DOI: 10.1007/s11099-013-0039-9 PHOTOSYNTHETICA 51 (3): 429-437, 2013

Photosynthesis and growth of winter wheat in response to waterlogging at different growth stages

G.C. SHAO[*,**,+], J.J. LAN[*,**], S.E. YU[*,**], N. LIU[***], R.Q. GUO[*,**], and D.L. SHE[*,**]

Key Laboratory of Efficient Irrigation-Drainage and Agricultural Soil-Water Environment in Southern China, Ministry of Education, Nanjing 210098, PR China[*]
College of Water Conservancy and Hydropower Engineering, Hohai University, Nanjing 210098, PR China[**]
School of Economics and Management, Nanjing University of Information Science & Technology, Nanjing 210044, PR China[***]

Abstract

A study on photosynthetic and yield effects of waterlogging of winter wheat at four stages of growth was conducted in specially designed experimental tanks during the 2007–2008 and 2008–2009 seasons. Compared with the control, waterlogging treatments at tillering and jointing-booting stages reduced photosynthetic rate (P_N) and transpiration (E) significantly, it also decreased average leaf water-use efficiency (WUE, defined as the ratio of P_N to E) by 3.3% and 3.4% in both years. All parameters returned quickly to the control level after soil was drained. Damage to the photosynthetic apparatus during waterlogging resulted in a lower F_v/F_m ratio, especially at the first two stages. A strong reduction in root length, root mass, root/shoot ratio, total dry mass, and leaf area index were observed. The responses from vegetative plants at tillering and jointing-booting stages were greater than in generative plants at onset of flowering and at milky stages. The number of panicles per hectare at tillering stage and the spikelet per panicle at the stages of jointing-booting and at onset of flowering were also significantly reduced by waterlogging, giving 8.2–11.3% decrease of the grain yield relative to the control in both years. No significant difference in yield components and a grain yield was observed between the control and treatments applied at milky stages. These responses, modulated by the environmental conditions prevailing during and after waterlogging, included negative effects on the growth, photosynthetic apparatus, and the grain yield in winter wheat, but the effect was strongly stage-dependent.

Additional key words: grain yield; wheat growth stages; milky stage; photosynthesis; plant growth; tillering; *Triticum aestivum*.

Introduction

Waterlogging is a common environmental stress in areas prone to high rainfall, poor soil drainage, and high water table fluctuations, influencing the survival, growth, and productivity of crops (Kozlowski 1997, Jackson and Colmer 2005). For instance, waterlogging has been shown to severely reduce grain yields of wheat (*Triticum aestivum* L.) in the UK (Belford and Cannell 1979, Cannell *et al*. 1980, 1984; Bellford *et al*. 1985), North America (Musgrave 1994, Musgrave and Ding 1998), and Australia (Dennis *et al*. 2000, Zhang *et al*. 2006) by about 20–50%. Due to the increased frequency of extreme climate events (Wollenweber *et al*. 2003), the possibility of waterlogging occurrence has become higher. In Asia, winter wheat could be cropped in a wide area with large climatic and edaphic variations. In the middle and lower reaches of Yangtze River, where the area of winter wheat accounts for about 12% of the whole plant area of China, this crop is commonly planted in paddy fields, which are frequently saturated with water due to excessive rainfall during the growing season. This results in a significant risk of intermittent waterlogging combined with the rainfall without any seasonal distribution.

Received
[+]Corresponding author; tel: +86-025-58099502, e-mail: sgcln@126.com
Abbreviations: C_i – intercellular CO_2 concentration; Ck – control; E – transpiration rate; g_s – stomatal conductance; JB – jointing-booting stage; MS – milky stage; OF – onset of flowering; PAR – photosynthetically active radiation; P_N – net photosynthetic rate; PSII – photosystem II; RM – root mass; RSR – root/shoot ratio; SM – shoot mass; TGM – thousand-grain mass; TS – tillering stage; WUE – leaf water-use efficiency.
Acknowledgements: This work was funded by the National Nature & Science Foundation of China (No. 51009047, No. 51279059, No. 50839002) and supported by the Supporting Program of the "Outstanding Young Creative Talents in Hohai University". We extend our gratitude to Abubaker Babikr Ahmed for considerable language improvements and technical assistance and support provided.

Maintaining agricultural production in areas, where periodic waterlogging occurs, requires the identification of growth response of crops to waterlogging at different growth stages (Setter and Waters 2003). Much of the work relating to waterlogging in crop species has involved the use of repeated and/or long (*i.e.* weeks or months) exposures to waterlogging (*e.g.* Orchard and Jessop 1984, Musgrave and Ding 1998), and those relating to different growth stages (*e.g.* wheat – Musgrave 1994, soybean – Linkemer *et al*. 1998) have been limited to considering effects on the final yield with little attention being paid to the underlying causes of the yield reduction. A direct physiological response of winter wheat to waterlogging might be expected. Waterlogging involves the rapid development of anoxia or hypoxia in the soil, with effects on water and nutrient absorption (Boru *et al*. 2003, Araki 2006). In most crops, O_2 used in root respiration is supplied from the soil (Drew 1997). Diffusion is the main mechanism of O_2 movement through the soil. Because O_2 has a very small diffusion rate in water, it almost stops when the soil is saturated with water. Then a lack of O_2 may limit crop growth due to alterations in metabolism (Drew 1997). One of alterations is a decline in nutrient uptake, as it has been found for N, P, K^+, and other nutrients in many crops (Mielke and Schaffer 2010). Waterlogging occurring at different development stages may reduce final grain yield of winter wheat to different degrees and the extent of yield reduction depends not only on the severity of the waterlogging, but also on the stage of the plant development. Waterlogging induces changes in soil physico-chemical properties, such as a reduction in oxygen level and an increase in CO_2 and ethylene concentration that affect several aspects of plant physiology, morphology, and anatomy (Kozlowski 1997, Pezeshki 2001, Kreuzwieser *et al*. 2004). Waterlogging adversely affects leaf expansion and leaf formation and leads to premature leaf senescence and abscission resulting in an inhibition of shoot growth (Kozlowski 1997). Root growth, on the other hand, is reduced from the lack of oxygen available for root respiration and the presence of soil phytotoxins inhibiting root formation and promoting root decay (Pezeshki 2001).

The physiological and biochemical responses of crops to waterlogging events have been extensively investigated. Photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration (E) decreased (Zheng *et al*. 2009) and it led to restrictions in carbohydrate metabolism in both shoots (Xie *et al.* 2003) and roots (Huang *et al*. 1995). This resulted in a decrease in both the accumulation of dry matter (Malik *et al*. 2002) and the amount of dry matter transferred into the grains (Tan *et al*. 2003). The decrease of plant biomass production may be directly related to stomatal limitations of net photosynthesis which reduces carbon assimilation (Mielke *et al*. 2003). Waterlogging occurring at any growth stage usually causes degradation of chlorophyll in leaves (Chen *et al*. 2005, Pociecha *et al*. 2008) and of protein content in grains (Mishra *et al*. 2008) and it decreases the concentrations of nitrogen, phosphorus, and potassium in plant shoots (Sharma and Swarup 1988). Chlorophyll fluorescence is a fast, nondestructive, and relatively simple technique for detecting the energetic/metabolic imbalance of photosynthesis, which can be damaged by waterlogging (Flagella *et al*. 1994). The ratio of variable to maximal fluorescence (F_v/F_m) is a good indicator of photoinhibitory impairment, when plants are subjected to a wide range of environmental stresses, including waterlogging. A reduction of F_v/F_m may represent either a reversible photoprotective downregulation or an irreversible inactivation of photosystem II (PSII) during waterlogging.

Waterlogging has been reported to cause a reduction in root growth, photosynthesis (Glinski and Stepniewski 1985), leaf area, and dry matter accumulation in wheat (Shao *et al*. 2010). However, little work has been done to assess the relative importance of these processes in an integrated way. The knowledge of the physiological effects of waterlogging on winter wheat is inadequate compared to that of the effects of water shortage on this crop. In the work, our objective was to use crop growth analysis to trace physiological mechanisms (at the crop level) by which the various effects of waterlogging at different stages impact the crop growth and yield. This study could be useful for the development of a more functional approach to simulate wheat response to waterlogging.

Materials and methods

Experimental conditions and plant material: The experiment was conducted in 15 concrete lysimeters, each of about 5 m^3 (surface area 2.5 × 2 m, depth 2 m), in Key Laboratory of Efficient Irrigation-Drainage and Agricultural Soil-Water Environment in Southern China, Ministry of Education (Nanjing, latitude 31°57'N, longitude 118°50'E, 144 m a.s.l.) during the wheat growing season of 2007–2008, and repeated in 2008–2009. The bottom of each lysimeter was filled with a 20 cm layer of coarse gravel, separated from the soil by a water-permeable membrane to allow free drainage. Drainage holes in the waterlogged lysimeters were blocked with rubber bungs. All lysimeters were painted with a waterproof material to prevent seepage through the concrete blocks (Fig. 1). The experimental site has subtropical humid climate with an annual mean temperature of 15.3°C. The mean annual precipitation is 1,051.4 mm and the mean annual surface water evaporation is 900 mm (data from Nanjing city of 1951–2009, 20 km northeast of experimental site). The soil type was clay with pH 7.78 and 2.40% of organic matter content, soil bulk density at 0–50 cm depth was 1.46 g cm^{-3}, field capacity was

26.47%, as mass of water on dried soil. During 2 years, plots were hand sown with winter wheat, *T. aestivum* L. cv. Yangmei 14, on 12 November 2007 (12 November 2008) using the 135 kg ha^{-1} template. A week before sowing, the experimental plots were dry-ploughed and harrowed. The soil was soaked 1 day before sowing to promote good crop burgeon. Basal application of fertilizers ($N:P_2O_5:K_2O$ = 15:10:15) at the rate of 1,200 kg ha^{-1} was applied in the soil. The heading date for wheat (50% of plants) was on 21 April 2008 (23 April 2009), and plants were harvested on 29 May 2008 (26 May 2009).

Treatments and experimental design: Five treatments (T1–T5) were used to evaluate effects of waterlogging at different phenological stages on physiological indicator parameters (E, P_N, g_s, and WUE), growth and wheat yields from 2007 to 2009, as shown in Table 1. The water depth of control treatments was kept 50 mm from the soil surface at the tillering stage (TS) and 100 mm at other stages. The water layer above the soil was maintained for 1 day, then it was drained to –400 mm below the surface within 3 days at TS and to –800 mm below the surface within 3 days at the other stages. The control (Ck) was the treatment, where the soil relative water content was maintained at 70–80% and the groundwater level was kept below –600 mm at TS, –800 mm at the jointing-booting stage (JB), and –1,000 mm at the onset of flowering (OF) and milky stage (MS). These treatments were arranged in a randomized complete block design with three replications and means were compared with a Least Squares Means procedure.

Table 1. Design of irrigation and drainage scheduling. I mm(K d)(M d –J mm): I mm fixed water level was kept with duration of K days at four stages from the soil surface and drained to –J mm in M days. < –N mm: –N mm was the upper limit of groundwater level, if groundwater level exceeded the value and drainage would be conducted. T1, T2, T3, T4 denote the waterlogging treatments at tillering, jointing-booting, onset of flowering, and milky stage, T5 is taken as the control.

Treatment	Tillering stage	Jointing-booting stage	Onset of flowering stage	Milky stage
T1	50 mm(1 d) (3 d –400 mm)	< –800 mm	< –1000 mm	< –1000 mm
T2	< –600 mm	100 mm(1 d) (3 d –800 mm)	< –1000 mm	< –1000 mm
T3	< –600 mm	< –800 mm	100 mm(1 d) (3d –800 mm)	< –1000 mm
T4	< –600 mm	< –800 mm	< –1000 mm	100 mm(1 d) (3 d –800 mm)
T5	< –600 mm	< –800 mm	< –1000 mm	< –1000 mm

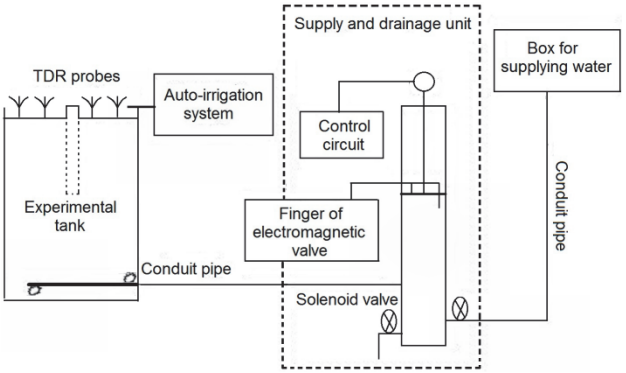

Fig 1. Schematic diagram of the experimental set-up placed in the lysimeter.

Drainage flow: A 2 m deep lysimeter of 2.5 × 2 m inside the instrument shed (Fig. 1) received the pipe drainage outflow from the plots. Fifteen automatic irrigation and drainage systems were installed inside the instrument shed to measure the drainage and irrigation volume of all the plots. The number of solenoid valve opening were counted and stored by a datalogger and the drainage and irrigation volume was subsequently calculated.

Soil samples, water table, and soil moisture: Fifteen soil samples from all the plots were collected, prior to planting. The soil samples were collected at the centre of the plot at 0–50 cm depth. One perforated PVC pipes (60 mm diameter) was installed to a depth of 180 cm over pipe in each plot and automated capacitive water level probes were inserted inside the PVC pipes to measure the water table depth. One tube was inserted to a depth of 180 cm of each plot. Soil water content measurements were made using the time-domain reflectometry (*TRIME-T3, USA*). The probe of time domain reflectometry was put into the tube and the values of soil water content at the depth of 0–100 cm were stored by a recorder. The measurements were taken twice a week during the growing season.

Plant and climatic measurements: Leaf area index (LAI) was measured in every stage with *LAI 2000* (*Li-Cor*, USA). In addition, plant development characteristics were determined including tillering number, height, and leaf number. During the course of the experiment, P_N, E, and g_s of the top, fully expanded leaves were determined at four stages. A gas-exchange analyzer (*Li-Cor 6400* portable photosynthesis measurement system, *Li-Cor*, Lincoln, NE, USA) was used for these measurements during 9:00–11:00 h, when photosynthetic active radiation above the canopy was 1,000–1,100 μmol m^{-2} s^{-1}. The maximum quantum yield of PSII photochemistry (F_v/F_m ratio) was measured at 23ºC in dark-adapted,

young, fully expanded leaves using *OS5-FL* (*Opti-Science*, USA) starting 1 day before flooding, till 7 days after cessation of flooding. For dark adaptation, a clip was placed on the leaf for 15 min. The saturating irradiance was 600 μmol m^{-2} s^{-1} PAR for 5 s. Six leaves were used for each treatment. The plants on each plot were randomly selected for each measurement. The average values of three leaves and plants were used to calculate the rates of E and g_s. The plants were harvested on May 29, 2008 (May 26, 2009). Panicle number per square meter, number of grains per panicle, percentage of filled spikelets, and mass of 1,000 dry grains for each individual plant per replicate were recorded. A random subsample of five stems was taken to measure straw length. Dry mass of all plant fractions was recorded after drying in an oven at 80°C for 48 h.

The air temperature, wind speed and direction, relative humidity, total solar radiation, and photosynthetically active radiation (PAR) were measured at the experimental site, using the automatic instrument for meteorological measurements. Precipitation was measured by a tipping bucket. Soil temperatures were measured at 5, 15, and 30 cm depths near the instrumentation site, using a temperature probe. All the meteorological parameters were stored using a datalogger and were downloaded weekly *via* a computer.

Statistical analysis: Experiments were designed as randomized complete blocks, with each replicate representing a separate block. Treatment effects in the experiment were analyzed using analysis of variance (*ANOVA*) procedure of *SPSS software*, version *14.0*. Treatment means were separated by least significant difference (LSD) test at $P \leq 0.05$ unless otherwise specified.

Results

Impact of waterlogging on vegetative growth: The inability of roots to acclimate to changes in soil water regimes might result in the reduced growth and function, thereby the reduction in total dry mass (TDM). In 2008, waterlogging during TS stages significantly decreased the root length (RL), root mass (RM), root/shoot ratio (RSR), and TDM of the plants but had no effect on shoot mass (SM) (Table 2). The T1 treatment had a significantly smaller SM and RSR compared with Ck. Waterlogging during TS stages induced excess moisture and depressed the vegetative growth. As shown in Table 2, waterlogging at JB stages also significantly reduced RL, RM, and RSR in 2008 compared with Ck, but it did not significantly reduce SM. During the OF stage, RL, RM, RSR, and TDM values were similar for control and T3 treatments. The plants exposed to waterlogging during the fourth growth phase exhibited more equilibrated plant growth throughout the experimental period (Table 2), no significant differences were found between T4 and Ck. Waterlogging at OF and MS stages had no significant impact on SM, RM, RSR, and TDM compared with Ck, as the peak of the vegetative growth was already completed at these growth stages. In 2009, the highest RM and RSR was obtained at T5, the lowest was at T1. These results indicated that waterlogging applied at TS stage could significantly decrease RM. RL and SM at each waterlogging treatment decreased by 10.7–24.0% and 6.7–11.7% at TS and JB stages, respectively. Furthermore, T2 treatment reduced TDM slightly. Waterlogging treatments at OF and MS stages had no significant effect on SM, RM, RSR, and TDM in 2009, as the peak of vegetative growth was already completed at these growth stages.

Table 2. Effect of waterlogging on root length (RL), shoot mass (SM), root mass (RM), root/shoot ratio (RSR) and total dry mass (TDM) of wheat at harvest in 2008 and 2009. T1, T2, T3, T4 denote the waterlogging treatments at tillering, jointing-booting, onset of flowering, and milky stage, T5 is taken as the control. The values of vegetative growth parameter are means of 3 replications. In the same column and in the same year, means followed by the *same letter* do not differ significantly at the 5% level by LSD.

Year	Treatment	RL [cm]	RM [g(DM) $plant^{-1}$]	SW [g(DM) $plant^{-1}$]	RSR [g g^{-1}]	TDM [mg $plant^{-1}$]
2008	T1	13.61 ± 0.75^{b}	0.28 ± 0.04^{b}	5.39 ± 0.58^{a}	0.052 ± 0.001^{b}	5.67 ± 0.64^{b}
	T2	12.82 ± 0.68^{b}	0.31 ± 0.06^{b}	5.28 ± 0.43^{a}	0.059 ± 0.001^{b}	5.59 ± 0.38^{b}
	T3	14.42 ± 0.56^{ab}	0.37 ± 0.05^{ab}	5.92 ± 0.37^{a}	0.063 ± 0.002^{ab}	6.29 ± 0.29^{ab}
	T4	15.23 ± 1.03^{a}	0.41 ± 0.02^{ab}	6.24 ± 0.89^{a}	0.066 ± 0001^{ab}	6.65 ± 0.41^{a}
	T5	17.65 ± 0.89^{a}	0.56 ± 0.05^{a}	6.71 ± 0.86^{a}	0.083 ± 0.002^{a}	7.27 ± 0.85^{a}
2009	T1	14.01 ± 1.23^{ab}	0.25 ± 0.03^{b}	6.32 ± 0.66^{a}	0.035 ± 0.001^{b}	6.57 ± 0.77^{b}
	T2	11.92 ± 0.67^{b}	0.31 ± 0.03^{b}	6.68 ± 1.02^{a}	0.046 ± 0.001^{b}	6.99 ± 1.02^{ab}
	T3	12.55 ± 0.62^{b}	0.42 ± 0.04^{ab}	7.13 ± 0.60^{a}	0.059 ± 0.002^{ab}	7.55 ± 0.64^{a}
	T4	14.21 ± 0.42^{ab}	0.38 ± 0.02^{ab}	7.06 ± 0.50^{a}	0.060 ± 0.001^{a}	7.44 ± 0.68^{a}
	T5	15.68 ± 1.12^{a}	0.58 ± 0.07^{a}	7.16 ± 0.67^{a}	0.076 ± 0.003^{a}	7.73 ± 0.47^{a}

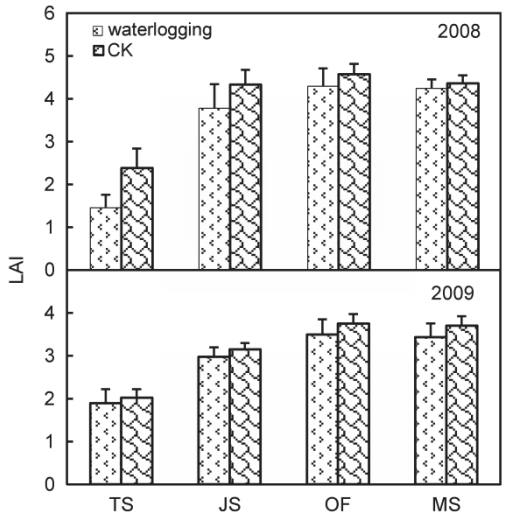

Fig. 2. Seasonal changes in leaf area index (LAI) of wheat under the waterlogging treatment. TS – tillering stage, JB – joint-booting stage, OF – onset of flowering, MS – milky stage, CK – control.The values are averages of the waterlogging treatment at each stage and the control of 3 replicates in 2008 and 2009. Vertical bars represent ± SE of the mean.

The stage-dependent changes of LAI are shown in Fig. 2 for five treatments as measured in both years. In 2008, waterlogging treatments reduced LAI of wheat significantly at TS and JB stages ($P \leq 0.05$), but insignificantly at OF and MS stages as compared with Ck (Fig. 2). Because the total number of tillering and growth of leaves reached its peak at the early OF stage, the period of the shoot growth at this stage had little impact on LAI. In 2009, however, no significant difference was observed among waterlogging treatments and Ck at all four stages. The discrepancy in LAI between 2008 and 2009 might be attributed to the difference in the weather. The effect was statistically significant only in plants at the vegetative stages and remained visible after cessation of waterlogging.

Effect of waterlogging at different stages on physiological parameters of wheat: Measurements of E, P_N, g_s, and WUE in uppermost leaves of winter wheat made at close to full sunlight in both years indicated that these physiological parameters were reduced after 4-d waterlogging and the reduction varied with the stage of waterlogging stress (Table 3). T1 and T2 treatments of waterlogging, which occurred at TS and JB stages, decreased average P_N by 13.6 and 12.2% relative to Ck in both years, and they decreased the average WUE by 4.2 and 3.9%, respectively. The effect on P_N and E was also statistically significant only in the plants at the vegetative stages (TS and JB) and it remained visible after cessation of waterlogging. In addition, the T1 and T2 treatments caused simultaneously a significant decrease of g_s, which could prevent CO_2 supply into leaf cells and resulted in decreasing of leaf C_i, indicating that the decline of photosynthesis was mainly due to the strong, reversible stomatal limitation, but the photosynthetic rate could be effectively resumed after waterlogging withdrawal. The T1 and T2 treatments reduced leaf g_s and P_N and damaged the membrane system and increased lipid peroxidation into superoxide radical, thus C_i remained unchanged or even increased (data not shown), which meant that the decline of photosynthesis was mainly due to a poor, reversible nonstomatal limitation, *i.e.* due to lower photosynthetic activity of leaf cells. After 3 days of recovery, E and P_N of the plants briefly exposed to waterlogging stress at TS and JB stages could enable the ''compensatory'' growth mechanism to act and they were not significantly different from that of the nonstressed, control plants. Both waterlogging treatments increased average WUE to about 97.8 and 97.5% of Ck in both years. Compared with Ck, T3, and T4 treatments of waterlogging, which occurred at OF and MS stages, decreased average WUE only by 1.6 and 2.7% in both years, moreover, P_N showed no significant difference. E, P_N, and g_s slightly restored 3 d after waterlogging withdrawal, when waterlogging treatments occurred at OF and MS stages, and no significant difference was found from Ck. This could help to stimulate the nutrition accumulation and life activity of wheat. A decline in chlorophyll fluorescence F_v/F_m values was observed

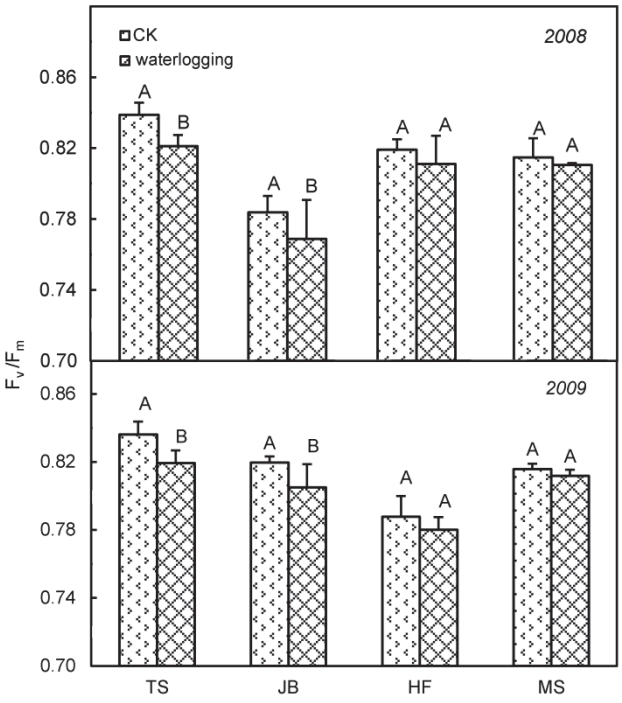

Fig. 3. Seasonal changes in F_v/F_m of wheat under the waterlogging treatments. TS – tillering stage, JB – joint-booting stage, OF – onset of flowering, MS – milky stage, CK – control. The values are averages of the waterlogging treatment at each stage and the control of 3 replicates in 2008 and 2009. Vertical bars represent ± SE of the mean. *Different letters* indicate significant difference ($P \leq 0.05$).

Table 3. Transpiration rate (E), photosynthetic rate (P_N), stomatal conductance (g_s), and water-use efficiency (WUE) of winter wheat leaves under different waterlogging treatments in 2008 and 2009. T1, T2, T3, T4 denote the waterlogging treatments at tillering, jointing-booting, onset of flowering, and milky stage, T5 is taken as the control. P_N [μmol(CO_2) m^{-2} s^{-1}]; E [mmol(H_2O) m^{-2} s^{-1}]; g_s [mol(H_2O) m^{-2} s^{-1}]; WUE [μmol(CO_2) mmol(H_2O)$^{-1}$]. Each value is the mean ± SD (n = 6). *Different letters* indicate significant difference ($P \leq 0.05$).

Year	Growth stage	Treatment	1 d after waterlogging				3 d after waterlogging withdrawal			
			P_N	E	g_s	WUE	P_N	E	g_s	WUE
2008	Tillering	T1	20.33 ± 0.82^b	2.93 ± 0.22^b	0.32 ± 0.00^b	6.94 ± 0.29^a	23.87 ± 0.38^a	3.52 ± 0.31^a	0.40 ± 0.00^a	6.78 ± 0.23^a
		T5	25.20 ± 0.42^a	3.49 ± 0.15^a	0.45 ± 0.00^a	7.22 ± 0.24^a	25.43 ± 1.02^a	3.64 ± 0.12^a	0.40 ± 0.01^a	6.99 ± 0.32^a
	Jointing-booting	T2	17.03 ± 1.52^b	3.14 ± 0.15^b	0.28 ± 0.02^b	5.42 ± 0.14^a	19.30 ± 0.47^b	3.19 ± 0.10^b	0.30 ± 0.02^a	6.05 ± 0.05^a
		T5	21.23 ± 0.46^a	3.77 ± 0.19^a	0.35 ± 0.07^a	5.63 ± 0.26^a	21.47 ± 1.25^a	3.43 ± 0.24^a	0.36 ± 0.09^a	6.23 ± 0.18^a
	Onset of flowering	T3	21.20 ± 0.43^a	3.98 ± 0.17^a	0.42 ± 0.02^a	5.33 ± 0.25^a	21.20 ± 0.72^a	3.06 ± 0.14^a	0.38 ± 0.01^a	6.93 ± 0.15^a
		T5	23.77 ± 0.68^a	4.34 ± 0.15^a	0.46 ± 0.02^a	5.48 ± 0.27^a	22.10 ± 1.27^a	3.12 ± 0.06^a	0.39 ± 0.01^a	7.08 ± 0.35^a
	Milky	T4	13.20 ± 1.75^a	2.07 ± 0.39^a	0.22 ± 0.01^a	6.38 ± 0.27^a	17.03 ± 0.12^a	2.33 ± 0.10^a	0.28 ± 0.01^a	7.31 ± 0.30^a
		T5	16.23 ± 0.99^a	2.50 ± 0.36^a	0.25 ± 0.02^a	6.49 ± 0.29^a	18.37 ± 1.02^a	2.47 ± 0.16^a	0.30 ± 0.01^a	7.44 ± 0.26^a
2009	Tillering	T1	22.63 ± 0.52^b	2.94 ± 0.13^b	0.26 ± 0.03^b	7.70 ± 0.33^a	22.38 ± 0.48^a	3.08 ± 0.23^a	0.32 ± 0.02^a	7.27 ± 0.21^a
		T5	24.50 ± 0.41^a	3.04 ± 0.05^a	0.38 ± 0.01^a	8.06 ± 0.15^a	23.25 ± 0.79^a	3.15 ± 0.21^a	0.38 ± 0.03^a	7.38 ± 0.56^a
	Jointing-booting	T2	20.70 ± 0.41^a	2.28 ± 0.15^a	0.21 ± 0.02^b	9.08 ± 0.72^a	20.83 ± 0.71^a	3.08 ± 0.13^a	0.31 ± 0.01^a	6.76 ± 0.66^a
		T5	21.73 ± 0.71^a	2.30 ± 0.13^a	0.32 ± 0.02^a	9.45 ± 0.75^a	21.98 ± 0.47^a	3.18 ± 0.23^a	0.33 ± 0.02^a	6.91 ± 0.47^a
	Onset of flowering	T3	18.55 ± 1.44^a	2.74 ± 0.08^a	0.22 ± 0.02^a	6.77 ± 0.34^a	20.70 ± 1.21^a	3.34 ± 0.16^a	0.32 ± 0.02^a	6.20 ± 0.49^a
		T5	20.73 ± 0.29^a	3.04 ± 0.12^a	0.29 ± 0.01^a	6.82 ± 0.24^a	21.80 ± 1.79^a	3.51 ± 0.30^a	0.35 ± 0.03^a	6.21 ± 0.17^a
	Milky	T4	13.98 ± 1.44^a	3.16 ± 0.13^a	0.25 ± 0.01^a	4.42 ± 0.44^a	16.03 ± 1.23^a	3.02 ± 0.34^a	0.26 ± 0.02^a	5.31 ± 0.09^a
		T5	14.88 ± 0.72^a	3.23 ± 0.15^a	0.26 ± 0.01^a	4.61 ± 0.11^a	17.19 ± 0.87^a	3.21 ± 0.25^a	0.28 ± 0.01^a	5.36 ± 0.20^a

Table 4. Grain yield and its components in wheat during two years under the treatments of waterlogging. T1, T2, T3, T4 denote the waterlogging treatments at tillering, jointing-booting, onset of flowering, and milky stage, T5 is taken as the control. The values of water consumption are means of 3 replications. In the same column and in the same year, means followed by the *same letters* do not differ significantly at the 5% level by LSD.

Year	Treatment	Panicles per hectare	Spikelet per panicle	1000-grain mass [g]	Grain yield [kg ha^{-1}]
2008	T1	420.00 ± 2.30^{b}	33.32 ± 0.47^{a}	42.28 ± 0.13^{a}	5.917 ± 45^{b}
	T2	490.00 ± 3.12^{a}	29.69 ± 0.23^{b}	41.58 ± 0.24^{a}	6.049 ± 72^{b}
	T3	492.00 ± 3.08^{a}	29.52 ± 0.36^{b}	40.37 ± 0.14^{a}	5.863 ± 65^{b}
	T4	502.00 ± 2.45^{a}	31.66 ± 0.48^{ab}	38.62 ± 0.26^{b}	6.138 ± 59^{b}
	T5	506.00 ± 1.89^{a}	31.92 ± 0.23^{ab}	40.90 ± 0.32^{a}	6.606 ± 79^{a}
2009	T1	407.57 ± 4.05^{b}	34.92 ± 0.56^{ab}	41.69 ± 0.21^{a}	5.932 ± 73^{b}
	T2	423.58 ± 4.05^{a}	34.33 ± 0.49^{ab}	41.68 ± 0.41^{a}	6.060 ± 88^{b}
	T3	424.89 ± 1.76^{a}	33.10 ± 0.19^{b}	41.54 ± 0.03^{a}	5.842 ± 56^{b}
	T4	432.24 ± 2.30^{a}	36.37 ± 0.17^{a}	40.16 ± 0.03^{a}	6.312 ± 69^{ab}
	T5	430.88 ± 3.33^{a}	36.65 ± 0.31^{a}	41.73 ± 0.36^{a}	6.589 ± 81^{a}

during waterlogging. Waterlogging occurring at TS and JB stages (Fig. 3) was characterized by a decrease of F_v/F_m, up until the fourth day of waterlogging, when they reached the lowest values (data not presented). When wheat was waterlogged at OF and MS stages (Fig. 3*B*), a weaker decline in F_v/F_m occurred and the difference was insignificant compared with Ck.

Grain yield and its components: Waterlogging at different growth stages had significant effects on wheat grain yield in both years with exception for T4 treatment in 2009, although their relative impacts varied between years. In 2008, the greatest effect resulted from treatment at OF stage. Compared with the nonstressed control, the waterlogging treatments decreased the grain yield by 7.1–11.2% (Table 4). In 2009, the effect of waterlogging at different growth stages was similar, but less deleterious than in 2008. These factors resulted in significant decreases in the grain yield of 4.2–11.3%, with exception for the T4 treatment compared with Ck.

In both years, the decrease in the yield at T1 was due to a significant decrease in the number of panicles per hectare, other yield components were unaffected. However, in 2008, the thousand-grain mass (TGM) of waterlogged plants was slightly higher than that of Ck, whereas it was less than that of Ck in 2009, although the differences were not significant. Waterlogging at OF stage had little effect on the number of panicles per ha and TGM, but it resulted in a significant decrease in number of spikelet per panicle in both years. In 2008, waterlogging at MS stage significantly decreased TGM whilst no significant difference occurred relative to Ck in 2009.

Discussion

The vegetative growth effects of waterlogging varied with treatments at various stages of the growth. This study showed that waterlogging at the early growth stage could reduce the shoot and root growth of wheat. During periods, when soil is saturated by water, oxygen deficiency can develop rapidly in the roots, causing root damage and subsequently death of the entire plant (Palta *et al*. 2010). In wheat, symptoms and injury can become evident in roots. Absence of soil oxygen initiates a sequence of chemical and biochemical reduction reactions, producing components that may be injurious to root metabolism (Jackson and Drew 1984). Lateral root extension and number are reduced by low concentrations of oxygen (Geisler 1967). Plants, which can survive in anoxic stress, decrease their root growth rate (Jackson and Campbell 1979). These anoxic conditions cause respiratory disturbances in the roots and this has injurious consequences for the shoot system. The reduced vegetative growth was also possibly a result of waterlogging restricting the supply of nitrogen to the shoot at different growth stages. Nitrogen deficiency would be expected to slightly slow down plant development (Mirshel *et al*. 2005). In this study, waterlogging delayed the shoot development slightly as waterlogged plants showed delayed ear emergence and maturity. This senescence caused earlier grain maturity (data not shown). Early waterlogging treatments delayed the flowering time of wheat and reduced flowers per panicle and flower dry mass. Because vegetative growth of wheat was almost ceased at OF stage, the activity of roots and shoots was less sensitive to waterlogging at OF and MS stages. The effects were not fully reversible after the stress was relieved. This and other damaging effects on stem and leaf expansion appeared to be more severe in plants of the vegetative stages than at the generative ones.

It is known that anaerobiosis induced by waterlogging can decrease root hydraulic conductivity resulting in a decreased leaf turgor and stomatal conductance and thus CO_2 deficiency inside the leaves. The decrease of plant biomass production may be directly related to stomatal

limitations of net photosynthesis that reduces carbon assimilation (Mielke *et al*. 2003). The restriction of photosynthetic activity has been attributed to stomata closure (Yordanova *et al*. 2005), decrease in leaf chlorophyll content (Bradford 1983), and disruption of the translocation of photosynthates (Sij and Swanson 1973, Chen *et al*. 2005). The present study showed that P_N was a very sensitive trait in response to waterlogging, which decreased in the initial 2 d of waterlogging. The decrease in g_s and the reductions in E values were observed, together with the simultaneous decline in P_N. Thus, the data obtained in our experiment led to the conclusion that the main effect of waterlogging on leaf gas exchange in winter wheat seemed to be a reduction in stomata opening that led to the reduction of P_N. It could be found in both years that under T1 treatment P_N, E, and g_s decreased by 13.6, 33.6, and 58.6% of the T5 treatment, while in the T4 treatment, this decrease was only 11.4, 7.0, and 33.6%, respectively. Thus, it could be concluded that the photosynthesis in leaves decreased more markedly at the early period than during the later growth stage under waterlogging stress.

Chlorophyll fluorescence is an efficient tool for detecting changes in functions of photosynthetic apparatus, which can be damaged by waterlogging (Waldhoff *et al*. 2002, Mielke *et al*. 2003). During the period of growth of wheat, declines in F_v/F_m were observed under waterlogging treatments compared with Ck, indicating damage to PSII. Thus, the use-efficiency of captured photon energy by PSII was reduced. Some damage to PSII seems to be independent of decreases in stomatal conductance and it may be caused by the changes within mesophyll cells and correlated with photoinhibition (Ahmed *et al*. 2002). The responses of photosynthetic apparatus observed in this study suggested that changes in the rate of CO_2 fixation depended more strongly on stomata closure than on the damage of PSII. However, other mechanisms may also play a part. In agreement with the findings of the former research (Tan *et al*. 2008, Zheng *et al*. 2009), these changes in fluorescence parameters further contributed to the decline in leaf P_N and consequently resulted in reductions of carbon assimilation. In conclusion, our data suggested that episodic waterlogging at different stages promoted stomata closure and also disturbed functioning of the photosynthetic apparatus of winter wheat plants in terms of depressing maximum quantum yield (F_v/F_m) of PSII.

Yield losses due to waterlogging reported in this paper were in the range of those found in the experiments using China cultivars of winter wheat in the south, grown in outdoor lysimeters (Shao *et al*. 2010). The decreased grain yield at TS stage resulted from a decrease in the number of ears per plant, rather than the number of grains per ear or TGM, in agreement with previous works (Belford *et al*. 1985). The reduction of ear number at harvest (Table 3) was due to the inhibition of tiller initiation rather than an increased rate of tiller abortion. It could be noticed that the effect of waterlogging on TGM was not significant. The decrease in grain yield at JB and OF stages was mainly due to the decrease in the number of grains per ear and TGM at MS stage. In both years, waterlogging significantly reduced the total number of tillers produced (Tables 3,4). OF stage was the most susceptible stage, followed by TS stage, JB stage, and MS stage. In contrast, the plants waterlogged at JB stage could withstand prolonged waterlogging. Waterlogging was the major physiological constraint during OF stage, and there was a significant correlation between seed yield and growth during this stage. The present experiment confirmed that physiological function of winter wheat plants was retarded during the time of waterlogging at OF stage and its adverse effect still remained afterwards, resulting in a highly significant decrease of the yield. The ability of wheat to withstand prolonged waterlogging at JB stage was partly due to the fact that water requirement during this stage was the greatest during the whole growth period.

References

Ahmed, S., Nawata, E., Hosokawa, M. *et al*.: Alterations in photosynthesis and some antioxidant enzymatic activities of mungbean subjected to waterlogging. – Plant Sci. **163**: 117-123, 2002.

Araki, H.: Water uptake of soybean (*Glycine max* L. Merr.) during exposure to O_2 deficiency and field level CO_2 concentration in the root zone. – Field Crop Res. **96**: 98-105, 2006.

Belford, R.K., Cannell, R.Q.: Effects of waterlogging of winter-wheat prior to emergence on crop establishment and yield. – J. Sci. Food Agr. **30**: 340-341, 1979.

Belford, R.K., Cannell, R.Q., Thomson, R.J.: Effects of single and multiple waterlogging on the growth and yield of winter wheat on a clay soil. – J. Sci. Food Agr. **36**: 142-156, 1985.

Boru, G., Van Toai, T., Alves, J. *et al*.: Responses of soybean to oxygen deficiency and elevated root-zone carbon dioxide concentration. – Ann. Bot. **91**: 447-453, 2003.

Bradford, K.J.: Effects of soil flooding on leaf gas exchange of tomato plants. – Plant Physiol. **73**: 475-479, 1983.

Cannell, R.Q., Belford, R.K., Gales, K. *et al*.: Effects of waterlogging at different stages of development on the growth and yield of winter wheat. – J. Sci. Food Agr. **31**: 117-132, 1980.

Cannell, R.Q., Belford, R.K., Gales, K. *et al*.: Effects of waterlogging and drought on winter wheat and winter barley grown on clay and a sandy soil. – Plant Soil **80**: 53-66, 1984.

Chen, H., Qualls, R.G., Blank, R.R.: Effect of soil flooding on photosynthesis, carbohydrate partitioning and nutrient uptake in the invasive exotic *Lepidium latifolium*. – Aquatic Bot. **82**: 250-268, 2005.

Dennis, E.S., Dolferus, R., Ellis, M. *et al.*: Molecular strategies for improving waterlogging tolerance in plants. – J. Exp. Bot. **51**: 89-97, 2000.

Drew, M.C.: Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. – Ann. Rev. Plant Physiol. Mol. Biol. **48**: 223-250, 1997.

Flagella, Z., Pastore, D., Campanile, R.G. *et al*.: Photochemical quenching of chlorophyll fluorescence and drought tolerance in different durum wheat (*Triticum durum*) cultivars. – J. Agric. Sci. **122**: 183-192, 1994.

Geisler, G.: Interactive effects of CO_2 and O_2 in soil on root and top growth of barley and peas. – Plant Physiol. **42**: 305-307, 1967.

Glinski, J., Stepniewski, W.: Soil Aeration and its Role for Plants. – CRC Press, Boca Raton 1985.

Huang, B., Johnson, J.W.: Root respiration and carbohydrate status of two wheat genotypes in response to hypoxia. – Ann. Bot. **75**: 427-432, 1995.

Jackson, M.B., Campbell, D.J.: Effect of benzyladenine and gibberellic acid on the responses of tomato plants to anaerobic root environments and to ethylene. – New Phytol. **82**: 331-340, 1979.

Jackson, M.B., Colmer, T.D.: Response and adaptation by plants to flooding stress. – Ann. Bot. **96**: 501-505, 2005.

Jackson, M.B., Drew, M.C.: Effects of flooding on growth and metabolism of herbaceous plants. – In: Kozlowski, T.T. (ed.): Flooding and Plant Growth. Pp. 47-128. Academic Press, New York 1984.

Kozlowski, T. T.: Responses of woody plants to flooding and salinity. – Tree Physiol. **17**: 1-29, 1997.

Kreuzwieser, J., Papadopoulou, E., Rennenberg, H.: Interaction of flooding with carbon metabolism of forest trees. – J. Plant Biol. **6**: 299-306, 2004.

Linkemer, G., Board, J.E., Musgrave, M.E.: Waterlogging effects on growth and yield components in late-planted soybean. – Crop Sci. **38**: 1576-1584, 1998.

Malik, A.I., Colmer, T.D., Lambers, H. *et al*.: Short-term waterlogging has long-term effects on the growth and physiology of wheat. – New Phytol. **153**: 225-236, 2002.

Mielke, M.S., Schaffer, B.: Photosynthetic and growth responses of *Eugenia uniflora* L. seedlings to soil flooding and light intensity. – Environ. Exp. Bot. **68**: 113-121, 2010.

Mielke, M.S., de Almeida, A.A.F., Gomes, F.P. *et al*.: Leaf gas exchange, chlorophyll fluorescence and growth responses of *Genipa americana* seedlings to soil flooding. – Environ. Exp. Bot. **50**: 221-231, 2003.

Mirshel, W., Wenkel, K.O., Schultz, A. *et al*.: Dynamic phenological model for winter rye and winter barley. – Eur. J. Agron. **23**: 123-135, 2005.

Mishra, S., Patro, L., Mohapatra, P., Biswal, B.: Response of senescing rice leaves to flooding stress. – Photosynthetica **46**: 315-317, 2008.

Musgrave, M.E.: Waterlogging effect on yield and photosynthesis in eight winter wheat cultivars. – Crop Sci. **34**: 1314-1318, 1994.

Musgrave, M.E., Ding, N.: Evaluating wheat cultivars for waterlogging tolerance. – Crop Sci. **38**: 90-97, 1998.

Orchard, P.W., Jessop, R.S.: The response of sorghum and sunflower to short-term waterlogging. I. Effects of stage of development and duration of waterlogging on growth and yield. – Plant Soil. **81**: 119-132, 1984.

Palta, J.A., Ganjeali, A., Turner, N.C., Siddique, K.H.M.: Effects of transient subsurface waterlogging on root growth, plant biomass and yield of chickpea. – Agr. Water Manage. **97**: 1469-1476, 2010.

Pezeshki, S.R.: Wetland plant responses to soil flooding. – Environ. Exp. Bot. **46**: 299-312, 2001.

Pociecha, E., Kóscielniak, J., Filek, W.: Effects of root flooding and stage of development on the growth and photosynthesis of field bean (*Vicia faba* L. minor). – Acta Physiol. Plant. **30**: 529-535, 2008.

Setter, T.L., Waters, I.: Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. – Plant Soil **253**: 1-34, 2003.

Shao, G.C., Yu, S.E., Liu, N. *et al*.: [Study on continuous days of water logging and excessive soil water as drainage index of wheat.] – Trans. CSAE **26**: 56-60, 2010. [In Chin.]

Sharma, D., Swarup, A.: Effects of short-term flooding on growth, yield and mineral composition of wheat on sodic soil under field conditions. – Plant Soil **7**: 137-143, 1988.

Sij, J.W., Swanson, C.A.: Effect of petiole anoxia on phloem transport in squash. – Plant Physiol. **51**: 368-371, 1973.

Tan, W., Liu, J., Dai, T. *et al*.: Alterations in photosynthesis and antioxidant enzyme activity in winter wheat subjected to post-anthesis waterlogging. – Photosynthetica **46**: 21-27, 2008.

Waldhoff, D., Furch, B., Junk, W.J.: Fluorescence parameters, chlorophyll concentration and anatomical features as indicators for flood adaptation of an abundant tree species in Central Amazonia: *Symmeria paniculata*. – Environ. Exp. Bot. **48**: 225-235, 2002.

Wollenweber, B., Porter, J.R., Schellberg, J.: Lack of interaction between extreme high-temperature events at vegetative and reproductive growth stages in wheat. – J. Agron. Crop Sci. **189**: 142-150, 2003.

Xie, Z., Jiang, D., Cao, W. *et al*.: Relationships of endogenous plant hormones to accumulation of grain protein and starch in winter wheat under different post-anthesis soil water statuses. – Plant Growth Regul. **41**: 117-127, 2003.

Yordanova, R.Y., Uzunova, A.N., Popova, L.P.: Effects of short-term soil flooding on stomata behavior and leaf gas exchange in barley plants. – Biol. Plant. **49**: 317-319, 2005.

Zhang, H., Turner, N.C., Poole, M.L. *et al*.: Crop production in the high rainfall zones of southern Australia – potential, constraints and opportunities. – Aust. J. Exp. Agr. **46**: 1035-1049, 2006.

Zheng, C., Jiang, D. Liu, F. *et al*.: Effects of salt and waterlogging stresses and their combination on leaf photosynthesis, chloroplast ATP synthesis, and antioxidant capacity in wheat. – Plant Sci. **176**: 575-582, 2009.