

Article

Waterlogging Priming at Tillering Stage Confers Stronger Tolerance to Wheat Plants Waterlogged During Anthesis

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Abstract

Waterlogging stress, particularly during flowering, severely constrains wheat production, yet the optimal timing and frequency of waterlogging priming and its linkage to post-stress nitrogen acquisition remain unclear. We conducted pot experiments under a glasshouse over two consecutive growing seasons (2022/23 and 2023/24) using the Japanese bread wheat cultivar Norin 61. Eight treatment combinations were established with or without waterlogging priming applied at the tillering, stem elongation, and booting stages, followed by waterlogging for 5 days (2022/23) and 4 days (2023/24) during the flowering stage. To quantify post-stress nitrogen dynamics, ¹⁵N-labeled ammonium sulfate was applied immediately after waterlogging termination at flowering, and ¹⁵N uptake and its allocation to plant organs and grains were determined during grain filling and at harvest. Compared to the non-primed treatment, treatments that included tillering-stage priming consistently maintained higher leaf SPAD values, photosynthetic performance, and increased thousand-grain weight across both seasons, and grain yield increased by 54.8–80.6% in 2022/23 and 125.8–159.7% in 2023/24. These treatments also showed higher post-stress ¹⁵N content and greater ¹⁵N allocation to grains. Overall, tillering-stage waterlogging priming was associated with improved tolerance to flowering-stage waterlogging in wheat through the maintenance of post-stress nitrogen uptake capacity and nitrogen allocation to grains.

Keywords: nitrogen stable isotope (¹⁵N); nitrogen uptake; stress hardening; stress imprint; stress memory; stress priming; waterlogging stress; wheat

1. Introduction

In the context of global climate change, the frequency of extreme weather events, including sudden torrential rainfall and flooding, has increased worldwide. As global warming progresses, extreme precipitation events are occurring more frequently [1]. Consequently, waterlogging stress induced by such events has become one of the major abiotic constraints on crop production [2].

Wheat is predominantly cultivated in arid to semi-humid regions, particularly in semi-arid environments, and exhibits limited adaptability to humid conditions. A global meta-analysis indicates that waterlogging significantly reduces wheat yield by 25.53%, suggesting that excess soil moisture is a serious environmental stress factor that diminishes global wheat productivity [3]. In Japan, the risk of waterlogging damage is particularly high because of abundant rainfall and the frequent cultivation of wheat in converted



Received: 31 December 2025

Revised: 27 January 2026

Accepted: 30 January 2026

Published: 2 February 2026

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paddy fields characterized by heavy clay soils and poor drainage capacity. As a result, waterlogging has been identified as the primary cause of wheat yield reduction in Japan, making its mitigation a critical agronomic challenge [4].

Although waterlogging damage can occur at various growth stages in wheat, the flowering stage is especially sensitive [5–7]. Waterlogging stress during flowering reduces nitrogen uptake capacity and leaf greenness, as reflected by decreased SPAD values, thereby accelerating premature leaf senescence through chlorosis and necrosis. This process suppresses photosynthesis and leads to poor grain filling [6,8]. Grain yield and quality in wheat are strongly influenced by nitrogen redistribution during the grain filling period [9,10]. Waterlogging stress has been shown to inhibit the remobilization of nitrogen and photosynthetic assimilates stored in vegetative organs to the grain, resulting in yield reduction [11]. Early senescence has been hypothesized to occur via leaf “self-destruction” as a compensatory response to reduced nitrogen uptake capacity [12,13]. Therefore, sustaining nitrogen uptake capacity is essential for mitigating premature senescence and yield loss in wheat under waterlogging conditions.

Plants have been reported to possess adaptive mechanisms described as “priming”, “hardening”, “imprint”, or “stress memory”, which enable them to respond more effectively to subsequent stress after prior exposure [14–18]. The strategic exploitation of these mechanisms has attracted increasing attention as a potential approach to enhance crop tolerance to abiotic stresses. Waterlogging priming has been documented in several crop species. In soybeans, exposure to waterlogging during the vegetative stage reduced oxidative damage and maintained yield when plants were subsequently subjected to waterlogging during the reproductive stage [19]. Similarly, in tomato, repeated waterlogging treatments applied at the seedling stage maintained photosynthetic capacity compared with non-primed plants [20].

In wheat, waterlogging priming has also been reported. Short-term (two days) waterlogging applied at multiple seven-leaf, nine-leaf, and heading stages enhanced tolerance to subsequent waterlogging at flowering by delaying leaf senescence and maintaining grain filling and yield [21]. In addition, waterlogging priming at early vegetative stages, such as the four- and six-leaf stages, mitigated declines in photosynthetic capacity and grain yield under later waterlogging stress, with clear varietal differences [22]. Waterlogging priming at the four-leaf stage has also been shown to increase antioxidant enzyme activity and soluble sugar content, and in a tolerant variety, to promote inducible aerenchyma formation, thereby alleviating reductions in SPAD values and grain yield under waterlogging stress [23].

Despite these advances, previous studies have generally examined waterlogging priming at limited growth stages. Consequently, the optimal growth stage and frequency of waterlogging priming treatments required to maximize waterlogging tolerance in wheat remain unclear. Moreover, although wheat grain yield is closely associated with nitrogen uptake capacity after flowering [24], the effects of waterlogging priming on post-flowering nitrogen uptake and subsequent nitrogen remobilization to grains have not been quantitatively evaluated.

The present study was conducted under controlled pot conditions over two consecutive growing seasons to address these knowledge gaps. Eight treatments were established by combining the presence or absence of waterlogging priming at three key growth stages: the tillering, stem elongation, and booting stages. This experimental design enabled a systematic evaluation of both the timing and frequency of priming treatments. The objectives of this study were (i) to determine whether waterlogging priming mitigates waterlogging damage during flowering, (ii) to identify the growth stage and its combination that most effectively enhances waterlogging tolerance, and (iii) to evaluate the stability of these

effects across two growing seasons to determine whether they are consistently observed. In addition, ammonium sulfate labeled with the nitrogen stable isotope (^{15}N) was applied after the removal of waterlogging stress at flowering to quantitatively evaluate the effects of waterlogging priming on nitrogen uptake capacity and nitrogen remobilization to grains.

2. Materials and Methods

2.1. Cultivation Environments and Waterlogging Priming Treatments

The representative Japanese bread wheat (*Triticum aestivum* L.) cultivar Norin 61 was used as the plant material. Pot experiments were conducted in a glasshouse at the Faculty of Agriculture, Tottori University, Japan (134.1705° E, 35.5146° N), over two consecutive growing seasons (2022/23 and 2023/24). The glasshouse allowed control of precipitation, thereby eliminating rainfall effects. Temperature and relative humidity exhibited fluctuations in the ambient atmosphere through natural ventilation, facilitated by maintaining all doors and side windows of the glasshouse in an open position. According to the meteorological data collected by the Tottori Local Meteorological Office, the daily mean temperature and relative humidity in Tottori during the experiments ranged from -1.0 to 24.0 °C and 43 to 98% in 2022/23 and -0.5 to 23.6 °C and 46 to 98% in 2023/24. To ensure the precise application of waterlogging stress at specific growth stages, well-drained sandy soil was used and filled into white HIPS (High-Impact Polystyrene) pots (1/2000 a Wagner pot; surface area, 500 cm^2 ; inner diameter, 25 cm; inner height, 30 cm).

In the 2022/23 growing season, basal fertilizers were applied at the following rates: 1.19 g pot^{-1} (5 g N m^{-2}) ammonium sulfate, 3.40 g pot^{-1} ($12\text{ g P}_2\text{O}_5\text{ m}^{-2}$) superphosphate, 1.20 g pot^{-1} ($12\text{ g K}_2\text{O m}^{-2}$) potassium sulfate, and 4.50 g pot^{-1} of the trace element fertilizer “Hama Green”. On 28 December 2022, six seeds per hill were sown at six equally spaced points arranged in a circle in each pot. In this study, a ‘hill’ was defined as a single planting unit (i.e., one sowing point). Plants were thinned on three occasions to retain three plants per hill (18 plants per pot). On 10 March 2023, ammonium sulfate was applied as a topdressing at 1.19 g pot^{-1} (5 g N m^{-2}). On 13 April 2023, the day after termination of the 5-day waterlogging stress during flowering, 1.20 g pot^{-1} (5 g N m^{-2}) of ammonium sulfate containing 2.5 atom% ^{15}N was dissolved in tap water and applied evenly around the base of each hill to assess nitrogen uptake capacity and its allocation after the removal of waterlogging stress.

In the 2023/24 growing season, basal fertilizers were applied at 1.19 g pot^{-1} (5 g N m^{-2}) ammonium sulfate, 1.40 g pot^{-1} ($12\text{ g P}_2\text{O}_5\text{ m}^{-2}$) heavy superphosphate lime, 1.00 g pot^{-1} ($12\text{ g K}_2\text{O m}^{-2}$) potassium chloride, and 4.50 g pot^{-1} “Hama Green”. Seeds were sown on 28 November 2023 as in 2022/23, and plants were thinned to 2 plants per hill (12 plants per pot). Ammonium sulfate (1.19 g pot^{-1} , equivalent to 5 g N m^{-2}) was applied as topdressing on 8 February and again on 11 March 2024. On 16 April 2024, the day after termination of the 4-day waterlogging stress during flowering, 1.20 g pot^{-1} (5 g N m^{-2}) of ammonium sulfate containing 2.5 atom% ^{15}N was applied to each pot, as in 2022/23.

Eight treatments were established by combining with waterlogging priming (W) or control (C, without priming) treatments at the tillering, stem elongation, and booting stages (Figure 1). In the 2023/24 season, a non-waterlogged treatment (NW) that received no waterlogging stress throughout the growing period was also included for physiological evaluation. Except for this NW, all treatments were subjected to waterlogging stress during flowering. The experiments were conducted in a randomized block design with five replicates in 2022/23 and six replicates in 2023/24 (treatments WCC and WCW in 2022/23 had four replicates because of poor growth before initiation of the tillering-stage priming treatment).

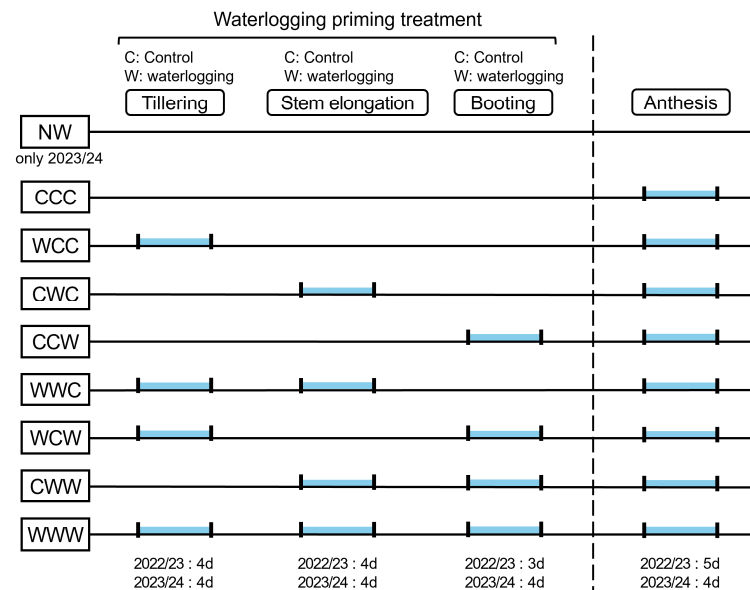


Figure 1. Schematic diagram of waterlogging priming treatments. Eight treatments were established by combining the presence or absence of waterlogging priming treatments at the tillering, stem elongation, and booting stages. Blue horizontal bars indicate periods during which waterlogging priming treatments were applied. Treatments were identified using three-letter codes, where “W” denotes the application of waterlogging priming treatment and “C” denotes control (i.e., without waterlogging). Treatment CCC represents the treatment without any waterlogging priming before anthesis. All treatments were subjected to 4 or 5 days (d) of waterlogging stress during the flowering stage. In the 2023/24 season, an additional non-waterlogged (NW) control that received no waterlogging stress throughout the growing period was included for physiological measurements.

In 2022/23, based on the evaluations of wheat visual responses in preliminary experiments, waterlogging priming treatments were applied for 4 days at the tillering stage (6–10 March 2023), 4 days at the stem elongation stage (20–24 March), and 3 days at the booting stage (27–30 March). All pots were subsequently subjected to 5 days of waterlogging stress during flowering (7–12 April). In 2023/24, treatments were applied for 4 days at the tillering stage (21–25 February 2024), 4 days at the stem elongation stage (6–10 March), and 4 days at the booting stage (22–26 March). During flowering, a 4-day waterlogging stress treatment (11–15 April) was applied to all pots except the non-waterlogged control. For all waterlogging treatments, the water level was maintained approximately 3 cm above the soil surface. After each treatment, water was rapidly drained through the drainage holes at the bottom of the pots, collected separately, and reused for subsequent irrigation to minimize nutrient leaching. In the experiments conducted in 2022/23 and 2023/2024, although the fertilizer source, plant density, flowering waterlogging duration, and topdressing differed slightly, the effects of the waterlogging priming treatment were independently investigated each year under these conditions.

2.2. Measurements and Sampling

SPAD values of the uppermost fully expanded leaf or flag leaf with an average leaf color in each pot were measured over time from the initiation of the tillering-stage priming treatment until complete leaf senescence using a portable chlorophyll meter (SPAD-502Plus, Konica Minolta Inc., Tokyo, Japan). Measurements were taken at the distal, medial, and basal regions of each leaf, and the mean of the three readings was used as the representative value.

The quantum yield of photosystem II (F_v'/F_m') was measured from the onset of waterlogging stress during flowering until leaf senescence using a portable chlorophyll fluorometer (PAR E-FP110/S, PSI (Photon Systems Instruments), spol. s r.o., Drásov, Czech

Republic). Measurements were conducted under natural light between 08:00 and 12:00 on the middle portion of the flag leaf in each pot. The photosynthetic rate was only measured in the 2023/24 season. On 17 April 2024, measurements were taken from the middle portion of the flag leaf using a photosynthetic rate measurement device (MIC-100, Masa International Corp., Kyoto, Japan).

Sampling was conducted twice, during grain filling and at harvest. In 2022/23, one average hill was sampled from each pot during grain filling on 24 April 2023 (12 days after removal of waterlogging stress during flowering). In 2023/24, the same sampling was conducted on 25 April 2024 (10 days after stress removal). Samples were oven-dried at 80 °C for 3 days. In 2022/23, culms + leaf sheaths, leaves, and grains were weighed. In 2023/24, culms + leaf sheaths, lower leaves, flag leaf, glumes + rachis, and grains were measured. At maturity, four hills (three and two plants per hill in 2022/23 and 2023/24, respectively) were sampled and air-dried at room temperature. For yield evaluation, two hills with average spike weight were selected from the four hills. In 2022/23, only the main culm was used since there was less appearance of tillers, whereas in 2023/24, both the main culm and tillers were used to assess grain yield per plant, grain number per spike, and thousand-grain weight.

After dry weight determination, dried samples of each component were ground with a pulverizer (WB-1, Osaka Chemical Co., Ltd., Osaka, Japan) to determine nitrogen concentration (Table A2) and a nitrogen stable isotope composition ($\delta^{15}\text{N}$, Table A3). Nitrogen concentration (%) was measured using a C/N analyzer (JM1000CN, J-Science Lab Co., Ltd., Kyoto, Japan) and multiplied by dry weight to calculate nitrogen content (mg plant^{-1}). Isotopic composition was determined using a stable isotope ratio mass spectrometer (EA IsoLink CN and DELTA V Advantage, Thermo Fisher Scientific Inc., Waltham, MA, USA) after the encapsulation of powdered samples in tin cups. Total ^{15}N content was calculated using $\text{atom}\% \text{ }^{15}\text{N}$ excess (difference between sample ^{15}N atom% and the natural abundance (0.3663 atom%)), following equation modification [25]. ^{15}N content (mg) = Nitrogen concentration (%) · dry weight (g) · $\text{atom}\% \text{ }^{15}\text{N}$ excess · 100. The ^{15}N allocation ratio for each component was calculated as the proportion of total aboveground ^{15}N content. The measurements of nitrogen-related parameters were performed by selecting three replicates from five to six replicates in each treatment area whose thousand-grain weight was close to the average value. This was performed with the aim of minimizing the impact of outliers caused by extreme individual variation while ensuring representativeness.

2.3. Statistical Analysis

Statistical analyses were performed using SPSS Statistics 29 (IBM (International Business Machines) Corporation, Armonk, NY, USA). Differences among treatments were evaluated by one-way analysis of variance (ANOVA), and Tukey's honestly significant difference (HSD) test was used for multiple comparisons when significant effects were detected. Pearson's correlation coefficient was used to assess relationships between variables. The significance level was set at $p < 0.05$ for all tests.

3. Results

3.1. Leaf Senescence (Chlorosis and Necrosis)

Treatment CCC, which did not receive any waterlogging priming treatment, showed the highest SPAD values in flag leaves at the onset of waterlogging stress during flowering in both seasons (2022/23 and 2023/24). However, SPAD values declined sharply after the onset of waterlogging, beginning 4 days after stress initiation in 2022/23 (Figure 2A) and throughout the stress period in 2023/24 (Figure 2B). As shown in Figure 3B,D, many leaves below the flag leaf in CCC had already yellowed or withered 10 days (2022/23) and 5 days

(2023/24) after the removal of waterlogging stress. CCC exhibited the second earliest complete leaf senescence in 2022/23 and the earliest in 2023/24 among all treatments.

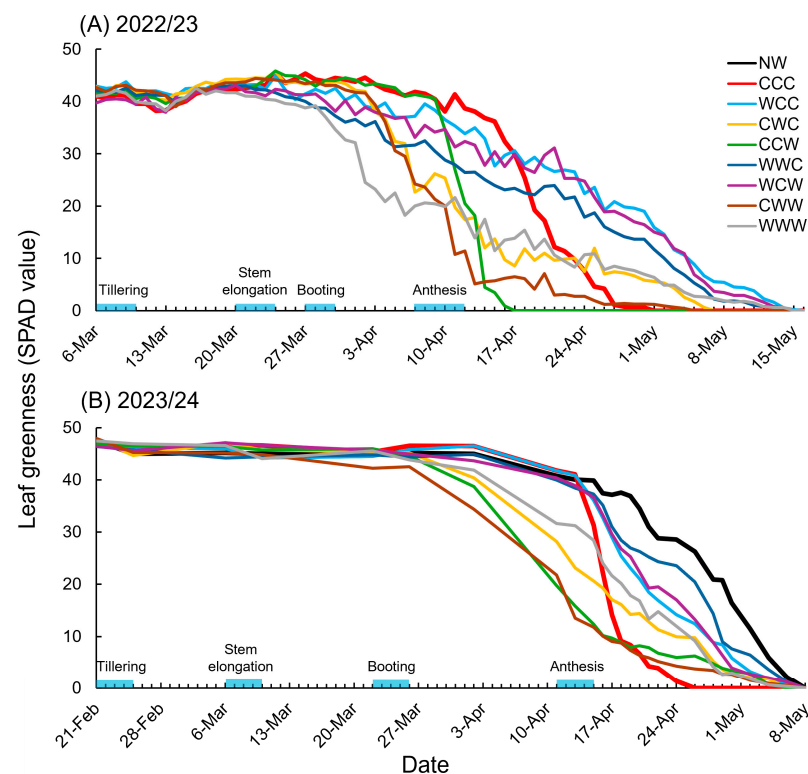


Figure 2. Changes in leaf greenness (SPAD values) of the uppermost fully expanded leaf or flag leaf from the tillering stage to complete senescence in 2022/23 (A) and 2023/24 (B). Blue horizontal bars indicate periods of waterlogging priming treatments and flowering-stage waterlogging stress. NW means non-waterlogged control during cultivation.

In contrast, treatments that received waterlogging priming maintained higher SPAD values for a longer period than CCC, resulting in delayed final senescence. In particular, treatments WCC, WWC, and WCW, which included waterlogging priming during the tillering stage, showed pronounced maintenance of SPAD values in both seasons. Conversely, treatments CCW and CWW, which received waterlogging priming during the booting stage but did not during the tillering stage, exhibited severe waterlogging damage before the onset of flowering (Figure 3A,B), followed by earlier declines in SPAD values than CCC. Treatment WWW, which received waterlogging priming at all three stages, showed shorter plant height and began senescence earliest during the booting-stage treatment period in 2022/23, since the interval of priming treatments between stem elongation and booting stages was only three days. It maintained low SPAD values for an extended period, although final senescence occurred later than in CCC. In 2023/24, SPAD values at the start of the flowering-stage waterlogging were lower in WWW than in CCC; however, WWW did not show the rapid wilting observed in CCC and retained leaf greenness for a longer period. Treatment CWC showed trends similar to those of WWW in both seasons.

3.2. Photosynthetic Performance

The quantum yield of photosystem II (F_v'/F_m') varied with weather conditions on measurement days; however, overall seasonal trends were similar to those observed for SPAD values in both years (Figure 4). Treatment CCC showed the highest F_v'/F_m' at the onset of flowering-stage waterlogging but exhibited a precipitous decline in both seasons and became the first treatment to reach complete senescence and become unmeasurable,

except for treatment CCW in 2022/23. In contrast, WCC, WWC, and WCW, which included tillering-stage priming, maintained relatively high F_v'/F_m' values throughout the observation period. CWC and WWW showed similar trends to each other, particularly in 2022/23. CCW and CWW exhibited consistently low values in both seasons and became unmeasurable later than treatment CCC, except for treatment CCW in 2022/23.

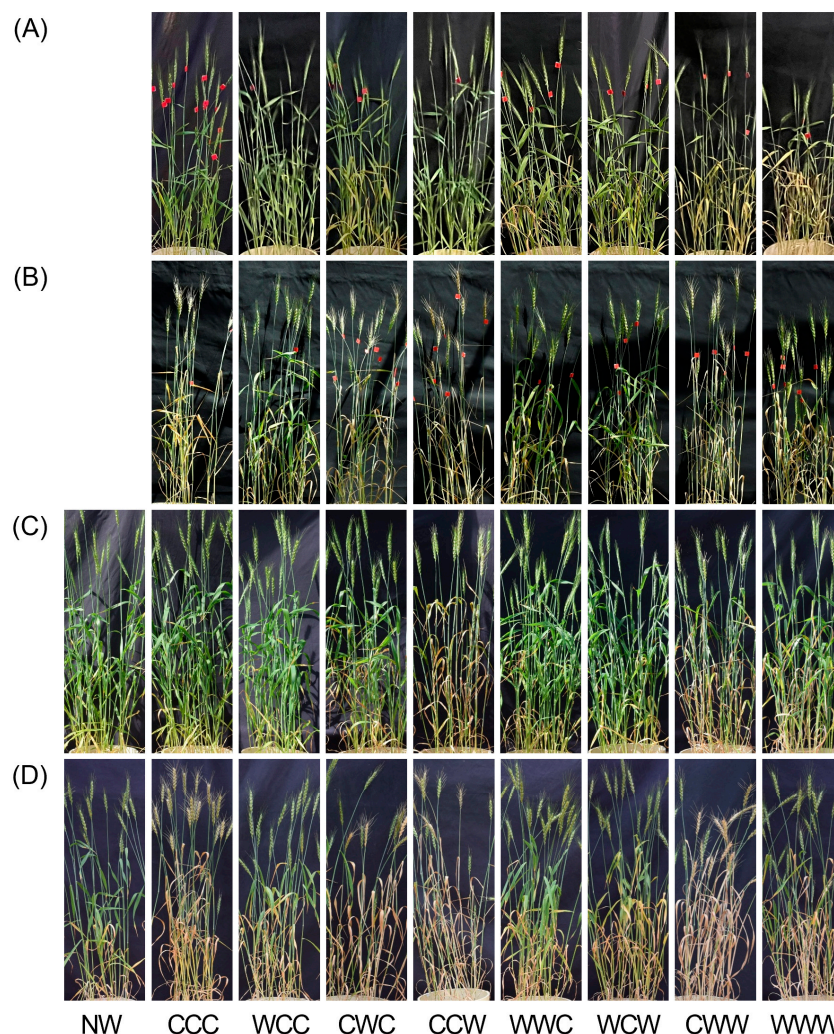


Figure 3. Aboveground appearance of wheat before and after flowering-stage waterlogging stress. (A) Aboveground appearance one day before the initiation of waterlogging stress during flowering in 2022/23. (B) Aboveground appearance 10 days after removal of waterlogging stress during flowering in 2022/23. (C) Aboveground appearance one day before the initiation of waterlogging stress during flowering in 2023/24. (D) Aboveground appearance 5 days after removal of waterlogging stress during flowering in 2023/24. NW means non-waterlogged control during cultivation.

The photosynthetic rate of the flag leaf was measured in 2023/24 on the second day after the removal of flowering-stage waterlogging stress (17 April 2024; Figure 5). A non-waterlogged control (NW) was included for comparison. The photosynthetic rate in CCC was reduced by 93.2% compared with the NW, confirming the strong negative effect of flowering-stage waterlogging on wheat photosynthesis. Although no significant differences were detected among the waterlogging priming treatments, all such treatments exhibited higher photosynthetic rates than CCC. WCC, WWC, WCW, and WWW, which included tillering-stage priming, showed photosynthetic rates that did not differ significantly from those of the NW.

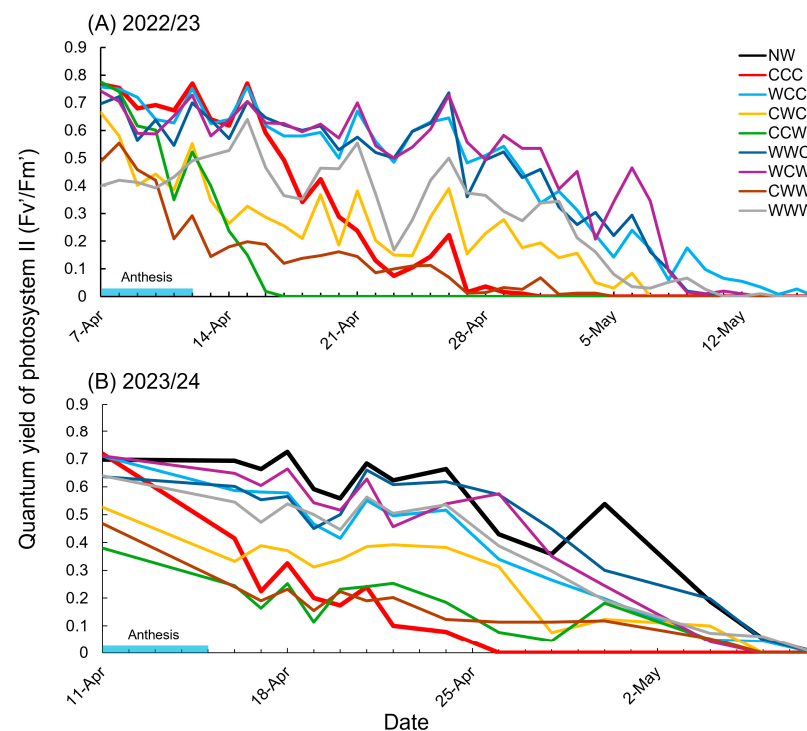


Figure 4. Changes in the quantum yield of photosystem II (F_v'/F_m') of flag leaf from anthesis to complete senescence in 2022/23 (A) and 2023/24 (B). Blue horizontal bars indicate periods of flowering-stage waterlogging stress. NW means non-waterlogged control during cultivation.

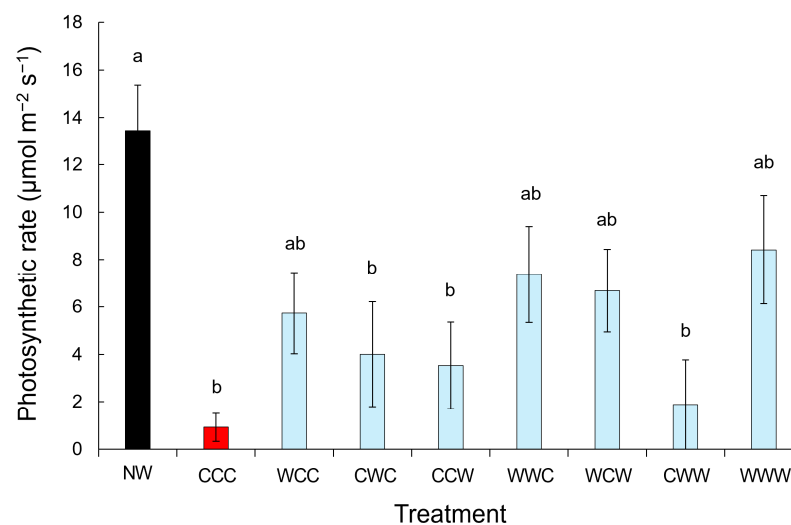


Figure 5. Photosynthetic rate of flag leaves after flowering-stage waterlogging stress in 2023/24. Photosynthetic rate was measured on 17 April 2024, two days after the removal of waterlogging stress during flowering. NW means non-waterlogged control during cultivation. Black and red bar indicate NW and CCC treatments, respectively as same as in Figures 2 and 4. Bares represent means $\pm$ standard error with six replicates. Different letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test.

3.3. Grain Yield and Its Components

Grain number per spike tended to be higher in waterlogging priming treatments than in CCC in 2023/24, although no significant differences were detected in either season (Table 1). In contrast, thousand-grain weight in WCC, WWC, and WCW, which included tillering-stage priming, was significantly higher than in CCC in 2022/23, with increases of 59.3%, 62.7%, and 55.9%, respectively (Table 1). In 2023/24, these treatments also

showed higher thousand-grain weight than CCC (increases of 32.0%, 45.9%, and 34.6%, respectively), although the differences were not statistically significant.

Table 1. Grain yield and its components under different waterlogging priming treatments in 2022/23 and 2023/24.

Treatment	Grain Number per Spike	Thousand-Grain Weight (g)	Grain Yield (g plant ^{−1})
2022/23			
CCC	14.17 ± 1.49 ¹	22.41 ± 3.55 b ²	0.31 ± 0.05 b
WCC	15.54 ± 1.57	35.69 ± 0.86 a	0.56 ± 0.07 a
CWC	13.73 ± 1.25	33.03 ± 0.86 a	0.45 ± 0.04 ab
CCW	15.40 ± 1.27	25.77 ± 3.72 ab	0.39 ± 0.05 ab
WWC	13.93 ± 0.53	36.45 ± 1.01 a	0.51 ± 0.03 ab
WCW	13.63 ± 1.53	34.94 ± 2.60 a	0.48 ± 0.07 ab
CWW	8.73 ± 1.62	31.88 ± 2.68 ab	0.28 ± 0.05 b
WWW	11.67 ± 2.07	27.40 ± 1.84 ab	0.32 ± 0.05 ab
2023/24			
CCC	15.93 ± 2.67	21.39 ± 3.45 ab	0.62 ± 0.15
WCC	23.80 ± 3.28	28.24 ± 1.58 ab	1.40 ± 0.27
CWC	24.82 ± 5.00	23.67 ± 2.12 ab	1.47 ± 0.38
CCW	25.18 ± 3.05	20.47 ± 4.18 ab	0.80 ± 0.18
WWC	24.95 ± 2.59	31.20 ± 1.30 a	1.61 ± 0.18
WCW	22.66 ± 3.58	28.80 ± 1.20 ab	1.49 ± 0.33
CWW	22.43 ± 3.34	17.24 ± 3.16 b	0.66 ± 0.16
WWW	20.71 ± 4.19	27.07 ± 1.85 ab	1.48 ± 0.41

In the 2022/23 season, measurements were conducted using the main culm of six plants per pot, whereas in the 2023/24 season, both the main culm and tillers of four plants per pot were included. ¹ Values represent means ± standard error with five replicates in 2022/23 and six replicates in 2023/24. ² Different letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test.

Grain yield was higher in all waterlogging priming treatments except CCW and CWW compared with CCC in both seasons (Table 1). This effect was most pronounced in WCC, WWC, and WCW. In 2022/23, grain yield increased by 80.6%, 64.5%, and 54.8% in WCC, WWC, and WCW, respectively, relative to treatment CCC. In 2023/24, corresponding increases were 125.8%, 159.7%, and 140.3%. WWW showed grain yield comparable to CCC in 2022/23 but exhibited a 138.7% increase in 2023/24.

3.4. Nitrogen Uptake Capacity and Remobilization

Nitrogen content in grains during grain filling (10–12 days after removal of flowering-stage waterlogging) tended to be higher in WCW and CWW than in CCC in 2022/23; however, no significant differences among treatments were detected in either season (Table A1). In contrast, ¹⁵N content, which shows nitrogen uptake capacity during grain filling, was consistently higher in treatments WCC, WWC, and WCW than in CCC across all components in both seasons. In 2023/24, CWC and WWW also showed higher ¹⁵N content than CCC in most components, except for lower leaves (Table A1). At harvest, nitrogen content in culms + leaf sheaths, flag leaves, and glumes + rachis was lower in waterlogging priming treatments than in CCC in both seasons, whereas nitrogen content in grains was higher in the priming treatments (Table 2). In grains, WCC, WWC, and WCW showed notably higher values than CCC in both years. Patterns of ¹⁵N content were similar to those of nitrogen content (Table 2). In 2022/23, ¹⁵N content in culms + leaf sheaths and leaves was lower in waterlogging priming treatments than in CCC, whereas grain ¹⁵N content was higher in treatments WCC, WWC, and WCW, with a significant increase in WCW. In 2023/24, grain ¹⁵N content was markedly higher in WCC, WWC, WCW, CWC, and WWW than in CCC,

and WCC, WWC, and WCW also showed higher ^{15}N content in other organs, including culms + leaf sheaths.

Table 2. Nitrogen content and ^{15}N content in plant components at harvest in 2022/23 and 2023/24.

2022/23					
Treatment	Culm + Leaf Sheath		Leaf	Grain	
Nitrogen Content (mg plant ⁻¹)					
CCC	8.67 ± 1.01 a ^{1,2}		2.24 ± 0.51	9.18 ± 1.45	
WCC	5.06 ± 0.49 b		1.53 ± 0.13	16.20 ± 1.59	
CWC	5.38 ± 0.60 b		2.51 ± 0.17	15.09 ± 1.24	
CCW	6.50 ± 0.31 ab		1.50 ± 0.25	14.67 ± 2.06	
WWC	5.33 ± 0.25 b		1.75 ± 0.07	14.86 ± 0.76	
WCW	4.50 ± 0.87 b		1.57 ± 0.12	16.98 ± 1.51	
CWW	4.87 ± 0.60 b		1.72 ± 0.16	10.29 ± 2.34	
WWW	4.26 ± 0.48 b		2.43 ± 0.12	11.50 ± 1.74	
¹⁵ N Content (µg plant ⁻¹)					
CCC	55.89 ± 5.48 a		5.33 ± 2.48	57.62 ± 13.33 bc	
WCC	37.58 ± 4.78 ab		4.18 ± 1.48	127.72 ± 12.18 ab	
CWC	28.70 ± 1.44 b		1.99 ± 0.19	70.68 ± 7.27 bc	
CCW	33.60 ± 5.08 ab		1.98 ± 0.89	84.99 ± 21.53 abc	
WWC	38.11 ± 6.92 ab		6.07 ± 2.29	117.63 ± 16.84 abc	
WCW	34.05 ± 7.36 ab		5.09 ± 0.48	143.72 ± 11.97 a	
CWW	26.14 ± 3.04 b		1.50 ± 0.16	55.10 ± 19.40 c	
WWW	15.45 ± 3.85 b		1.74 ± 0.41	50.91 ± 3.85 c	
2023/24					
Treatment	Culm + Leaf Sheath	Lower Leaves	Flag Leaf	Glume + Rachis	Grain
Nitrogen Content (mg plant ⁻¹)					
CCC	11.90 ± 2.52 a	2.62 ± 0.37 b	2.70 ± 0.54	7.38 ± 1.41	20.37 ± 5.55
WCC	6.92 ± 0.87 ab	2.83 ± 0.58 b	1.47 ± 0.23	5.06 ± 0.67	45.84 ± 1.69
CWC	7.66 ± 1.58 ab	4.03 ± 1.28 ab	1.55 ± 0.06	3.08 ± 0.65	35.12 ± 6.32
CCW	9.20 ± 1.88 ab	3.75 ± 0.66 ab	2.02 ± 0.26	5.72 ± 1.94	22.07 ± 4.28
WWC	3.83 ± 0.39 b	1.97 ± 0.15 b	0.96 ± 0.20	2.33 ± 0.42	38.12 ± 3.04
WCW	7.62 ± 0.27 ab	2.10 ± 0.29 b	2.04 ± 0.55	5.07 ± 1.36	40.02 ± 3.55
CWW	9.94 ± 1.91 ab	6.16 ± 0.87 a	2.09 ± 0.61	6.02 ± 1.76	20.06 ± 4.92
WWW	6.56 ± 0.60 ab	2.07 ± 0.16 b	1.43 ± 0.17	3.85 ± 1.41	32.90 ± 11.09
¹⁵ N Content (µg plant ⁻¹)					
CCC	15.16 ± 4.71	1.06 ± 0.70	0.15 ± 0.06	2.18 ± 1.04	25.88 ± 7.79 bc
WCC	19.54 ± 9.34	1.73 ± 0.43	0.51 ± 0.22	7.63 ± 4.60	178.23 ± 42.65 a
CWC	14.22 ± 5.76	1.59 ± 1.03	0.11 ± 0.06	1.84 ± 0.38	114.19 ± 57.48 abc
CCW	10.53 ± 2.95	0.88 ± 0.24	0.62 ± 0.60	4.59 ± 3.61	24.25 ± 15.85 bc
WWC	7.00 ± 1.20	0.75 ± 0.15	0.62 ± 0.33	3.97 ± 1.30	174.72 ± 6.95 ab
WCW	24.63 ± 4.80	1.57 ± 0.14	1.06 ± 0.84	9.76 ± 6.67	141.50 ± 31.63 abc
CWW	7.48 ± 1.18	2.58 ± 0.51	0.04 ± 0.03	0.97 ± 0.22	12.96 ± 6.26 c
WWW	16.87 ± 5.82	1.14 ± 0.31	0.38 ± 0.20	9.43 ± 7.56	123.16 ± 33.72 abc

In 2022/23, analyses were conducted for culms + leaf sheaths, leaves, and grains; in 2023/24, analyses included culms + leaf sheaths, lower leaves, flag leaves, glumes + rachis, and grains. ¹ Values represent means $\pm$ standard error, with three replicates in 2022/23 and 2023/24. ² Different letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test.

^{15}N allocation ratios among plant components are shown in Figure 6. During grain filling in 2022/23, no clear differences among treatments were detected, with grain allocation ratios ranging from 35% to 50% (Figure 6). In contrast, during grain filling in 2023/24, treatments CCC and CWW showed grain allocation ratios of approximately

35–40%, whereas WWC, WCW, and WWW exhibited higher ratios of 55–70% (Figure 6). At harvest, grain allocation ratios increased in most treatments in both seasons. In 2022/23, the grain allocation ratio was 48% in CCC and 65–75% in waterlogging priming treatments (Figure 6). A similar pattern was observed in 2023/24, with low grain allocation ratios in CCC, CCW, and CWW and higher ratios (80–90%) in the remaining treatments, including those that received tillering-stage priming (Figure 6).

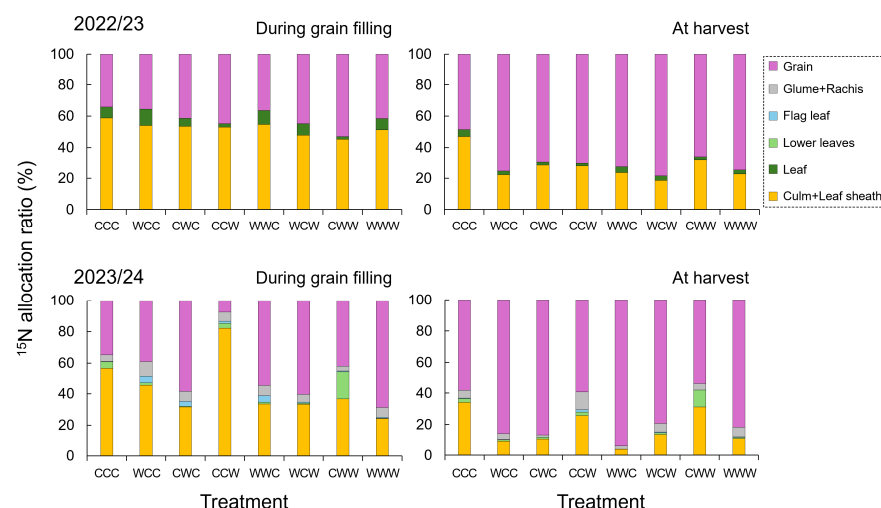


Figure 6. Allocation ratio of ^{15}N to plant components during grain filling and at harvest in 2022/23 and 2023/24. ^{15}N allocation ratios among plant components were evaluated for the wheat plants sampled in the grain filling stage 12 days after removal of flowering-stage waterlogging in 2022/23, and 10 days after removal of flowering-stage waterlogging in 2023/24.

3.5. Correlation Between SPAD and Leaf $\delta^{15}\text{N}$ or Thousand-Grain Weight

Significant positive correlations were observed between SPAD values of the terminal leaf during grain filling and leaf $\delta^{15}\text{N}$ values (all leaves in 2022/23 and flag leaf in 2023/24), as well as between SPAD values of the flag leaf and thousand-grain weight, in both seasons (Figure 7).

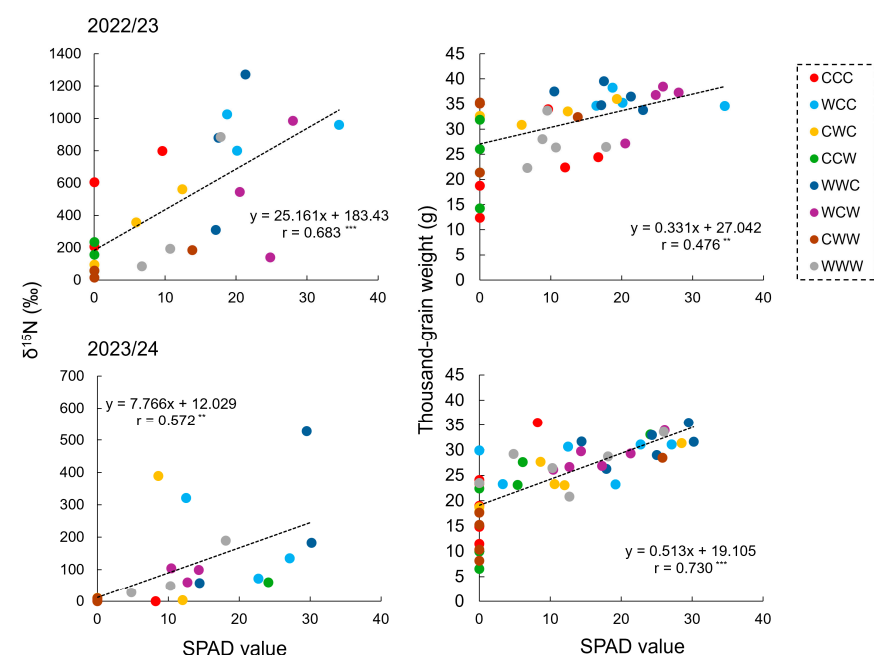


Figure 7. Relationships between SPAD values and leaf $\delta^{15}\text{N}$ or thousand-grain weight in 2022/23 and 2023/24. Relationship between SPAD values of the flag leaf at 12 days after removal of flowering-stage

waterlogging stress and $\delta^{15}\text{N}$ of all leaves in 2022/23. Relationship between SPAD values of the flag leaf at 9 days after removal of the stress and $\delta^{15}\text{N}$ of the flag leaf in 2023/24. Pearson's correlation coefficient was used to test relationships between variables. ** and *** indicate significance at $p < 0.01$ and $p < 0.001$, respectively.

4. Discussion

The objective of this study was to identify the most effective growth stage and optimal frequency for applying pre-flowering waterlogging priming treatments to mitigate waterlogging damage in wheat during flowering and to clarify the physiological basis of this priming effect with particular emphasis on nitrogen uptake after stress removal. In this context, waterlogging priming is defined as the persistent physiological capacity of plants to respond more effectively to subsequent waterlogging stress following an earlier, non-lethal exposure, without implying permanent acclimation or genetic adaptation. In the current study, the use of sandy soil enabled precise and reproducible imposition of waterlogging at defined growth stages, and consistent responses were observed across two consecutive pot experiment seasons.

Plants subjected only to waterlogging during flowering (treatment CCC) exhibited pronounced declines in SPAD values and accelerated senescence of the flag leaf after stress removal in both seasons (Figures 2–4). This early loss of photosynthetically active leaf area was associated with impaired grain filling, leading to reduced thousand-grain weight and consequently lower grain yield (Table 1). These responses are consistent with previous reports indicating that wheat is particularly vulnerable to waterlogging stress during flowering, when premature senescence constrains assimilate supply to developing grains [6,7].

In contrast, several treatments receiving waterlogging priming prior to flowering, most notably treatment WCC, WWC, and WCW, showed a markedly attenuated decline in SPAD values when exposed to waterlogging at flowering, thereby delaying senescence (Figures 2 and 3). Grain yield responses closely mirrored SPAD trends, with significant yield increases observed in these treatments. In the 2022/23 season, grain yield in WCC was significantly higher than in CCC, while in 2023/24, additional treatments (CWC and WWW) also showed higher yields (Table 1). Across both seasons, increased thousand-grain weight was the most consistent contributor to yield improvement, particularly in WCC, WWC, and WCW. Similar increases in thousand-grain weight following waterlogging stress priming have been reported previously [21,22]. Collectively, these results indicate that waterlogging priming can alleviate the deterioration of grain filling typically induced by flowering-stage waterlogging.

Waterlogging stress has been posited as a potential factor contributing to the delay in subsequent plant development [26]. However, the present study, employing a 3–4 day waterlogging priming treatment, observed a consistent flowering date across all treatments. Moreover, given that prior studies involving two days of waterlogging priming in wheat also did not report delayed phenology [21,22], this suggests that the impact of short-term waterlogging priming on growth process delays is extremely limited. Conversely, the application of short-term waterlogging priming did not result in enhanced plant growth. In the 2022/2023 growing season, the aboveground biomass was recorded at $1.37 \text{ g plant}^{-1}$ in the CCC. The primed treatments exhibited slightly lower values, ranging from 0.944 to $1.39 \text{ g plant}^{-1}$. While waterlogging priming led to a modest decrease in biomass, it did not result in a delay in phenology or promoting growth. Consequently, it was deduced that the mitigation of waterlogging stress during the flowering stage was attributable to the effect of waterlogging priming itself.

By comparing eight treatments that combined the presence or absence of waterlogging priming at tillering, stem elongation, and booting stages, this study demonstrated that priming effects were strongest when waterlogging priming was applied during tillering, either alone (treatment WCC) or in combination with subsequent stages (WWC and WCW). Although the tillering-stage priming in this study corresponded to the five–six leaf stage, earlier studies have shown that brief waterlogging at the four-leaf stage can reduce declines in SPAD values and grain yield under later stress at heading [23], and that repeated priming at the four- and six-leaf stages can mitigate reductions in photosynthesis and yield under booting-stage waterlogging [22]. In contrast, treatments primed at the booting stage (CCW and CWW) exhibited accelerated leaf senescence prior to flowering in both seasons (Figures 3 and 4), with no corresponding increases in thousand-grain weight or yield (Table 1). While it was reported that repeated waterlogging at multiple later stages had beneficial effects, differences in growth stage timing and stress duration likely account for these discrepancies [20]. Notably, WWW, which received priming at all three stages, tended to maintain leaf greenness and grain yield in 2023/24 (Figures 3 and 4; Table 1). Taken together, these findings suggest that exposure to waterlogging stress during tillering is a critical component for establishing effective priming that enhances tolerance from booting through flowering.

Waterlogging stress is known to reduce the quantum yield of photosystem II (F_v'/F_m') and the photosynthetic rate in wheat [27]. Consistent with this, CCC showed pronounced declines in both parameters (Figures 4 and 5). In contrast, treatments receiving tillering-stage priming (WCC, WWC, and WCW) maintained relatively high F_v'/F_m' values during flowering-stage waterlogging, paralleling SPAD responses (Figure 4). Although differences in the photosynthetic rate were not statistically significant, primed treatments exhibited values approximately 6–8 times higher than CCC (Figure 5). In previous studies, it is documented that a modest increase in the photosynthetic rate in flowering-stage waterlogging was observed in response to waterlogging priming in vegetative stages of wheat [21]. These results indicate that tillering-stage waterlogging priming contributes to maintaining photosystem II function under subsequent stress, which is consistent with improved grain filling and higher thousand-grain weight.

Previous studies have proposed antioxidant capacity and/or aerenchyma formation as key components of waterlogging priming in wheat [21–23,28,29]. Given that waterlogging after flowering is known to suppress nitrogen uptake [11], this study focused on nitrogen dynamics after stress removal using stable isotope tracers. Treatments primed during tillering (WCC, WWC, and WCW) consistently showed higher $\delta^{15}\text{N}$ values in multiple organs during grain filling than CCC (Table A3), indicating greater nitrogen acquisition after flowering-stage waterlogging. Correspondingly, the total ^{15}N content, calculated from $\delta^{15}\text{N}$ values and dry weight, was higher in these treatments both during grain filling and at harvest (Tables 2, A1 and A3). These results demonstrate that tillering-stage waterlogging priming allows wheat to sustain nitrogen uptake capacity long after the priming event, even when later exposed to waterlogging at flowering.

The persistence of this effect observed more than 4–6 weeks after tillering-stage priming suggests the involvement of relatively stable morphological or physiological modifications in the root system. Under waterlogged conditions, wheat roots can form aerenchyma through programmed cell death, facilitating oxygen transport to root tips and supporting root function under hypoxia [30]. Previous studies have shown that brief waterlogging at early leaf stages can induce aerenchyma formation in wheat, particularly in tolerant varieties [23], and that priming accelerates aerenchyma development during subsequent stress events [28,29]. While root anatomy was not directly assessed in the present study, the enhanced post-flowering nitrogen uptake and higher SPAD values observed in tillering-

primed treatments (Table 2; Figure 2) are consistent with improved root function under waterlogging, potentially mediated by such structural adaptations.

Nitrogen demand in wheat grains increases substantially after flowering [31], yet waterlogging from stem elongation to flowering can impair nitrogen uptake, translocation, and remobilization to grains [32]. A comparable phenomenon has been documented in the context of maize [33]. An analysis of ^{15}N allocation revealed that, although treatment differences were minimal during grain filling in 2022/23, allocation to grains at harvest was consistently lower in CCC than in primed treatments (Figure 6). In 2023/24, treatments lacking tillering-stage priming (CCW and CWW) showed little improvement over CCC, whereas treatments including tillering priming exhibited higher ^{15}N allocation to grains. These results indicate that waterlogging priming at the tillering stage contributes not only to sustained nitrogen uptake but also to the maintenance of nitrogen translocation to grains, supporting grain nitrogen accumulation under flowering-stage waterlogging.

5. Conclusions

Overall, the two-year precision pot experiments demonstrate that waterlogging priming at the tillering stage enhances wheat tolerance to subsequent waterlogging during flowering. This enhanced tolerance is closely associated with the maintenance of nitrogen uptake capacity and delayed leaf senescence, as supported by the significant positive correlations between flag-leaf SPAD values and $\delta^{15}\text{N}$ during grain filling and between SPAD values and thousand-grain weight (Figure 7). Despite differences in sowing dates, fertilization regimes, weather conditions, etc., between seasons, treatments WCC, WWC, and WCW consistently showed superior performance, indicating that tillering-stage priming produces robust and reproducible effects. From an agronomic perspective, these findings suggest that early-stage waterlogging events, if managed appropriately, may condition wheat plants to better withstand later waterlogging during flowering. Further studies under field conditions, particularly in waterlogging-prone systems such as converted paddy fields, will be essential to evaluate the practical applicability of waterlogging priming-based wheat management strategies.

Author Contributions: Conceptualization, W.T.; Methodology, W.T.; formal analysis, M.K.; investigation, M.K.; resources, W.T.; data curation, M.K.; writing—original draft preparation, M.K. and W.T.; writing—review and editing, W.T.; visualization, M.K. and W.T.; supervision, W.T.; project administration, W.T.; funding acquisition, W.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partly supported by JSPS KAKENHI Grant Numbers JP20K05999 and JP24K08859.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: We are deeply grateful to Takeshi Yamaguchi, Tottori University and Tadashi Takahashi, Yamaguchi University, for their valuable comments and suggestions before and during the experiment. We also express our thanks to Yukiko Kano and Masako Iwashita, Arid Land Research Center, Tottori University for their supports of $\delta^{15}\text{N}$ and nitrogen concentration measurements. During the preparation of this manuscript, the authors partly used DeepL Write for the purposes of text editing. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

^{15}N	Nitrogen stable isotope
$\delta^{15}\text{N}$	Nitrogen stable isotope composition
Fv'/Fm'	Quantum yield of photosystem II

Appendix A

Table A1. Nitrogen content and ^{15}N content in plant components during grain filling in 2022/23 and 2023/24.

2022/23					
Treatment	Culm + Leaf Sheath	Leaf			Grain
Nitrogen Content (mg plant ⁻¹)					
CCC	11.33 ± 0.47 ¹	2.83 ± 0.47			5.60 ± 1.17
WCC	11.80 ± 2.13	3.60 ± 1.10			5.53 ± 0.73
CWC	10.17 ± 1.27	2.53 ± 0.27			4.57 ± 2.13
CCW	10.00 ± 0.60	2.17 ± 0.10			6.00 ± 0.27
WWC	11.20 ± 0.07	3.10 ± 0.20			5.50 ± 0.73
WCW	10.47 ± 0.77	3.07 ± 0.20			6.57 ± 0.60
CWW	11.30 ± 1.17	2.77 ± 0.20			7.50 ± 0.43
WWW	7.47 ± 0.53	2.13 ± 0.17			4.90 ± 1.17
¹⁵ N Content (µg plant ⁻¹)					
CCC	42.17 ± 5.03	5.10 ± 1.47 ab ²			24.43 ± 8.93
WCC	62.80 ± 14.63	12.10 ± 3.97 a			41.13 ± 6.70
CWC	30.80 ± 10.93	2.97 ± 1.00 ab			23.83 ± 17.70
CCW	27.70 ± 4.73	1.23 ± 0.47 b			23.37 ± 5.73
WWC	54.83 ± 9.20	8.87 ± 2.63 ab			36.33 ± 5.53
WCW	42.60 ± 13.93	6.53 ± 3.03 ab			39.80 ± 12.27
CWW	23.70 ± 4.23	0.90 ± 0.57 b			27.77 ± 1.07
WWW	19.67 ± 8.30	2.77 ± 1.63 ab			15.90 ± 7.37
2023/24					
Treatment	Culm + Leaf Sheath	Lower Leaves	Flag Leaf	Glume + Rachis	Grain
Nitrogen Content (mg plant ⁻¹)					
CCC	18.55 ± 2.70	1.15 ± 0.17	2.22 ± 0.21	7.48 ± 1.00	11.82 ± 3.47
WCC	18.87 ± 5.03	1.27 ± 0.39	2.49 ± 0.82	6.27 ± 1.84	10.91 ± 2.74
CWC	17.43 ± 1.81	1.80 ± 0.35	2.85 ± 0.64	8.36 ± 1.20	12.26 ± 3.25
CCW	21.18 ± 3.50	2.21 ± 0.68	3.62 ± 0.68	9.86 ± 1.63	7.66 ± 3.72
WWC	13.41 ± 2.06	1.95 ± 0.60	3.11 ± 0.70	6.16 ± 1.08	13.03 ± 4.41
WCW	19.41 ± 2.86	1.42 ± 0.14	2.34 ± 0.36	8.79 ± 1.21	18.57 ± 3.66
CWW	13.78 ± 1.98	1.89 ± 0.37	1.98 ± 0.48	8.33 ± 1.35	11.25 ± 3.43
WWW	16.26 ± 2.62	1.28 ± 0.29	1.75 ± 0.53	7.76 ± 2.01	19.24 ± 5.93
¹⁵ N Content (µg plant ⁻¹)					
CCC	7.58 ± 1.37	0.54 ± 0.28	0.02 ± 0.02	0.59 ± 0.14	4.62 ± 1.87
WCC	19.74 ± 8.84	0.65 ± 0.21	1.81 ± 0.91	4.30 ± 2.14	16.91 ± 7.44
CWC	16.52 ± 7.14	0.32 ± 0.09	1.65 ± 1.60	3.28 ± 2.42	30.67 ± 22.71
CCW	9.30 ± 2.22	0.35 ± 0.25	0.16 ± 0.18	0.66 ± 0.37	0.85 ± 0.50
WWC	25.99 ± 14.01	0.93 ± 0.15	3.10 ± 2.13	5.09 ± 2.49	21.41 ± 13.83
WCW	33.41 ± 1.97	0.66 ± 0.15	0.72 ± 0.03	4.85 ± 0.84	60.56 ± 3.19
CWW	1.01 ± 0.14	0.49 ± 0.33	0.01 ± 0.01	0.09 ± 0.09	1.16 ± 0.99

Table A1. Cont.

2023/24					
Treatment	Culm + Leaf Sheath	Lower Leaves	Flag Leaf	Glume + Rachis	Grain
WWW	27.19 ± 12.23	0.20 ± 0.02	0.76 ± 0.58	6.91 ± 3.70	76.98 ± 36.37

¹ Values represent means ± standard error, with three replicates in 2022/23 and 2023/24. ² Different letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test.

Table A2. Nitrogen concentration in plant components during grain filling and at harvest in 2022/23 and 2023/24.

2022/23					
Treatment	Nitrogen Concentration (%)				
	Culm + Leaf Sheath	Leaf	Grain		
During Grain Filling					
CCC	1.27 ± 0.08 ¹	1.61 ± 0.25	2.93 ± 0.08		
WCC	1.26 ± 0.06	1.97 ± 0.30	2.63 ± 0.06		
CWC	1.43 ± 0.24	1.51 ± 0.13	2.87 ± 0.29		
CCW	1.24 ± 0.08	1.47 ± 0.05	2.90 ± 0.02		
WWC	1.29 ± 0.02	1.86 ± 0.17	2.79 ± 0.10		
WCW	1.26 ± 0.07	1.73 ± 0.15	2.73 ± 0.03		
CWW	1.37 ± 0.05	1.69 ± 0.14	2.80 ± 0.05		
WWW	1.25 ± 0.09	1.35 ± 0.04	2.68 ± 0.21		
At Harvest					
CCC	1.21 ± 0.08	1.59 ± 0.20	3.26 ± 0.11		
WCC	0.89 ± 0.11	1.14 ± 0.11	2.89 ± 0.19		
CWC	0.97 ± 0.10	1.62 ± 0.12	3.07 ± 0.10		
CCW	0.97 ± 0.05	1.23 ± 0.07	3.21 ± 0.13		
WWC	0.97 ± 0.08	1.20 ± 0.10	3.13 ± 0.07		
WCW	0.89 ± 0.03	1.11 ± 0.07	3.18 ± 0.15		
CWW	1.01 ± 0.13	1.43 ± 0.13	3.34 ± 0.15		
WWW	0.99 ± 0.10	1.43 ± 0.06	3.26 ± 0.21		
2023/24					
Treatment	Culm + Leaf Sheath	Lower Leaves	Flag Leaf	Glume + Rachis	Grain
During Grain Filling					
CCC	0.75 ± 0.10	0.86 ± 0.08 bc ²	1.69 ± 0.22 ab	1.26 ± 0.16	2.66 ± 0.17
WCC	1.07 ± 0.12	1.00 ± 0.07 abc	1.87 ± 0.17 ab	1.26 ± 0.02	2.51 ± 0.07
CWC	0.91 ± 0.10	1.10 ± 0.11 abc	1.70 ± 0.14 ab	1.57 ± 0.33	3.07 ± 0.56
CCW	1.07 ± 0.13	1.25 ± 0.15 ab	2.16 ± 0.16 a	1.71 ± 0.18	3.05 ± 0.24
WWC	0.74 ± 0.13	1.08 ± 0.06 abc	1.81 ± 0.29 ab	1.15 ± 0.18	2.20 ± 0.25
WCW	0.61 ± 0.01	0.71 ± 0.02 c	1.15 ± 0.09 b	1.16 ± 0.06	2.33 ± 0.06
CWW	0.89 ± 0.04	1.31 ± 0.07 a	1.35 ± 0.18 ab	1.75 ± 0.14	3.12 ± 0.21
WWW	0.75 ± 0.18	0.83 ± 0.10 bc	1.14 ± 0.20 b	1.18 ± 0.12	2.36 ± 0.12
At Harvest					
CCC	0.62 ± 0.13	0.81 ± 0.14 ab	1.74 ± 0.25 a	1.33 ± 0.23	2.82 ± 0.11
WCC	0.32 ± 0.05	0.67 ± 0.08 b	0.78 ± 0.07 b	0.68 ± 0.11	2.38 ± 0.15
CWC	0.45 ± 0.15	0.91 ± 0.12 ab	1.12 ± 0.24 ab	0.63 ± 0.25	2.32 ± 0.33
CCW	0.59 ± 0.08	1.19 ± 0.14 a	1.78 ± 0.07 a	1.09 ± 0.26	3.15 ± 0.25
WWC	0.25 ± 0.02	0.70 ± 0.03 ab	0.73 ± 0.09 b	0.44 ± 0.07	2.32 ± 0.15
WCW	0.29 ± 0.01	0.59 ± 0.03 b	0.91 ± 0.16 ab	0.63 ± 0.16	2.10 ± 0.12
CWW	0.59 ± 0.07	1.19 ± 0.06 a	1.29 ± 0.13 ab	1.17 ± 0.28	2.96 ± 0.32

Table A2. Cont.

2023/24					
Treatment	Culm + Leaf Sheath	Lower Leaves	Flag Leaf	Glume + Rachis	Grain
WWW	0.49 ± 0.23	0.74 ± 0.14 ab	1.02 ± 0.36 ab	0.98 ± 0.63	2.39 ± 0.38

¹ Values represent means ± standard error, with three replicates in 2022/23 and 2023/24. ² Different letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test.

Table A3. Nitrogen stable isotope composition ($\delta^{15}\text{N}$) in each plant component during grain filling and at harvest in 2022/23 and 2023/24.

2022/23					
Treatment	$\delta^{15}\text{N}$ (‰)				
	Culm + Leaf Sheath	Leaf		Grain	
During Grain Filling					
CCC	1022.81 ± 107.10 ¹	538.00 ± 174.16		1123.88 ± 219.88	
WCC	1145.21 ± 101.67	928.87 ± 66.55		2046.54 ± 93.79	
CWC	809.00 ± 213.77	338.69 ± 134.69		1153.42 ± 405.28	
CCW	779.18 ± 172.00	150.74 ± 51.56		1058.34 ± 213.78	
WWC	1348.05 ± 231.52	821.36 ± 278.96		1838.84 ± 184.79	
WCW	1077.52 ± 296.83	557.20 ± 243.87		1599.44 ± 407.20	
CWW	567.85 ± 42.79	87.03 ± 51.17		1022.32 ± 60.32	
WWW	689.37 ± 248.16	388.93 ± 250.50		824.91 ± 215.22	
At Harvest					
CCC	1791.43 ± 102.88 ab ²	602.52 ± 150.91		1684.05 ± 165.08 ab	
WCC	2046.98 ± 137.81 a	732.22 ± 215.02		2233.92 ± 369.62 ab	
CWC	1489.05 ± 99.21 ab	218.19 ± 13.62		1285.27 ± 28.22 b	
CCW	1409.97 ± 153.52 ab	395.13 ± 207.38		1557.26 ± 189.04 ab	
WWC	1948.78 ± 258.98 a	930.59 ± 313.80		2187.61 ± 313.99 ab	
WCW	2078.62 ± 100.35 a	892.71 ± 65.95		2342.49 ± 46.80 a	
CWW	1483.49 ± 29.16 ab	239.11 ± 7.67		1399.51 ± 169.63 ab	
WWW	1040.20 ± 286.05 b	198.12 ± 49.60		1250.79 ± 111.89 b	
2023/24					
Treatment	Culm + Leaf Sheath	Lower Leaves	Flag Leaf	Glume+Rachis	Grain
During Grain Filling					
CCC	113.39 ± 17.44	130.95 ± 56.87	2.76 ± 2.20	20.77 ± 2.36	94.84 ± 24.78
WCC	259.65 ± 87.54	140.45 ± 30.60	176.22 ± 74.67	166.95 ± 55.05	393.63 ± 126.97
CWC	273.15 ± 135.92	56.54 ± 24.98	134.43 ± 127.25	138.94 ± 114.62	523.18 ± 361.66
CCW	127.48 ± 39.12	35.67 ± 17.47	19.19 ± 20.70	22.50 ± 15.55	48.86 ± 23.86
WWC	504.07 ± 211.60	148.65 ± 38.80	256.25 ± 140.47	228.43 ± 90.66	1046.13 ± 295.19
WCW	500.49 ± 94.68	130.12 ± 30.70	88.08 ± 13.81	149.99 ± 6.20	948.42 ± 135.29
CWW	20.99 ± 4.34	60.04 ± 34.76	3.30 ± 3.30	3.08 ± 2.69	20.64 ± 14.40
WWW	405.60 ± 169.59	46.89 ± 10.68	88.84 ± 50.85	205.64 ± 109.92	886.58 ± 369.78
At Harvest					
CCC	371.90 ± 105.21	99.71 ± 55.83	14.17 ± 3.44	84.75 ± 35.77	363.52 ± 111.12 bc
WCC	713.29 ± 252.70	166.23 ± 26.54	91.95 ± 39.58	370.29 ± 181.71	1053.50 ± 221.98 ab
CWC	476.49 ± 103.27	91.64 ± 35.29	20.62 ± 11.54	172.49 ± 46.24	817.74 ± 302.48 abc
CCW	328.76 ± 79.99	61.88 ± 7.59	67.29 ± 64.40	178.42 ± 96.20	284.07 ± 144.27 bc
WWC	518.69 ± 122.23	108.88 ± 28.88	201.45 ± 122.13	460.99 ± 147.45	1280.49 ± 124.28 a
WCW	888.05 ± 165.75	207.94 ± 16.95	130.75 ± 94.77	440.41 ± 218.05	950.69 ± 142.02 abc
CWW	217.77 ± 44.75	116.89 ± 21.67	7.85 ± 5.27	51.81 ± 16.09	147.57 ± 65.66 c
WWW	675.44 ± 173.67	148.33 ± 30.72	67.99 ± 29.00	477.93 ± 269.93	1090.80 ± 90.96 ab

¹ Values represent means ± standard error, with three replicates in 2022/23 and 2023/24. ² Different letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test.

References

- Donat, M.G.; Angéilil, O.; Ukkola, A.M. Intensification of precipitation extremes in the world's humid and water-limited regions. *Environ. Res. Lett.* **2019**, *14*, 065003. [\[CrossRef\]](#)
- Zhang, Y.; Chen, X.; Geng, S.; Zhang, X. A review of soil waterlogging impacts, mechanisms, and adaptive strategies. *Front. Plant Sci.* **2025**, *16*, 1545912. [\[CrossRef\]](#) [\[PubMed\]](#)
- Tian, L.; Zhang, Y.; Chen, P.; Zhang, F.; Li, J.; Yan, F.; Dong, Y.; Feng, B. How does the waterlogging regime affect crop yield? A global meta-analysis. *Front. Plant Sci.* **2021**, *12*, 634898. [\[CrossRef\]](#)
- Kawaguchi, K. Wet Injury of Wheat in Upland Field Converted from Paddy Field in Japan. In Proceedings of the 7th Asian Crop Science Association Conference, Bogor, Indonesia, 27–30 September 2011; pp. 147–152.
- Cannell, R.Q.; Belford, R.K.; Gales, K.; Dennis, C.W.; Prew, R.D. Effects of waterlogging at different stages of development on the growth and yield of winter wheat. *J. Sci. Food Agric.* **1980**, *31*, 117–132. [\[CrossRef\]](#)
- Araki, H.; Hamada, A.; Hossain, M.A.; Takahashi, T. Waterlogging at jointing and/or after anthesis in wheat induces early leaf senescence and impairs grain filling. *Field Crops Res.* **2012**, *137*, 27–36. [\[CrossRef\]](#)
- de San Celedonio, R.P.; Abeledo, L.G.; Miralles, D.J. Identifying the critical period for waterlogging on yield and its components in wheat and barley. *Plant Soil* **2014**, *378*, 265–277. [\[CrossRef\]](#)
- Hossain, M.A.; Araki, H.; Takahashi, T. Poor grain filling induced by waterlogging is similar to that in abnormal early ripening in wheat in Western Japan. *Field Crops Res.* **2011**, *123*, 100–108. [\[CrossRef\]](#)
- Distelfeld, A.; Avni, R.; Fischer, A.M. Senescence, nutrient remobilization, and yield in wheat and barley. *J. Exp. Bot.* **2014**, *65*, 3783–3798. [\[CrossRef\]](#)
- Zhou, B.; Serret, M.D.; Pie, J.B.; Shah, S.S.; Li, Z. Relative contribution of nitrogen absorption, remobilization, and partitioning to the ear during grain filling in Chinese winter wheat. *Front. Plant Sci.* **2018**, *9*, 01351. [\[CrossRef\]](#)
- Jiang, D.; Fan, X.; Dai, T.; Cao, W. Nitrogen fertiliser rate and post-anthesis waterlogging effects on carbohydrate and nitrogen dynamics in wheat. *Plant Soil* **2008**, *304*, 301–314. [\[CrossRef\]](#)
- Sinclair, T.R.; de Wit, C.T. Analysis of the carbon and nitrogen limitations to soybean yield. *Agron. J.* **1976**, *68*, 319–322. [\[CrossRef\]](#)
- Sakuraba, Y. Molecular basis of nitrogen starvation-induced leaf senescence. *Front. Plant Sci.* **2022**, *13*, 1013304. [\[CrossRef\]](#)
- Bruce, T.J.A.; Matthes, M.C.; Napier, J.A.; Pickett, J.A. Stressful memories of plants: Evidence and possible mechanisms. *Plant Sci.* **2007**, *173*, 603–608. [\[CrossRef\]](#)
- Liu, H.; Able, A.J.; Able, J.A. Priming crops for the future: Rewiring stress memory. *Trends Plant Sci.* **2022**, *27*, 699–716. [\[CrossRef\]](#)
- Sharma, M.; Kumar, P.; Verma, V.; Sharma, R.; Bhargava, B.; Irfan, M. Understanding plant stress memory response for abiotic stress resilience: Molecular insights and prospects. *Plant Physiol. Biochem.* **2022**, *179*, 10–24. [\[CrossRef\]](#) [\[PubMed\]](#)
- Siddique, A.; Parveen, S.; Rahman, M.Z.; Rahman, J. Revisiting plant stress memory: Mechanisms and contribution to stress adaptation. *Physiol. Mol. Biol. Plants* **2024**, *30*, 349–367. [\[CrossRef\]](#) [\[PubMed\]](#)
- Aswathi, K.P.R.; Ul-Allah, S.; Puthur, J.T.; Siddique, K.H.M.; Frei, M.; Farooq, M. The Plant Mind: Unraveling Abiotic Stress Priming, Memory, and Adaptation. *Physiol. Plant.* **2025**, *177*, e70372. [\[CrossRef\]](#)
- Agualongo, D.A.P.; Da-Silva, C.J.; Garcia, N.; de Oliveira, F.K.; Shimoia, E.P.; Posso, D.A.; de Oliveira, A.C.B.; de Oliveira, D.D.C.; do Amarante, L. Waterlogging priming alleviates the oxidative damage, carbohydrate consumption, and yield loss in soybean (*Glycine max*) plants exposed to waterlogging. *Funct. Plant Biol.* **2022**, *49*, 1029–1042. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zhou, R.; Jiang, F.; Yu, X.; Abdelhakim, L.; Li, X.; Rosenqvist, E.; Ottosen, C.; Wu, Z. Dominant and priming role of waterlogging in tomato at e[CO₂] by multivariate analysis. *Int. J. Mol. Sci.* **2022**, *23*, 12121. [\[CrossRef\]](#)
- Li, C.; Jiang, D.; Wollenweber, B.; Li, Y.; Dai, T.; Cao, W. Waterlogging pretreatment during vegetative growth improves tolerance to waterlogging after anthesis in wheat. *Plant Sci.* **2011**, *180*, 672–678. [\[CrossRef\]](#)
- Zhang, Y.F.; Wang, X.; Malko, M.M.; Zhou, Q.; Cai, J.; Zhong, Y.; Huang, M.; Jiang, D. Unravelling the long-term beneficial effects of stress priming-induced tolerance to waterlogging stress in wheat. *Plant Growth Regul.* **2025**, *105*, 2067–2084. [\[CrossRef\]](#)
- Jin, N.; Cai, Z.; Ye, L.; Shen, Q.; Zhang, G.; Xu, Z. Improvement of waterlogging tolerance in wheat by the stress priming through inducing aerenchyma formation. *Plant Growth Regul.* **2025**, *105*, 245–255. [\[CrossRef\]](#)
- Taulemesse, F.; Le Gouis, J.; Gouache, D.; Gibon, Y.; Allard, V. Post-flowering nitrate uptake in wheat is controlled by N status at flowering, with a putative major role of root nitrate transporter NRT2.1. *PLoS ONE* **2015**, *10*, e0120291. [\[CrossRef\]](#) [\[PubMed\]](#)
- Martínez-Alcántara, B.; Quiñones, A.; Polo, C.; Primo-Millo, E.; Legaz, F. Use of nitrification inhibitor DMPP to improve nitrogen uptake efficiency in citrus trees. *J. Agric. Sci.* **2013**, *5*, 1–18. [\[CrossRef\]](#)
- de San Celedonio, R.P.; Abeledo, L.G.; Brihet, J.M.; Miralles, D.J. Waterlogging affects leaf and tillering dynamics in wheat and barley. *J. Agron. Crop Sci.* **2016**, *202*, 409–420. [\[CrossRef\]](#)
- Pais, I.P.; Moreira, R.; Semedo, J.N.; Ramalho, J.C.; Lidon, F.C.; Coutinho, J.; Maças, B.; Scotti-Campos, P. Wheat crop under waterlogging: Potential soil and plant effects. *Plants* **2023**, *12*, 149. [\[CrossRef\]](#)
- Li, L.; Dong, X.; He, M.; Huang, M.; Cai, J.; Zhou, Q.; Zhong, Y.; Jiang, D.; Wang, X. Unravelling the role of adventitious roots under priming-induced tolerance to waterlogging stress in wheat. *Environ. Exp. Bot.* **2023**, *216*, 105516. [\[CrossRef\]](#)

29. Zhang, Y.; Wang, X.; Malko, M.M.; Zhou, Q.; Cai, J.; Zhong, Y.; Huang, M.; Jiang, D. The physiological mechanisms of waterlogging priming on aerenchyma formation in secondary roots of wheat under waterlogging stress. *Environ. Exp. Bot.* **2025**, *237*, 106207. [[CrossRef](#)]
30. Herzog, M.; Striker, G.G.; Colmer, T.D.; Pedersen, O. Mechanisms of waterlogging tolerance in wheat—A review of root and shoot physiology. *Plant Cell Environ.* **2016**, *39*, 1068–1086. [[CrossRef](#)]
31. Fradgley, N.S.; Bentley, A.R.; Swarbreck, S.M. Defining the physiological determinants of low nitrogen requirement in wheat. *Biochem. Soc. Trans.* **2021**, *49*, 609–616. [[CrossRef](#)]
32. de San Celedonio, R.P.; Abeledo, L.G.; Miralles, D.J. Nitrogen accumulation and remobilisation in wheat and barley plants exposed to waterlogging at different developmental stages. *Crop Pasture Sci.* **2022**, *73*, 615–626. [[CrossRef](#)]
33. Ren, B.; Dong, S.; Zhao, B.; Liu, P.; Zhang, J. Responses of nitrogen metabolism, uptake and translocation of Maize to water logging at different growth stages. *Front. Plant Sci.* **2017**, *8*, 01216. [[CrossRef](#)] [[PubMed](#)]

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