Evaluating genetic contribution to mitigation of barley grain yield penalty caused by soil waterlogging

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Abstract

In-crop soil waterlogging caused by extreme rainfall events, high ground water tables, excessive irrigation and lateral ground water flow inhibit potential grain yields. However, the extent to which yield is influenced by the timing and duration of waterlogging relative to crop phenology is unknown. To investigate this, we conducted various waterlogging treatments on a range of modern barley genotypes varying in their waterlogging tolerance, with tolerance conferred through aerenchyma formation under oxygen deficit conditions. Results showed that yield was reduced by 35% in W1 (waterlogging at Zadoks stage (ZS) 12.5 for one month) to 52% in WL3 (waterlogging at ZS 15 for two months) due to fewer spikes/m2 and kernels/spike. Two weeks waterlogging at ear emergency stage had the greatest impact on yield (70% reduction) due to its effect on spikelet fertility and grain filling. Phenology was delayed 1-8 ZS at the end of waterlogging treatments, with the waterlogging-susceptible cultivar Franklin showing the greatest delays, and waterlogging tolerant genotypes capable of AF (Macquarie+, TAMF169) having the least delays (0-4 ZS). Genotypes with the AF QTL (Macquarie+) showed a slight and nonsignificant yield reduction compared with unwaterlogged controls and mitigated around 23% yield loss under early phenological waterlogging stress.

1. Introduction

Crop waterlogging is increasingly a global problem due to increased frequencies of extreme climate events (Wollenweber, Porter, & Schellberg, 2003). Globally, excessive water and poor soil drainage constraints adversely affect ˜10% of arable land area (Setter & Waters, 2003), with average annual economic losses caused by crop waterlogging amounting to tens of billions of US dollars from 2004 to 2013 (Hirabayashi et al., 2013). With climate change, more than 10% of agricultural regions will have greater risk of waterlogging due to higher frequencies and greater magnitudes of extreme rainfall events (Chang-Fung-Martel, Harrison, Rawnsley, Smith, & Meinke, 2017; Hirabayashi et al., 2013).

Waterlogging is a 'wicked problem' in that it is highly complex and multi-faceted. In field crop experimental trials, waterlogging driven by excessive rainfall or subsurface or lateral flooding may have poor reproducibility, because waterlogging-prone environments have considerable complexity, including variable dimensions of time, space, biology and chemistry. Thus, methods with which such events are analysed and quantified in a farming systems context requires careful consideration (Harrison, Cullen, & Armstrong, 2017; Harrison, Cullen, & Rawnsley, 2016).

Barley crops (*Hordeum vulgare L.*) are currently cultivated in more than 100 countries for use as animal feed and human consumption (Zhou, 2009). Global barley production has diminished over the last two past decades, decreasing from 155 Mt tons in 2008-9 to 142 Mt in 2017-18 (Statista, 2020). Part of this decline is due to increased frequency of waterlogging and susceptibility of barley to waterlogging stress damage

(Setter & Waters, 2003). In many contexts, improving crop tolerance to minor waterlogging is generally cost effective, however under severe waterlogging, combined agronomic, engineering and genetic solutions are more effective (Manik et al., 2019).

Defined physiologically, waterlogging tolerance is the survival or maintenance of growth under waterlogging relative to non-waterlogged conditions (Gibbs & Greenway, 2003; van der Moezel, Pearce-Pinto, & Bell, 1991). Oxygen deficiency in soil pores caused by waterlogging reduces root growth, leading to premature leaf senescence and tillering, inhibition of dry matter accumulation and production of sterile florets. In combination, such effects stun kernel number and weight, ultimately penalising potential grain yield (de San Celedonio, Abeledo, Brihet, & Miralles, 2016; de San Celedonio, Abeledo, & Miralles, 2014, 2018; Masoni, Pampana, & Arduini, 2016).

Defined agronomically, waterlogging tolerance is less grain loss relative to susceptible control genotype (Setter & Waters, 2003). Past studies have measured yield declines of 40–79% in waterlogged barley, depending on genotype, growth stage, soil type and duration of waterlogging (de San Celedonio et al., 2014). Yield loss in barley is also likely to be sensitive to the phenological stage with which waterlogging occurs (de San Celedonio et al., 2014). One of the few reports that examined the relationship between yield loss and phenological stage reported that barley was most susceptible during grain filling, moderately susceptible during tillering and least susceptible during seedling stage (Setter & Waters, 2003). However, there are few reports confirming this observation. It is also likely that post-waterlogging growth recovery is a function of genotype of environmental interactions, analogous to crop recovery following defoliation (Harrison, Evans, Dove, & Moore, 2011a, 2011b).

Waterlogging tolerance is likely to be a complex trait related to many morphological and physiological traits that are under strong environmental influence (Zhou, Li, & Mendham, 2007). Lack of oxygen causes roots to shift energy metabolism from an aerobic to anaerobic mode, resulting in cellular energy crises (Gibbs & Greenway, 2003). Apart from tolerance to secondary metabolic compounds associated with anaerobic soil conditions (Huang et al., 2015; Pang et al., 2007), tolerant genotypes of barley may adapt to transient waterlogging via development of morphological mechanisms allowing plants to cope with the stress (Herzog, Striker, Colmer, & Pedersen, 2016; Hossain, Araki, & Takahashi, 2011; Kreuzwieser & Rennenberg, 2014). Morphological adaptations include development of adventitious roots with well-formed aerenchyma (Pang, Zhou, Mendham, & Shabala, 2004; Zhang et al., 2015). An aerenchyma is a continuous gas filled channel that occurs under flooded or hypoxic conditions. This enhances internal diffusion of atmospheric and photosynthetic oxygen from the aerial parts to the flooded roots, allowing roots to maintain aerobic respiration (Armstrong, 1979). Waterlogging tolerant barley genotypes such as the wild barley TAM407227 show not only higher adventitious root porosity than sensitive barley genotypes (e.g. Franklin, Naso Nijo), but also have faster development of aerenchyma (Zhang et al., 2015) under waterlogging conditions. Metabolically, tolerance mechanisms in barley include enhanced activities of glycolytic and fermentative enzymes that increase availability of soluble sugars, and involvement of antioxidant defence mechanisms (e.g. superoxide radicals, hydroxyl radicals and hydrogen peroxide) against post-stress oxidative damages under anaerobic conditions (Armstrong, Brandle, & Jackson, 1994; Davies, 1980; Drew, 1997; Mittler, Vanderauwera, Gollery, & Van Breusegem, 2004; Pan et al., 2019; Setter et al., 1997). Contemporary crop breeders are targeting genetic tolerance mechanisms including aerenchyma formation using molecular marker assisted selection. In barley, a major QTL for AF under waterlogging conditions was identified from several waterlogging tolerance genotypes (Broughton et al., 2015; Zhang et al., 2017; Zhang et al., 2016). This QTL was located in the same position as a QTL for waterlogging tolerance on chromosome 4H (Li, Vaillancourt, Mendham, & Zhou, 2008; Zhang et al., 2017; Zhou, 2010; Zhou, Johnson, Zhou, Li, & Lance, 2012). However, allelic differences exist in different parents, with the contribution of AF to field waterlogging tolerance ranging from around 5% to over 80%. A prospective allele from a wild barley identified in past work (Zhang et al., 2016) has been introgressed to a commercial variety, Macquarie, and this new line, Macquarie+, will be used in this study.

In this study, we imposed four waterlogging treatments on six barley genotypes differing in waterlogging tolerance; two genotypes (Macquarie+ and TAMF169) had the allele for AF under waterlogging stress from the wild barley. The objectives of this study were to examine (1) the impact of timing and period of waterlogging on grain yield and yield components, and (2) the contribution of the QTL for AF under waterlogging stress to mitigate yield loss.

2. Materials and methods

Two experiments with six barley genotypes were conducted at Mt Pleasant Laboratories (41°28'S, 147°08'E) in Launceston, Tasmania during 2019. Genotypes included four commercial varieties: Macquarie, Franklin, Planet, Westminster, a backcross line, Macquarie+ (Macquarie/TAM407227//Macquarie), and a double haploid line from the cross of TAM407227/Franklin, TAMF169 (Table 1).

For experiment 1, seeds were sown in six rows in stainless steel tanks (200 cm x·100 cm x 45 cm) filled with sandy loam soil with and bottom of each tank contained 50 mm coarse gravel overlaid with drainage matting. Each row was sown with 30 seeds on 30 May 2019. Each of the three blocks were randomised with one plot of each treatment (three plots per block) three replications. Plants were fertilised with 24 kg/ha of YaraMila Complex (12%N:11%P_2O_5:18% K_2O, Yara Company). During the growth periods, all treatments were topdressed with equal amounts of 50 kg/ha of YaraMila Complex at jointing (GS32) and booting (GS45), respectively. No signs of nutrient deficiencies were observed.

Experiment 2 was conducted in a field screening facility with a waterlogging controlling system. Each genotype was sown in 1.2 m x 2 m plots with a 1.2 m row spacing of 20 cm and 30 seeds per row. The controls were sown in well drained beds. Four replicates were applied for both waterlogging treatment and controls. The trial was sown on 28th April 2019. The waterlogging treatment began at Zadoks stage 12.5 and continued for two months. All treatments were topdressed with equal amounts of 50 kg/ha of YaraMila Complex at jointing (GS32) and booting (GS45), respectively.

2.1 Waterlogging treatments

For experiment 1, a water tray was used to supply water to the bottom of each tank (Fig. 1). The water level of each container was maintained at 75 mm depth by fitting a float valve to a reservoir. Excess water from rainfall flowed back to the reservoir and out an overflow. Any water lost from the plant containers through evapotranspiration that reduced the water level below 75 mm was resupplied by the reservoir to maintain the water level. Control plots were watered near to field capacity until grain filling. Waterlogging was achieved by raising the reservoir above the soil surface such that the water level increased to 400 mm and the soil was completely saturated (lower panel in Fig. 1).

Waterlogging treatments for barley genotypes used in experiment 1 are shown in Table 2. Waterlogging treatment WL2 and a non-waterlogged control were conducted in experiment 2. For both experiments, the leaf number at which waterlogging was applied was measured on the main stem. After each waterlogging treatment concluded, treated plots were watered near to field capacity until grain filling, after which watering was ceased. Weed control was performed from emergence to harvesting by hand hoeing. No incidence of pest or disease infection were observed in either experiment.

2.2 Measurements

Phenology

Crop phenology was measured every two weeks following the decimal code of (Zadoks, Chang, & Konzak, 1974).

Biomass

For each treatment, three plants were selected for biomass measurement before and after waterlogging treatments. At maturity, total above-ground biomass was harvested from three plants in each pot and separated into stem, leaf and panicle. Samples were then oven dried at 65°C for at least 48 hrs until constant weight.

Grain yield and yield components

At maturity, plants in the middle three rows of each tank (90 plants per treatment) and plot in the field were selected for determination of grain yield and yield components. Spike number was enumerated in each plot and recorded prior to harvest. All spikes were manually harvested, threshed and weighed to calculate grain yield. One thousand random kernels from each harvested grain were weighed to calculate a 1000-kernel weight. Grain moisture was measured using a Grain Analyser (InfratecTM 1241, Foss, Denmark). Grain yield and 1000-kernel weight were adjusted to 13% moisture. The average number of kernels per spike was enumerated from 30 spikes.

Grain size parameters, including 1000 kernel weight, grain length, width and thickness were measured using SeedCount SC5000 (Next Instruments, Condell Park, NSW, Australia) and a digital balance.

2.3. Statistical analysis

Statistical differences between treatments were tested using analysis of variance (ANOVA) with SAS9.2 (SAS Institute Inc., Cary, NC, United States). Mean treatment values were compared using least significant differences (LSD), assuming a significance level of 0.05.

3. Results

3.1 Grain yield

In experiment 1, across genotypes, the average grain yield reduction for WL1, WL2, WL3 and WL4 was 35%, 46%, 52% and 70%, respectively (Fig. 2a). Yield loss in the waterlogging-susceptible variety Franklin was 47% in WL2; this genotype died completely in WL3. Yield loss in the waterlogging tolerant genotype (Macquarie+) was 17% and 21% in WL1 and WL3, respectively, which was lower than other genotypes. In experiment 2, the average grain yield reduction for WL2 was 25-59% (Fig. 2b). Franklin and Planet showed the greatest yield reduction, while TAMF169 and Macquarie+ were the least reduced by waterlogging. Yield loss in Macquarie+ was 17-21% in comparison with 43-52% in Macquarie under continuous one or two-month waterlogging conditions in experiment 1, and was 18% (Macquarie+) vs 38% (Macquarie) in experiment 2.

3.2 Yield components

Spikes per m^2 and kernels per spike were reduced by waterlogging treatments (Fig. 3). WL1-3 reduced spikelet per m^2. No death of tillers was recorded for WL4, as this treatment was applied after ear emergence (spikelets per m^2 were not affected). WL2 caused the highest spike number reduction across genotypes (average decline of 37%). All treatments reduced kernels per spike except WL1 and WL2 for Westminster. WL4 caused the greatest reduction in kernels per spike for all genotypes (except TAMF169) by increasing numbers of infertile spikelets. Waterlogging that occurred relatively early in crop phenology (WL1, WL2, WL3) led to an increase in 1000-kernel weight of Franklin, Macquarie+ and Westminster. In contrast, waterlogging in later crop development stages (WL4) reduced 1000-kernel weight by more than 50%. These results indicated that yield penalty was primarily associated with either (1) plant survival and reduced tillering when waterlogging was applied at early growth stages or (2) with reduced spikelet fertility and grain filling when waterlogging was applied at ear emergence. An extreme example is that none of the Franklin plant survived under WL3 thus led to 100% yield reduction.

To better understand how waterlogging affected 1000 kernel weight, further measurements were conducted on grain size. Waterlogging reduced grain length for all barley genotypes except for Franklin, where grain length increased by 5% in WL1 and 10% in WL2 (Fig. 4). In contrast with grain length, all waterlogging treatments except WL4 increased grain width and grain thickness. WL4 reduced grain width across genotypes, with an average reduction of 20%. Similarly, grain thickness in Planet, Macquarie, Macquarie+ and TamF169 was reduced, decreasing by 5%, 11%, 13% and 14%, respectively, compared with the controls.

3.3 Total above-ground biomass

Franklin had the greatest capacity to recover from WL1; relative to control biomass of Franklin after waterlogging was 56% (Fig. 5a) but relative to control biomass at harvest was 79% (Fig. 5b). Westminster had the greatest capacity to recover from WL2; relative to control biomass of Westminster after waterlogging was

29% (Fig. 5a) and relative to control biomass at harvest was 78%. Macquarie+ had the greatest capacity to recover from WL3; There was no significant effect on above-ground biomass excluding grains of WL4. At harvest, apart from Macquarie+, W3 showed much great effect on the biomass with all plant Franklin failing to survive.

Across genotypes, the average biomass reduction at maturity for WL1, WL2, WL3 and WL4 was 28%, 41%, 52% and 55% respectively (Fig. S1). Generally, the greatest biomass reduction at maturity caused by waterlogging was in treatment WL4, with biomass reductions ranging from 50 to 68%. The main effect of waterlogging on above-ground biomass was on dry spike weight and to a lesser extent dry stem and leaf weight, particularly WL4. Franklin did not recover from WL3 (WL4 was not conducted on Franklin and Westminster).

3.4 Effects of waterlogging stress on phenology

At the end of each waterlogging treatment, phenology was delayed (Fig. 6a). Phenology was delayed to the greatest extent in the WL2 treatment and the least in WL3. WL4 began after ear emergence thus had no effect on phenology. Franklin was delayed the most by waterlogging, while TAMF169 and Macquarie+ were the least delayed. Maturity dates were delayed by 8-15 days by waterlogging at early growth stages across genotypes (Fig. 6b). In contrast, WL4 resulted in premature, and maturity dates were 5 - 8 days earlier in WL4 compared with controls.

4. Discussion

The purpose of this study was to examine the physiological effects of waterlogging at different phases of phenology, and the mechanisms and extent to which these physiological effects influenced yield. The contribution of AF to mitigation of yield reduction under waterlogging and ability to recover from waterlogging stress are also discussed.

4.1 Effects of waterlogging on phenology and implications for yield

Waterlogging caused transient reductions in biomass accumulation, but the final impact on grain yield depended on the capacity of the plant to recover after waterlogging and before maturity (Romina, Abeledo, Mantese, & Miralles, 2017). In this study, Franklin showed a better ability to recovery from short-term waterlogging treatment (WL1) compared with other barley genotypes. WL1 caused a 26% yield loss in Franklin compared with around 40% in the other sensitive genotypes. This is because Franklin has a longer growth duration, thus a longer period of shoot biomass recovery. It is worth mentioning that this capacity to recover decreased the later waterlogging was imposed in the phenological cycle. Previous studies have shown that the capacity of barley to recover shoot biomass after waterlogging is related to genotypic and environmental propensity to produce new tillers (de San Celedonio et al., 2016; Robertson, Zhang, Palta, Colmer, & Turner, 2009). Thus, when barley plants are waterlogged late in their lifecycle (e.g. beginning of stem elongation), they are not able to produce new tillers and compensate for the lost shoot biomass caused by waterlogging (Romina et al., 2017).

Waterlogging treatments (WL1-3) delayed maturity (Fig. 6b) across genotypes, with WL3 having the greatest effect on phenology. As well, WL3 had the greatest effect on biomass, suggesting that imposition of waterlogging later in the crop lifecycle has the greatest implications for yield. Such yield penalisation can occur either via reductions in biomass accumulation (WL3) or in yield components if waterlogging is imposed very late in the crop lifecycle (WL4).

In barley, flowering date is primarily a function of temperature, photoperiod and vernalisation (Liu et al., 2020). The rate of leaf emergence and final leaf number determine the duration of the period between emergence and anthesis (Alzueta, Abeledo, Mignone, & Miralles, 2012). Here, waterlogging at early growth stages (WL1-3) inhibited leaf appearance rate and reduced final leaf number, delaying maturity date. This is because oxygen deficiency predisposes to denitrification with the consequent rapid loss of nitrate in waterlogged soils. Since nitrate is essential for physiological function, plants' growth and appearance are quickly affected. In this study, all barley genotype leaves started to become yellow 5 days after being waterlogged, and leaf yellowing area increased with waterlogging durations. The early yellowing of basal leaves during waterlogging coincides with lower photosynthetic rate (Hossain et al., 2011) and water-soluble carbohydrates, and this could be the possible reason that shoot growth was reduced in this study. The phenology of two waterlogging-tolerant genotypes, TAMF169 and Macquarie+, were the least delayed by waterlogging, indicating that the ability to avoid phenological delay may be regarded as a criterion to evaluate waterlogging tolerance. There is little information available in the literature to support this claim. As such, we call for further work on the relationships between waterlogging tolerance and the impact of waterlogging on phenology.

4.2 Relationship between yield and yield components

We found that grain yield in barley was reduced by waterlogging regardless of genotype or treatment imposed (Fig. 2a, b). Yield reductions were mainly caused by reduced spikes per $m^2$when waterlogging occurred in early phenology. WL1-3 had similar effects on grain yield and yield components, with reduction in spikes per m^2. This is likely to be due to the growth stage when waterlogging treatments were imposed. WL1-3 were applied prior to/at tillering stages (ZS12.5 and ZS15); all three treatments caused reductions in tiller numbers (data not shown), such that spike numbers were reduced at the end of waterlogging for all treatments except WL4. The final number of fertile spikes at maturity depends on tiller appearance rate (Alzueta et al., 2012) and the percentage of tiller mortality (Baethgen, Christianson, & Lamothe, 1995; García del Moral & García del Moral, 1995). Thus, reduced spike number in WL1-3 was mainly a consequence of lower tillering under waterlogging similar to that seen with nutrient deficiency (Alzueta et al., 2012) and water deficits (Cossani, Slafer, & Savin, 2009).

Fewer kernels per spike under waterlogging was a function of reduced spike length (Arisnabarreta & Miralles, 2006; García del Moral & García del Moral, 1995). Westminster was the only barley genotype that did not show a reduction in kernels per spike under waterlogging treatments WL1-3. This may be because Westminster has relatively fewer grain numbers per spike than other genotypes under control conditions (Fig. 3). In contrast to spikes per m^2 and kernels per spike, waterlogging induced a higher grain weight compared with controls in Franklin, Westminster and Macquarie+. The increase in grain weight under waterlogging was attributed to increased grain length in Franklin, and grain width and thickness for Westminster and Macquarie+ (Fig. 4). Fewer kernel numbers per spike induced by waterlogging could result in more assimilate for grain growth and kernel weight, thus compensating for the detrimental effects of waterlogging on other yield components to some degree. Similar effects have been observed for wheat defoliated in vegetative stages in which more assimilate is partitioned to kernels of grazed crops (Harrison et al., 2011a, 2011b).

4.3 Physical mechanisms of grain yield penalty caused by WL4

We found that the greatest reductions in grain yield occurred when waterlogging treatment was applied close to heading, even though the duration of waterlogging was very short (WL4; Fig. 2). This finding is in line with previous results (de San Celedonio et al., 2014; Setter & Waters, 2003). For WL4, lower grain yield was attributed lower grain weight and to a lesser extent, lower kernels per spike. In this treatment, waterlogging caused premature leaf senescence in waterlogging sensitive genotypes. It has been reported that reduced leaf greenness coincides with lower stomal conductance (Araki, Hamada, Hossain, & Takahashi, 2012), photosynthetic rate (Hossain et al., 2011) and water-soluble carbohydrates (Araki et al., 2012). Assimilate from photosynthesis and remobilization of culm residual water-soluble carbohydrates reserves are important for grain filling in crops (Kamran et al., 2020; Schnyder, 1993). Therefore, reduced carbon assimilation rates and lower remobilization of culm reserves in waterlogged plants may have resulted in lower grain growth rate during the grain-filling period of WL4.

4.4 Genetic understanding of waterlogging tolerance

Our results showed that most of currently available Australia barley genotypes are intolerant to waterlogging. It is thus crucial that further scientific endeavour is undertaken to develop more waterlogging tolerant genotypes that alleviate yield losses caused by waterlogging.

Our previous studies have identified QTL controlling root AF under waterlogging stress, which is one of the major mechanisms for waterlogging tolerance in barley (Zhang et al., 2016). This gene was introgessed into a commercial variety Macquarie through repeated backcrossing. Although we did not measure AF (e.g. scored the proportion of aerenchyma based on digital images), because this has been done in many previous studies (Zhang et al., 2015; Zhang et al., 2016). Our results showed that Macquarie+ was the most tolerant to waterlogging (Fig. 2a, b); this result was mainly a consequence of higher numbers of spikes/m^2 and to a lesser extent maintenance of grain weight under waterlogging (Fig. 3). Macquarie+ outperformed other varieties in most case and for most waterlogging treatments. The QTL for AF mitigated around 23% yield loss under waterlogging stress, suggesting that the QTL is effective in improving waterlogging tolerance of commercial varieties and can be used in breeding programs.

5. Conclusions

Here we examined the physiological impacts of waterlogging on susceptible and tolerant waterlogging barley varieties. We also examined how the timing of waterlogging relative to phenology physiologically impacted on yield. Our analysis suggests that waterlogging close to heading is the most susceptible period, with yield losses primarily attributed to reductions in spikelet fertility and grain weight. Yield loss caused by waterlogging at earlier growth stages was mainly a consequence of reduced spike number and to a lesser extent kernels per spike. With regards to waterlogging tolerance, we found that the phenologies of waterlogging tolerant genotypes were less delayed compared with controls, and AF helps mitigate yield losses under waterlogging.

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Author contributions

M.Z., H.M.T and X.T conceived and designed the research. K.L., I.A., M.S.M.N., and J.P. conducted the experiments and collected the data. K.L., H.M.T and M.Z. analysed the data and wrote the paper.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Table 1 . Barley cultivars used in the study, year of release, pedigree and commercialising organisation.

Genotype	**Pedigree**	**Source**
Macquarie	Alexis/Gairdner//Gairdner	Commercial variety released by the University of Tasmania
Macquarie+	Macquarie/TAM407227//Macquarie[3]	A backcross lines with the background of Macquarie and the waterlo
Planet	Tamtam/Concerto	A commercial variety released by Seed Force Pty Ltd
Franklin	Shannon/Triumph	A commercial variety released by the University of Tasmania
Westminster	NSL97-5547/Barke	A commercial variety released by GrainSearch
TamF169	TAM407227/Franklin	A DH line from the cross between TAM407227 and Franklin, by the

Table 2 . Waterlogging time and durations for each barley genotype.

Genotypes	**Treatments**	**Growth stage**	**Waterlogging durations**
Franklin/Westminster	Control	-	-
	WL1	ZS12.5	One month
	WL2	ZS12.5	Two months
	WL3	ZS15	Two months
Macquarie/ Macquarie+/ TamF169/Planet	Control	-	-
	WL1	ZS12.5	One month
	WL2	ZS12.5	Two months

Genotypes	Treatments	Growth stage	Waterlogging durations
	WL3	ZS15	Two months
	WL4	ZS59	15 days

Figure Captions

Figure 1. Location of water reservoir relative to the plant tanks to achieve waterlogging. Upper diagram: watering of control plants; lower diagram, waterlogged treatments.

Figure 2. Effect of waterlogging treatments on grain yield. WL1: waterlogging exposed at ZS12.5 for one month; WL2: waterlogging exposed at ZS12.5 for two months; WL3: waterlogging exposed at ZS15 for two months; WL4: waterlogging exposed at ZS59 for 15 days. WL4 treatment was not conducted on Franklin and Westminster. Vertical bars indicate ± standard error of the mean.

Figure 3. Effect of waterlogging treatments on yield components. WL1: waterlogging exposed at ZS12.5 for one month; WL2: waterlogging exposed at ZS12.5 for two months; WL3: waterlogging exposed at ZS15 for two months; WL4: waterlogging exposed at ZS59 for 15 days. WL4 treatment was not conducted on Franklin and Westminster. Vertical bars indicate ± standard error of the mean.

Figure 4. Grain dimensions of six barley genotypes in response to waterlogging. Vertical bars indicate ± standard error of the mean. WL1: waterlogging exposed at ZS12.5 for one month; WL2: waterlogging exposed at ZS12.5 for two months; WL3: waterlogging exposed at ZS15 for two months; WL4: waterlogging exposed at ZS59 for 15 days. WL4 was not conducted on Franklin or Westminster.

Figure 5 . Relative to control biomass of each genotype after waterlogging (a) and relative to control biomass at harvest of each genotype (b) under different waterlogging treatments. WL1: waterlogging exposed at ZS 12.5 for one month; WL2: waterlogging exposed at ZS12.5 for two months; WL3: waterlogging exposed at ZS 15 for two months; WL4: waterlogging exposed at ZS59 for 15 days. WL4 was not conducted on Franklin or Westminster.

Figure 6. Delay phenology at the end of waterlogging treatments (a), and delay maturity (b) under different waterlogging treatments. WL1: waterlogging exposed at ZS 12.5 for one month; WL2: waterlogging exposed at ZS12.5 for two months; WL3: waterlogging exposed at ZS 15 for two months;

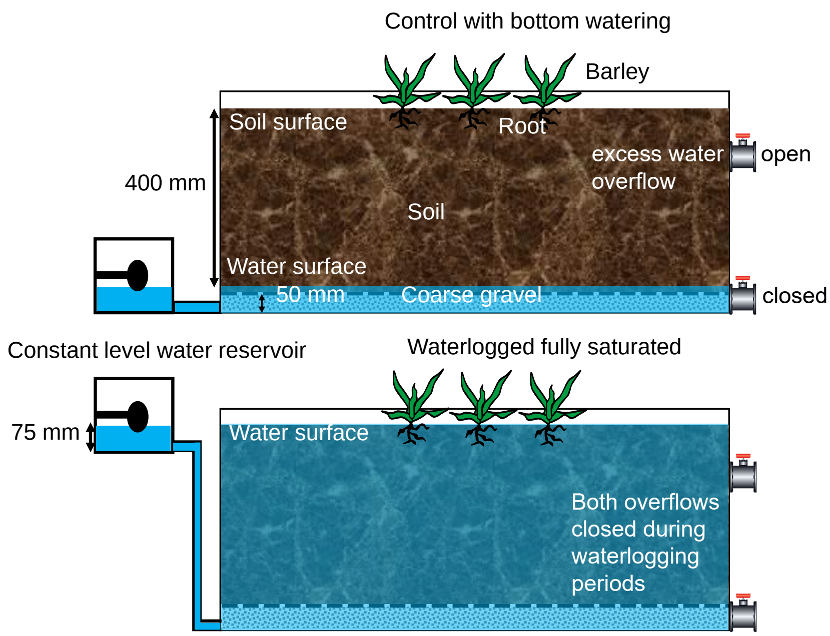

Figure 1 Location of water reservoir relative to the plant tanks to achieve waterlogging. Upper diagram: watering of control plants; lower diagram, waterlogged treatments.

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