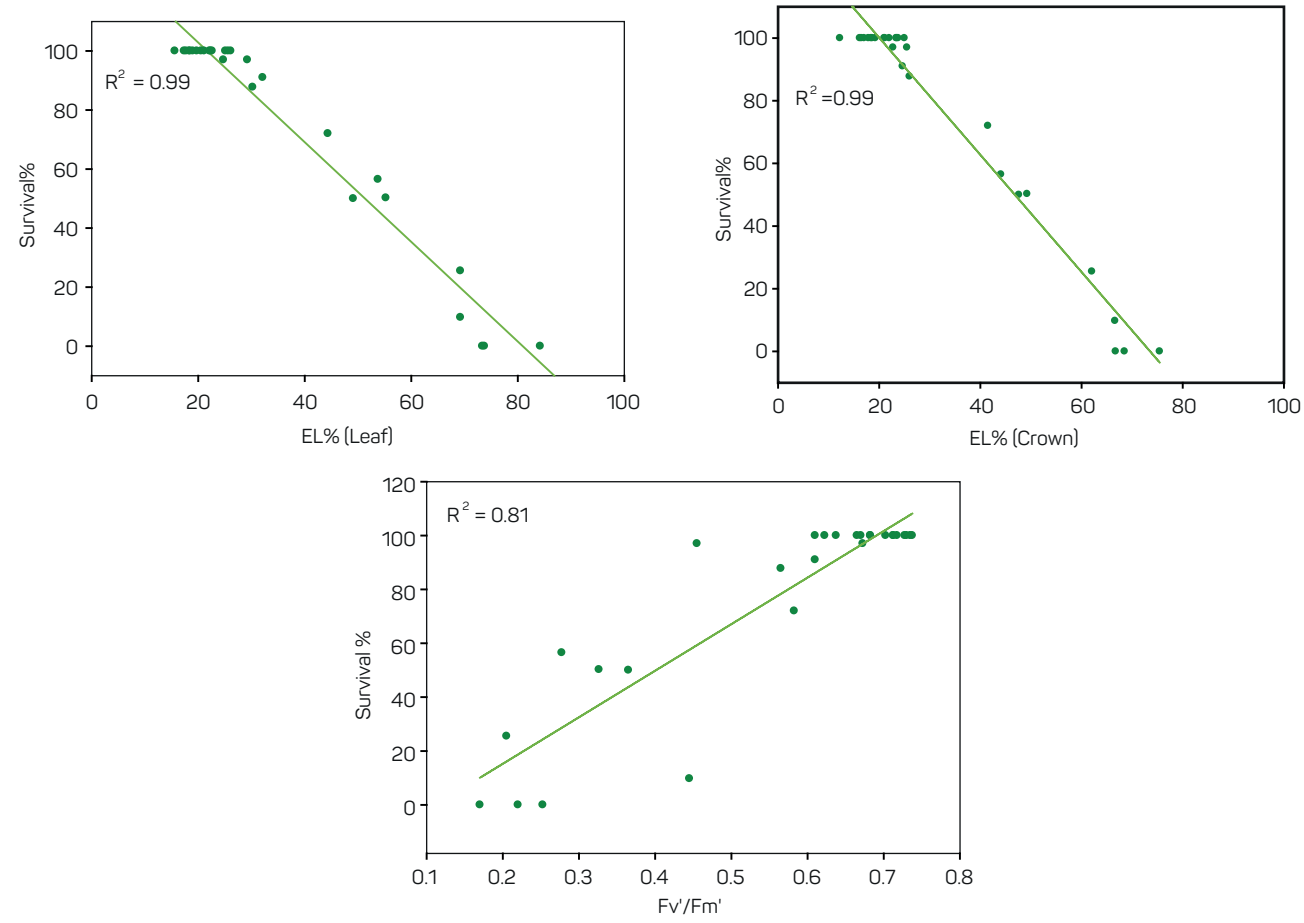

Figure 7 - Relationship between plant species survival and leaf and crown electrolyte leakage percentage (EL%) and maximum photochemical efficiency of photosystem II (Fv'/Fm') under different freezing levels

As global warming continues, transitional agroecosystems and marginal lands are expected to become increasingly susceptible to invasion by such species. The reduced cold tolerance in many cultivated crops renders them vulnerable even to mild frost episodes, thereby limiting their survival and productivity in colder environments. In contrast, winter annual weeds such as wild barley and feral rye exhibit a pronounced capacity for cold acclimation and physiological adaptation, which significantly enhances their freezing tolerance. This improved tolerance enables them to survive, regenerate, and complete their reproductive cycles under adverse conditions. Their efficient acclimation mechanisms facilitate gradual physiological adjustments to declining temperatures, enhancing cellular resilience against cold and freezing stresses (Hekneby et al., 2006).

Cell membrane stability is a critical factor for plant survival under freezing stress, as any disruption to membrane integrity can lead to cellular damage. Among the species studied, feral rye exhibited the lowest EL% across all freezing temperatures, indicating a high tolerance to freezing stress. This tolerance may be attributed to the plant's ability to regulate cellular water content, thereby minimizing ice formation and associated membrane damage, which results in reduced EL%. Yadav (2010) emphasized that cold stress primarily affects the plasma membrane in plants, making EL a valid and reliable indicator for assessing membrane stability after freezing stress. Similarly, Zhang et al. (2019) reported that a temperature decrease from approximately -6°C to -14°C caused an increase in EL% in six cultivars of tall fescue (*Festuca arundinacea*). Cold acclimation enhances a plant's ability to maintain cell membrane integrity and withstand freezing stress. Xuan et al. (2009) demonstrated that Zoysia grass (*Zoysia* spp.) ecotypes with lower $T_{EL_{50}}$ exhibited lower EL% and greater freezing tolerance. The higher freezing tolerance observed in feral rye may result from more effective cold acclimation compared to wild barley. Therefore, in cold regions, implementing careful management strategies for feral rye is crucial, particularly given its ability to tolerate temperatures as low as -15°C.

In weed species, maintaining survival after freezing stress and consequently continuing seed production facilitates their spread and contributes to widespread invasion. The results of this study suggest that feral rye exhibits greater freezing tolerance than wild barley, a trait that may enhance its success as an invasive species. This increased tolerance could lead to broader establishment and spread of feral rye in colder regions, where it might outcompete agricultural crops and native plant species. The SU% after freezing stress in crops and their common weeds provides valuable insight into the dynamics of their distribution and competitiveness in low-temperature environments (Cici, Van Acker, 2009).

According to the results, feral rye, which showed the lowest LT_{50}su, was the most tolerant species, maintaining 50% survival at lower temperatures than wild barley, and likely has a wider distribution range in colder areas. Thus, the ability to survive at lower temperatures may promote better establishment and increased seed production, thereby enhancing the invasive potential of feral rye. In other words, feral rye is more resilient to frost than wild barley, which helps preserve its survival and strengthens its competitive ability. For comparison, Arisz et al. (2018) reported LT_{50}su values of -5.7°C and -7.2°C for the SAD12 and LTM ecotypes of *Boechera stricta* seedlings, respectively, indicating higher freezing tolerance in the LTM ecotype.

In densely populated plant communities and under abiotic stresses such as freezing temperatures, increased plant height serves as a critical trait contributing to enhanced competitive ability, particularly in terms of light interception and spatial dominance (Hasanfard et al., 2023). Accordingly, the relatively shorter stature of wheat cultivars compared to feral rye has been identified as a limiting factor in their competitive performance. Similarly, the reduced height of wild barley likely compromises its ability to compete effectively with wheat cultivars for light acquisition. In light of the current lack of selective herbicides for effective control of wild barley, breeding for traits that enhance the competitive capacity of wheat such as increased plant height could provide a strategic advantage in integrated weed management programs.

Reduction in leaf development has been closely associated with diminished photosynthetic capacity and biomass accumulation (Qu et al., 2017). Consequently, evaluating the deleterious effects of freezing stress via LA measurements provides a robust indicator for assessing freeze-induced physiological damage. In this context, wild barley exhibited significant reductions in LA at relatively milder freezing temperatures (-12°C), whereas feral rye maintained higher leaf area until lower temperatures (-15°C), suggesting greater cold sensitivity in wild barley and enhanced freeze tolerance in feral rye. The observed declines in LA, photosynthesis, and biomass are directly linked to reductions in the plant's ability to capture and convert light energy into chemical energy, thereby impairing key metabolic processes.

Feral rye has demonstrated a remarkable capacity for DM accumulation during post-freezing recovery, particularly under suboptimal thermal conditions. Its physiological resilience is underscored by the maintenance or even enhancement of photosynthetic activity at temperatures as low as -15°C. This species efficiently converts absorbed light into organic compounds, such as carbohydrates and proteins, thus ensuring superior biomass production. The ability to store and utilize these photosynthetic products under stress confers a significant adaptive advantage for growth and survival in cold environments.

When compared to wild barley and various wheat cultivars, feral rye exhibits superior photosynthetic

performance and cold tolerance, enabling it to outcompete these species under low-temperature regimes. Its increased DM accumulation reflects elevated metabolic efficiency and supports more vigorous growth and resource acquisition, including light, water, and nutrients. This competitive superiority enhances its ecological fitness and contributes to its invasive potential and persistence in regions characterized by harsh winter climates.

Chlorophyll plays an essential role in the plant's photosynthetic machinery, functioning as the primary pigment in light energy capture (Li et al., 2018). Under freezing stress, chlorophyll degradation negatively impacts light absorption and photosynthetic efficiency. Therefore, sustaining chlorophyll stability under low-temperature conditions is critical for maintaining photosynthetic function and ensuring biomass and yield production (Østrem et al., 2018). In this study, Fv'/Fm' the effective quantum yield of PSII was identified as a reliable physiological marker for assessing the functional integrity of the photosynthetic apparatus post-freezing. A pronounced decline in Fv'/Fm' in wild barley was observed at temperatures as early as -6°C, indicating a higher susceptibility to cold-induced photoinhibition compared to other species. Progressive reductions in temperature were associated with diminished electron transport through PSII, thereby disrupting overall photosynthetic performance.

Feral rye, by contrast, demonstrated a higher capacity to maintain photosynthetic efficiency under freezing stress, likely due to its enhanced tolerance of PSII electron transport chain disruption. Supporting findings by Hasanfard et al. (2021) revealed that a temperature drop below -12°C impaired carbon exchange and electron flow in PSII, as evidenced by a 28% decrease in Fv'/Fm' in turnipweed (*Rapistrum rugosum* (L.) All.).

Although average temperatures are rising due to climate change, this warming does not occur uniformly across all seasons or regions. In many areas particularly at higher latitudes and in mountainous regions greater temperature variability is observed during autumn and winter. For example, prolonged warm periods may suddenly be followed by sharp frost events, and autumns may become longer, exposing plants to low temperatures later in the season before they have fully entered dormancy. Sudden and short-term cold events (cold snaps) can still recur, even under a generally warmer climate. These short-term freezes, especially during extended autumns or unseasonal temperature drops, can severely impact crops that have lost their cold acclimation capacity. In contrast, winter annual weeds such as wild barley and feral rye maintain a strong ability to acclimate to cold and survive such frost events, giving them a competitive advantage in transitional and marginal ecosystems. At the physiological level, correlations between shoot and crown EL% and survival rate (SU%) with chlorophyll fluorescence efficiency (Fv'/Fm') underscore the critical role of membrane integrity and PSII function in determining freezing tolerance. These findings highlight the importance of integrating cellular and physiological indicators when evaluating plant resilience to freezing stress, particularly under increasingly variable climatic conditions.

5. Conclusions

Wild species and weedy relatives often retain or even enhance their cold tolerance due to long-term evolutionary adaptation to harsh and variable environments, including colder climates. In contrast, cultivated wheat has undergone intensive breeding and selection primarily for yield and agronomic traits under more controlled conditions. This process may have inadvertently reduced its tolerance to abiotic stresses such as freezing. As winter temperatures rise due to climate change, many crops' ability to acclimate to cold diminishes, increasing their vulnerability to even mild freezing stress. This environmental may shift favors cold-tolerant weeds such as wild barley and feral rye, which are inherently better adapted for survival and spread in colder conditions. Feral rye, in particular, poses a significant threat to wheat cultivars because of its robustness, high freezing tolerance, and prolific seed production. Its potential for invasion is likely to increase under changing climatic conditions, especially in regions like Khorasan Razavi province in Iran. To mitigate the competitive impact of these invasive species, it is crucial to implement effective weed management strategies. Cultural practices such as crop rotation, tillage, and other integrated approaches can play a vital role in reducing the spread of feral rye and wild barley, thereby ensuring more effective control of their competition with wheat in autumn-sown cereals.

Authors' contributions

All authors read and agreed to the published version of the manuscript. EID: conceptualization and methodology. AH: analyzed and interpreted data. AH: writing-orginal draft. EID: writing-review & editing. AH and EID: data collection and curation. AH: data analysis. AH and EID: data interpretation.

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Data availability

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

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