

Effects of prolonged seed submersion on seedling establishment and early growth in wheat

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Research Article

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Abstract

Wheat seedlings are widely used as a fresh food source for herbivorous insects and as a model for studies of early plant development. Although the effects of seed soaking on wheat germination have been extensively investigated, much less is known about how prolonged pre-germination submersion influences subsequent seedling establishment and early growth. In this study, we examined the effects of seed submersion duration on germination, seedling growth, and biomass accumulation in two wheat cultivars under controlled laboratory conditions. Seeds were submerged in water for 0 to 336 h at 25°C before germination. Germination remained high after submersion for up to 48 h but declined markedly after 168 h or longer. More importantly, prolonged submersion delayed subsequent seedling development, increasing the time required for seedlings to reach leaf lengths of 5, 10, and 15 cm. Following a 24 h submersion treatment, leaf length increased rapidly until days 11–12 before reaching a plateau, whereas seedling dry weight continued to increase until approximately day 16. These results demonstrate that the effects of pre-germination water exposure extend beyond germination success and influence subsequent seedling establishment and early growth. Our findings provide practical guidance for optimizing wheat seedling production and emphasize the importance of evaluating post-germination performance when assessing the effects of prolonged seed submersion.

1. Introduction

Understanding the biology of pest insects is essential for developing effective management strategies, and laboratory rearing experiments play a central role in this process. For herbivorous insects, maintaining a stable food supply under controlled conditions is critical. Wheat seedlings are widely used as a convenient food source for insect rearing because of their rapid growth and ease of production.

Certain grasshopper species, including locusts, are among the most destructive agricultural pests worldwide. The desert locust, *Schistocerca gregaria*, is particularly well known for causing severe crop damage (Brader et al. 2006; Sword et al. 2010; Le Gall et al. 2019). Establishing a reliable system for producing wheat seedlings is therefore a fundamental requirement for laboratory studies of locusts. However, methods for maintaining a constant supply of seedlings with consistent quality and at defined developmental stages have rarely been quantified.

Although wheat germination has been extensively studied, most previous work has focused on germination success rather than subsequent seedling development (Nonogaki et al. 2010; Finch-Savage and Bassel 2016). Germination begins with water uptake, which activates metabolic processes essential for growth (Nonogaki et al. 2010). While water is required, excessive exposure can induce hypoxic stress and reduce seed vigor (Al-Ani et al. 1985; Drew 1997; Bailey-Serres and Voesenek 2008). Although the effects of prolonged water exposure on germination have been widely documented, comparatively little attention has been given to its influence on subsequent seedling establishment and early growth.

In addition to germination, uniform seedling establishment and subsequent growth are essential for obtaining reproducible experimental outcomes, as insect performance depends strongly on food quality.

It is therefore important to identify conditions that produce seedlings of uniform quality and to determine the time window during which seedlings maintain this state. Accordingly, this study evaluated how seed submersion duration influences not only germination but also subsequent seedling establishment, early growth, and biomass accumulation. We further identified the developmental stage at which wheat seedlings are most suitable for experimental use.

2. Materials and Methods

2.1. Seed materials

Two wheat cultivars, Satonosora and Yumeakari, were commercially obtained in November 2023. Seeds were stored in paper bags at 4°C until use. All experiments were conducted at the Japan International Research Center for Agricultural Sciences, Tsukuba, Japan, from July to August 2024.

2.2. Germination experiment

To evaluate the effects of submersion duration on germination, twenty seeds were submerged in 20 mL of distilled water for 0, 6, 24, 48, 72, 168, and 336 hours at 25°C. After each treatment, seeds were sown on moist soil (commercial horticultural substrate) in plastic trays (30 × 20 × 5 cm). Seeds were evenly spaced to avoid density effect. Trays were covered with a transparent plastic sheet for the first two days to reduce water loss and maintained in a controlled environment at 25 ± 1°C, 25 to 35 percent relative humidity, and a 12 h light and 12 h dark photoperiod. Light was provided by LED lamps at an intensity of approximately 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. After day 2, the plastic cover was removed, and seedlings were watered every other day. Germination was recorded daily. A seed was considered germinated when the radicle emerged from the seed coat (Yazdi et al. 2013). Each treatment was replicated three times.

2.3. Effect of submersion duration on seedling development

Following sowing, seedlings were grown for up to 20 days under the same environmental conditions. Seedling development was assessed by measuring the maximum length of the longest leaf for each individual. Measurements were taken daily. The number of days required for seedlings to reach 5, 10, 15, and 20 cm in length was recorded. When seedlings exceeded a threshold length between observation days, the day of attainment was estimated based on the closest observation. Seedlings that showed abnormal growth or physical damage were excluded from the analysis. Due to experimental constraints, some trials were terminated when seedlings reached 15 cm.

2.4. Daily development of seedlings

Twenty seeds from each of the two cultivars were submerged for 24 hours and then sown in moist soil. Seedling development was monitored under the same laboratory conditions as described above. There were three replicate sets prepared for each cultivar. In each set, the maximum leaf length of seedlings was measured daily. In addition, one replicate set was destructively sampled every two days to determine

seedling dry weight. Seedlings were individually oven dried at 60°C for 48 hours and weighed using an electronic balance (± 0.1 mg). Seedlings that did not develop normally were excluded from the analysis.

2.5. Statistical analysis

Seedling developmental period, maximum leaf length and dry weight were compared among treatments using analysis of variance followed by Tukey Kramer honestly significant difference tests. Germination rates were analyzed using Fisher's exact test with Bonferroni correction. All analyses were performed using the JMP software (SAS Institute, Cary, NC, USA) and the software R, version 4.3.3 (R Development Core T. 2020)..

3. Results

3.1. Effects of submersion duration on germination and seedling development

Germination rate was significantly affected by submersion duration in both wheat cultivars (Fig. 1). Germination remained high up to 48 hours of submersion but declined significantly with longer submersion, particularly beyond 72 hours (Fisher's exact test with Bonferroni correction, $P < 0.002$). Almost no seedlings emerged when seeds were submerged for 168 hours or longer.

Submersion duration also affected subsequent seedling development (Fig. 2). Seedlings originating from seeds submerged for 0 to 72 hours exhibited similar developmental patterns, reaching target leaf lengths within comparable time frames. In contrast, seedlings from seeds submerged for 168 hours or longer required substantially more time to reach the same developmental stages. In addition, only a limited number of seedlings emerged under these treatments, and those that did often exhibited delayed growth and abnormal morphology.

A more detailed analysis of developmental timing showed that time to germination was significantly shorter in the 24 and 48 hour treatments than in the 0 and 6 hour treatments, as well as in treatments longer than 72 hours (Fig. 3a, b; Tukey Kramer HSD test, $P < 0.05$). However, no significant differences were detected among treatments up to 72 hours in the number of days required to reach leaf lengths of 5, 10, 15, or 20 cm (Fig. 3c to j). In contrast, although sample sizes were limited, seedlings from the 168 and 336 hour treatments showed markedly delayed development across all growth thresholds.

3.2. Daily seedling development

Seedling developmental patterns were similar between the two cultivars following the 24 hour submersion treatment (Fig. 4). Maximum leaf length increased rapidly from days 2 to 10, followed by only slight increases after days 11 to 12, reaching a plateau (Fig. 4). Although leaf length plateaued around day 11, dry weight continued to increase and reached a plateau around day 16 (Fig. 5). These

results suggest that seedling development was closely associated with biomass accumulation in both cultivars.

4. Discussion

The present study demonstrated that prolonged seed submersion influences not only germination success but also subsequent seedling establishment and early growth. Germination remained stable up to 48 hours of submersion but declined sharply with longer exposure, particularly beyond 72 hours, with almost no emergence after 168 hours. These results indicate that excessive water exposure prior to sowing negatively affects seed viability, likely due to oxygen limitation during prolonged submersion. Hypoxic conditions are known to disrupt aerobic metabolism and reduce the energy available for germination and early growth (Drew 1997; Bailey-Serres and Voesenek 2008), which is consistent with the reduced germination observed in this study.

Previous studies have shown that moderate seed soaking or hydropriming can enhance germination speed and seedling vigor in wheat, with optimal durations typically around 12 to 24 hours, whereas longer treatments often reduce performance (Sethar et al. 2024). Although these studies primarily evaluated germination performance, our results show that prolonged submersion also has persistent effects on seedling establishment and subsequent early growth. In contrast, the present study extended the range of submersion duration up to 336 hours and demonstrated clear threshold effects not only on germination but also on subsequent seedling development. Even when seeds germinated after prolonged submersion, seedling growth was delayed and abnormal morphology was frequently observed. This suggests that pre germination conditions can have lasting effects on post germination development, possibly due to impaired reserve mobilization or metabolic imbalance during early developmental stages. In contrast, seeds submerged for up to 72 hours showed comparable growth trajectories, indicating that moderate water exposure does not negatively affect subsequent development. Our findings indicate that the effects of prolonged seed submersion cannot be fully evaluated by germination success alone, because post-germination seedling performance may be affected even when seeds successfully germinate.

Germination occurred more rapidly in the 24 and 48 hour treatments than in shorter or longer submersion treatments. This pattern is consistent with priming effects, in which controlled hydration facilitates physiological activation prior to sowing and accelerates germination (Bradford 1986; Paparella et al. 2015). However, no clear differences in germination rate or subsequent seedling development were observed between the 24 and 48 hour submersion treatments and the non submerged control. This may be explained by the use of soil as a growth substrate, which allowed seeds to absorb water while maintaining sufficient oxygen availability. Under such conditions, seeds may already experience partial hydration similar to priming, thereby reducing the relative benefit of prior submersion treatment. In contrast, in laboratory systems where seeds are germinated on paper or in substrates such as vermiculite, water availability and oxygen conditions differ from those in soil. In these environments, pre sowing hydration treatments may play a more important role in ensuring sufficient

water uptake without negatively affecting subsequent development. These findings suggest that the effects of seed submersion should be evaluated in relation to both the germination environment and subsequent seedling performance.

Beyond germination success, daily measurements revealed that leaf elongation and biomass accumulation followed distinct temporal patterns. The analysis of daily seedling development further revealed that leaf elongation and biomass accumulation followed distinct temporal patterns. Leaf length increased rapidly until approximately days 11 to 12 and then plateaued, whereas dry weight continued to increase until around day 16. The continued increase in dry weight after the cessation of leaf elongation suggests ongoing physiological processes such as tissue thickening or reserve allocation (Poorter et al. 2012). These patterns were consistent across cultivars, indicating that early seedling development follows a robust trajectory under optimal submersion conditions.

From a practical perspective, submersion durations of 24 to 48 hours provide a suitable balance between reliable hydration and uniform seedling establishment. In addition, the observed growth patterns suggest that seedlings reach a stage of maximal elongation around days 10 to 12, while biomass accumulation continues thereafter. Therefore, the appropriate harvest stage should be selected according to the intended experimental purpose, whether maximizing leaf elongation or biomass accumulation. Overall, these conditions are likely to support stable and efficient seedling production under controlled laboratory conditions.

In conclusion, the present study demonstrates that evaluating germination alone is insufficient to assess the effects of prolonged seed submersion. Pre-germination water exposure has lasting consequences for subsequent seedling establishment and early growth, highlighting the importance of considering post-germination performance when developing standardized seedling production protocols.

Declarations

AI usage

The authors used ChatGPT (OpenAI) to assist with English language editing and improve grammar and readability. All scientific content, interpretation, and conclusions were developed and verified by the author, who takes full responsibility for the manuscript.

Financial Support

Not applicable.

Conflicts of Interest

The author declares none.

Ethical Standards

Not applicable

Author Contribution

Koutaro Ould Maeno: Conceptualization, investigation, formal analysis, writing the original draft, resources, methodology, review & editing.

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Data Availability

Data are available as electronic supplementary material.<https://figshare.com/s/0182538824d5a5f8f9b6>

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Figures

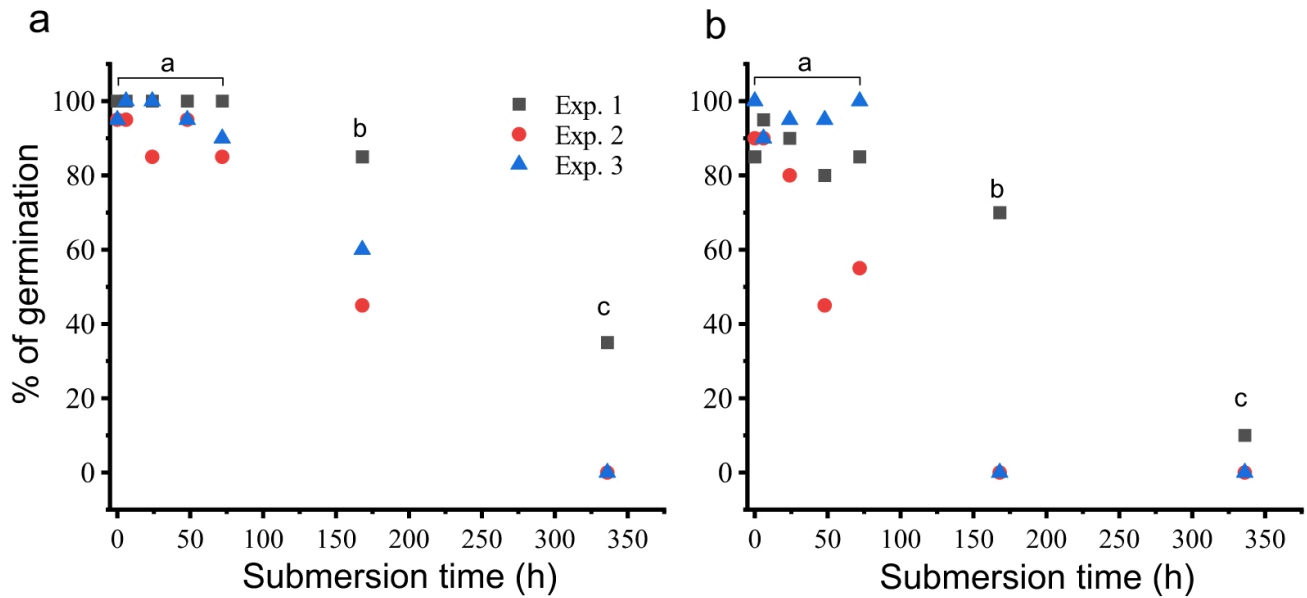


Figure 1

Effects of submersion duration on germination rate in two wheat cultivars, Satonosora (a) and Yumeakari (b). Seeds were submerged for 0 to 336 hours prior to sowing. Values represent means of three replicates. Different letters indicate significant differences among treatments at $P < 0.002$ (Fisher's exact test with Bonferroni correction). Sample size is 20 each.

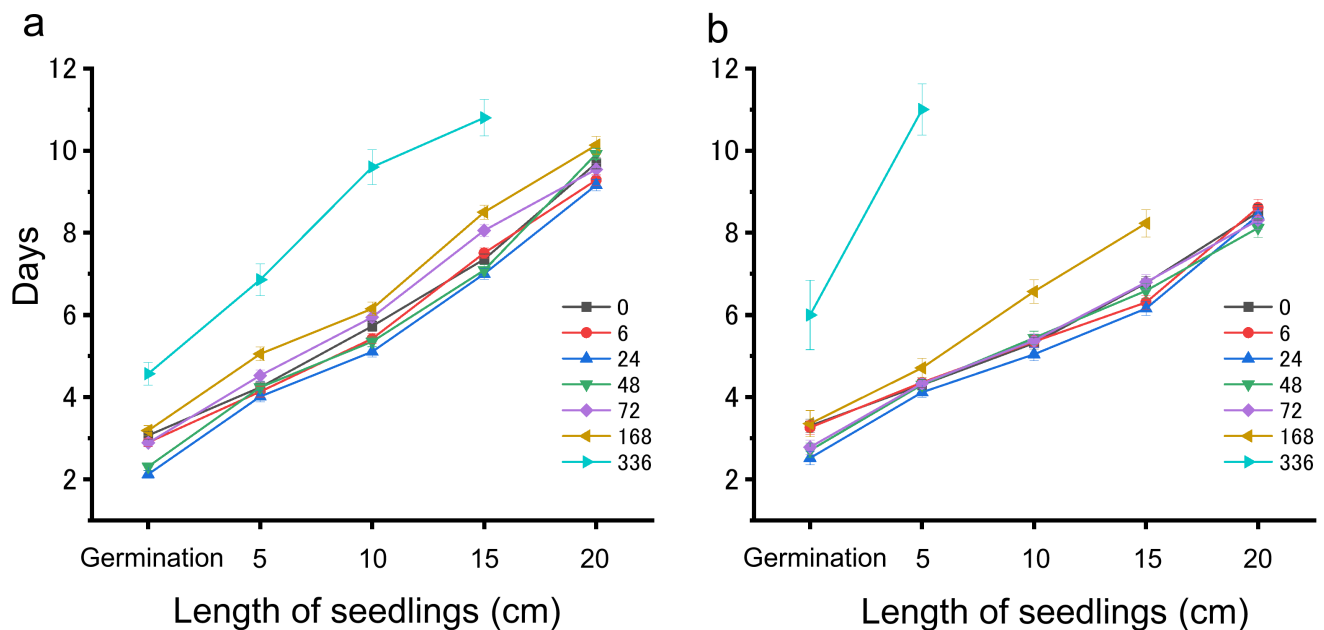


Figure 2

Effects of submersion duration on seedling development in two wheat cultivars, Satonosora (a) and Yumeakari (b). Time required for seedlings to reach target leaf lengths under different submersion treatments is shown. Values represent mean $\pm$ SE. Sample sizes are shown in Fig. 3. Values represent means of three independent experiments, each using 20 seeds. Each experiment used 20 seeds, and data from three independent experiments were pooled.

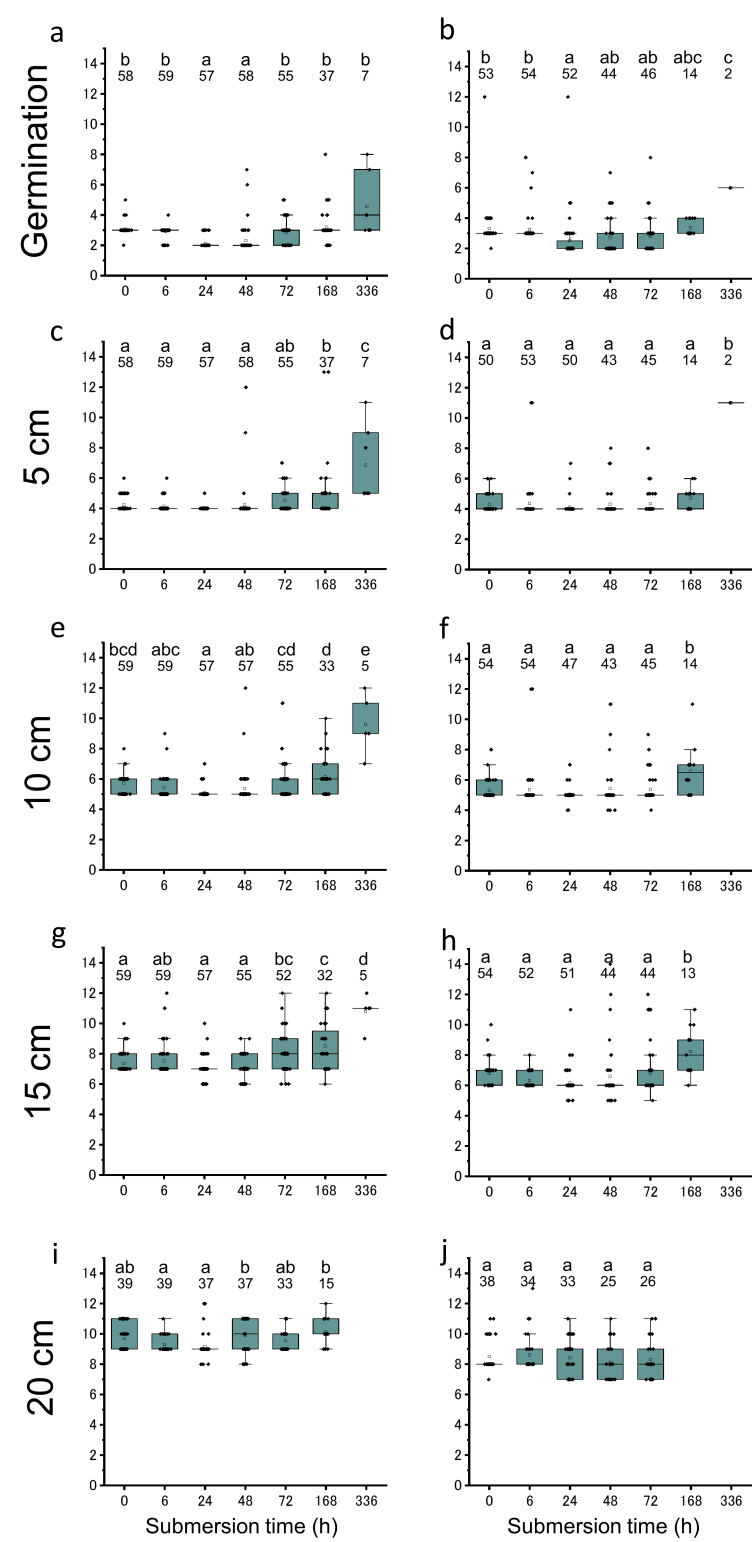


Figure 3

Effects of submersion duration on germination timing and seedling development in two wheat cultivars, Satonosora (a, c, e, g and i) and Yumeakari (b, d, f, h and j). (a, b) Time to germination under different submersion durations. (c–j) Number of days required for seedlings to reach leaf lengths of 5, 10, 15, and 20 cm. Each box plot displays the median value with the ends of the boxes representing the 25th and 75th percentiles, and the ends of the lines representing the 10th and 90th percentiles. Different letters above each box indicate significant differences at $P < 0.05$ (Tukey–Kramer HSD test). Numbers in figures indicate sample sizes

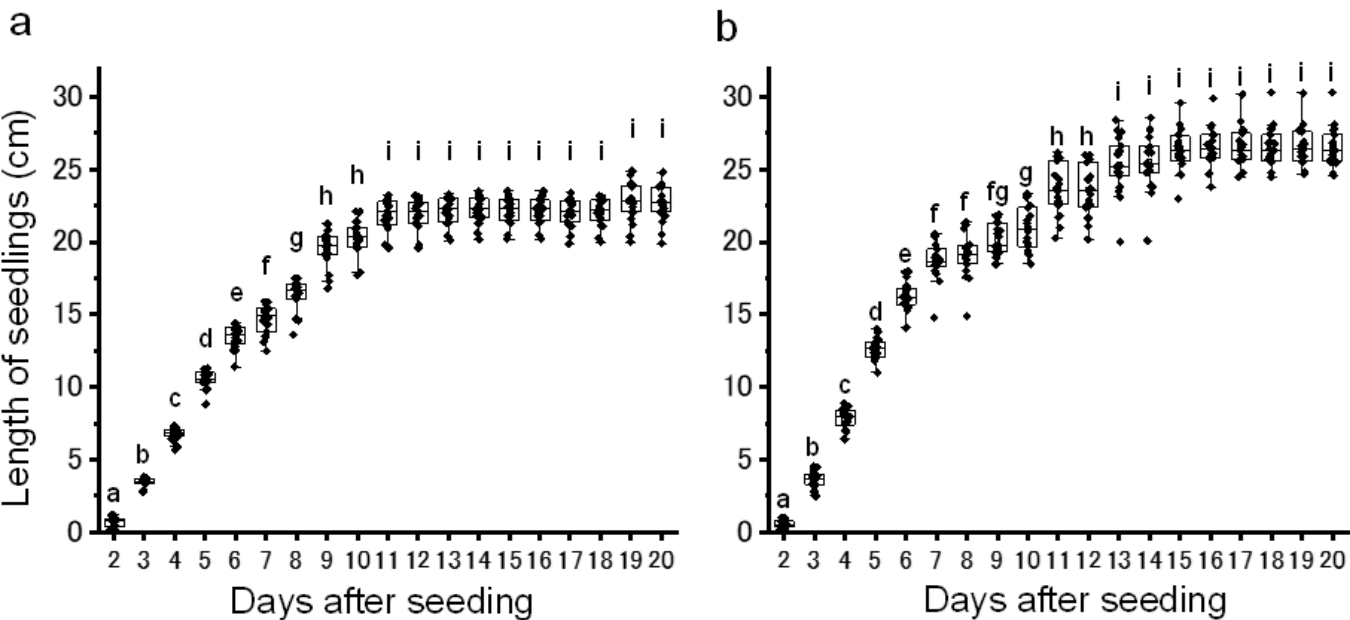


Figure 4

Daily changes in maximum leaf length of wheat seedlings following 24 hour submersion treatment in two wheat cultivars, Satonosora (a) and Yumeakari (b). Each box plot displays the median value with the ends of the boxes representing the 25th and 75th percentiles, and the ends of the lines representing the 10th and 90th percentiles. Different letters above each box indicate significant differences at $P < 0.05$ (Tukey–Kramer HSD test). Each experiment used 20 seeds, and data from three independent experiments were pooled.

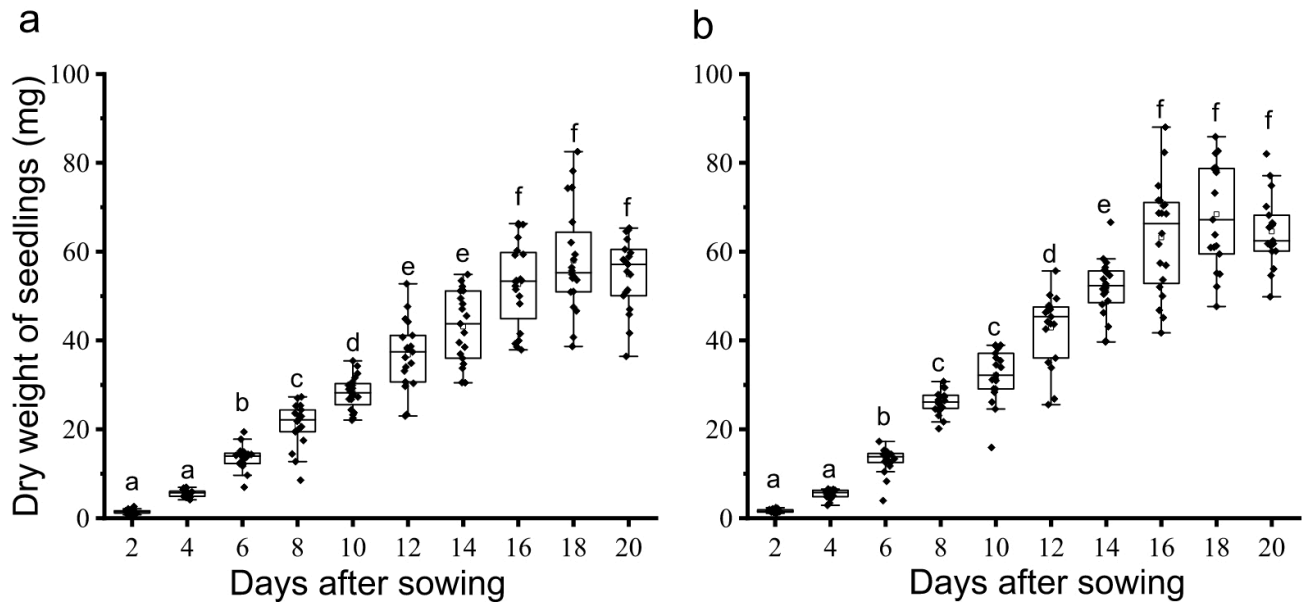


Figure 5

Daily changes in dry weight of wheat seedlings following 24 hour submersion treatment in two wheat cultivars, Satonosora (a) and Yumeakari (b). Each box plot displays the median value with the ends of the boxes representing the 25th and 75th percentiles, and the ends of the lines representing the 10th and 90th percentiles. Different letters above each box indicate significant differences at $P < 0.05$ (Tukey-Kramer HSD test). Each experiment used 20 seeds, and data from three independent experiments were pooled.