



Full Length Article

Identification of Growth Stages Sensitive to Waterlogging during Seedling Emergence and Establishment for Winter Oilseed Rape (*Brassica napus*)

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Abstract

Waterlogging often occurs after winter oilseed rape (*Brassica napus* L.) is sown in Yangtze River basin, China, which leads to poor seedling establishment and seed yield penalties. Identification of the growth stage/s sensitive to waterlogging is critical for developing appropriate management strategies and improving the productivity of direct-sown winter oilseed rape. In this study, the impact of waterlogging on the seedling emergence and establishment of winter oilseed rape over a range of initial growth stages was assessed in replicated pot experiments. Waterlogging was imposed for 1, 3 or 5 days at various phases using a completely randomized design (CRD). The results showed that, pre-emergence waterlogging reduced seedling emergence significantly, whereas post-emergence waterlogging for 5 days did not affect seedlings survival although the seedling dry matter production was reduced in most cases. The seriously reduced seedling emergence, dry matter production and the shorter hypocotyl elongation after waterlogging at the radicle growth indicate that this phase was probably most sensitive to waterlogging. Nearly half of the seedlings failed to emerge after waterlogging at the radicle growth for 1 day, and more than 90% did not emerge after waterlogging for up to 3 days. The hypocotyl became longer after waterlogging at cotyledon emergence and expansion, resulting in taller seedlings with low resistance to stress during winter. Therefore, waterlogging from the radicle growth to cotyledon expansion was critical for seedling emergence and growth. The seedlings had a higher compensatory growth in dry matter accumulation after mild waterlogging at cotyledon emergence. For nutrient uptake, N, P and Na decreased but K increased in plants after waterlogging. © 2019 Friends Science Publishers

Keywords: Waterlogging tolerance; Seedling emergence; Young seedling growth; Seedling recovery; Nutrient uptake

Introduction

Waterlogging occurs in many regions worldwide, affects about 10% of the global land area (Setter and Waters, 2003) and reduces crop yields by up to 80% (Shabala, 2011). It will probably become more prevalent in certain areas in the years to come, which may further reduce crop yields (Li *et al.*, 2014). Oilseed rape (*Brassica napus*) is a widely cultivated oil crop in the world and has been proven being susceptible to waterlogging (Boem *et al.*, 1996; Lü *et al.*, 2013; Zhang *et al.*, 2013). It has been documented that 17–42% of seed yield is lost due to the negative impacts of waterlogging on plant growth and yield formation of the crop (Zhou and Lin, 1995; Boem *et al.*, 1996). Yangtze River basin is the largest oilseed rape production region in China as well as in the world, with about 6.67 M ha of winter oilseed rape grown each year, producing 90% of the total rapeseed in China (Yu *et al.*, 2010). Oilseed rape is primarily grown as a break crop from late September to May in single- or double-season rice cropping systems

(Fu *et al.*, 2001) and direct sowing has been recently developed as a major establishment method due to the increasing labor shortage in this region (Li *et al.*, 2016). However, frequent occurrence of rainfall that exceed the water requirement of the crop over this period, combined with heavy soil texture, poor air permeability and shallow ground water table in paddy fields, often result in severe waterlogging during the cropping season especially at its early and late growth stages of oilseed rape (Li *et al.*, 2012; Lü *et al.*, 2013). For instance, over the past 18 years in Wuhan where the experiments were conducted, there were 14.7 rainy days and 3.02 continuous rainy days with the daily rainfall of 8.7 mm on average and the longest continuous raining lasted for 11 days in the period from September 20 to October 31, when winter oilseed rape is sown.

Developing appropriate management strategies to alleviate waterlogging stress depends largely on knowledge about when and how severe waterlogging affects crop growth and development; however, there has been

ambiguous information on these from various studies (Boem *et al.*, 1996). Cannell and Belford (1980) observed that the seed yield of winter oilseed rape was unaffected by waterlogging for 10 to 42 days during the seedling (4.5-leaf) stage. In contrast, Boem *et al.* (1996) found that both aboveground biomass and seed yield of winter oilseed rape were lower when waterlogging was imposed at the vegetative (4-leaf) stage than at the seed filling. Zhou and Lin (1995) also found that more seed yield was lost when oilseed rape was subjected to waterlogging at seedling stage than at the floral bud appearance. However, no significant seed yield loss was found in winter oilseed rape when plants were waterlogged for 30 days at flowering and silique formation (Zhou and Lin, 1995). These suggest that vegetative growth could be more sensitive to waterlogging than reproductive growth in oilseed rape.

Seedling growth is critical for the yield formation of oilseed rape (Li *et al.*, 2016). This can be affected by waterlogging, resulting in weaker seedlings such as fewer leaves, smaller leaf size, shorter roots and eventually lower biomass and seed yields (Boem *et al.*, 1996; Lü *et al.*, 2013). Waterlogging damage to crop growth is also ascribed to the reduced uptake of nutrients in plants (Boem *et al.*, 1996; Milroy *et al.*, 2009; Irfan *et al.*, 2010; Shabala, 2011). Under the hypoxia conditions and lower redox potentials caused by waterlogging in soils, the nutrient uptake by plant and the active transport of nutrients in plants are inhibited (Boem *et al.*, 1996; Barrett-Lennard, 2003). Insufficient nutrient uptake may affect plant metabolism and ultimately reduce the growth of roots and shoots (Zeng *et al.*, 2013).

High crop production requires rapid and high seedling emergence in the field (Bewley, 1997). The seed size of oilseed rape is small, and its germination is more likely to be affected by environmental conditions especially water stress (Liu *et al.*, 2014). The optimum soil moisture for rapeseed germination and young seedling growth ranges 60–70% of fully saturated soil moisture (Hu and Ding, 2008). In practice, however, the soil moisture is often well over this optimum range at the time of sowing, which leads to very low seedling emergence of winter oilseed rape in the Yangtze River basin (Zhang *et al.*, 2008; Li *et al.*, 2012). This indicates that the seed germination and seedling emergence of oilseed rape is likely subjected to waterlogging. Unfortunately, there is little information on the sensitivity of oilseed rape to waterlogging during seed germination and seedling development. The objectives of this study were to investigate the impact of waterlogging on young seedlings of oilseed rape and identify their most sensitive stage/s to waterlogging from sowing to seedling establishment. It is expected that the findings from this study will provide information for developing management techniques to combat waterlogging for direct-sown oilseed rape.

Materials and Methods

Materials and Media

An oilseed rape cultivar, *viz.*, Fengyou 737 commonly sown in the Yangtze River basin, was used as material. Seed was provided by the breeder, Institute of Crop Science, Hunan Academy of Agricultural Sciences, Changsha, China, with germination rate $\geq 93\%$ at 25°C .

Plastic pots (13 cm in upper diameter and 6.5 cm in depth) with 5 holes of 5 mm in diameter at the bottom were used as incubators and a mixture of yellowish-brown sandy loam and sand as culture medium. Two layers of filter paper were laid at the bottom of individual pots to prevent culture medium outflow before filled with 500 g of culture medium. The yellowish-brown sandy loam was taken from an experimental field of Huazhong Agricultural University (HZAU; $30^{\circ}37' \text{N}$, $114^{\circ}21' \text{E}$, 27 m above sea level), Wuhan, China, which contained 5.2% clay, 27.1% silt and 67.7% sand. The sand was taken from Yangtze River bank at Wuhan and then cleaned thoroughly with tap water. The cleaned sand and the soil were air dried, sieved through a 1 mm mesh and then mixed at a ratio of 1: 2. The medium mixture contained 2.2% of water, 0.94% of organic matter, 0.21 g kg^{-1} of total nitrogen (N), 0.73 g kg^{-1} of total phosphorus (P), 7.63 g kg^{-1} of total potassium (K), 44.47 mg kg^{-1} of alkali-hydrolysable N, 34.92 mg kg^{-1} of Olsen-P and $121.62 \text{ mg kg}^{-1}$ of available K with pH of 5.79. The water content at full saturation for the medium mixture was 28.5% on average.

Experimental Design and Treatments

Two experiments were conducted using a completely randomized design (CRD) with five replicates to determine the effects of waterlogging at pre- (Exp. 1) and post-emergence (Exp. 2) on the seedling emergence, survival and growth. In Exp. 1, waterlogging was imposed at 3 phases: seed imbibition (after sowing), radicle growth (radicle length was 2–5 mm; 2 days after sowing) and cotyledon emergence (about 10% of cotyledons emerged; 3 days after sowing). In Exp. 2, waterlogging was imposed at 4 phases: cotyledon expansion (about 60% of cotyledons expanded; 2 days after emergence), 1-leaf (9 days after emergence), 2-leaf (16 days after emergence) and 3-leaf (20 days after emergence) stages.

Each phase included 3 waterlogging durations: 1, 3 and 5 days for both experiments. A treatment with about 65% of fully saturated soil moisture in the culture medium, which is among the optimum soil moisture range for rapeseed germination and young seedling growth (Hu and Ding, 2008), was used as control for each experiment throughout the course of the experiments. Therefore, there were 10 and 13 treatments with 50 and 65 pots in total (replicated 5 times) for Exp. 1 and 2, respectively.

When waterlogging was being imposed, pots with seeds or seedlings were placed into large plastic tanks (10 pots per tank) and tap water was then gradually filled into the tanks to 1–2 mm above the medium surface. During the treatment periods, the water level was closely monitored and maintained on a daily basis. Soil redox potential and temperature at 4 cm depth in the center of each pot were measured using ORP30 redox recorder (Shanghai ZhenMai Instruments Co., Ltd.) and pH using CT6021A pH meter (Shenzhen Kedida Electronics Co., Ltd.). Soil redox potentials were adjusted to at pH 7.0 and 30°C according to the standard of M.E.P.C. (2015).

After treatment, the pots were taken out and drained freely to 65% of the fully saturated soil moisture (28.5%) in culture medium through weighing, which was approximately $65\% \times 28.5\% = 18.5\%$ of soil moisture. From then on, the medium in pots was kept at this soil moisture level by weighing individual pots daily.

Plantation and Management

The experiments were conducted under a transparent rainout shelter at HZAU. Seeds were sown into individual pots on October 17, 2014 for both experiments. Prior to sowing, fertilizer (N, P, K) was applied to the culture medium based on optimum soil nutrient recommendations and chemical analysis of the medium, *i.e.*, 0.1 g N kg⁻¹, 0.05 g P₂O₅ kg⁻¹ and 0.07 g K₂O kg⁻¹ were thoroughly mixed into the culture medium in each pot. After the medium was adjusted to 65% of fully saturated soil moisture, 30 seeds were sown in each pot and then covered with 5 mm of clean sand. Therefore, there were 30 seeds in each pot when waterlogging was imposed in Exp. 1. After the treatments and measurements on seedling counts etc. in Exp. 1, seedlings were thinned to 5 plants per pot at 1-leaf stage to monitor seedling growth, but pots with ≤ 5 seedlings were not thinned.

For Exp. 2, ten uniform and healthy seedlings were kept at the cotyledon expansion and 5 at the 1-leaf stage in each pot before imposition of treatments. Therefore, there were 10 young seedlings in each pot in the subset of cotyledon expansion treatments and 5 in the other subsets of treatments in Exp. 2, when waterlogging was imposed. Outside the treatment periods for both experiments, soil moisture was maintained at approximately 18.5%.

When the seedlings in the control treatments grew to 5-leaf stage on November 30, 2014, all plants/seedlings were harvested to determine the seedling growth for both experiments. The daily average temperature over the experimental period was 14.9°C (9.5–24.5°C) and average midday light intensity was 180.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (89–309 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

Measurements

Seedling emergence was recorded daily until constant number of emerged seedlings for all pots at 12 days after sowing (DAS) and the final emergence rate was calculated

(Zhou *et al.*, 2010) for all treatments in Exp. 1. The seedling survival rate was calculated by counting the survived seedlings after waterlogging treatments in Exp. 2. At harvest, the hypocotyl length, leaf number, the width and length of the 1st and 4th leaf from the bottommost of each seedling were measured. All harvested seedlings including roots were then cleaned with tap water followed with distilled water carefully. The clean seedlings were oven-dried at 70°C until constant dry weight achieved to determine dry biomass.

The dried samples were ground into fine powder using mortar and pestle and 0.2 g of dried sample powder was weighed and digested with concentrated H₂SO₄ (98%, v/v) and HClO₄ (70%, v/v). The digestion solution was used to determine plant N, P, K and Na uptakes. Nitrogen and P were analyzed using an Automatic Discrete Analyzer (SMART CHEM 200, Alliance International Co., Ltd., Frépillon, France) (Han *et al.*, 2015) and K and Na using a flame photometer (FP6410, Shanghai Precise Instrument Co., Ltd., Shanghai, China) (Xu and Yang, 2000).

Statistical Analyses

Data were analyzed using 2-way analysis of variance (ANOVA) with a CRD design and Duncan's multiple comparison test to determine significance amongst treatments at $P = 0.05$. Prior to ANOVA, the data for

emergence rate were transformed as $x' = \arcsin \sqrt{x}$ (x' is the transformed data and x is the observed data), to meet assumptions for ANOVA. All data analyses were performed using IBM S.P.S.S. Statistics 20 (IBM, New York, U.S.A.).

Results

Exp. 1: Effects of Waterlogging Prior to Emergence

Soil redox potential: Redox potentials in waterlogged soils decreased with the stress durations regardless of imposition timings except for waterlogging for 5 days at the cotyledon emergence, but increased in the controls at the pre-emergence. After one day of waterlogging, the redox potential was significantly lower at the radicle growth but slightly higher at the seed imbibition and cotyledon emergence than in the control. When waterlogged stress continued up to 3 days at various phases, soil redox potentials decreased below 350 mV that was significantly ($P < 0.01$ or 0.001) lower than in the control (Fig. 1a–c).

Seedling Emergence

Seedling emergence delayed by imposition of waterlogging at the seed imbibition but not at other phases, compared to the control (Table 1). Seedling emergence rate was significantly ($P < 0.01$) affected by the timing and duration of waterlogging and their interactions prior to seedling emergence (Table 1). Seedling emergence rate significantly

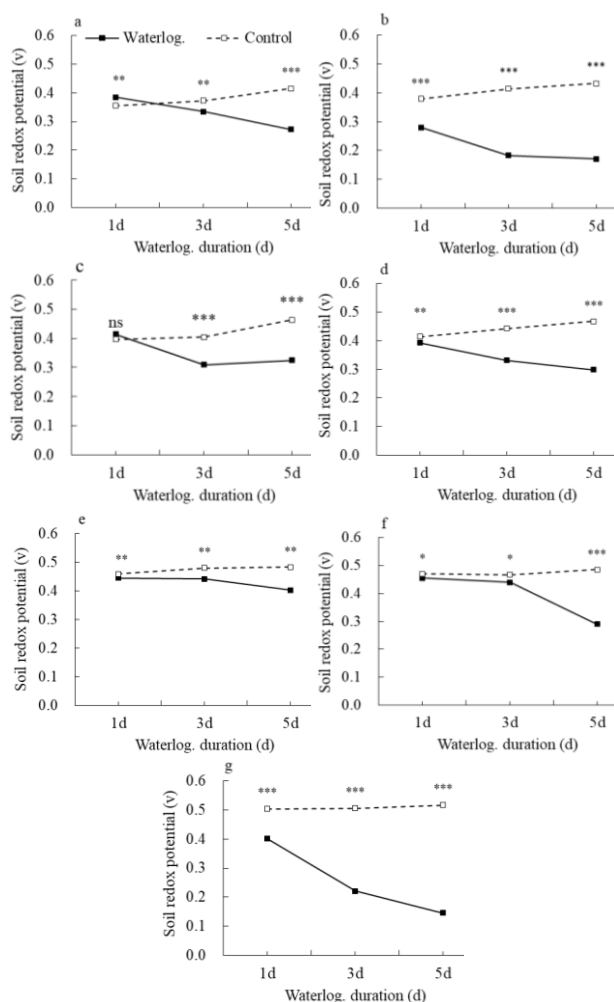


Fig. 1: Soil redox potentials after waterlogging treatment at various phases for different durations: **a**, treatments at seed imbibition; **b**, treatments at radicle growth; **c**, treatments at cotyledon emergence; **d**, treatments at cotyledon expansion; **e**, treatments at 1-leaf stage; **f**, treatments at 2-leaf stage; **g**, treatments at 3-leaf stage. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns - not significant

decreased with the prolonged waterlogging durations at different phases. The most pronounced effect was found in the treatments at the radicle growth. Nearly 50% of the seeds did not emerge after waterlogging for 1 day at this phase and this increased to over 90% with increasing time of waterlogging up to 3 days (Table 1).

Seedling Growth

The hypocotyl length of the emerged seedlings was significantly ($P < 0.01$) influenced by pre-emergence waterlogging timing alone (Table 2). Waterlogging at the seed imbibition did not change the hypocotyl length compared to the control. However, the hypocotyl became significantly shorter than control after

Table 1: Seedling emergence after pre-emergence waterlogging treatment at various phases for different durations. Data are given as means \pm SE. The relative emergence rates of treatments to the control are given in the brackets. Different letters within a column indicate significant differences at $P < 0.05$. ** $P < 0.01$. DAS: days after sowing

Waterlogging phase	Waterlogging duration (days)	Initial emergence time (DAS)	Final emergence rate at 12 DAS (%)
Imbibition	1	5	82.0 \pm 3.8b (87.2)
	3	7	82.0 \pm 3.5b (87.2)
	5	7	78.0 \pm 2.6bc (83.0)
Radicle growth	1	3	50.0 \pm 2.0d (53.2)
	3	3	9.0 \pm 2.5e (9.6)
	5	3	6.0 \pm 1.2e (6.4)
Cotyledon emergence	1	3	76.0 \pm 3.7bc (80.9)
	3	3	74.0 \pm 3.5bc (78.7)
	5	3	72.0 \pm 2.8c (76.6)
Control		3	94.0 \pm 1.2a (100)
ANOVA results			
Source of variation			
Waterlog. timing (T)	-		**
Waterlog. duration (D)	-		**
T \times D	-		**

Table 2: Analysis of variance for hypocotyl length and uptakes of nitrogen (N), phosphorus (P), potassium (K) and sodium (Na) in seedlings after pre-emergence or post-emergence waterlogging. * $P < 0.05$; ** $P < 0.01$; ns - not significant

Source of variation	Pre-emergence waterlogging					Post-emergence waterlogging				
	Hypocotyl length	N	P	K	Na	Hypocotyl length	N	P	K	Na
Waterlog. timing (T)	**	*	**	**	**	**	ns	**	ns	**
Waterlog. duration (D)	ns	**	**	*	**	*	*	**	**	**
T \times D	ns	*	*	*	**	ns	ns	**	ns	**

waterlogging at the radicle growth. Nevertheless, waterlogging up to 3 days at the cotyledon emergence promoted the hypocotyl growth (Fig. 2).

The leaf number per plant, the 1st leaf size and the dry weight of seedlings were all significantly ($P < 0.01$) affected by pre-emergence waterlogging timing, and the seedling dry weight affected ($P < 0.05$) by duration (Table 3). When waterlogging was imposed at the cotyledon emergence, the leaf number per plant, the 1st leaf size and the dry weight of seedlings were not significantly different between the treatments and the control. However, seedling dry weight declined with increasing durations of waterlogging at the seed imbibition and so did that at the radicle growth with a sharper decline (Table 3). The leaf number per plant of the seedlings was less than control either after waterlogging up to 3 days at the seed imbibition or at the radicle growth. Smaller leaf size was only found in the treatments at the radicle growth (Table 3).

Uptake of Mineral Elements by the Seedlings

The uptake of N, P, K and Na by seedlings were significantly ($P < 0.05$ or 0.01) influenced by the timing and duration of pre-emergence waterlogging and their

Table 3: Leaf number per plant, 1st leaf size and seedling dry weight after pre-emergence waterlogging treatment at various phases for different durations. The investigation was performed when the control seedlings grew to 5-leaf stage. Data are given as means \pm SE. The relative values of treatments to the control are given in the brackets. Different letters within a column indicate significant differences at $P < 0.05$. * $P < 0.05$; ** $P < 0.01$; ns - not significant

Waterlog. phase	Waterlog. duration (days)	Leaf no. per plant	Leaf length (cm)	Leaf width (cm)	Dry weight (g plant ⁻¹)
Imbibition	1	4.6 \pm 0.2ab (92.0)	9.2 \pm 0.3a (98.9)	3.5 \pm 0.1a (100)	0.18 \pm 0.01ab (94.7)
	3	4.0 \pm 0.3bc (80.0)	8.7 \pm 0.3b (93.5)	3.4 \pm 0.2a (97.1)	0.14 \pm 0.02bc (73.7)
	5	4.0 \pm 0.3bc (80.0)	8.6 \pm 0.1abc (92.5)	3.5 \pm 0.1a (100)	0.12 \pm 0.01cd (63.2)
Radicle growth	1	4.6 \pm 0.2ab (92.0)	8.2 \pm 0.2bcd (88.2)	3.1 \pm 0.2ab (88.6)	0.13 \pm 0.02c (68.4)
	3	3.6 \pm 0.5c (72.0)	7.8 \pm 0.2cd (83.9)	2.5 \pm 0.1c (71.4)	0.10 \pm 0.03cd (52.6)
	5	3.8 \pm 0.1bc (76.0)	7.4 \pm 0.3d (79.6)	2.9 \pm 0.3bc (82.9)	0.08 \pm 0.00d (42.1)
Cotyledon emergence	1	5.0 \pm 0.0a (100)	9.1 \pm 0.2ab (97.8)	3.4 \pm 0.1a (97.1)	0.21 \pm 0.01a (110.5)
	3	5.0 \pm 0.0a (100)	9.1 \pm 0.4ab (97.8)	3.6 \pm 0.2a (102.9)	0.22 \pm 0.01a (115.8)
	5	5.0 \pm 0.0a (100)	8.4 \pm 0.4abc (90.3)	3.1 \pm 0.1ab (88.6)	0.20 \pm 0.02a (105.3)
Control		5.0 \pm 0.0a (100)	9.3 \pm 0.3a (100)	3.5 \pm 0.2a (100)	0.19 \pm 0.01a (100)

ANOVA results				
Source of variation				
Waterlog. timing (T)	**	**	**	**
Waterlog. duration (D)	ns	ns	ns	*
T \times D	ns	ns	ns	ns

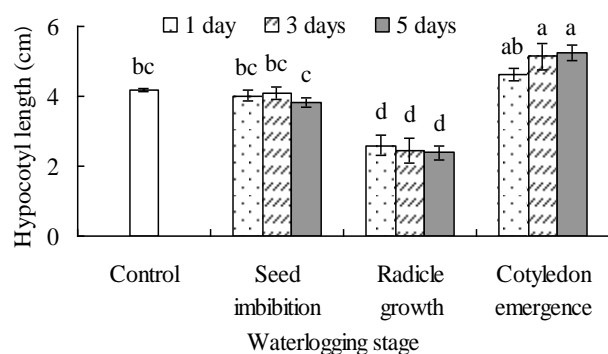


Fig. 2: Hypocotyl length after pre-emergence waterlogging treatment at various phases for different durations. The investigation was performed when the control seedlings grew to 5-leaf stage. Data are means \pm SE. Different letters above SE bars indicate significant differences among treatments at $P < 0.05$

interactions (Table 2). Plant N uptake significantly decreased after waterlogging up to 3 days at the seed imbibition, 1 day at the radicle growth and 5 days at the cotyledon emergence. Plant P uptake was significantly reduced by waterlogging for 5 days at the seed imbibition, up to 3 days at the radicle growth, but did not change at the cotyledon emergence, in comparison

with the control (Fig. 3). Plant N/P ratio decreased along with prolonged waterlogging durations when the stress was imposed at the seed imbibition. When waterlogging was imposed at the radicle growth, plant N/P ratio in all stress treatments was lower than in the control, but it rose slightly with increasing waterlogging durations. Plant N/P ratio was higher in 1 day waterlogging treatment but lower in 3 day treatment than in the control when waterlogging was imposed at the cotyledon emergence (Fig. 3).

Plant K uptake significantly increased in waterlogging treatments for 3 days at the seed imbibition and the cotyledon emergence, but did not change significantly in the other treatments including waterlogging for various days at the radicle growth, in comparison with the control (Fig. 4). Plant Na uptake decreased with waterlogging durations both at the seed imbibition and radicle growth, but maintained at a similar level to the control after waterlogging for various days at the cotyledon emergence. Pre-emergence waterlogging led to higher plant K/Na ratios, compared to the control, regardless of imposition timing and durations. The K/Na ratio kept increasing with the stress durations when waterlogging was imposed at seed imbibition or up to 3 days at the radicle growth (Fig. 4).

Exp. 2: Effects of Waterlogging Post Emergence

Soil Redox Potential

Soil redox potentials decreased in all waterlogging treatments but increased in the control with increasing durations at various post-emergence phases. The treatments of waterlogging for 1 day at the cotyledon expansion, 1- and 2-leaf stages had slightly lower soil redox potentials than the control, although significant ($P < 0.05$ or 0.01) differences were detected. The 3 day waterlogging treatments had similar responses in soil redox potential at 1- and 2-leaf stages. However, the redox potentials were much lower in other waterlogging treatments than in the control (Fig. 1d–g).

Seedling Survival and Growth

All seedlings survived post-emergence waterlogging up to 5 days at various phases (data not shown). The timing and duration of waterlogging had a significant ($P < 0.01$ or 0.05) impact on the hypocotyl length of the seedlings measured when seedlings in the control treatment reached 5-leaf stage, but no significant interactions were detected between them (Table 2). The seedling hypocotyl became significantly longer than the control after being exposed to waterlogging for various days at the cotyledon expansion. However, post-emergence waterlogging at other phases did not significantly change the hypocotyl length, except for the treatment at the 1-leaf stage for 3 days, in which the hypocotyl became shorter than in the control (Fig. 5).

The 4th leaf size and dry weight of the recovered seedlings were significantly ($P < 0.01$) influenced by post-

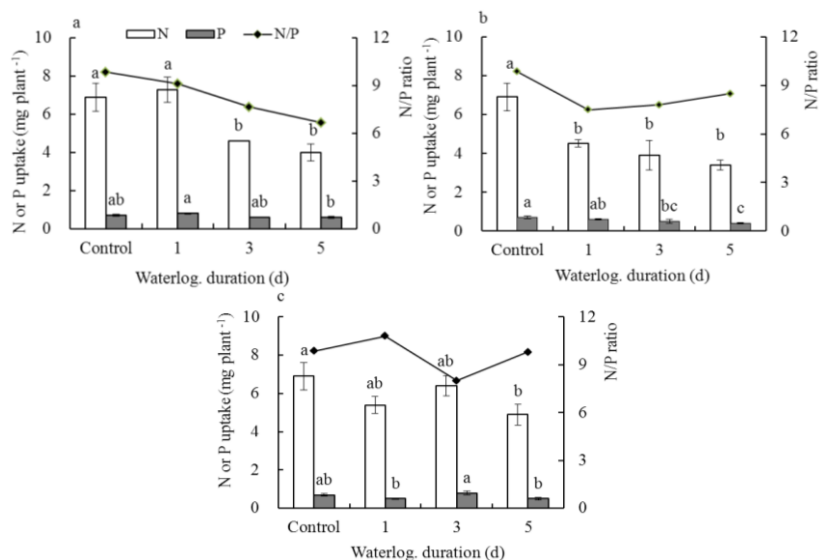


Fig. 3: Plant nitrogen (N) and phosphorus (P) uptakes after pre-emergence waterlogging treatment at various phases for different durations: **a**, treatments at seed imbibition; **b**, treatments at radicle growth; **c**, treatments at cotyledon emergence. The investigation was performed when the control seedlings grew to 5-leaf stage. Data are means \pm SE. Different letters above SE bars indicate significant differences among treatments at $P < 0.05$

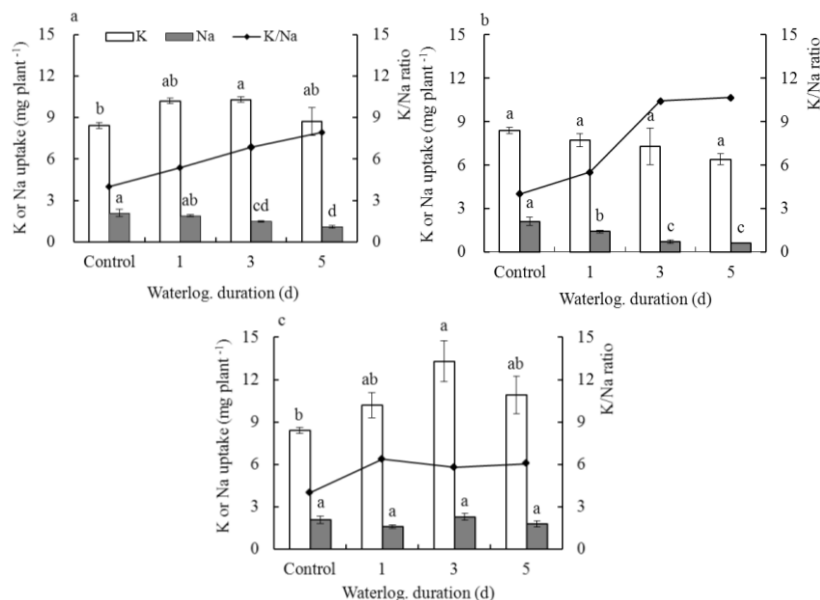


Fig. 4: Plant potassium (K) and sodium (Na) uptakes after pre-emergence waterlogging treatment at various phases for different durations: **a**, treatments at seed imbibition; **b**, treatments at radicle growth; **c**, treatments at cotyledon emergence. The investigation was performed when the control seedlings grew to 5-leaf stage. Data are means \pm SE. Different letters above SE bars indicate significant differences among treatments at $P < 0.05$

emergence waterlogging timing alone but the leaf number per plant was not (Table 4). The length and width of 4th leaves in all waterlogged seedlings were not significantly different from those in the control. However, the 4th leaf length became significantly shorter in waterlogging for 3 and 5 days at the 2-leaf stage than at the 1-leaf stage. The width of 4th leaves was also shorter in the treatments of various days at the 2-leaf and 1 and 3 days

at the 3-leaf stage than in the treatments of 1 and 3 days at the 1-leaf stage (Table 4). The highest dry weight of seedlings was found in the treatment of 3 day waterlogging at the cotyledon expansion, but this was not significantly different from the control. The lowest seedling dry weight was found in the treatments of 5 days at both 1- and 2-leaf stages and 1–5 days of waterlogging at the 3-leaf stage (Table 4).

Table 4: Leaf number per plant, 4th leaf size and seedling dry weight after post-emergence waterlogging at various stages for different durations. The investigation was performed when the control seedlings grew to 5-leaf stage. Data are given as means \pm SE. The relative values of treatments to the control are given in the brackets. Different letters within a column indicate significant differences at $P < 0.05$. ** $P < 0.01$; ns - not significant

Waterlog. phase	Waterlog. duration (days)	Leaf no. per plant	Leaf length (cm)	Leaf width (cm)	Dry weight (g plant ⁻¹)
Cotyledon expansion	1	5.0 \pm 0.0a (100)	11.3 \pm 0.5abcd (100.9)	3.6 \pm 0.1ab (97.3)	0.18 \pm 0.01abc (100)
	3	4.8 \pm 0.2a (96.0)	11.4 \pm 0.3abc (101.8)	3.6 \pm 0.1ab (97.3)	0.19 \pm 0.01a (105.6)
	5	4.6 \pm 0.2a (92.0)	11.0 \pm 0.6abcd (98.2)	3.7 \pm 0.2ab (100)	0.18 \pm 0.01ab (100)
1-leaf	1	5.0 \pm 0.0a (100)	11.8 \pm 0.5a (105.4)	3.9 \pm 0.1a (105.4)	0.16 \pm 0.01bcd (88.9)
	3	4.8 \pm 0.2a (96.0)	11.6 \pm 0.5ab (103.6)	3.9 \pm 0.1a (105.4)	0.16 \pm 0.01bcd (88.9)
	5	4.6 \pm 0.2a (92.0)	11.8 \pm 0.3a (105.4)	3.5 \pm 0.1ab (94.6)	0.14 \pm 0.01d (77.8)
2-leaf	1	4.6 \pm 0.2a (92.0)	10.6 \pm 0.3abcd (94.6)	3.4 \pm 0.1b (91.9)	0.15 \pm 0.01cd (83.3)
	3	4.4 \pm 0.2a (88.0)	10.2 \pm 0.4cd (91.1)	3.3 \pm 0.1b (89.2)	0.16 \pm 0.01bcd (88.9)
	5	4.2 \pm 0.2a (84.0)	9.9 \pm 0.1d (88.4)	3.4 \pm 0.1b (91.9)	0.15 \pm 0.00d (83.3)
3-leaf	1	4.8 \pm 0.2a (96.0)	11.3 \pm 0.1abcd (100.9)	3.4 \pm 0.1b (91.9)	0.15 \pm 0.01d (83.3)
	3	4.8 \pm 0.2a (96.0)	10.7 \pm 0.5abcd (95.5)	3.5 \pm 0.1b (94.6)	0.15 \pm 0.01d (83.3)
	5	4.6 \pm 0.2a (92.0)	10.3 \pm 0.4bcd (92.0)	3.5 \pm 0.2ab (94.6)	0.14 \pm 0.00d (77.8)
Control		5.0 \pm 0.0a (100)	11.2 \pm 0.5abcd (100)	3.7 \pm 0.1ab (100)	0.18 \pm 0.01abc (100)

ANOVA results				
Source of variation				
Waterlog. timing (T)	ns	**	**	**
Waterlog. duration (D)	ns	ns	ns	ns
T \times D	ns	ns	ns	ns

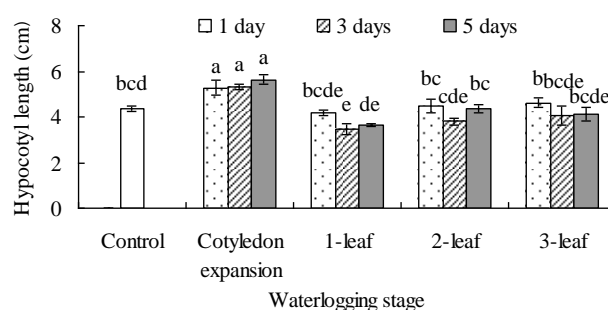


Fig. 5: Hypocotyl length after post-emergence waterlogging treatment at various phases for different durations. The investigation was performed when the control seedlings grew to 5-leaf stage. Data are means \pm SE. Different letters above SE bars indicate significant differences among treatments at $P < 0.05$

Uptake of Mineral Elements

Plant P and Na uptakes were all significantly ($P < 0.01$) influenced by the timing and duration of post-emergence

waterlogging and their interactions, but N and K only by the duration ($P < 0.01$ or 0.05 ; Table 2). Plant N uptake in all waterlogging treatments was similar to the control but lower in 3 and 5 day treatments than in 1 day waterlogging at the cotyledon expansion (Fig. 6). Plant P uptake changed inconsistently amongst the treatments of waterlogging at various phases. When waterlogging was imposed at the cotyledon expansion, it was not significantly different from the control (Fig. 6a); When waterlogging was imposed at the 1-leaf stage, it was lower in 1 and 5 day treatments than in the control (Fig. 6b); When waterlogging was imposed at the 2-leaf stage, it was lower in 1 day waterlogging treatment than in the control (Fig. 6c); When waterlogging was imposed at the 3-leaf stage, plant P decreased in all waterlogging durations compared to in the control (Fig. 6d). Plant N/P ratio showed a trend that it increased initially and then fell with increasing waterlogging durations, but rose slightly again when waterlogging duration was close to 5 days at 1- to 3-leaf stages (Fig. 6).

Post-emergence waterlogging at various phases significantly increased plant K uptake with 1 or 3 day waterlogging treatment being the highest, compared to the control (Fig. 7). Plant Na uptake did not change significantly after waterlogging at the cotyledon expansion (Fig. 7a), but was significantly lower at 1- to 3-leaf stages than in the control (Fig. 7b–d). Plant K/Na ratio increased to a varying degree after post-emergence waterlogging at various phases, compared to the control (Fig. 7).

Discussion

The adverse impact of waterlogging on oilseed rape not only depends on duration but also crop developmental stages of stress occurrence (Boem *et al.*, 1996). In this study, the seedling emergence of oilseed rape declined after seed was waterlogged at various phases prior to seedling emergence. However, the young seedlings survived relatively well under the post-emergence waterlogging. The seedling dry matter production was reduced more in most pre-emergence treatments than in post-emergence, as measured when the seedlings of the control treatment grew to 5-leaf stage. In this regard, the damage to winter oilseed rape is more apparent when waterlogging occurs prior to seedling emergence than post emergence. Among the treatments at pre-emergence, the lowest seedling emergence and the highest dry matter reduction of seedlings were found at the radicle growth, although seedling emergence was delayed at the seed imbibition. This suggests that the most sensitive stage to waterlogging in the early growth/establishment of oilseed rape was probably at radicle growth and irremediable damage could happen to the crop if heavy rainfall events occur about 2 days after sowing. Therefore, severe waterlogging at radicle growth must be strictly avoided for direct-sown winter oilseed rape.

Waterlogging is characterized by low free oxygen (O_2) (hypoxia) in soils, because of the low solubility of oxygen in

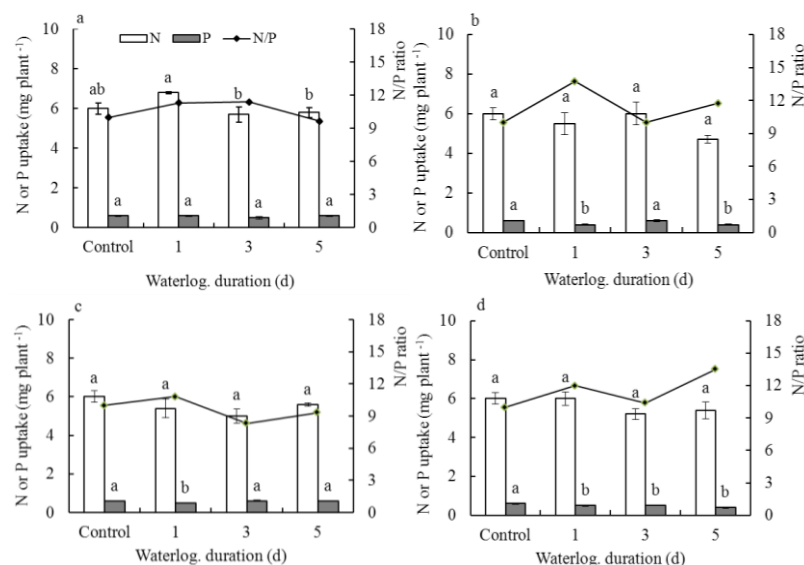


Fig. 6: Plant nitrogen (N) and phosphorus (P) uptakes after post-emergence waterlogging treatment at various phases for different durations: **a**, treatments at cotyledon expansion; **b**, treatments at 1-leaf stage; **c**, treatments at 2-leaf stage; **d**, treatments at 3-leaf stage. The investigation was performed when the control seedlings grew to 5-leaf stage. Data are means \pm SE. Different letters above SE bars indicate significant differences among treatments at $P < 0.05$

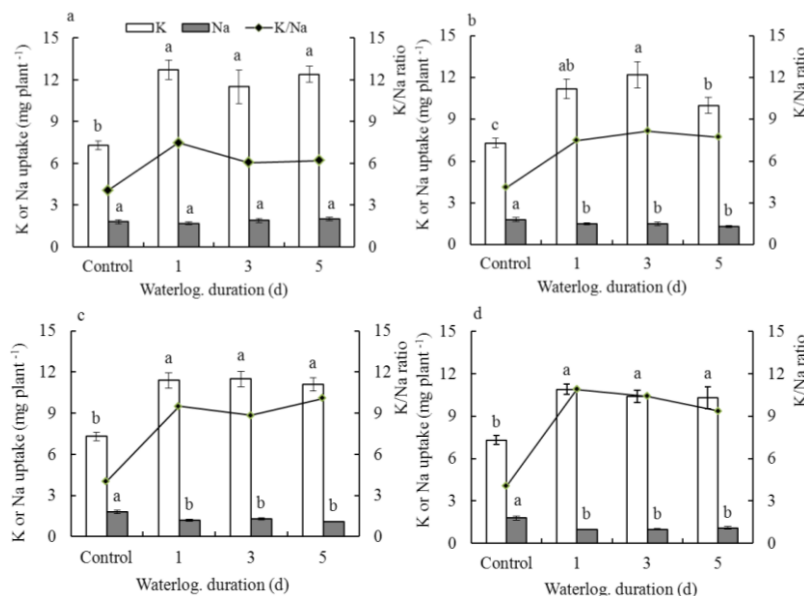


Fig. 7: Plant potassium (K) and sodium (Na) uptakes after post-emergence waterlogging treatment at various phases for different durations: **a**, treatments at cotyledon expansion; **b**, treatments at 1-leaf stage; **c**, treatments at 2-leaf stage; **d**, treatments at 3-leaf stage. The investigation was performed when the control seedlings grew to 5-leaf stage. Data are means \pm SE. Different letters above SE bars indicate significant differences among treatments at $P < 0.05$

water and the rapid use of dissolved oxygen by bacteria and roots (Barrett-Lennard, 2003). The redox potential has been effectively used to assess the aeration status in waterlogged soils (Patrick, 1966) and a value of 350 mV at pH 7 is generally regarded as the threshold that free oxygen disappears in soil (Musgrave, 1994). In the present study, soil redox potentials were higher than 350 mV in all non-

waterlogged controls and 1 day waterlogging treatments at various phases with the only exception at the radicle growth. This indicates sufficient aerations in the well-drained and short-term waterlogged soils. Among the pre-emergence waterlogging treatments, soil redox potentials declined mostly at the radicle growth. During the seed germination of oilseed rape, the oxygen demand sharply increases to the

peak after the radicle breaking through testa because its respiration converts to aerobic metabolism from the anaerobic for the oxidation of rich lipid and protein reserves (Li, 1981). This may lead to more and rapid consumption of free oxygen in soil and consequently severe damage to seedling emergence under waterlogging. After seedling emergence, the above-ground parts of young seedlings can absorb aerial oxygen to meet part of the oxygen demand (Sung *et al.*, 1965), thereby relieve the stress. Soil redox potential and seedling emergence did not change much when waterlogging was experienced from 3 to 5 days at the cotyledon emergence in this study. This may have been attributed to some aerial oxygen that entered into the soil when seedlings broke through the surface. Li (1981) reported that the oxygen demand reduced to a relatively low and stable level after the cotyledon expansion during the germination of oilseed rape, which may lead to less change in soil redox potentials under waterlogging for a short term at 1- and 2-leaf stages in this study. Nevertheless, soil redox potentials declined remarkably with increasing waterlogging durations at the 3-leaf stage. These may have been due to the high oxygen consumption by rapid root growth at this stage. This warrants further investigation.

Leaf number of seedlings was reduced after waterlogging for 3 and 5 days at the seed imbibition in this study, probably because of the delayed seedling emergence by waterlogging at this phase. The significant reduction in the leaf number per plant and leaf sizes at the radicle growth may have been due to the damage to morphological growth by waterlogging at the sensitive stage of oilseed rape. Zhang *et al.* (2008) reported that the hypocotyl length was reduced by waterlogging at seed imbibition. In this study, the significantly shorter hypocotyls were only found at the radicle growth in pre-emergence waterlogging, which may have partially been attributed to the lowest emergence rate at this phase. What causes the difference between the study by Zhang *et al.* (2008) and present study are not known and deserve further investigation. However, the hypocotyl of seedlings became longer after being waterlogged at the cotyledon emergence and expansion, which could have been an adaptive mechanism of the plant to have taller seedlings over waterlogging at these phases. Waterlogging can cause the rapid accumulation of ethylene in soil (Talbot *et al.*, 1987; Barrett-Lennard, 2003). Ethylene is well known to induce short hypocotyls of etiolated seedlings (Hua and Meyerowitz, 1998) but has been shown to stimulate the hypocotyl elongation of *Arabidopsis* in the light (Smalle *et al.*, 1997). Whether there is a development-specific ethylene release or development-dependent ethylene response in oilseed rape under waterlogging remains largely unknown. However, taller seedlings of oilseed rape with long hypocotyl have lower resistance to stress especially at low temperature during the winter (Wang, 2002). Therefore, waterlogging at cotyledon emergence and expansion should be also avoided to support healthy seedlings in winter oilseed rape production.

Waterlogging not only affects the seedling growth of oilseed rape but also seedling recovery (Zhang *et al.*, 2013). In this study, the dry weight reduction of the waterlogged seedlings was found in the treatments for various durations at radicle growth, 3-leaf stages and only for a longer waterlogging period, *i.e.*, up to 3 days at seed imbibition and 5 days at 1- and 2-leaf stages. The more penalties to seedling dry matter accumulation at the radicle growth and 3-leaf stages is in line with the pronounced declines in soil redox potentials under waterlogging. In addition, the dry matter loss in the treatments at the 3-leaf stage was also partially due to the shorter recovery period after waterlogging in this study. The reduction of photoassimilates accumulation in the seedlings after post-emergence waterlogging was probably attributed to stomatal closure, decreased activity of Rubisco, high respiration rate and the blockage of photosynthates transport during the stress (Irfan *et al.*, 2010). However, the effects of pre-emergence waterlogging may have been attributed to the alterations in metabolism because of low O₂ (Boem *et al.*, 1996). In contrast, the seedling dry weight increased after waterlogging at the cotyledon emergence despite of insignificant differences from the control. This was probably due to compensatory growth after stress (Zhou *et al.*, 2011), although it has not been reported yet in oilseed rape. Compensatory growth appeared to be evident for young oilseed rape seedlings that suffered mild waterlogging in this study. However, the compensatory growth capacity varied depending on the developmental phases and waterlogging durations, which is consistent with the previous findings in drought- stressed wheat, maize and rice (Zhou *et al.*, 2011).

Under waterlogging stress, the ATP (Adenosine triphosphate) synthesis is restricted in roots and the toxic organic and inorganic compounds may accumulate in soil under long-term lower redox potentials, which hamper plants to take up nutrients (Barrett-Lennard, 2003; Irfan *et al.*, 2010; Zeng *et al.*, 2013). In this study, plant N uptake after pre-emergence waterlogging and P after both pre- and post-emergence waterlogging were lower than or similar to those in the control, which is generally consistent with the findings of Boem *et al.* (1996), Zhou *et al.* (1997) and Zhang *et al.* (2013). However, plant N uptake was not significantly different from that in the control after waterlogging at post-emergence even though seedling dry matter reduced in some cases. This indicates less impact on N uptake of short-term waterlogging after seedling emergence during the early development of oilseed rape. Plant N/P ratio showed contrasting changes under waterlogging during different periods in this study, *i.e.*, it was lower in most pre-emergence waterlogging but higher in most post-emergence waterlogging treatments, in comparison with in the control. This suggests that N uptake of seedlings was more affected by pre-emergence waterlogging while P uptake more affected by post-emergence waterlogging in oilseed rape.

In the present study, Na uptake in seedlings after waterlogging at both pre- and post-emergence was lower than or similar to controls, which is in general in line with the findings in oilseed rape at the vegetative stage by Boem *et al.* (1996). Contrasting to Na, K uptake by seedlings after waterlogging at post-emergence was higher than control. The similar trend was also shown in pre-emergence waterlogging, except for the cases at the radicle growth, the most sensitive stage. Seedling K/Na ratio increased to various levels in all cases. Our results suggest that seedling K uptake was stimulated but Na inhibited by waterlogging during the early development of oilseed rape. The maintenance of ionic balance is vital for plant growth and development under stresses (Bressan *et al.*, 2008; Lin *et al.*, 2009). Whether the changes in seedling N/P and/or K/Na ratios can be used to assess the waterlogging tolerance at different stages during the early development of oilseed rape remains unclear and needs to be studied further. Moreover, only one cultivar was tested in this study and further studies are necessary to examine more cultivars and their responses to waterlogging in the early development phases.

Conclusion

Pre-emergence seed/seedlings of winter oilseed rape are more sensitive to waterlogging than post-emergence seedlings during the early development of the crop. Pre-emergence waterlogging significantly decreased the seedling emergence rate whereas post-emergence waterlogging up to 5 days did affect the seedlings survival. The seedling emergence and dry matter production decreased most and the hypocotyl elongation declined when waterlogging was imposed at the radicle growth. The seedling emergence was delayed by waterlogging at seed imbibition. The hypocotyl elongation was enhanced by waterlogging at the cotyledon emergence and expansion, which could lead to lower resistance of seedlings to stress during the winter. Waterlogging from the radicle growth to the cotyledon expansion must be avoided in direct-sown winter oilseed rape. Long term post-emergence waterlogging brought about penalties to the dry matter accumulation of seedlings. There was a compensatory growth characteristic in dry matter accumulation of oilseed rape seedlings after mild waterlogging, but the compensatory growth capacity varied with developmental phases. Waterlogging resulted in less N, P and Na but more K uptakes by seedlings during the early development phases in oilseed rape.

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