Chlorophyll Fluorescence and Yield Responses of Winter Wheat to Waterlogging at Different Growth Stages

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Abstract: The agronomic and physiological effects of waterlogging in winter wheat were examined at four growth stages in the 2011/2012 and 2012/2013 seasons. In both seasons, the greatest yield penalties occurred by waterlogging at the tillering stage (10%–15% decrease), followed by the jointing stage; however, waterlogging at the grain filling stage had less effect on the yield. The lower grain yield caused by waterlogging at the tillering stage was primarily reflected in reductions in spike and grain numbers per m². Waterlogging at the jointing and booting stages reduced grain weight through reduced dry matter translocation. In addition, waterlogging at the tillering stage significantly reduced chlorophyll content and thus photosynthetic capacity, resulting in a lower $F_v/F_m$ ratio, apparent electron transport rate (ETR), effective quantum yield of photosystem II ($\Phi_{PSII}$) and photochemical quenching ($q_P$). However, waterlogging at the grain filling stage improved the leaf photosynthetic capacity and grain yield. We found that the tillering stage was most the susceptible to waterlogging in wheat; therefore, the maintenance of photosynthetic performance after anthesis could be a reasonable strategy for increasing grain yield.

Key words: Agronomic, Grain yield, Physiological, Tillering, Wheat growth stages.

Waterlogging is a global constraint on wheat (Triticum aestivum) production worldwide, particularly in irrigated areas and high rainfall environments (Shao et al., 2013; de San Celedonio et al., 2014). Waterlogging affects approximately 1–15 million ha of wheat globally, representing 15–20% of the 70 million ha of wheat cultivated annually (Setter and Waters, 2003). The effects of waterlogging are most widespread in the rice-wheat rotation regions of south and southeast Asia, including Bangladesh, Pakistan, India, Nepal and China (Samad et al., 2001). In recent years, due to the increase in extreme climate events (Wollenweber et al., 2003; Shao et al., 2013), the occurrence of waterlogging has increased. Approximately one-third of the winter wheat area in southwestern China is devoted to tillage in rice-wheat rotation. This area is frequently saturated with water from the excessive rainfall during the growing season, especially in autumn, and waterlogging often exceeds 4 weeks. This results in poor seeding stand establishment and weak growth. These conditions represent a significant risk for intermittent waterlogging resulting from irregular and unseasonal rainfall.

At the agronomic level, the typical response of waterlogging is; restricted root growth, reduced dry matter accumulation, premature leaf senescence, increased wilting and sterile floret production, and reduced tillering, kernel weight and grain yield (Zhang et al., 2006; Jiang et al., 2008; Hussain et al., 2011; Hussain and Uddin, 2011; Shao et al., 2013). Sairam et al. (2008) and Zheng et al. (2009) reported induced chlorophyll degradation and reduced photosynthesis and chlorophyll fluorescence under waterlogged conditions. Mineral nutrient deficiencies and microelement toxicities at the physiological level were reported by Setter and Waters (2003) and Setter et al. (2009). Waterlogging can reduce winter wheat grain yield by approximately 20 to 50%, depending on several factors, which includes the stage of crop development (Setter and Waters, 2003) and severity and duration of waterlogging (Collaku and Harrison, 2002; Malik et al., 2002). Several studies have shown that the variations in wheat yield were primarily reflected in grain number, with little influence on individual grain weight (González et al., 2005; Peltonen-Sainio et al., 2007). The reduced plant biomass production could be directly associated with limited stomatal conductance and photosynthesis, which reduces carbon assimilation (Mielle et al., 2005). Many studies have also been reported on waterlogging impacts at different growth stages. For
example, Li et al. (2001) concluded that waterlogging treatment during vegetative growth improves tolerance to waterlogging after anthesis in wheat. de San Celedonio et al. (2014) concluded that the greatest yield reduction occurred when the plant was waterlogged during the period from seven leaves on the main stem to anthesis. In their studies, reduction in grain number was primarily reflected in the reduced grain numbers per spike. Saqib (2002) demonstrated that waterlogging for 25 days at the tillering stage did not significantly reduce the grain yield of bread wheat genotypes in Pakistan. However, the largest reductions in grain yield and 1000 grain weight were observed when waterlogging was applied at the stem elongation and grain filling stages. In southwest China, little is known about the critical period of sensitivity to waterlogging in wheat. Additionally the agronomic and physiological changes in response to waterlogging have not been characterized.

The aim of this study was to examine the effects of applied waterlogging at different growth stages, on the agronomic and physiological traits of wheat to identify the most waterlogging-sensitive period of this plant. This study was conducted to help in the development of a more functional approach to simulate wheat responses to waterlogging and its mitigation.

Materials and Methods

1. General conditions, treatments and experimental designs

Field experiments were conducted in Guan County, Sichuan Province, China (104°25′N 30°99′E, 450 m above sea level) over two growing seasons (2011/2012 and 2012/2013). Two popular wheat varieties were used, Neimai836 and Chuanmai104. Neimai836 was bred by Neijiang Academy of Agriculture Science and released in 2008. It was widely used in the hill areas of southwest Sichuan. Chuanmai104, a high yielding variety, was bred by Crop Research Institute of Sichuan Academy of Agriculture Science and released in 2012. The field soil was a clay loam with 43.84 g kg⁻¹ organic matter, 186 mg kg⁻¹ alkali-hydrolyzable N, 9.6 mg kg⁻¹ Olsen-P, and 113 mg kg⁻¹ exchangeable K in the 0–20 cm soil profile. Wheat was sown on 31 October 2011 and 2 November 2012 in plot areas of 13.68 m² (3.6 m wide × 3.8 m long in 18 rows, 20 cm apart) with line socket spacing of 20 cm × 10 cm. Ten seeds per socket in Neimai836 and 7 seeds per socket in Chuanmai104 were sown according to the seed germination rate (Chuanmai104 was 99%, Neimai836 was 76%). Nitrogen basal fertilizer was applied at 135 kg ha⁻¹. Other cultivation and management measures were consistent with the field production.

The experiments were arranged using a split plot design with three replicates, with the main plots as wheat varieties, while the waterlogging treatments were split plots. Four treatments (T1–T4) were used to evaluate the effects of waterlogging (WL) at Feekes’ stages 2 (treatment T1 at the tillering stage, tillering initiated), 4 (T2 at the jointing stage, stem elongation approximately 1 cm), 9 (T3 at the booting stage, heads emerging through flag leaf sheaths) and 10.5 (T4 at the grain filling stage, flowering initiated) after Large (1954) based on physiological indicators (SPAD and chlorophyll fluorescence parameters), growth, and yield from 2011 to 2013. All waterlogged treatments were conducted for 35 days, which was achieved by pumping water onto the plots 2–3 times per day to maintain a 1–2 cm layer of free water on the field surface in the daytime during the entire waterlogging period. The control (CK) was not irrigated.

2. Agronomic and physiological measurements

Two rows of wheat in each plot were tagged for tiller number observations, which were taken at the three-leaf, jointing and maturity stages. The percentage of productive tillers was defined as the number of spikes that developed from tillers expressed as a percentage tiller numbers at the jointing stage. At the end of the waterlogging treatment, leaf area index (LAI) was determined using the formula of leaf area divided by ground area. Plants from three rows in each plot (except those in border rows) were harvested at maturity (early to mid May) for grain yield determination, which was adjusted to 13% moisture. The yield components, spike number per m⁻², grain number per spike, grain number per m⁻², and 1000-kernel weight (TKW), were determined by sampling randomly three sockets from each plot (excluding those in border plants).

The total above-ground dry mass was measured at the end of waterlogging at the tillering, jointing, booting and grain filling stages. Two sockets of plants from each plot were sampled from the third row to minimize the border effect. Total dry matter (DM) was determined after drying at 70°C to a constant weight and by noting the final weight. In addition, DM at anthesis and maturity was also determined. DM remobilized into grain, remobilization efficiency and DM contribution to grain, were calculated using the following formula: DM remobilized into grain = above ground DM at anthesis minus the accumulated non-grain DM at harvest; Remobilization efficiency = DM remobilized into grain / DM at anthesis × 100; and DM contribution to grain weight = DM remobilized into grain / total grain yield × 100.

The greenness of the topmost, fully expanded lamina, was measured in both cultivars during waterlogging and after anthesis, using a non-destructive, hand-held chlorophyll meter (SPAD-502; Konica Minolta Sensing Inc., Osaka, Japan). The SPAD readings were obtained from the one-third and two-third positions of each lamina. Ten lamina were measured in each subplot, and these values were averaged. The chlorophyll fluorescence was

\[ \text{DM remobilized into grain} = \text{above ground DM at anthesis} - \text{accumulated non-grain DM at harvest} \]

\[ \text{Remobilization efficiency} = \text{DM remobilized into grain} / \text{DM at anthesis} \times 100 \]

\[ \text{DM contribution to grain weight} = \text{DM remobilized into grain} / \text{total grain yield} \times 100 \]
also measured in the topmost leaf at the end of waterlogging at different stages and at 15, 25, and 35 days after anthesis (DAA) using a hand-held fluorometer, FluorPen FP100 (Photon Systems Instruments, Czechoslovakian Republic). The chlorophyll fluorescence data were collected on three occasions. At the onset of flowering, five plants in each plot were tagged and successive readings were obtained from the same plant. Prior to each measurement, a clip was placed on the leaf for 30 min for dark adaptation. The weak-modulated irradiance, “actinic light”, and saturating pulses were 0.8, 1200, and 4,000 μmol m⁻² s⁻¹, respectively. The parameters; Fv/Fm, ETR, qP and NPQ, were calculated according to Maxwell and Johnson (2000).

### Table 1. Yield and yield components responses of winter wheat to waterlogging at different growth stages in 2011 / 2012 and 2012 / 2013.

| Year / Cultivar | Waterlogging | Grain yield (kg ha⁻¹) | Spikes number (m⁻²) | Grain number per spike (× 10⁻³ m⁻²) | Grain number (× 10⁴) | 1000-grain weight (g) | Days to jointing | Days to anthesis | Days to maturity | Anthesis to maturity |
|----------------|--------------|-----------------------|--------------------|-------------------------------------|-----------------------|-----------------------|------------------|-----------------|-----------------|---------------------|
| 2011 / 2012    | Neimai836    | CK                    | 8583               | 350                                | 46.7                  | 16.73                 | 48.4             | 73              | 154             | 190                 |
|                |              | T1                    | 7778               | 312                                | 45.9                  | 14.31                 | 50.5             | 69              | 156             | 192                 |
|                |              | T2                    | 8273               | 401                                | 49.5                  | 19.81                 | 43.4             | 73              | 156             | 192                 |
|                |              | T3                    | 8897               | 411                                | 48.3                  | 19.90                 | 43.4             | 73              | 154             | 193                 |
|                |              | T4                    | 8682               | 393                                | 42.8                  | 16.80                 | 49.4             | 73              | 154             | 193                 |
|                | Chuanmai104  | CK                    | 9186               | 434                                | 46.9                  | 20.35                 | 48.7             | 71              | 153             | 192                 |
|                |              | T1                    | 8390               | 350                                | 46.5                  | 16.65                 | 48.9             | 67              | 155             | 196                 |
|                |              | T2                    | 8978               | 461                                | 49.3                  | 22.67                 | 42.5             | 71              | 155             | 196                 |
|                |              | T3                    | 9254               | 434                                | 51.1                  | 22.10                 | 42.7             | 71              | 154             | 196                 |
|                |              | T4                    | 9479               | 469                                | 45.5                  | 21.32                 | 46.5             | 71              | 154             | 196                 |
| 2012 / 2013    | Neimai836    | CK                    | 8597               | 339                                | 53.2                  | 17.98                 | 48.0             | 89              | 140             | 182                 |
|                |              | T1                    | 7526               | 299                                | 58.5                  | 17.45                 | 50.6             | 82              | 143             | 185                 |
|                |              | T2                    | 7974               | 345                                | 55.0                  | 18.99                 | 45.8             | 83              | 140             | 182                 |
|                |              | T3                    | 7998               | 377                                | 48.9                  | 18.39                 | 43.2             | 89              | 140             | 182                 |
|                |              | T4                    | 8091               | 327                                | 50.1                  | 16.38                 | 50.1             | 89              | 140             | 182                 |
|                | Chuanmai104  | CK                    | 9776               | 426                                | 46.3                  | 19.72                 | 50.3             | 87              | 138             | 185                 |
|                |              | T1                    | 8366               | 377                                | 49.3                  | 18.56                 | 50.4             | 80              | 141             | 188                 |
|                |              | T2                    | 8880               | 437                                | 44.7                  | 19.58                 | 48.0             | 85              | 138             | 185                 |
|                |              | T3                    | 8709               | 506                                | 47.2                  | 23.79                 | 41.2             | 87              | 138             | 185                 |
|                |              | T4                    | 10179              | 447                                | 45.5                  | 20.34                 | 50.4             | 87              | 138             | 185                 |

Combined analysis of variance

| Cultivar       | 123.3*       | 3253.0**          | NS   | NS   | NS   | NS   |
|----------------|--------------|-------------------|------|------|------|------|
| Waterlogging   | 9.1*         | 7.94**            | NS   | NS   | 40.4** |     |
| Cultivar × waterlogging | NS | NS | NS | NS | NS | NS |

CK is taken as the control, T1, T2, T3, T4 denote the waterlogging treatments at tillering, jointing, booting, grain filling stage. *and ** denote significance F values at P ≤ 0.05 and 0.01 level, respectively. NS means non-significant at P ≤ 0.05 level.

3. Statistical analysis

The treatment effects were analyzed using analysis of variance (ANOVA) in the SAS statistical analysis package (SAS version 8.0 for Windows, SAS Inc., IL, USA). Treatment means were compared using the least significant difference (LSD) test at P ≤ 0.05, unless specified otherwise.

Results

1. Grain yields and yield components

Significant differences in the grain yields were observed between varieties. The grain yield of Chuanmai104 was
higher than that of Neimai836 across all treatments in both years (Table 1). Waterlogging at the tillering stage showed the greatest effect on grain yield, with a 10–15% reduction compared with the control (CK), followed by jointing, booting and grain filling stages. In 2011/2012, grain yield in treatments T3 and T4 showed a slight increase compared with CK, although not statistically different. The effect of waterlogging on Neimai836 in 2012/2013 was similar to that observed in 2011/2012. Chuanmai104 grain yield was significantly reduced by all waterlogging treatments, except for T4. In both years and varieties, the decrease in the yield in T1 was due to a significant decrease in spike numbers m$^{-2}$ and grain numbers m$^{-2}$, however other yield components remained unaffected.

In both years, the spike numbers m$^{-2}$ and grain numbers m$^{-2}$ in Chuanmai104 were larger than those in Neimai836, reflecting the higher grain yield in Chuanmai104. The spike numbers m$^{-2}$ were significantly reduced in T1 in both cultivars. The 1000-kernel weight in both varieties was significantly reduced in treatments T2 and T3 compared with CK. Waterlogging treatments did not significantly affect the grain number per spike in either cultivar.

The phenological events were significantly affected by waterlogging treatments in both cultivars and seasons. In 2011/2012, days to jointing was 4 days shorter in T1 than...
3. Dry matter at anthesis and maturity and DM remobilization

There was no significant genotype variation ($p > 0.05$) in DM accumulation was observed in either season (Table 4). In 2011/2012, T2 treatment significantly increased DM at anthesis and maturity in Neimai836, while reduced DM accumulation into grain, remobilization efficiency, DM contribution to grain and harvest index compared with CK. DM accumulation at maturity was significantly reduced in T1, T3 and T4 in Neimai836. In Chuanmai104, DM at anthesis and maturity, and the harvest index, were significantly reduced in T1 treatment, while other DM parameters were increased compared with CK. DM remobilized into grain, remobilization efficiency and DM contribution to the grain were decreased in T2 and T4 treatments.

In 2012/2013, both cultivars exhibited similar responses to waterlogging at different growth stages. T1 reduced DM accumulation and remobilization parameters except DM at maturity, but had no effect on harvest index. Treatments T2 and T3 did not affect dry matter parameters compared

### Table 3. The effect of waterlogging on leaf area index (LAI) and dry matter accumulation at different growth stages in 2011/2012 and 2012/2013.

| Year / Cultivar | Treatment | Leaf area index | Dry matter accumulation (g stem$^{-1}$) |
|----------------|-----------|-----------------|----------------------------------------|
|                |           | Tillering | Jointing | Booting | Grain filling | Tillering | Jointing | Booting | Grain filling |
| 2011/2012      | Neimai836 | CK        | 3.42 ± 0.18 | 6.38 ± 0.21 | 4.97 ± 0.20 | 1.94 ± 0.10 | 0.26 ± 0.01 | 0.73 ± 0.05 | 3.70 ± 0.13 | 4.57 ± 0.15 |
|                |           | WL        | 2.95 ± 0.11 | 6.61 ± 0.28 | 5.91 ± 0.19 | 3.57 ± 0.22 | 0.24 ± 0.00 | 0.95 ± 0.05 | 3.37 ± 0.12 | 4.74 ± 0.15 |
|                | Chuanmai104 | CK       | 4.10 ± 0.25 | 6.71 ± 0.32 | 5.33 ± 0.51 | 1.69 ± 0.00 | 0.32 ± 0.01 | 0.91 ± 0.03 | 3.21 ± 0.12 | 4.32 ± 0.17 |
|                |           | WL        | 3.14 ± 0.27 | 7.63 ± 0.29 | 6.50 ± 0.29 | 3.83 ± 0.12 | 0.32 ± 0.01 | 0.97 ± 0.02 | 3.27 ± 0.10 | 4.06 ± 0.19 |
| 2012/2013      | Neimai836 | CK        | 2.13 ± 0.17 | 4.62 ± 0.11 | 4.30 ± 0.11 | 2.21 ± 0.01 | 0.16 ± 0.00 | 0.46 ± 0.01 | 3.56 ± 0.16 | 4.87 ± 0.16 |
|                |           | WL        | 1.55 ± 0.09 | 5.06 ± 0.19 | 4.56 ± 0.18 | 2.63 ± 0.08 | 0.15 ± 0.00 | 0.64 ± 0.02 | 3.16 ± 0.14 | 4.64 ± 0.11 |
|                | Chuanmai104 | CK       | 1.93 ± 0.10 | 3.15 ± 0.15 | 5.38 ± 0.20 | 2.06 ± 0.05 | 0.15 ± 0.00 | 0.45 ± 0.01 | 3.09 ± 0.13 | 3.84 ± 0.10 |
|                |           | WL        | 1.79 ± 0.08 | 3.01 ± 0.20 | 4.13 ± 0.15 | 3.84 ± 0.06 | 0.16 ± 0.01 | 0.74 ± 0.27 | 2.45 ± 0.14 | 3.37 ± 0.09 |

Means ± SD, n = 3. CK is taken as the control, WL denote the waterlogging.
with CK, T4 decreased DM at anthesis in Neimai836, and DM remobilized into grain, remobilization efficiency, and DM contribution to the grain in Chuanmai104.

4. SPAD reading and Chlorophyll fluorescence

(1) SPAD reading

SPAD readings during the 35 days of waterlogging significantly varied with the treatment (T1~T4) in both wheat varieties (Fig. 1). Significant reductions in SPAD readings were observed at 15 days in T1, but at 20 days in T2. Responses to waterlogging were not observed in T3 and T4 had no effect on the SPAD readings until the end of the treatment period (35 days), suggesting that waterlogging during vegetative growth improved leaf SPAD reading attenuation, while waterlogging during reproductive growth delayed leaf senescence.

Changes in SPAD readings after anthesis are shown in Fig. 2, which exhibited a sharp reduction at 15 days after anthesis in both varieties; however, the reduction in T1 and T4 was less pronounced than in T2 and T3. Genotype differences were observed at 35 days after anthesis, at which T1 had the least effect on the SPAD readings in Neimai836, while T4 showed the least effect on the SPAD readings for Chuanmai104.
(2) Chlorophyll fluorescence

Waterlogging at the tillering stage significantly decreased $F_v/F_m$, $\Phi_{PSII}$, ETR and qP, but increased NPQ parameters (Table 5). Differences in the chlorophyll fluorescence parameters between the two varieties were observed at the jointing stage: the chlorophyll fluorescence parameters in Neimai836 were not affected by waterlogging, while the $F_v/F_m$, $\Phi_{PSII}$ and qP were significantly reduced by waterlogging in Chuanmai104. Waterlogging at the grain filling stage increased $F_v/F_m$ and reduced NPQ in both cultivars, while it had no significant effect on $\Phi_{PSII}$, ETR and qP compared with CK.

There were no significant differences in chlorophyll fluorescence parameters ($F_v/F_m$, $\Phi_{PSII}$ and qP) at the early grain filling stage (15 days after anthesis, DAA) among the waterlogging treatments (T1–T4) in either wheat varieties, except for $F_v/F_m$ in Chuanmai104 in T4 (Table 6). At the mid-grain filling stage (25 DAA), a reduction in the $F_v/F_m$ was observed in T4 in comparison with the other waterlogging treatments, in both varieties, but no significant difference was observed in $\Phi_{PSII}$ among waterlogging treatments. Moreover, Neimai836 showed no difference in qP with the waterlogging treatment, while Chuanmai104 was markedly affected by treatments T2, T3 and T4 compared with CK. A significant decrease in the $F_v/F_m$ and $\Phi_{PSII}$ was observed at the late grain filling stage (35 DAA), compared with early and mid-grain filling.

![Fig. 1. The change in leaf SPAD readings of winter wheat during waterlogging treatment at different growth stages in 2012/2013. n = 30. CK denotes the control treatment, T1, T2, T3, and T4 denote the waterlogging treatments at tillering, jointing, booting, and grain filling stages, respectively.](image1)

![Fig. 2. The change in flag leaf SPAD after anthesis in each waterlogging treatment in 2012/2013. Means ± SD, n = 30. Where CK is the control and T1, T2, T3 and T4 denote the waterlogging treatments at tillering, jointing, booting, and grain filling stages, respectively.](image2)

Table 5. The effect of waterlogging on chlorophyll fluorescence parameters ($F_v/F_m$, $\Phi_{PSII}$, ETR, qP and NPQ) at different growth stage in 2012/2013.

| Growth stage | Variety       | Treatment | $F_v/F_m$ | $\Phi_{PSII}$ | ETR       | qP        | NPQ       |
|--------------|---------------|-----------|-----------|---------------|-----------|-----------|-----------|
|              | Neimai836     | CK        | 0.761 ± 0.024 | 0.525 ± 0.007 | 289 ± 12.5 | 1.349 ± 0.044 | 0.583 ± 0.092 |
|              |               | WL        | 0.638 ± 0.010 | 0.395 ± 0.004 | 251 ± 11.7 | 0.989 ± 0.028 | 1.247 ± 0.016 |
|              | Chuanmai104   | CK        | 0.767 ± 0.022 | 0.523 ± 0.002 | 288 ± 12.8 | 1.226 ± 0.043 | 0.607 ± 0.097 |
|              |               | WL        | 0.650 ± 0.032 | 0.381 ± 0.002 | 267 ± 10.0 | 1.023 ± 0.015 | 1.255 ± 0.007 |
|              | Neimai836     | CK        | 0.795 ± 0.009 | 0.625 ± 0.010 | 255 ± 9.9  | 1.023 ± 0.073 | 1.465 ± 0.023 |
|              |               | WL        | 0.791 ± 0.006 | 0.615 ± 0.008 | 255 ± 9.5  | 1.026 ± 0.055 | 1.427 ± 0.037 |
|              | Chuanmai104   | CK        | 0.796 ± 0.013 | 0.661 ± 0.003 | 258 ± 10.3 | 1.051 ± 0.021 | 1.155 ± 0.013 |
|              |               | WL        | 0.767 ± 0.022 | 0.614 ± 0.005 | 253 ± 10.4 | 1.006 ± 0.023 | 1.152 ± 0.022 |
|              | Neimai836     | CK        | 0.773 ± 0.010 | 0.644 ± 0.000 | 250 ± 9.8  | 0.987 ± 0.035 | 1.375 ± 0.009 |
|              |               | WL        | 0.795 ± 0.010 | 0.655 ± 0.012 | 246 ± 8.0  | 0.952 ± 0.011 | 1.191 ± 0.001 |
|              | Chuanmai104   | CK        | 0.789 ± 0.004 | 0.657 ± 0.009 | 251 ± 10.1 | 0.990 ± 0.009 | 1.354 ± 0.039 |
|              |               | WL        | 0.799 ± 0.016 | 0.689 ± 0.005 | 241 ± 9.9  | 0.915 ± 0.019 | 1.181 ± 0.016 |

Means ± SD, n = 15. CK is taken as the control, WL denote the waterlogging.
stages. Furthermore, chlorophyll fluorescence parameters were significantly increased in T1 and T4, (Fv/Fm, ΦPSII and qP) compared with other waterlogging treatments in both varieties.

**Discussion**

Waterlogging occurring at different developmental stages could reduce the final grain yield of winter wheat, and the extent of the yield reduction depends not only on the severity of the waterlogging, but also on the stage of plant development. Several studies examined the critical period of waterlogging on wheat grain yield, suggesting that the reproductive stages was more adversely affected than the vegetative growth stages (Li et al., 2001; Setter and Waters, 2003). However, some studies have demonstrated that the period from the beginning of stem elongation to anthesis were most sensitive to waterlogging, in terms of yield penalties (Shao et al., 2013; de San Celedonio et al., 2014). In the present study, tillering was most susceptible stage, followed by jointing, booting, and grain filling stages (Table 1).

In this area, shorter growth period, earlier tillering stage and longer grain filling stage often results in fewer spikes per m², so higher yield depends largely on more spikes per m² (Tang et al., 2006). Waterlogging at the tillering stage reduced yield as a consequence of reduction in spike number per m² and grain number per m², rather than grain number per spike or the 1000-grain weight. The observed reduction in spike number per m² was due to the inhibition of tiller initiation and increased rate of tiller abortion which is in agreement with Shao et al. (2013). Nevertheless, several other studies have demonstrated that waterlogging at the early phase of growth significantly reduced neither biomass nor yield of wheat because waterlogging occurring early in the crop cycle allowed plants to recover from stress by different mechanisms. This would be related to the waterlogging duration and other factors. The duration of waterlogging in most previous studies were less than 20 days (Cannell et al., 1980; Jiang et al., 2008; Hossain et al., 2011; de San Celedonio et al., 2014), but our study showed that after 35 d of waterlogging at different stages, the wheat was still alive and had some photosynthetic capacity. This was due to the soil texture of the experimental area. In our study, the soil was clay loam in the topmost 20 cm of soil, while in the deeper layer, the soil was sandy with weak water retention, which was good for water infiltration. On the other hand, waterlogging at the seeding stage has long been the major constraint, which resulted in production of waterlogging-tolerant varieties by the breeder. In the present study, waterlogging at the early crop phases (seeding to tillering) significantly reduced biomass at the reproductive stage (Table 4), consistent with the results of previous studies of Malik et al. (2002) and Pang et al. (2004). Waterlogging at the early

| Variety | CK | T1 | T2 | T3 | T4 | CK | T1 | T2 | T3 | T4 |
|---------|----|----|----|----|----|----|----|----|----|----|
| Neimai836 | 0.786 ± 0.010 | 0.781 ± 0.006 | 0.780 ± 0.004 | 0.778 ± 0.002 | 0.773 ± 0.000 | 0.783 ± 0.010 | 0.790 ± 0.015 | 0.787 ± 0.001 | 0.784 ± 0.006 | 0.765 ± 0.010 |
| Chuanmai104 | 0.783 ± 0.006 | 0.782 ± 0.005 | 0.782 ± 0.004 | 0.782 ± 0.004 | 0.782 ± 0.004 | 0.787 ± 0.005 | 0.789 ± 0.006 | 0.787 ± 0.005 | 0.786 ± 0.006 | 0.785 ± 0.006 |

Means ± SD, n = 15. CKs taken as the control, T1, T2, T3, T4 denote the waterlogging treatments at tillering, jointing, booting, and grain filling stages.
phases reduced tiller appearance, leaf area index, wheat growth and dry matter content at the middle or later phases of wheat growth. In wheat, symptoms and injury are initially evident in the roots. When the soil is saturated with water, oxygen deficiency rapidly develops in the roots, causing a sequence of chemical and biochemical reactions, producing components that might be injurious to root metabolism (Palta et al., 2010). In addition, waterlogging at the tillering stage delayed the time of anthesis and maturity, which is consistent with previous studies (Robertson et al., 2009). Since anthesis was delayed by the events at early stages of development, this is likely associated with higher order tiller appearance (i.e., secondary and tertiary tillers). However, the higher-order tillers could not compensate for the early tiller mortality, because the final number of spikes per m² was markedly reduced in this experiment.

Waterlogging at the jointing and booting stages also reduced grain yield, although no significant difference was observed compared with CK. This response was reflected in the reduction in grain weight, which is not consistent with the results of Belford et al. (1985), who reported that waterlogging at the jointing stage reduced yield through decreased grain number per spike. In the present study, the reduction in 1000-grain weight by waterlogging at the jointing stage was attributed to poor accumulation of dry matter per kernel, and reduced the remobilization of stored carbohydrates from stems/leaves to grains, which is consistent with the previous reports of Jiang et al. (2008) and Hossain et al. (2011). Waterlogging at the jointing stage in this study was reflected in reduced SPAD readings and photosynthesis which affected remobilization and grain weight. Therefore, most of the photosynthetic available to complete grain weight was largely derived from carbohydrate accumulated in the stems and its translocation to the grains, since the photosynthetic active leaf area was severely affected (Serrago et al., 2011). However, the reduction in the grain weight suggests that the translocation was not sufficient to fulfill the previously established grains. In this sense, it was shown that reduction in grain growth due to waterlogging at the booting stage was attributable to decreased current assimilation and the poor remobilization of water-soluble carbohydrates (Jiang et al., 2008; Hossain et al., 2011). Moreover, Calderini et al. (2001) demonstrated that reduction in the carpel size determined reduction in the potential grain weight. Thus, waterlogging around anthesis was likely to reduce grain size potential, through reductions in the carpel size and the number of endospermatic cells, resulting in a lighter grain weight. Notably, in the previous treatment (water logging at the booting stage) had an additional adverse effect on the photosynthetically active leaf area.

The anaerobiosis induced by waterlogging could reduce water uptake by roots, resulting in decreased leaf turgor and stomatal conductance, leading to CO₂ deficiency in the leaves. The decrease in plant biomass production in this case could be directly associated with reduced photosynthesis. The restriction of photosynthetic activity has been attributed to stomata closure (Yordanova et al., 2005), a decrease in leaf chlorophyll content (Bradford, 1983), and the disruption of the translocation of photosynthates (Chen et al., 2005). In the present study, senescence, leaf yellowing and a reduction in total chlorophyll content was observed during waterlogging at the vegetative stages (tillering and jointing), but not at all or only slightly during waterlogging at the reproductive stages. This indicates that waterlogging during the early growth phase reduces photosynthetic performance (reduced LAI and SPAD reading), while waterlogging during later phases may improve the performance (increase LAI and SPAD readings), which is consistent with Shao et al. (2013). Thus, waterlogging at the vegetative stages could lead to leaf yellowing, reflecting a reduction in leaf nitrogen (Bacanamwo and Purcell, 1999), N fixation and the production of toxic substances, such as nitrates and sulfides, which move upward from the soil through the roots to the leaves in large quantities (Ezin et al., 2010). In addition, waterlogging at the grain filling stage delayed the full maturity of the plant, indicating that waterlogging at the vegetative stages could lead to leaf yellowing, reflecting a reduction in leaf nitrogen (Bacanamwo and Purcell, 1999), N fixation and the production of toxic substances, such as nitrates and sulfides, which move upward from the soil through the roots to the leaves in large quantities (Ezin et al., 2010). In addition, waterlogging at the grain filling stage delayed the attenuation of the post-anthesis chlorophyll content, improving the translocation of photosynthates to the grain from leaves and stems, prolonging grain filling duration. Therefore, waterlogging at the grain filling stage had a positive effect on grain yield.

Chlorophyll fluorescence has been suggested as a sensitive indicator of stress-induced damage to photosystem II (Maxwell and Johnson, 2000). Fv/Fm was thought to indicate the effects of environmental stress on photosynthesis (Lavinsky et al., 2007; Jing et al., 2009). For example, a reduction in Fv/Fm is a good indicator of photosynthetic impairment resulting from waterlogging stress. Throughout our study, reduction in the Fv/Fm and SPAD values were observed after waterlogging at the tillering and jointing stages, indicating impairment of PSII. Thus, damage to PSII occurred together with reduction in the pigment content and a decrease in ΦPSII after waterlogging. Thus, the use-efficiency of captured photon energy through PSII was reduced. Consistent with the findings of Zheng et al. (2009), a decrease in qP and ETR was also observed during waterlogging at the tillering stage, mainly due to a decrease in the efficiency of excitation energy capture of the open PSII reaction centers (Roháček and Barták, 1999). However, waterlogging at the grain filling stage improved Fv/Fm particularly at 35 days after anthesis, which was likely associated with higher chlorophyll content, indicating that waterlogging at the grain filling stage delayed leaf senescence, prolonged the green leaf stage and improved photosynthetic translocation. Furthermore, a similar result in SPAD values at 35 days after anthesis was
observed for the $F_v$/$F_m$, ϕPSII and qP. Waterlogging at the tillering stage also improved SPAD values and chlorophyll fluorescence parameters at 35 days after anthesis, thus it is reasonable to speculate that waterlogging at the tillering stage inhibited tiller initiation and improved the photosynthetic capacity of the stem leaf, but this higher photosynthetic capacity could not compensate for the decline in grain yield resulting from the reduction in number of spikes per m$^2$.

In summary, the tillering stage was identified as the most susceptible to waterlogging in wheat, followed by the jointing, booting, and grain filling stages. Waterlogging at the tillering stage reduced yield through a reduction in the spike number per m$^2$ and grain numbers per m$^2$, while waterlogging at the jointing and booting stages also reduced grain yield, primarily through reduction in grain weight. Waterlogging at the grain filling stage improved leaf photosynthetic capacity and grain yield, which could be considered a strategy for further increases in grain yield.

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