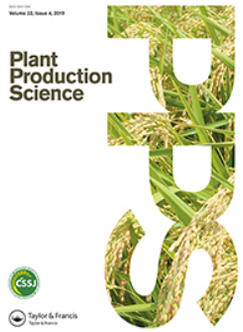

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# Growth and yield response of common buckwheat (*Fagopyrum esculentum* Moench) to waterlogging at different vegetative stages

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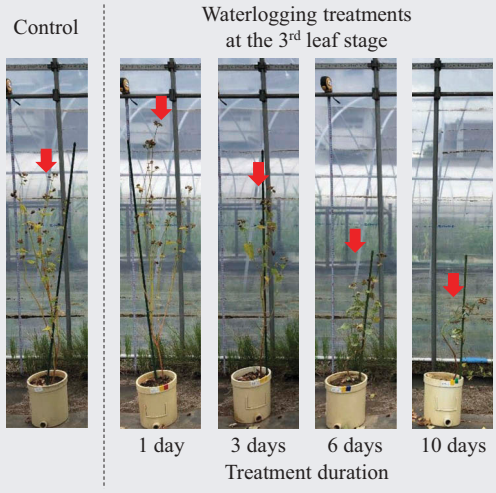
**ABSTRACT**

This study identified timing (1st, 3rd, or 5th leaf stage) and duration (1, 3, 6, or 10 days) of waterlogging treatment during the vegetative stage that had the most severe effect on yield and elucidated yield determining process. Yield was reduced the most by the waterlogging treatments at the 3rd leaf stage. Among stages, yield was significantly depressed, when the treatment duration was longer than 6 days. Seed weight of the 1st branches declined more under waterlogging treatments than did that of main stem and 2nd branches, accounting for approximately 55% of total seed weight in all treatments. On the other hand, the decline in node and branch numbers was more pronounced for 2nd branches than 1st branches at the ripening stage. The development of the 2nd branches during ripening did not contribute much to increase sink capacity. Development of the main stem and the 1st branches was almost complete until the full flowering stage and shoot dry weight did not increase from the full flowering stage to the ripening stage. Shoot dry weight at the full flowering stage was determined by both leaf number and net assimilation rate (NAR). Flower cluster number at that stage was significantly correlated with total seed weight. These results showed that the critical timing was at the 3rd leaf stage and the critical duration was longer than 6 days and indicated that maintenance of leaf number and NAR and development of flowers on the 1st branches until the full flowering stage would ensure the yield.

**Abbreviations**: NAR - net assimilation rate, SLA - specific leaf area, $g_s$ - stomatal conductance.

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## Introduction

Common buckwheat (*Fagopyrum esculentum* Moench) is a traditional Japanese crop and is nutritional, containing high levels of starch, protein, flavonoids, and dietary fiber in the grain (Giménez-Bastida & Zielinski, 2015). Recently, the cultivated areas of common buckwheat in Japan have been increased, especially in converted paddy fields (MAFF, 2019). In these fields, excess water or waterlogging is likely to decrease the growth and productivity of common buckwheat (Sugimoto & Sato, 2000). To improve waterlogging tolerance of common buckwheat agronomically and genetically, we need to comprehensively understand the

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The supplementary data for this article can be accessed here.

effects of the timing and duration of waterlogging treatments on yield-related traits.

The impact of waterlogging on plant growth varies depending on the developmental stage (Mano & Oyanagi, 2009). In common buckwheat, waterlogging at different growth stages negatively impacted seed yield by hindering seed germination, seedling emergence, branch and flower development, and 1000 seed weight (Sakata & Ohsawa, 2005; Sugimoto & Sato, 2000). Sakata and Ohsawa (2005) suggested that the seedling emergence stage was the most vulnerable to waterlogging. Thus, many researchers have tried to increase the seedling emergence rate agronomically (Kudo & Sone, 2016; Mizushima, 2015) and genetically (Murayama et al., 2004; Sakata & Ohsawa, 2005, 2006). Mizushima (2015) raised seedling emergence rate by seed coating with $CaO_2$. Sakata and Ohsawa (2006) evaluated the varietal differences in waterlogging tolerance at the seedling emergence stage in 17 Japanese local and bred varieties of common buckwheat. They succeeded in increasing seedling emergence rate in five of six selected lines by mass selection after four generations. These studies have paved the way to overcome detrimental waterlogging effects at the seedling stage.

However, a few studies have examined the effects of waterlogging treatments on buckwheat after the seedling stage (Sakata & Ohsawa, 2005; Sugimoto & Sato, 2000). In maize, common wheat, barley, and soybean, varietal differences in waterlogging tolerance and quantitative trait loci associated with waterlogging tolerance after the seedling emergence stage have been found (Mustroph, 2018). To approach them in buckwheat, we need to identify the critical timing and duration for waterlogging on yield and to reveal the yield determining process. Clarification of them leads to develop the screening methods and identify responsible traits for waterlogging tolerance after the seedling stages. Therefore, the objectives of this study were to identify the timing and duration of waterlogging treatment during the vegetative stage that had the most severe effect on yield and to elucidate the yield determining process by analyzing yield components and conducting growth analysis.

## Materials and methods

### Experimental design

We used a Japanese common buckwheat cultivar (*F. esculentum* Moench cv. kitawasesoba). Pots (height 200 mm, diameter 167 mm), each of which had a 24 mm diameter hole at the bottom end of a wall, were placed in a greenhouse at the School of Agriculture, Utsunomiya University. The pots were filled with 3 L of a mixture of 83% artificial soil (200 mg N $L^{-1}$, 2500 mg $P_2O_5$ $L^{-1}$, and 200 mg $K_2O$ $L^{-1}$, Kumiai Nippi engeibaido, Nihon Hiryo, Tokyo) and 17% vermiculite. The soil was well-watered and drained one day before sowing. Fifteen seeds were sown at 5 mm depth in the soil on 8 May 2018. Plants were watered daily. The plants were thinned to three per pot, when the primary leaves appeared on 18 May. Then, 0.2 L of the soil was added to each pot to prevent lodging. A pole (height 1.2 m, diameter 8 mm) was set for each plant to prevent lodging on 1 June. A total of 108 pots, in each of which three plants were cultivated, were prepared.

When seedlings had either the 1st (19 May, I), 3rd (24 May, II), or 5th leaf (29 May, III), 32 pots were selected and were subjected to either 1, 3, 6, or 10-day waterlogging treatments. In this study, the plants that were treated at the 1st, 3rd, 5th leaf stage for 1, 3, 6, 10 days are abbreviated as I-1, 3, 6, and 10, II-1, 3, 6, and 10, and III-1, 3, 6, and 10. In the waterlogging treatments, the hole was filled with a rubber plug and the plants were flooded to approximately 20 mm above the soil surface, whereas 8 pots remained as drained controls and were watered daily. After the treatment, the pots were drained and subsequently watered daily. All pots were arranged in a completely randomized design ($n = 4$).

Weather data were cited from the nearest observatory (Utsunomiya local meteorological office, Japan Meteorological Agency, www.data.jma.go.jp), approximately 4.4 km apart from the greenhouse and were analyzed by the authors.

### Measurements

At the 1st leaf stage (19 May) before waterlogging treatments, four pots were used for the following analyses. At each of the full flowering stage (19 June) and the ripening stage (5 July), 52 pots, four pots from each treatment, were used for the following analyses. At each stage, one plant per pot, which showed similar growth to the others in each treatment, was selected and harvested. After measuring SPAD values of the top fully expanded leaf with a SPAD meter (SPAD-502, KONIKA MINOLTA, Tokyo, Japan), the plants were divided into the main stem, branches, leaves, and seeds. The number of nodes on each of the main stem, 1st branches, and 2nd branches, and the number of 1st branches and 2nd branches was recorded. 3rd branches hardly developed and thus the number of nodes and seed number and weight on 3rd branches and the number of 3rd branches were included in those of 2nd branches. Leaf area was determined with an area meter (AAM-8, Hayashi denko, Japan). Digital images of all seeds on each of main stem and the 1st branch and 2nd branch were obtained with a digital camera (Tough TG-5, Olympus, Tokyo, Japan). Sterility and maturity of seeds were determined by the color of

seeds: red as sterile seeds, green as un-matured seeds, and black as matured seeds (Supplemental Figure 2). The number of sterile, un-matured, and matured seeds was recorded. The weight of each plant part was measured after oven-drying at 80°C for 72 h. Net assimilation rate (NAR) (1) and specific leaf area (SLA) (2) were calculated by the following equations:

$$NAR = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{\ln L_2 - \ln L_1}{L_2 - L_1} \quad (1)$$

$$SLA = \frac{L_2 - L_1}{\ln L_2 - \ln L_1} \times \frac{\ln WL_2 - \ln WL_1}{WL_2 - WL_1} \quad (2)$$

where W, WL, and L were the dry weight of the shoot, dry weight of the leaves, and the total leaf area per plant, respectively; $_1$ and $_2$ occurred at the 1st leaf stage and full flowering stage, respectively. All statistical analyses were performed with statistical software (JMP 12.2, SAS Institute Inc., USA). All data were analyzed by analysis of variance (ANOVA) at significant levels of $p < 0.001$, 0.01, and 0.05 and the Tukey HSD test at a significance level of $p < 0.05$. Simple linear regression models were applied to analyze correlations among parameters at significant levels of $p < 0.001$, 0.01, and 0.05.

## Results

Most of the daily mean, minimum, and maximum air temperatures during cultivation period in 2018 were extremely higher than the means of those from 1981 to 2010 (Supplemental Figure 1). From 27 June to 4 July, the daily minimum temperatures in 2018 exceeded the means of the daily mean temperatures from 1981 to 2010. The daily maximum temperature reached 35.3°C on 2 July 2018. The rainy season was unusually short in 2018. Thus, most of the sunshine duration during cultivation period in 2018 was longer than the mean of those from 1981 to 2010.

We first examined the timing and duration of waterlogging treatment that had the most severe effect on yield production (Figure 1). There was a significant two-way interaction between timing and duration of waterlogging treatment on total seed weight ($p < 0.001$) (Supplemental Table 1). Though the total seed weight of II-1 was the highest among the treatments, that of II drastically decreased compared to that of I and III when the treatment duration lasted longer than 3 days. As a result, the average of total seed weight of C, I, II, and III were 4.24, 2.90, 2.47, and 3.73 g plant$^{-1}$, respectively. The waterlogging treatments at the 3rd leaf stage (II) were the most severe in terms of timing. At any leaf stages (I, II, and III), waterlogging treatments significantly reduced total seed weight compared to the control when the treatment duration lasted longer than 6 days.

To clarify the yield determining process, we next evaluated the seed weight on the main stems, 1st branches, and 2nd branches (Figure 1). Although a declining trend observed in total seed weight was also found in the seed weight on the main stems, 1st branches, and 2nd branches, the decline was more pronounced on the 1st branches. The highest and lowest seed weight on the main stem, 1st branches, and 2nd branches were 2.23 and 0.21 g plant$^{-1}$, 3.18 and 0.22 g plant$^{-1}$, and 0.71 to 0 g plant$^{-1}$, respectively (Supplemental Table 1). In addition, the seed weight on the 1st branches accounted for approximately 55% of total seed weight, regardless of the treatment. In contrast, the seed weight on the 2nd branches contributed a small amount to the total seed weight. Regardless of the treatment, they only accounted

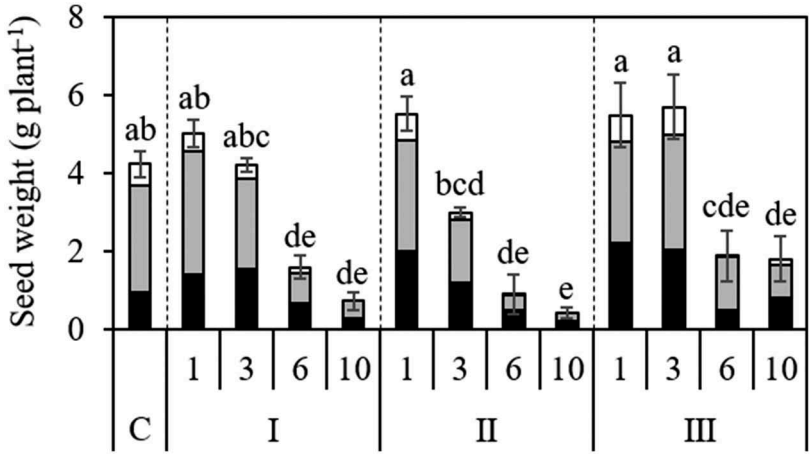


Figure 1. Effects of timing and duration of waterlogging treatment on seed weight on main stem (black bar), 1st branches (grey bar), and 2nd branches (white bar). C, control. I, II, and III indicate the 1st, 3rd, or 5th leaf stage, respectively. Values are means ($n = 4$). The different letters indicate significant differences among the treatments (Tukey HSD test, $p < 0.05$). The seed weight on 3rd branches was included in that on 2nd branches.

for approximately 6% of total seed weight. The seed weight on the 1st branches contributed more to total seed weight than did that of the main stem and 2nd branches combined.

Total node and branch numbers at the ripening stage also showed the same declining trend as did total seed weight, but the decline in nodes and branch numbers was more pronounced for the 2nd branches rather than the 1st (Figure 2(b,d)). The node number on the 1st branches ranged from 22.8 to 49.5 no. plant$^{-1}$, but that on the 2nd branches ranged from 0 to 62.5 no. plant$^{-1}$ (Supplemental Table 1). Likewise, the number of the 1st branches ranged from 3.5 to 6.0 no. plant$^{-1}$, but the number of the 2nd branches ranged from 0 to 12.8 no. plant$^{-1}$. The development of the 2nd branches and the nodes on the 2nd branches did not contribute to an increase in total seed weight. In addition, the node number on the main stem and the 1st branches and the number of the 1st branches at the ripening stage did not increase much from the full flowering stage, except for I-10 (Figure 2).

As mentioned above, the node number and branch number cannot explain well the seed weight, especially on the 2nd branches, and thus we examined the flower cluster number and seed number (Figures 3 and 4). There was a significantly positive correlation between flower cluster number at the full flowering stage and total seed

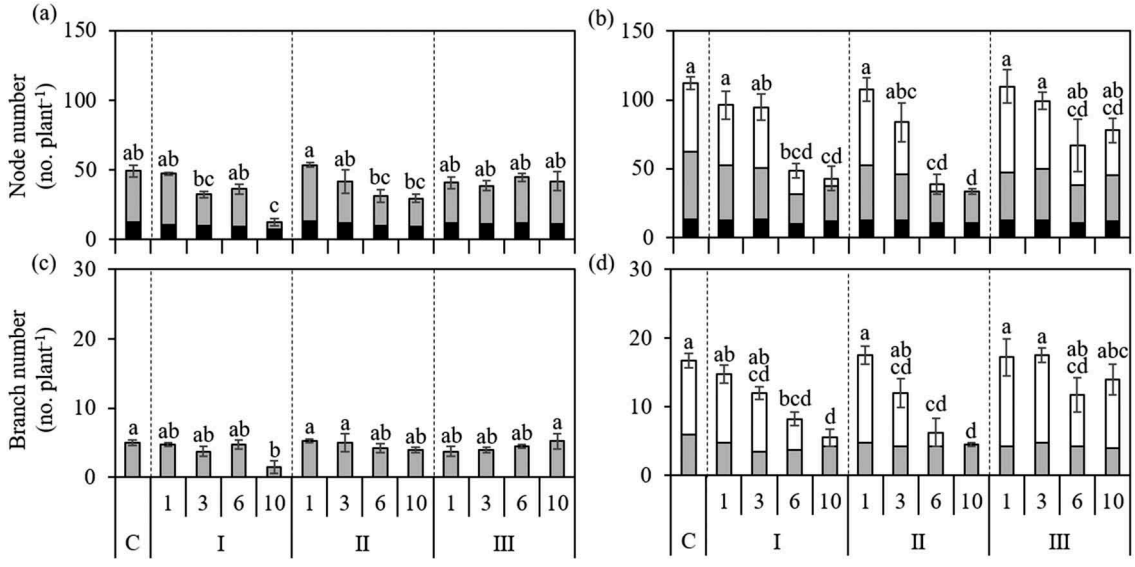


**Figure 2.** Effect of different timing and duration of waterlogging treatment on node number (a, b) and branch number (c, d) on main stem (black bar), 1st branches (grey bar), and 2nd branches (white bar) at the full flowering (a, c) and ripening stage (b, d). C, control. I, II, and III indicate the 1st, 3rd, or 5th leaf stage, respectively. Values are means ($n$ = 4). The different letters indicate significant differences among the treatments (Tukey HSD test, $p < 0.05$). The node number and branch number on 3rd branches were included in those on 2nd branches.

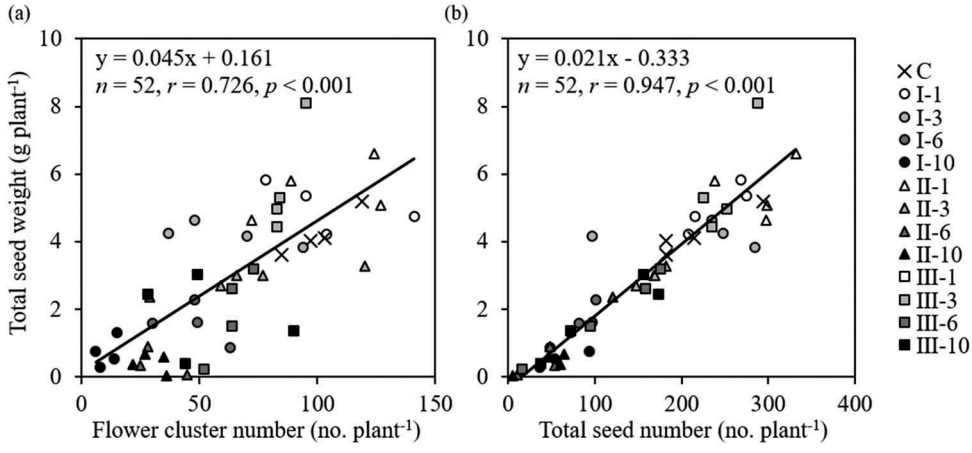


**Figure 3.** Correlation of total seed weight with flower cluster number at the full flowering stage (a) and total seed number (b). C, control. I, II, and III indicate the 1st, 3rd, or 5th leaf stage, respectively.

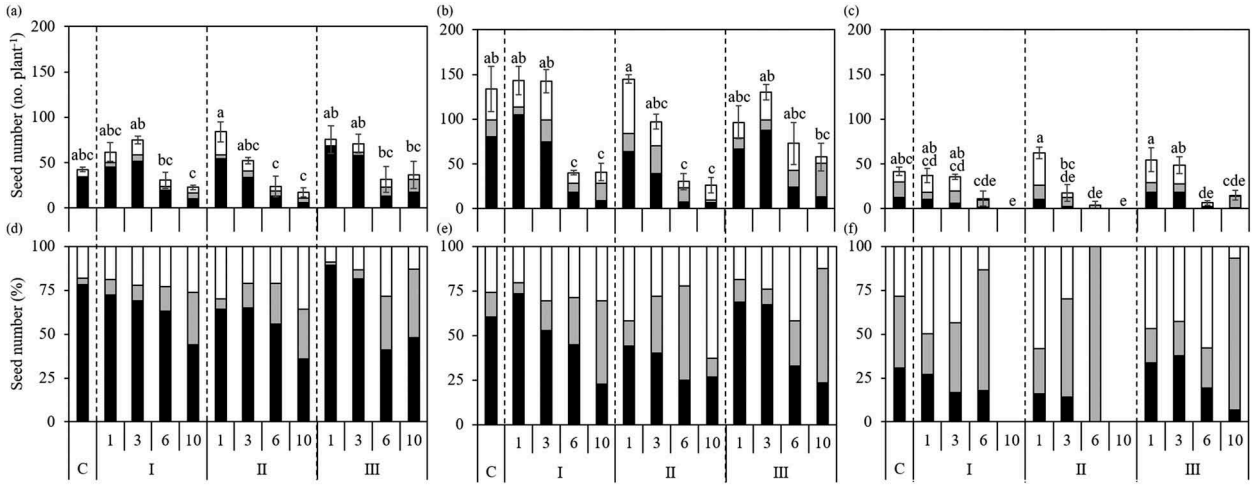


**Figure 4.** Effects of timing and duration of waterlogging treatments on sterile (white bar), unmatured (grey bar), and matured (black bar) seed number on the main stem (a, d), 1st branches (b, e), and 2nd branches (c, f). C, control. I, II, and III indicate the 1st, 3rd, or 5th leaf stage, respectively. Values are means ($n$ = 4). The different letters indicate significant differences among the treatments (Tukey HSD test, $p < 0.05$). The seed number on 3rd branches was included in that on 2nd branches.

weight ($n$ = 52, $r$ = 0.726, $p < 0.001$) (Figure 3(a)). Total seed number was correlated more strongly with total seed weight ($n$ = 52, $r$ = 0.947, $p < 0.001$) (Figure 3(b)). To examine in detail, we separately investigated seed number on the main stem, 1st branches, and 2nd branches and separated harvested seeds into three developmental stages (matured, unmatured, and sterile) (Supplemental Figure 2 and Figure 4). Similar to seed weight, seed number on the 1st branches accounted for approximately half of the total seed number regardless of treatment (Figure 4(a–c)). In addition, the decline in seed number by treatments was more pronounced on the 1st branches. The highest and lowest seed number on the main stem, 1st branches, and 2nd branches were 84 and 18 no. plant$^{-1}$, 145 and 26 no. plant$^{-1}$, and 63 and 0 no. plant$^{-1}$, respectively. On the other hand, the highest percentage of matured seed was observed on the main stem, followed in order by the 1st branches and 2nd branches, regardless of treatment (Figure 4(d–f)). The highest and lowest percentage of matured seed on the main stem, 1st branches, and 2nd branches were 89 and 36%, 73 and 23%, and 38 and 0%, respectively. In addition, the percentages of sterile seeds on the 2nd branches were higher than that on the main stem and 1st branches in most treatments (Figure 4(d–f)). These results showed that the 1st branches contributed more to increase the sink capacity than did the main stem or 2nd branches.

Shoot dry weights at the full flowering stage and the ripening stage were similar, except for I-3 and 10 (Figure 5). The plants of I-3 and 10 recovered growth during ripening. The highest and lowest shoot dry weight at the full flowering stage and ripening stage were 13.1 and 0.8 g plant$^{-1}$, and 12.3 and 1.6 g plant$^{-1}$, respectively. There was a significantly positive correlation between the shoot dry weight at the full flowering stage and that at the ripening stage ($n$ = 52, $r$ = 0.848, $p < 0.001$) and between seed weight and shoot dry weights at the ripening stage ($n$ = 52, $r$ = 0.969, $p < 0.001$)

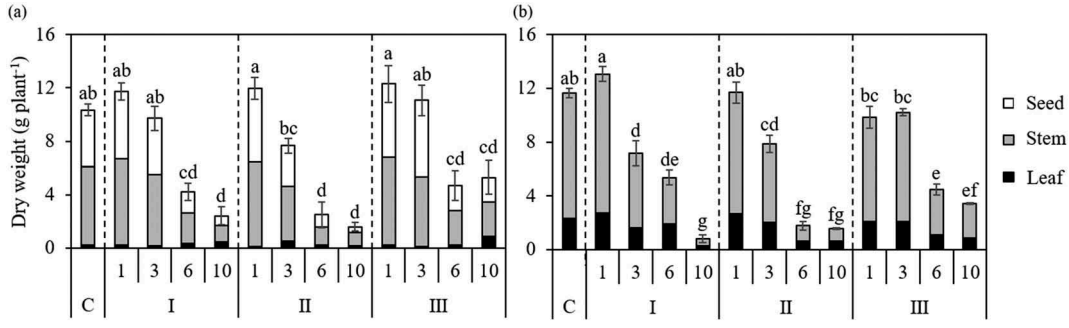


**Figure 5.** Effects of different timing and duration of waterlogging treatment on the dry weight of seeds, stems, and leaves at the ripening (a) and full flowering stage (b). C, control. I, II, and III indicate the 1st, 3rd, or 5th leaf stage, respectively. Values are means ($n$ = 4). The different letters indicate significant differences among the treatments (Tukey HSD test, $p < 0.05$).

(Supplemental Figure 3). Although the 2nd branches developed during ripening, the stem and leaf weight decreased from the full flowering stage to the ripening stage.

To elucidate the trait determining vegetative growth until the full flowering stage, we evaluated leaf traits (Figure 6 and Table 1). There were significant positive correlations between shoot dry weight at the full flowering stage and both leaf area and NAR (Figure 6(a,b)). The leaf area of the I-10 treatment significantly decreased compared to that of the control (Table 1). In the II and III treatments, the leaf areas significantly decreased when treatment duration was longer than 6 days. These decreasing trends were not observed for individual leaf area but were for leaf number. The NAR was lower, when at the earlier stage the treatments conducted and the longer the treatment duration became. However, SLA and SPAD values were not significantly different among treatments.

## Discussion

The first objective of this study was to determine the timing and duration of waterlogging treatment that is most detrimental to yield production and growth. Regardless of the treatment at all stages, yield production and growth were significantly depressed as treatment duration was longer than 6 days (Figures 1 and 5). In addition, the waterlogging treatment effects at the 3rd leaf stage (II) were the most severe on yield production and growth relative to those at the other stages. At this stage, the plants began to form flower buds. In wheat, plants at the seedling and flowering stages are most susceptible to waterlogging (Setter & Waters, 2003). de San Celedonio et al. (2014) also determined the most detrimental stage for wheat and barley plants to waterlogging and they observed the highest yield reduction during stem elongation to anthesis. Michiyama and Hayashi (1998) reported that main stem

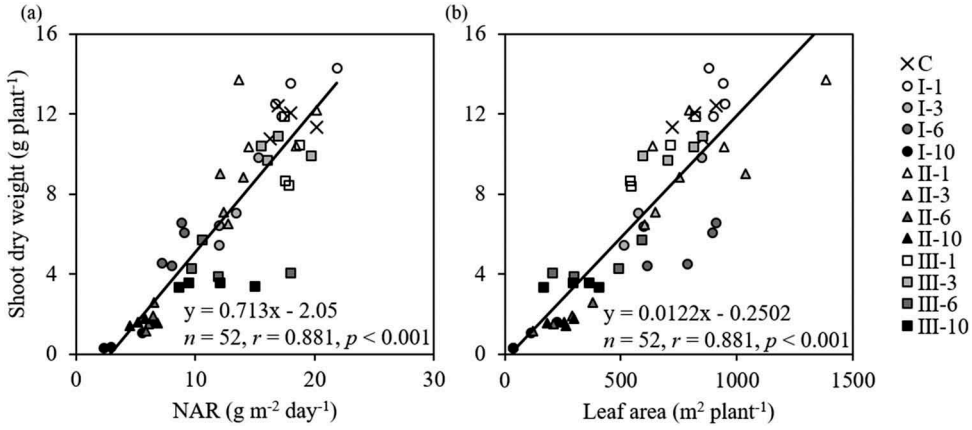


**Figure 6.** Correlation of shoot dry weight with NAR (a) and leaf area (b) at the full flowering stage. C, control. I, II, and III indicate the 1st, 3rd, or 5th leaf stage, respectively.

**Table 1.** Effect of different timing and duration of waterlogging treatment on leaf area, leaf number, individual leaf area, net assimilation rate (NAR), specific leaf area (SLA), and SPAD value.

| T | D | Leaf area cm$^2$ plant$^{-1}$ | Leaf number no. plant$^{-1}$ | Individual leaf area cm$^2$ | NAR g m$^{-2}$ day$^{-1}$ | SLA cm$^2$ g$^{-1}$ | SPAD value |
|---|---|---|---|---|---|---|---|
| C | | 825 ± 39.0a | 76.8 ± 8.64a | 11.3 ± 1.76 | 17.8 ± 0.85ab | 316 ± 7.7 | 34.0 ± 3.17 |
| I | 1 | 918 ± 16.5a | 84.3 ± 6.26a | 11.0 ± 0.62 | 18.5 ± 1.17a | 307 ± 6.06 | 31.8 ± 2.50 |
| | 3 | 635 ± 74.1abc | 56.0 ± 6.72abcd | 11.4 ± 0.55 | 13.2 ± 0.79bcd | 334 ± 8.6 | 33.9 ± 2.59 |
| | 6 | 802 ± 67.6a | 72.5 ± 12.1abc | 11.6 ± 1.09 | 8.3 ± 0.43de | 357 ± 15.2 | 36.2 ± 3.59 |
| | 10 | 103 ± 44.1d | 20.5 ± 5.74d | 4.46 ± 0.75 | 4.31 ± 0.99e | 318 ± 16.3 | 42.7 ± 4.13 |
| II | 1 | 940 ± 160a | 96.0 ± 5.15a | 9.99 ± 2.04 | 16.7 ± 1.56abc | 319 ± 20.5 | 35.3 ± 3.51 |
| | 3 | 761 ± 97.4a | 75.3 ± 12.4ab | 10.3 ± 0.51 | 12.8 ± 0.42cd | 328 ± 15.2 | 38.7 ± 0.67 |
| | 6 | 249 ± 55.5d | 33.0 ± 3.72cd | 7.49 ± 1.26 | 6.22 ± 0.17e | 322 ± 4.24 | 42.9 ± 0.99 |
| | 10 | 249 ± 24.4d | 34.3 ± 4.07bcd | 7.33 ± 0.39 | 5.58 ± 0.50e | 319 ± 17.3 | 43.6 ± 5.34 |
| III | 1 | 656 ± 68.8abc | 70.3 ± 6.52abc | 9.34 ± 0.47 | 17.9 ± 0.29ab | 300 ± 21.9 | 37.1 ± 5.55 |
| | 3 | 741 ± 58.2ab | 67.5 ± 5.45abc | 11.4 ± 1.78 | 17.1 ± 0.92abc | 310 ± 11.5 | 36.0 ± 3.74 |
| | 6 | 396 ± 88.1bcd | 61.0 ± 5.83abcd | 6.28 ± 0.95 | 12.5 ± 1.88cd | 319 ± 13.1 | 37.6 ± 4.38 |
| | 10 | 307 ± 52.3cd | 55.8 ± 12.4abcd | 5.78 ± 0.73 | 11.3 ± 1.44d | 298 ± 20.8 | 35.1 ± 3.87 |
| T | | *** | ** | ** | *** | ns | ns |
| D | | *** | *** | *** | *** | ns | ns |
| T × D | | *** | *** | ns | *** | ns | ns |

T, timing; D, duration, C, control. I, II, and III indicate the 1st, 3rd, or 5th leaf stage, respectively. Values are means ($n$ = 4). The different letters indicate significant differences among the treatments (Tukey HSD test, $p < 0.05$). ANOVA: ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

elongation increased and peaked at the start of the flowering stage regardless of the cultivar or ecotypes for buckwheat. Similar to that for wheat and barley, our results suggested that the critical stage of buckwheat plants to waterlogging is during the transition from the vegetative to reproductive phase.

Sugimoto and Sato (2000) exposed plants to excess soil moisture treatments (water table of 5–7 cm below the soil surface) or waterlogging treatments at the first leaf, flowering, and ripening stages for the same duration as in the present study. They showed that the earlier the excess soil moisture treatment was conducted and the longer the treatment duration, the more the yield decreased. This was similar to the observation in this study (Figure 1). However, under the waterlogging treatment, they reported that yield of the I-1 and 3 treatments was less than half of the control plants and all of I-6 and 10 withered and died (Sugimoto & Sato, 2000). These findings differed from our results. In our experiment, we added soil to the pots to prevent lodging when the seedlings had their 1st leaf. Kawamura et al. (2006) showed that higher than 2 cm ridging at the flower bud appearance stage improved the growth of plants that were waterlogged for 2 days at the flowering stage. Thus, the addition of soil might have improved growth in this study. This implies that ridging at an earlier growth stage than the flower bud appearance stage might be effective in the improvement of the growth of waterlogged plants. This should be the subject of further study.

The second objective of this study was to clarify the yield determining process. Sugimoto and Sato (2000) reported that yield reduction caused by excess soil moisture and waterlogging treatments at the 1st leaf stage and the flowering stage was caused by the decrease in the number of seeds on branches. However, they did not separately examine the 1st and 2nd branches. Our study showed that the seed weight on the 1st branches was reduced the most by waterlogging treatment and accounted for approximately 55% of total seed weight, regardless of the treatments (Figure 1). This decreasing trend was also observed in seed number (Figure 4(b)). In contrast, though the seed number on the 2nd branches was reduced by the treatments, it accounted for less than 23% of the total seed number (Figure 4(c)). In addition, the percentage of sterile seeds on the 2nd branches was higher than that on the main stem and 1st branches in most of the treatments (Figure 4(d–f)). These results indicated that the number of matured seeds on the 1st branches, not that on the 2nd branches, would determine the yield reduction caused by waterlogging treatment.

Sugimoto and Sato (2000) showed that yield reduction caused by excess soil moisture and waterlogging treatments at the 1st leaf stage and the flowering stage was caused by the reduction in the number of flowers on branches. This study also showed that the same decreasing trend of flower cluster number and total seed number (Figure 3). In addition, the node number on the main stem and 1st branches and the 1st branch number at the ripening stage did not increase much from the full flowering stage (Figure 2). This indicated that the development of the main stem and the 1st branches would finish at the full flowering stage. Michiyama and Hayashi (1998) also reported that summer agroecotype cultivars finished the elongation of the main stem, 1st branches, and flower development approximately 2 weeks after the beginning of anthesis. Sugimoto (2004a) examined the effect of nitrogen fertilizer application on the growth and yield of the same cultivar as used in this study and reported that high NAR and crop growth rate at the late ripening stage did not contribute to yield increase. These findings suggested that sink capacity would be determined until the full flowering stage and high source ability at the late ripening stage contributes little to yield increase. In addition, the shoot weights at the full flowering stage and ripening stage were almost same, although the stem and leaf weight decreased from the full flowering stage to the ripening stage (Figure 5). Sugimoto (2004a) reported that shoot dry weight stopped increasing from the late flowering stage, except when under high nitrogen application treatment (10 g N $m^{-2}$), and showed the same dry matter allocation patterns as in the present study. Plants allocated approximately 80% of total dry matter to stems at the early ripening stage and then allocated it to seeds (Sugimoto, 2004a). These findings indicated that translocation of carbon from the stems would contribute to seed weight. Sugimoto and Masaoka (2001) examined sink activity by applying $^{13}CO_2$ to the whole plant and reported the sink capacity of the stems was high at the start of flowering and that of seeds was high at the full flowering stage. Carbon allocation from stems to seeds should be studied in the future.

There were significant positive correlations between shoot dry weight at the full flowering stage and both total leaf area and NAR (Figure 6(a,b)). Both total leaf area and NAR significantly decreased, except the total leaf area in the I-6 treatment, when the treatment duration was longer than 6 days (Table 1). These results suggested that both the total leaf area and NAR determine vegetative growth. Total leaf area was attributed to leaf number and individual leaf area, and the reduction caused by waterlogging treatment was more apparent in leaf number (Table 1). Thus, leaf number was more susceptible to waterlogging and it mostly determined the differences in total leaf area. Sugimoto and Sato (1999) suggested that high SLA, which is indicative of a thin leaf, resulted in a low SPAD value, indicating low chlorophyll content. However, the SLA and SPAD values

were not significantly different among treatments (Table 1). This suggested that the stomatal conductance ($g_s$), not chlorophyll content, limits photosynthesis. In fact, the leaf temperature, which reflects $g_s$ and transpiration (Jones, 2004), of the waterlogged plants was higher than that of the control plants (Supplementary Figure 3). It can be measured easily with thermocouples and an infrared thermometer. In particular, thermal imaging is a useful tool to screen plants for differences in $g_s$. Further studies should examine whether photosynthesis and $g_s$ are reduced by waterlogging treatments and whether thermal imaging can be used to screen for plants tolerant to waterlogging.

In addition to the waterlogging treatment, the air temperature and the sunshine duration affect yield components of buckwheat. In this study, the high air temperature and the long sunshine duration were observed (Supplemental Figure 1). However, the seed number and 1000 seed weight of this study were compatible with other studies. The highest seed number and 1000 seed weight of pot experiments using the same cultivar (cv. kitawasesoba) as this study were 124 no. plant$^{-1}$ and 40.9 g (Sugimoto & Sato, 1999), 119 no. plant$^{-1}$ and 30.2 g (Sugimoto & Sato, 2000), 280 no. plant$^{-1}$ and 30.6 g (Sugimoto, 2004b), and 188 no. plant$^{-1}$ (the sum of unmatured and matured seeds) and 30.3 g in this study. Sugimoto and Sato (1999) showed that the higher the daily minimum air temperature during the period from the beginning of flowering to the ripening became, the more flowers produced and the lower seed setting rate and 1000 seed weight became. The high air temperature and the long sunshine hours in this study might have increased the flower number and would have increased seed number compared to other studies (Sugimoto & Sato, 1999, 2000), though they did not affect much on 1000 seed weight. The two-way interaction between air temperature and waterlogging treatment on yield components should be studied in the future.

The objectives of this study were to identify the timing and duration of waterlogging treatment during the vegetative stage that had the most severe effect on yield and to elucidate the yield determining process. We demonstrated that critical timing occurs during the transition from vegetative to reproductive phase and the critical duration is longer than 6 days. We also found that yield can be ensured by the maintenance of leaf number and NAR and the development of flowers on 1st branches until the full flowering stage. These findings contribute to the development of screening methods after the seedling stage, which can identify genetic locus (loci) associated with waterlogging tolerance.

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