Drought escape during the late growth stage through early recovery from initial drying stress by hydropriming of upland rice

Yoshihiro Nakaoa, Minoru Yoshino, Kisho Miyamoto, Shin Yabuta, Rieko Kamiok, Keisuke Hatanaka and Jun-Ichi Sakagami

International Cooperation Agency, Uganda Office, Kampala, Uganda; 3Department of Agricultural Sciences and Natural Resources, Graduate School of Agriculture, Kagoshima University, Kagoshima, Japan

ABSTRACT

This study investigated the interactions between soil moisture conditions and seed priming on initial and late growth over 2 years (2017–2018) through field trials and container experiments with regulated soil moisture. Field trials were conducted on rainfed upland rice fields in Uganda, East Africa, where primed and control seeds were planted in triplicate and cultivated. In 2017, the percentage of first heading (head emergence) of hills was higher in priming treatments, and the time for 20% of the hills to achieve the first heading (H20) was significantly earlier than in controls. Additionally, grain ratio (number of fertile grains to sterile grains) and H20 were negatively correlated (P < 0.05). However, the difference in growth parameters between control and priming was not found in the case of 2018. The results suggested that priming reduces the growth period under certain conditions and improves drought escape. Therefore, we investigated the relationship between priming effects on initial growth under soil moisture treatments and subsequent development under waterlogged conditions. After transfer to waterlogged conditions, plants of the primed seeds initially grown in low soil moisture conditions recovered earlier than control plants. Our research concludes that the agronomical impact of hydropriming on upland rice prevents prolonged plant growth under drought by early recovery. Furthermore, it could decrease the yield loss caused by reduced rainfall in the late growth stage.

ARTICLE HISTORY

Received 23 August 2021
Revised 7 January 2022
Accepted 1 March 2022

KEYWORDS

Rice; rainfed; africa; direct sowing; NERICA

Introduction

Rice is the most grown food commodity in sub-Saharan Africa (SSA), and demand is expected to grow (Africe Rice Center, 2011). Therefore, it is essential to develop optimal rice cultivation methods that suit local environmental, agricultural, and economic conditions. In SSA, rice is cultivated on dry land (38%), rainfed wetlands (33%), deep-water or mangrove swamps (9%), and irrigated wetlands (20%: Balasubramanian et al., 2007), meaning that 71% of rice cultivation is conducted in rainfed fields. When water availability is low, and the cost of labor is high, direct seeding is recommended for rice cultivation (Pandey & Velasco, 2005). However, the poor establishment of rice plants restricts the
approval of this technique (Farooq et al., 2011). Also, rainfall distribution can be inadequate in good dryland rice-growing areas because of unpredictable drought (Balasubramanian et al., 2007). In central Uganda, rainfall averages 400–700 mm per rice cultivation season (Miyamoto et al., 2012). The minimum water requirement of rice varieties used in East Africa is 311–400 mm per season (Matsumoto et al., 2014), but the productivity can be low. Therefore, technical improvement is required for rainfed rice cultivation.

Seed priming is a simple, low-cost, low-risk method that improves seedling growth (Harris et al., 2001, 2002) by promoting enzyme activity, starch degradation (Farooq et al., 2006a; Lee & Kim, 2000), and stress responses (Chen & Arora, 2013; Jisha et al., 2013). There are many pre-sowing treatments for enhancing the initial plant growth, but hydrop Dimitrion is preferred for its low-cost and beneficial effects (Soltani & Soltani, 2015). Therefore, researchers have applied hydrop Dimitrion techniques in SSA to improve rice growth and yield (Bina et al., 2012; Harris et al., 2001; Nakao et al., 2018). Previous studies revealed that seed-priming affects initial and late growth stages. The higher yield of rice plants from primed seeds was attributed to earlier seedling establishment, which correlated with subsequent plant growth, allometry, and early flowering in field trials (Farooq et al., 2006a, 2006b; Harris et al., 2002). However, recent studies on the initial growth stage suggested that hydrop Dimitrion efficacy on initial growth depends on the soil moisture (Matsushima & Sakagami, 2013; Nakao et al., 2020), suggesting that mechanisms causing higher yields of primed seeds are more diverse and complicated. It is not well known how plants from primed seeds in middle and late growth stages are affected by differential priming effects of initial growth in an upland field. Hence, Harris et al. (2001) reported that hydrop Dimitrion treatment could not always bring related results in on-farm trials. Therefore, this study clarified the interaction between soil moisture conditions and hydro-seed-priming effects on plant growth. Further, we investigated the relationship between hydrop Dimitrion effects on initial and late growth stages through field trials and container experiment under-regulated soil moisture conditions.

Materials and methods

Plant material and seed hydrop Dimitrion treatment

Interspecific hybrids between Oryza sativa and Oryza glaberrima, known as NERICA4, were used. First, seed quality was determined using a salt solution with 1.17 specific gravity. Next, seeds were washed in water and air-dried. Then, as Nakao et al. (2018) explained, hydrop Dimitrion was performed, consisting of soaking the seeds in 25°C tap water for 24 h and air drying to their original weight.

Field trials in Uganda

Field trials were conducted during the second rainy season of 2017 and 2018 at rainfed upland rice fields in Uganda (0°30′46.1"N, 32°38′03.3"E). Basal fertilizers were applied before sowing (N:P:K = 60:60:60 kg ha⁻¹). By randomised block design, primed and control seeds were planted in triplicate on September 7, 2017, and September 14, 2018. Each block was 3 × 3 m², containing five seeds sown 1 cm deep in the hole at a 15 × 30 cm density, and weeds were manually controlled. Daily temperatures and rainfall were recorded. Then, five hills were used to measure plant length and tiller number in each treatment, and 21 hills were used to measure the first heading (head emergence) and yield. Plant length and tiller number of five hills were measured fortnightly 4 weeks after sowing. The first heading was determined by the date of the first emergence of the panicle. Time taken for 20% and 50% of hills to achieve the first heading (H20 and H50, respectively) was calculated using the formula of time to achieve 50% germination with modification (Matsushima & Sakagami, 2013):

\[ H20 = t_1 + \frac{(N/5 - n_i) \times (t_j - t_1)}{(n_j - n_i)} \]
\[ H50 = t_1 + \frac{(N/2 - n_i) \times (t_j - t_1)}{(n_j - n_i)} \]

Where N is the total number of hills, and \( n_i \) and \( n_j \) are the cumulative number of hills headed, as determined by adjacent counts at times \( t_1 \) and \( t_j \) respectively, when \( n_i < N/5 < n_j \) and \( n_i < N/2 < n_j \).

Rice plants were harvested on January 4, 2018, and January 23, 2019, and yield properties of all 21 hills were recorded. Panicles with stem length less than 50% of the average stem length or sterile grains more than 80% of the total grains were separated as late emerged heads. The grain ratio was calculated as the ratio of fertile grains to sterile grains.

Container experiment in regulated soil moisture conditions

A container experiment was conducted using plastic containers in a greenhouse at Kagoshima University, Japan. Twelve plastic containers (length: 107 cm, width: 73 cm, height: 30 cm) were filled with air-dried mountain soil after removing gravel using a sieve. The three soil moisture treatments were repeated four
times each by randomized block design. Each container was divided in half: one side for primed seeds and the other for control seeds. Five seeds were sown 1 cm deep into a hole at a 17.5 × 24.0 cm density (nine hills in each area). Primed and control seeds were planted on June 4, and plants were harvested on October 8. The average volumetric moisture content of soil in containers was 11.2% ± 0.4% at 4 days before sowing, and 8, 10, and 14 L of water was irrigated in low, middle, and high treatments by the sowing date. For the first 21 days after sowing, soil moisture conditions were maintained at three levels: low, middle, and high treatments through irrigation with 2–5, 4, and 5–7 L every 2 days, respectively. All containers were gradually saturated from 22 DAS and ultimately waterlogged at 24 DAS. Then, soil water potential (pF) and volumetric moisture content (%) were recorded using DIK8343 (Daiki Rika Kogyo Co., Ltd.) and STE sensor (Decagon Inc.), respectively, with the sensor buried 10 cm underground. The average pF of each treatment was 1.69, 2.51, and 2.76, respectively. The daily air temperature and relative humidity were recorded using a wireless thermometer RTR502 (T&D Corporation). The average temperature and relative humidities were 27.5°C and 74.2%, respectively. Seedling emergence was observed every day until 14 DAS, and the seedling establishment observed was 21 DAS. Mean seedling emergence time (MET) was calculated according to the following formula from Ranal and De Santana (2006):

\[
\bar{t}(MET) = \frac{\sum_{i=1}^{k} (n_i \times t_i)}{\sum_{i=1}^{k} n_i}
\]

Where \(t_i\) is the time from the start of the experiment to the \(i^{th}\) observation (day or hour), \(n_i\) is the number of seedling emergence in time \(i\) (not the accumulated number, but the number corresponding to the \(i^{th}\) observation), and \(k\) is the last time of seedling emergence.

Growth parameters of all nine hills were recorded. Plant length and tiller number were determined weekly. From 28 DAS, plant length, tiller number, and leaf age were noted fortnightly until 56 DAS. We noted the total number of leaves on the main stem and calculated the leaf age index as a percentage of leaf age to the final leaf number on the main stem at 28, 42, and 56 DAS. The growth parameter is the multiplication of plant length and tiller number (estimated biomass production), corresponding to biomass production (Suenobu et al., 1994). The first heading was also noted as before. The heading date was determined as the date when 50% of effective tiller headed. The yield properties of the nine hills were recorded on October 8.

**Data analysis**

We conducted t-tests, Tukey’s tests, and regression analyses using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) Statistics v. 26 (International Business Machines Corp.) and Excel 2016 (Microsoft Corp.).

**Results**

**Field trials**

Daily rainfall and average temperature from September to January 2017 and 2018 are shown in Figure 1. Total rainfall was lower in 2017 than in 2018 (Table 1). Regarding 2017, cumulative rainfall 1–15 and 16–30 DAS was 89 mm and 5 mm (Table 1). In 2018, cumulative rainfall 1–15 DAS was 3 mm. Subsequently, 45 mm of rainfall was found during 16–30 DAS. Cumulative rainfall levels 31–60, 61–90, and 91–120 DAS were lower in 2017 than in 2018 (Table 1; Figure 1). In the field trials, plant length and tiller number did not differ significantly between

![Figure 1. Daily rainfall and average temperature from September to January 2017 (A) and 2018 (B).](image-url)
Table 1. Amount of rainfall (mm) in 15-day increments from the first day after planting to 30 days after planting, and 30-day increments from 31 days after planting to 4 months after planting of field trials in 2017 and 2018.

| Year | 1–15 | 16–30 | 31–60 | 61–90 | 91–120 | Total |
|------|------|-------|-------|-------|--------|-------|
| 2017 | 89   | 5     | 68    | 131   | 14     | 308   |
| 2018 | 3    | 45    | 191   | 142   | 64     | 444   |

This table shows the results of field experiments.

Primed and control treatments in 2017 and 2018 when counted 4 weeks after sowing (Figure 2). The plant length and tiller number increased at larger rates in 2018 than in 2017, with a significant difference in the rate seen 6 weeks after sowing (Figure 2). Figure 3 compares the first heading of primed and control seeds in 2017 (A) and 2018 (B) by the percentage of the first heading of hills over time. In 2017, the first heading of hills of primed seeds started earlier than the control plants, but significant differences were not found between priming and control (Figure 3A). Table 2 shows the time taken for 20% (H20) and 50% (H50) of primed and control hills to achieve the first heading. Also, the plants of primed seeds reached H20 significantly earlier in 2017 compared to the control plants. The number of late emerged heads per hill was higher in control hills than primed, with a significant difference in 2017 (Table 2). Control plants also displayed a higher occurrence of sterile grains than plants of primed seeds, with a significant difference found in 2017 (Table 2). Grain ratios (number of fertile versus sterile grains) and yields were higher in primed seeds than controls, although no statistically significant differences were discovered in 2017 (Table 2). A significant negative correlation (P < 0.05, r = −0.840) was found between H20 and grain ratio in 2017 (Figure 4). Table 3 shows the amount of rainfall that occurred within 14 days before and after H20 (H20 ± 14) and between 14 and 42 days after sowing.
during and H20 treatments both experienced after H20 (H20 + 14 ~ +42). In 2017, the control experienced less rainfall than the priming treatments during H20 ± 14. After that, the amount of rainfall in both treatments was reduced. The total rainfall at H20 ± 14 was similar in the control and priming treatments in 2018. In 2018, rainfall between H20 + 14 and H20 + 42 was equal (61 mm) in control and priming treatments.

### Table 2. Time for 20% (H20) and 50% (H50) hills to achieve the first heading, late emerged head, number of fertile grains, number of sterile grains, grain ratio (number of fertile grains per sterile grains), and yield of field trials in 2017 and 2018.

|          | 2017 | 2018 |
|----------|------|------|
|          | Control | Priming | Control | Priming |
| H20 (days) | 80    | 76   | 87    | 86    | ns |
| H50 (days) | 83    | 80   | 91    | 91    | ns |
| Late emerged head (/hill) | 1.94 | 0.78 | 1.11 | 0.38 | ns |
| Number of sterile grains (/hill) | 363 | 214 | 516 | 451 | ns |
| Number of fertile grains (/hill) | 228 | 272 | 1737 | 1422 | ns |
| Grain ratio (fertile/ sterile grains) | 0.63 | 1.36 | 3.37 | 3.23 | ns |
| Yield (g/m²) | 86 | 121 | 581 | 428 | ns |

* indicates significant difference (P < 0.05), and ns indicates the non-significant differences between control and priming treatments, determined by t-test.

**Figure 4.** Relationship between time for 20% of hills to achieve the first heading (H20) and grain ratio of fertility to sterility in 2017. * indicates that the correlation coefficient is significant (P < 0.05). This figure shows results of field trials.

**Container experiment**

Information on the growing environment, including daily average air temperature, humidity, and soil moisture conditions, is shown in Figures 5 and 6. In the container experiment, seedling emergence percentage at 7 and 14 DAS and seedling establishment percentage at 21 DAS were higher in priming treatments than controls for all moisture conditions (Table 4). Plant length at 7, 14, and 21 DAS also appeared to be higher in priming treatments than controls for all soil moisture conditions. However, significant differences in plant length were found only at 7 DAS and 21 DAS in high soil moisture and 21 DAS in middle soil moisture conditions. Regarding plant lengths 28 and 42 DAS, seedlings previously grown in low soil moisture conditions for 21 days were significantly shorter than seedlings grown in other soils (Table 5). However, plants of primed seeds grown in low soil moisture conditions were notably longer than their control counterparts (Table 5). Low soil moisture seedlings had significantly fewer tillers than other soil types 28 DAS (Table 5). At 42 DAS, tiller number of plants of primed seeds in low soil moisture conditions improved, but control plants still had the least (P < 0.05; Table 5). Daily estimated biomass production was determined by the multiplication of plant length and tiller number. Under low soil moisture conditions, the estimated biomass production of seedlings was lower at 21–28 DAS relative to other soil treatments (Table 5). Estimated biomass production of the low soil moisture seedlings increased over time, but the control was still significantly lower than other seedlings grown under higher soil moisture within 28–42 DAS. However, at 42–56 DAS, all seedlings grown under low soil moisture conditions maintained a significantly higher estimated biomass production than seedlings grown in
Figure 6. Change of soil volumetric moisture content (A) and water potential (B) during treatment period of container experiment. Triangles, squares, and circles indicate high, middle, and low soil moisture treatment, respectively. Bars indicate standard deviation.

Table 4. Seedling emergence percentage 7, 14 DAS, seedling establish percentage 21 DAS, mean seedling emergence time (MET), and plant length 7, 14, and 21 DAS of control and priming treatments in high, middle, and low soil moisture conditions of container experiment.

|                         | High soil moisture | Middle soil moisture | Low soil moisture |
|-------------------------|--------------------|----------------------|------------------|
|                         | Control Priming    | Control Priming      | Control Priming  |
| Seedling emergence (%)  | 7 DAS 85.0 a       | 14 DAS 86.7 ab       | 21 DAS 86.7 a    |
|                         | 42 DAS 58 a        | 55 a                 | 42 c             |
| Seedling establishment  | 21 DAS 86.7 a      | 21 DAS 89.6 a        | 21 DAS 89.6 a    |
| ( % )                  | 5.5 a              | 6.0 a                | 5.4 a            |
| MET ( DAS )            | 21 DAS 36.8 a      | 21 DAS 38.3 **       | 21 DAS 31.4 a    |
| Plant length (cm)      | 7 DAS 5.9 a        | 4.1 a                | 1.2 c            |
|                         | 14 DAS 21.4 a      | 17.6 a               | 11.7 ns          |
|                         | 21 DAS 36.8 a      | 29.7 a               | 19.4 ns          |

* and ** indicate a significant difference (P < 0.05 and P < 0.01) and ns indicate non-significant difference between control and priming treatments, determined by t-test.

Table 5. Plant length and tiller number after 7, 14, and 21 DAS, and plant length × tiller number 1–28, 28–42, and 42–58 DAS of control and priming plots in high, middle, and low soil moisture conditions of container experiment.

|                          | High soil moisture | Middle soil moisture | Low soil moisture |
|--------------------------|--------------------|----------------------|------------------|
|                          | Control Priming    | Control Priming      | Control Priming  |
| Plant length (cm)        | 28 DAS 58 a        | 55 a                 | 42 c             |
|                         | 42 DAS 58 a        | 62 a                 | 42 c             |
|                         | 56 DAS 58 a        | 125 a                | 42 c             |
| Tiller number (/hill)    | 28 DAS 13 a        | 10 b                 | 6 c              |
|                         | 42 DAS 21 a        | 12 ab                | 16 b             |
|                         | 56 DAS 19 a        | 19 a                 | 18 a             |
| Plant length × Tiller number (/day) | 21–28 DAS 58 a | 42 abc               | 23 c             |
|                         | 28–42 DAS 91 ab    | 98 ab                | 61 b             |
|                         | 42–56 DAS 32 b     | 22 b                 | 72 a             |

Different alphabet characters indicate significant differences (P < 0.05) between all treatments as determined by Tukey’s test on each day after sowing.

high and middle soil moisture treatments (Table 5). Leafage index (leaf age/final number of leaves on the main stem) and percentage of the first heading of hills were higher in priming treatments than controls in low soil moisture conditions (Figure 7). Leaf age index was lower in the controls than the primed treatments, with significant differences at 28 and 42 DAS (Figure 7A). Similarly, H20 and H50 values of priming were lower than control in low soil moisture conditions, with a significant difference between the two treatments at H20 (Table 6). The yield difference between control and priming was not found in soil moisture treatment (Table 7). As soil moisture decreased from high to low, the percentage of ripened grains and yields increased and cumulative temperature decreased (Table 7).

Discussion
Through the 2 years of field trials, the weather was completely different between 2017 and 2018. Therefore, we expected that seed priming is effective in certain conditions. In the field trials, cumulative
Figure 7. Change of leaf age index (leaf age/final leaf number of the main stem) (A) and the percentage of the first heading of hills (B) over time (days after sowing). White triangles = high soil moisture control (HC), black triangles = high soil moisture priming (HP), white squares = middle soil moisture control (MC), black squares = middle soil moisture priming (MP), white circles = low soil moisture priming (LP), and black circles = low soil moisture control (LC). * indicates a significant difference \( P < 0.05 \) between the control and priming in the low soil moisture treatment, determined by the t-test. This figure shows results of container experiment.

Table 6. Time for 20% (H20) and 50% (H50) hills to achieve the first heading in high, middle, and low soil moisture treatments of container experiment.

|          | High soil moisture | Middle soil moisture | Low soil moisture |
|----------|--------------------|----------------------|------------------|
|          | Control            | Priming              | Control          | Priming |
| H20 (days) | 68.8               | 68.4                 | ns               |         |
| H50 (days) | 70.0               | 69.7                 | ns               |         |

* indicates a significant difference \( P < 0.05 \) and ns indicates non-significant difference between control and priming treatments, determined by t-test.

Table 7. Days to heading, yield components, and cumulative temperature and maximum temperature during 10 days from heading of control and priming treatments in high, middle, and low soil moisture conditions of the container experiment.

|          | Days to heading | Panicle number (hill) | Grain number (panicle) | Ripened grains (%) | 1000 grains weight (g) | Yield (g/m²) | Cumulative temperature during 10 days from heading (°C) | Maximum temperature during 10 days from heading (°C) |
|----------|-----------------|-----------------------|-----------------------|---------------------|------------------------|-------------|------------------------------------------------------|--------------------------------------------------|
| High soil moisture | Control    | 73.1               | b                     | 10.0                | a                      | 91.3        | a                                     | 0.38                           | 24.8       | a           | 196.9                | 284.8       | a           | 39.9                                     |
|          | Priming        | 72.9               | b                     | 10.9                | a                      | 98.6        | a                                     | 0.33                           | 24.9       | b           | 207.5                | 285.1       | a           | 39.9                                     |
|          | Control        | 75.4               | b                     | 9.1                 | a                      | 81.3        | a                                     | 0.44                           | 25.1       | ab          | 211.8                | 279.3       | ab          | 38.6                                     |
|          | Priming        | 75.1               | b                     | 9.7                 | a                      | 85.0        | a                                     | 0.43                           | 25.3       | ab          | 189.5                | 279.5       | ab          | 38.6                                     |
| Middle soil moisture | Control | 81.7               | a                     | 11.3                | a                      | 104.1       | a                                     | 0.57                           | 25.4       | a           | 383.4                | 275.9       | b           | 37.6                                     |
|          | Priming        | 79.9               | a                     | 11.2                | a                      | 90.7        | a                                     | 0.58                           | 26.0       | a           | 331.5                | 276.9       | b           | 37.6                                     |

Different alphabet characters indicate significant differences \( P < 0.05 \) between all treatments as determined by Tukey’s test.

Rainfall 1–15 DAS and 16–30 DAS were completely different between 2017 and 2018. In 2017, plants that emerged were exposed to less rainfall conditions during 16–30 DAS followed by less rainfall during 31–60 DAS. However, in 2018, cumulative rainfall 1–15 DAS was 3 mm, and plants could not emerge. Subsequently, most plants started growing from 16 DAS. As mentioned above, rainfall patterns affected plant growth, but it was complicated in the field conditions. In 2017, 68 mm of rain fell between 31 and 60 DAS, which is about one-third of 191 mm that fell in 2018 during that period (Table 1), suggesting that tiller number and plant length are affected by the amount of rainfall that occurs during the plant’s vegetative stage. In 2017, there was less rain 91–120 DAS than in 2018 (Table 1), showing that rice plants were exposed to more severe drought during the reproductive stage in 2017 than in 2018. This explains why the grain yield was lower in 2017 than in 2018 (Table 2). Drought in the grain-filling period negatively affects rice yield (Zhang et al., 2018). Drought stress-
induced by reduced rainfall between H20 + 14 and H20 + 42 during the grain-filling period may have negatively affected grain yield in 2017. On the other hand, rainfall may have mitigated the drought effect on yields in 2018 than 2017.

Differences in growth and grain properties between priming treatments and controls were not observed in 2018, but in 2017 (Table 2): H20, late emerged head, and the number of sterile grains showed significantly lower values in priming treatments than in the controls, likely because of priming. Rice plants are most vulnerable to drought stress 20–30 days before heading and 10 days after heading (Matsushima, 1962). Further, soil moisture stress during the booting stage partly overlapped at H20 ± 14 days in this study, which decreases the ripening ratio and yield of NERICA varieties (Iwata-Higuchi et al., 2019). H20 control and priming treatments differed significantly in 2017 (Table 2) and total rainfall at H20 ± 14 was lower in control than priming treatments in 2017 (Table 3), suggesting that rainfall affects different grain properties of control and priming treatments. The negative correlation between grain ratio and H20 in 2017 (Figure 4) possibly suggests that earlier heading of priming reduced grain yield loss. Drought avoidance derived from a short-growing variety is used in areas with limited water availability (Bernier et al., 2008). In NERICA varieties, a short-growth period is an essential trait for escaping drought stress (WARDA, (Africa Rice Center), FAO, SAA, 2008). Binang et al. (2012) suggested that early plant maturation by seed priming is advantageous for escaping drought. The growth-shortening effects of seed priming on an early ripening cultivar were observed in our study, suggesting that the treatment allowed the plants to avoid severely dry conditions in a late rainy season, which overlapped with the period of heading in rainfed uplands.

Container experiments were conducted to investigate the differential efficacy of primed seed of field trials in 2017 and 2018. As mentioned above, the rainfall pattern during 1–30 DAS was complicated in the field trials. In the container experiment, completely different plant growth behavior was made by soil moisture treatments. The low soil moisture treatment simulates the drought of field trials during 16–30 DAS in 2017. This low soil moisture treatment for 21 days sufficiently suppressed the initial and middle-stage plant growth behavior, and traits affected by priming differed depending on soil moisture conditions (Tables 4, 5). The effects of priming on seedling emergence diminish with increasing or decreasing soil moisture conditions (Matsushima & Sakagami, 2013). However, even when there was no significant difference in seedling emergence between control and priming treatments in severely dry conditions, primed seeds grew better than control seeds after seedling emergence (Nakao et al., 2018). This suggests that priming contributed to root elongation before seedling emergence (Matsushima & Sakagami, 2013; Nakao et al., 2018). Alternatively, seed-priming efficacy diminished with increasing soil moisture content (Matsushima & Sakagami, 2013; Nakao et al., 2020). When seeds were grown in excess water conditions, priming affected the scavenging of reactive oxygen species and mobilization of carbohydrates (Ella et al., 2011). Therefore, priming efficacy and mechanism on initial growth depends on soil moisture. In this study, seedling emergence and growth appeared to be morphologically improved by priming treatment in all soil moisture conditions at 21 DAS, with significant differences found in middle and high soil moisture conditions (Table 4). In the low soil moisture condition, priming appeared to improve seedling emergence percentage and plant length, although the difference was not significant (Table 4). Priming may not enhance shoot growth, but the root elongation in diminished soil moisture conditions as supported by references (Matsushima & Sakagami, 2013; Nakao et al., 2018), which is important for extracting water from dry soils and adjusting osmotic status (Fukai & Cooper, 1995). This implies priming can mitigate low soil moisture stress.

When plants were subsequently subjected to waterlogged conditions, growth differences between priming and control appeared only in low soil moisture conditions significantly, which was subjected to 21 days low soil moisture at the initial growth stage (Table 5, Table 6, Figure 7). However, in high and middle soil moisture conditions, late plant growth was not affected by the initial plant growth difference. In the low soil moisture condition, which was subjected to 21 days of low soil moisture at the initial growth stage, tiller number, plant length, and the product thereof recovered to the same level as high and middle soil moisture conditions significantly earlier in priming treatments than in controls (Table 5). The growth stage, the progression of vegetative growth, and final leaf number of the main stem were different between treatments. Therefore, we showed growth stage as leaf age index calculated from the ratio of leaf age and final leaf number of the main stem. The higher leaf age index indicates further progression of vegetative growth. Under low soil moisture treatment, leaf age index was lower than other soil moisture treatments and leaf age index in priming was higher than in the control (Figure 7A). Further, the percentage of the first heading of hills (Figure 7B) was higher in priming treatments than in controls. Hence, H20 of priming treatments was also significantly earlier than controls (Table 6), similarly with the field trial result.
in 2017 (Figure 3A). However, priming effects on initial growth in middle and high soil moisture conditions do not affect late growth in waterlogged conditions, similarly with the result of the field trial in 2018. Recent field trials with continuous irrigation suggested that earlier establishment of seedlings affected subsequent plant growth and allometry (Farooq et al., 2006a) and led to improved yield and yield-related factors (Farooq et al., 2006b). Plants of primed seeds flowered and matured earlier, contributing to a higher yield (Binang et al., 2012; Farooq et al., 2006a; Harris et al., 2002). In our research, low soil moisture during initial growth reduced the length and tiller number and prolonged rice plant growth period even if soil moisture conditions improved in the late growth stage (Table 5). However, our research recently suggested that plant length and tiller number recovered earlier, and the growth period was reduced in the plant from primed seeds (Table 5, Table 6, and Figure 7). The yield difference was not found between control and priming in any soil moisture treatment (Table 7) because environmental factors are strongly affected plant growth. The yield difference between soil moisture treatments is explained by the ratio of ripened grain affected by high temperatures during the reproductive stage. High temperature is a well-known factor related to sterility (Krishnan et al., 2011). In the case of indica rice varieties, grain fertility decreased as the temperature increased from 29°C to 41°C at flowering (Satake & Yoshida, 1978). In this study, the ratio of the ripened grain of low soil moisture treatment was higher than other soil moisture treatments because the plants of low soil moisture treatment escaped high temperature during 10 days after heading, caused by their delayed growth. If plants were faced with drought at the beginning of the late growth period as rainfed upland field trials, the yield of low soil moisture treatment would decrease drastically caused by delayed plant growth.

If plants were affected by drought stress during the initial growth stage, priming primarily affected seed metabolism (e.g. enzyme activity, starch degradation, and drought resistance; Chen & Arora, 2013; Farooq et al., 2006a; Jisha et al., 2013; Lee & Kim, 2000) and root development (Matsushima & Sakagami, 2013; Zheng et al., 2016). In this study, plants of primed seeds grown under low soil moisture conditions of container showed earlier recovery (Table 5) and shortened plant growth period (Table 6, Figure 7), similarly with the field trial result in 2017. However, plants grew better in high and middle soil moisture conditions regardless of priming, similar to the field trial result in 2018. These results suggested that priming effects on late growth stage plant growth appear under certain conditions. Hydropriming treatments enhance plant growth under moderately dry conditions (Matsushima & Sakagami, 2013), but the relationship between priming effects and soil moisture conditions was slightly different depending on genotypes (Nakao et al., 2020). Further, the potential of higher priming efficacy was shown in the dry condition in the case of interspecific progeny between O. sativa and O. glaberrima (Nakao et al., 2020). Therefore, the relationship between priming effects on late growth and the environment would also differ depending on the varieties. Consequently, the agronomical significance of hydropromising may be not only to improve plant growth and yield but to prevent prolonged plant growth by early recovering.

Conclusion

We conducted rainfed field trials and container experiments with regulated soil moisture conditions to investigate the effects of seed hydropromising on initial and late-stage growth in upland rice. Field trials in 2017 suggested that the H20, late emerged head, and the number of sterile grains was improved by seed priming. However, priming effects did not appear on the field trial in 2018, indicating that the effects of priming seem to vary depending on the environment. Container experiments suggested that priming affects all soil moisture conditions at the initial growth stage; still, the improved initial growth of plants of primed seeds under low soil moisture conditions led to better subsequent development under waterlogged conditions. Both growth of plants from control and primed seeds are satisfactory in middle and high soil moisture conditions in container experiments, like the field trial in 2018. However, plants of primed seeds initially grown in low soil moisture conditions recovered well after re-watering. They showed more significant improvements in leaf age index and percentage of the first heading of hills than controls, echoing the field experiment results in 2017. This study concludes that priming prevents prolonged plant growth under drought stress by earlier recovering.

Acknowledgments

We are grateful to Drs. Godfrey Asea, Jiro Nozaka, and Mrs. Nobuki Kojima and Hiroyuki Hanada for their continuous support and helpful comments during the early drafting of this paper. Additionally, we would like to thank Enago (www.enago.jp) for the English language review.

Disclosure statement

No potential conflict of interest was reported by the author(s).
Funding

This research was conducted with financial support from the ‘Tobitate Ryugaku Japan’ program by the Ministry of Education, Culture, Sports, Science, and Technology of Japan; the Japan International Cooperation Agency internship program; ‘Shinshu no Seishin’ support fund of Kagoshima University; and Japan Society for the Promotion of Science (JSPS) KAKENHI [Grant Number JP20J11707].

ORCID

Yoshihiro Nakao http://orcid.org/0000-0001-7597-6962

References

Africe Rice Center. (2011). Boosting Africa’s Rice Sector: A research for development strategy 2011–2020. Africa Rice Center.

Balasubramanian, V., Sie, M., Hjimans, R. J., & Otsuka, K. (2007). Increasing rice production in Sub-Saharan Africa: Challenges and opportunities. Advances in Agronomy, 94, 55–133. https://doi.org/10.1016/S0065-2113(06)94002-4

Bernier, J., Atlin, G. N., Serra, R., Kumar, A., & Spaner, D. (2008). Breeding upland rice for drought resistance. Journal of the Science of Food and Agriculture, 88(6), 927–939. https://doi.org/10.1002/jsfa.3153

Binang, W. B., Shiyam, J. O., & Ntia, J. D. (2012). Effect of seed priming method on agronomic performance and cost effectiveness of rainfed, dry-seeded NERICA rice. Research Journal of Seed Science, 5(4), 136–143. https://doi.org/10.3923/rjss.2012.136.143

Chen, K., & Arora, R. (2013). Priming memory invokes seed stress-tolerance. Environmental and Experimental Botany, 94, 33–45. https://doi.org/10.1016/j.envexpbot.2012.03.005

Ella, E. S., Dionisio-Sese, M. L., & Ismail, A. M. (2011). Seed pre-treatment in rice reduces damage, enhances carbohydrate mobilization and improves germination and seedling establishment under flooded conditions. AoB Plants, 2011, 1–11. https://doi.org/10.1093/aobpla/plr007

Farooq, M., Barsa, S. M. A., & Wahid, A. (2006a). Priming of field-sown rice seed enhances germination, seedling establishment, allometry and yield. Plant Growth Regulation, 49(2–3), 285–294. https://doi.org/10.1007/s10725-006-9138-y

Farooq, M., Barsa, S. M. A., Tabassum, R., & Afzal, I. (2006b). Enhancing the performance of direct seeded fine rice by seed priming. Plant Production Science, 9(4), 446–456. https://doi.org/10.1626/pps.9.446

Farooq, M., Siddique, K. H. M., Rehman, H., Aziz, T., Lee, D. J., & Wahid, A. (2011). Rice direct seeding: Experiences, challenges and opportunities. Soil and Tillage Research, 111(2), 87–98. https://doi.org/10.1016/j.still.2010.10.008

Fukai, S., & Cooper, M. (1995). Development of drought-resistant cultivars using physiological traits in rice. Field Crops Research, 40(2), 67–86. https://doi.org/10.1016/0378-4290(94)00096-U

Harris, D., Pathan, A. K., Gothkar, P., Joshi, A., Chivasa, W., & Nyamudeza, P. (2001). On-farm seed priming: Using participatory methods to revive and refine a key technology. Agricultural Systems, 69(1–2), 151–164. https://doi.org/10.1016/S0308-521X(01)00023-3

Harris, D., Tripathi, R. S., & Joshi, A. (2002). On-farm seed priming to improve establishment and yield in dry direct seeded rice. In S. Pandey, M. Mortimer, & L. Wade (Eds.), Direct seeding: Research strategies and opportunities p. 231–240. International Rice Research Institute.

Iwata-higuchi, M., Sakagami, J., & Maruyama, S. (2019). Effect of soil moisture stress at panicle development stage on growth and yield of upland NERICA cultivars. Tropical Agriculture and Development, 63(3), 140–149. https://doi.org/10.11248/jsta.63.140

Jisha, K. C., Vijayakumari, K., & Puthur, J. T. (2013). Seed priming for abiotic stress tolerance: An overview. Acta Physiologica Plantarum, 35(5), 1381–1396. https://doi.org/10.1007/s11738-012-1186-5

Krishnan, P., Ramakrishnan, B., Reddy, K. R., & Reddy, V. R. (2011). High-temperature effects on rice growth, yield, and grain quality. Advances in Agronomy, 111, 87–206. https://doi.org/10.1016/B978-0-12-387689-8.00004-7

Lee, S. S., & Kim, J. H. (2000). Total sugars, α-amylase activity, and germination after priming of normal and aged rice seeds. Korean Journal of Crop Science, 45(2), 108–111. https://www.koreascience.or.kr/article/JAKO200001192227398.pdf

Matsumoto, S., Tsuboi, T., Asea, G., Maruyama, A., Kikuchi, M., & Takagaki, M. (2014). Water response of upland rice varieties adopted in sub-Saharan Africa: A water application experiment. Rice Research: Open Access, 2(1), 121. https://doi.org/10.4172/jrr.1000121

Matsushima, S. (1962). Some experiments on soil water-plant relationship in the cultivation rice. Proceedings of the Crop Science Society of Japan, 31(2), 115–121. https://doi.org/10.1626/jcs.31.115

Matsushima, K.-I., & Sakagami, J.-I. (2013). Effects of seed hydropriming on germination and seedling vigor during emergence of rice under different soil moisture conditions. American Journal of Plant Sciences, 4(8), 1584–1593. https://doi.org/10.4236/ajps.2013.48191

Miyamoto, K., Maruyama, A., Haneishi, Y., Matsumoto, S., Tsuboi, T., Asea, G., Okello, S., Takagaki, M., & Kikuchi, M. (2012). NERICA cultivation and its yield determinants: The case of upland rice farmers in Namulonge, Central Uganda. Journal of Agricultural Science, 4(6), 120–135. https://doi.org/10.5539/jas.v4n6p120

Nakao, Y., Asea, G., Yoshino, M., Kojima, N., Hanada, H., Miyamoto, K., Yabuta, S., Kamioka, R., & Sakagami, J.-I. (2018). Development of hydropriming techniques for sowing seeds of upland rice in Uganda. American Journal of Plant Sciences, 9(11), 2170–2182. https://doi.org/10.4236/ajps.2018.911157

Nakao, Y., Sone, C., & Sakagami, J.-I. (2020). Genetic diversity of hydro Priming effects on rice seed emergence and subsequent growth under different moisture conditions. Genes (Basel), 11(9), 994–1006. https://doi.org/10.3390/genes11090994

Pandey, S., & Velasco, L. (2005). Trends in crop establishment methods in Asia and research issues. In K. Toriyama, K. L. Heong, & B. Hardy (Eds.), Rice is life: Scientific perspectives for the 21st century. Proceedings of the Woerd Rice Research Conference. International Rice Research Institute. Japan International Research Center for Agricultural Sciences, 4–7 November 2004.
Ranal, M. A., & De Santana, D. G. (2006). How and why to measure the germination process? *Revista Brasileira de Botânica*, 29(1), 1–11. https://doi.org/10.1590/S0100-84042006000100002

Satake, T., & Yoshida, S. (1978). High temperature -induced sterility in indica rice at flowering. *Japanese Journal of Crop Science*, 47(1), 6–17. https://doi.org/10.1626/jcs.47.6

Soltani, E., & Soltani, A. (2015). Meta-analysis of seed priming effects on seed germination, seedling emergence and crop yield: Iranian studies. *International Journal of Plant Production*, 9(3), 413–432. https://doi.org/10.22069/ijpp.2015.2224

Suenobu, S., Kadoshige, K., Yamamoto, T., & Inoue, K. (1994). Nitrogen nutritional diagnosis of rice cultivar “HINOHIKARI”. *Bull. Fukuoka Agriculture Research Center*, A-13, 5–8. http://www.farc.pref.fukuoka.jp/farc/kenpo/A-13.pdf

Zhang, J., Zhang, S., Cheng, M., Jiang, H., Zhang, X., Peng, C., Lu, X., Zhang, M., & Jin, J. (2018). Effect of drought on agronomic traits of rice and wheat: A meta-analysis. *International Journal of Environmental Research and Public Health*, 15(5), 839. https://doi.org/10.3390/ijerph15050839

Zheng, M., Tao, Y., Hussain, S., Jiang, Q., Peng, S., Huang, J., Cui, K., & Nie, L. (2016). Seed priming in dry direct-seeded rice: Consequences for emergence, seedling growth and associated metabolic events under drought stress. *Plant Growth Regulation*, 78(2), 167–178. https://doi.org/10.1007/s10725-015-0083-5