

## Effect of water priming on the seed moisture content and the rooting of soybean [*Glycine max* (L.) Merr.] 'Fukuyutaka' seeds under non-irrigated conditions

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### Abstract

This study aimed to clarify the effect of water priming on the seed moisture and the rooting rate of soybean [*Glycine max* (L.) Merr.] 'Fukuyutaka' for developing a cultivation method, where soybean seeds can directly be sown on the meadow or grassland for livestock production. The weight of each soybean seed was measured before and after they were subjected to: no treatment (control); and, two kinds of priming treatments, soaking (2 and 4 h), and moisture absorption (3, 12, and 24 h). The weight of seeds was additionally measured after keeping them at a specified temperature (28–30°C) and humidity (58–60%) for 4 h, 1, 2, and 3 days. The moisture content of soybean seeds was calculated based on their weights pre- and post-treatment. The rooting rate of the seeds was investigated after each priming (control, soaking, and moisture absorption), 48 h of standing and irrigation treatment. The seed moisture content of soybean seeds increased immediately after priming (soaking or moisture absorption treatment), however, after standing, the moisture content in both treatments decreased to the same level as that of control. The rooting rate in both priming pre-treatments was similar to or lower than that in control, indicating that neither soaking nor moisture absorption of soybean seeds had a keeping moisture content of seeds for long time which promoting effect on rooting of soybean under dry condition on meadow or grassland.

**Keywords:** Fukuyutaka; Priming; Rooting; Seed moisture; Soybean.

**Abbreviations:** CP\_crude protein.

### Introduction

Tropical grasses mainly utilized in southwestern Japan. Though it increased the yield of dry matter as they advanced through the growth stages, the crude protein (CP) content decreased remarkably (Minson, 1950). The reduction CP content is one of causes of reproductive disorders and retardation of growth and development in livestock. Therefore, it is desirable to search for and introduce new crops that can be used as high-protein roughages in Japan. Recently, soybean [*Glycine max* (L.) Merr.] silage has attracted attention as a new high-protein source in cold regions of Japan, and a cultivation method has been established for growing soybean by using the temperate Italian ryegrass (*Lolium multiflorum*) as a living mulch (Kaneko et al., 2011, Uchino et al., 2016). In the case of the southwestern region of Japan, the previous studies reported that soybean can potentially be used as a forage by living mulch (Prasojo et al., 2019) and for intercropping with Rhodes grass (*Cloris gayana* kunth.), which is one of the tropical grasses (Prasojo et

al., 2022). Since the nutritional value of the tropical grasses decreases after 2nd cutting, soybean is expected to improve the CP content of tropical grass after 1st cutting.

Tillage cultivation, partial or non-till cultivation, requires machinery, which is an economic burden on the farmer. Thus, developing a cultivation method where soybean seeds can directly be sown on the meadow or grassland (overseeding) after the 1st cutting without tillage would easily produce soybean for fodder with broadcast spreader that are only used in the normal forage production system.

When seeds are spread on the surface layer of meadow or grassland, the soil does not adhere closely to the seeds. Thus, the area around the seeds is prone to dry conditions, unless there is a prolonged natural rainfall that maintains the moisture content of the seeds at a level required for germination. Therefore, to regulate the moisture content of seeds, soaking or moisture absorption as a priming treatment before

**Table 1.** Rooting rates of soybean seeds after series of each priming, standing and irrigation treatments.

Priming treatment	Rooting rate (%)
Control	40.0±0.0 <sup>a</sup>
Moisture absorption	
M3h	26.7±5.8 <sup>b</sup>
M12h	40.0±0.0 <sup>a</sup>
M24h	30.0±0.0 <sup>b</sup>
Soaking	
S2h	30.0±0.0 <sup>b</sup>
S4h	33.3±5.8 <sup>ab</sup>

Means values with the different letters in the column are significantly different at  $P < 0.05$  among priming treatments by Tukey's HSD test. Priming treatments were moisture absorption for 3h(M3h), 12h(M12h) and 24h(M24h) and soaking for 2h(S2h) and 4h(S4h).

sowing on grassland could be effective for rooting and germination. Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops, particularly seeds of vegetables and small seeded grasses (Heydecker and Coolbaer 197; Bradford 19867). Harris et al., (2001a) reported that the direct benefits of seed priming in all crops included faster emergence, better, more and uniform status, less need to re-sow, more vigorous plants, better drought tolerance, earlier flowering, earlier harvest and higher grain yield. However, there is no previous study focus on the effect of water priming on the soybean seed under non-irrigated conditions.

The aim of this study is to identify the effect of water priming on the moisture content and the rooting of soybean 'Fukuyutaka' seeds when sown on the surface of ground under non-irrigated conditions.

## Results and Discussion

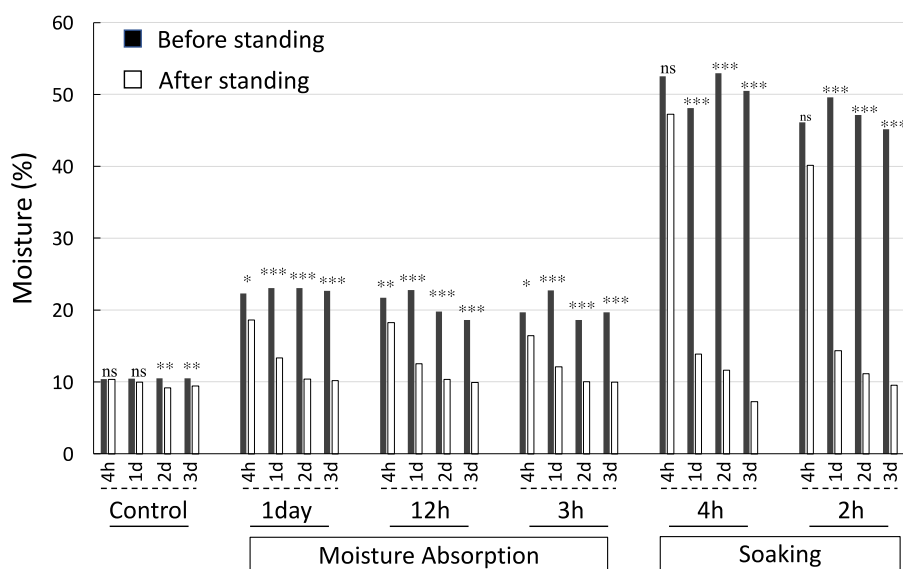
Figure 1 shows the transition of seed moisture content in soybean seeds after priming (soaking and moisture absorption treatment) and after standing treatment. In the case of soaking treatments (S2h and S4h), the seed moisture content of soybean seeds reached to approximately 50%. The absorbed moisture content by soaking treatment decreased after 4 h of standing, however there was no significant difference between before and after the seed standing. In the case of 1 day of standing, the moisture content in seeds decreased drastically, and after 3 days of incubation, the seed moisture content was approximately 10%, which was the same as at pre-pretreatment. In the case of treatment with moisture absorption treatment for 3, 12, and 24 h (M3h, M12h and M24h), moisture content of soybean seeds increased to approximately 20%. The moisture content then decreased with time after the seeds were allowed to stand, and after 3 days of standing, moisture content decreased up to 10%. Thus, the seed moisture content of soybean seeds increased immediately after priming (soaking or moisture absorption treatment), however, after 3 days of incubation, the moisture content in both treatments decreased to the same level as that of control

(untreated soybean seeds). If moisture in soil is

adequate, the size of seed doubles within 24 hours of planting, and seed moisture content increases up to 50% (Larry et al., 2014). Subsequently, the radical (root) is the first structure to emerge from the germinating seed, which usually occurs within 48 hours of planting under optimum conditions (Larry et al., 2014). The radical grows downward rapidly and can provide moisture to the germinating seed if the soil surrounding the seed becomes dry. If the soil dries around the radical before it reaches soil moisture, the seed will most likely die (Larry et al., 2014).

In Table 1, the rooting rate of soybean seeds after series of each priming (soaking, moisture absorption, and control), 2 days (48 h) of standing, and irrigation treatment are summarized. The rooting rate at M3h, M12h, and M24h moisture absorption treatments was 26.7%, 40.0% and 30.0%, respectively. The moisture content at control (40%) was significantly higher at M12h than in the M3h and M24h treatments. The rooting rate at S2h and S4h was 30% and 33.3%, respectively, which was significantly lower at S2h than in control. The rooting rate in both priming pre-treatments was similar to or lower than that in control, indicating that neither soaking nor moisture absorption of soybean seeds had a keeping moisture content of seeds for long time which promoting effect on rooting of soybean.

Harris and Jones (1997) showed that water priming for 12-24 h made the germination time reduced as 50% in rice cultivars from West Africa. Similarly, the early seeding emergence as an effect of water priming were reported in several other crops such as sorghum, millet, cotton, beans and maize (Harris 1996; Harris et al., 1999; Murungu et al., 2004). On the other hand, in case of soybean, Kazem et al (2011) reported biological and grain yields per unit area and also harvest index were statistically similar for plants from water-primed and unprimed seeds. They reported germination percentage, seedling dry weight and field emergence percentage decreased, and mean emergence time increased, due to seed priming in soybean. Kering et al. (2015) also reported that water priming led to a high reduction in seeding emergence



**Figure 1.** Seed moisture transition under non-irrigated condition (standing treatment). Asterisk indicates significantly difference (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; ns, not significant) in the paired t-test between pre- and post-treatment; and dotted- and solid line shows the period of standing and priming, respectively. Priming treatments were moisture absorption for 3h, 12h and 24h and soaking for 2h and 4h as solid underlined. Standing treatment time were 4h, 1, 2, and 3 days under a specified room temperature (28–30°C) and humidity (58–60%) as broken underlined.

in large-grain variety (>20 g/100 seeds; MFL-159, and V07-1897). Small-grain (between 12 and 16 g/100 seeds; Glenn, and V03-4705) and medium-grain variety (<10g/100 seeds; MFS-561 and V08-4773), on the other hand, showed no reduction in seeding emergence. One hundred seeds of soybean ‘Fukuyutaka’ (Japanese cultivar) used in this study weighed 32 g, which is classified as medium-grain by national standards in Japan, but is larger than the large-grain variety according to a previous study (Kering et al., 2015). In the case of this large-grain variety, even if seed moisture increases with water or moisture absorption treatments before sowing, the seed moisture content will decrease when the seeds are subsequently exposed to dry conditions (Figure 1), resulting in a lower rooting rate (Table 1).

Based on the above, in this study, we demonstrated that when soybean seeds are treated for moisture regulation by priming and seed dispersal to the surface layer of grassland was assumed, the seed moisture content required (50%) for germination decreases less than 20% (Figure 1), and subsequently limits the rooting and germination process, unless a continuous rainfall for a longer period is ensured. These results indicate the moisture retention capacity of soybean is important factor for germination after the water priming. Therefore, it is necessary to investigate the seed moisture content of different grain sizes (small-grain lines) and their germination traits under non-irrigated conditions, and thus, develop a novel moisture retention technology that enables keeping moisture content, make rooting and establishment after sowing seeds on the grassland surface.

## Material and Methods

### Plant material

In this study, the soybean cultivar ‘Fukuyutaka’ was

used. The cultivar has been promoted in the warm regions of southwest Japan. The selected cultivar had a medium-grain variety (32g/100seeds) with a late maturity trait.

### Water priming treatments

For the moisture content, to identify the effect of water priming, the weight of each ten soybean seeds as replicates was measured and then subjected to two priming treatments: priming treatment with soaking (2 and 4 h: named “S2h” and “S4h”); and priming treatment with moisture absorption (3, 12, and 24 h: named “M3h”, “M12h” and “M24h”) and non-treatment named as “control”. In the soaking priming treatment, the seeds were placed in a water-filled plastic container (8 cm × 11.5 cm × 6.5 cm), such that all the seeds were submerged. In the moisture absorption priming treatment, a water-filled plastic container was used, where a 60-hole divider was placed at a height of 5.5 cm such that the test seeds placed on the divider would not touch the water, and covered with a lid. After both soaking and moisture absorption, the weight of each seed was measured again. The seeds were allowed to stand for 4 h, 1, 2, and 3 days under a specified room temperature (28–30°C) and humidity (58–60%), and were weighed again after each standing. The moisture content of seeds was calculated based on the pre- and post-treated weight. The trial was conducted for 3 times as replication.

As rooting rate test, ten seeds was placed on soil-filled in trays (volume 20ml) and irrigated with 10 ml of water. The soil type was andosol consist of nitrogen (0.2% N), double superphosphate (0.13% P<sub>2</sub>O<sub>5</sub>) and potassium chloride (0.18% K<sub>2</sub>O). The number of seeds with roots was counted after 2 days. The trial was conducted for 3 times as replications.

### Statistical analysis

Seed moisture content was analyzed at each level of treatment level with a paired t-test between pre-standing treatment (after priming treatment) and post-standing treatment. A Tukey test was applied for rooting rates among all levels. Seed moisture content and rooting rate as a percentage were transferred to angular figures according to Claringbold (1953) before stactical analysis. Statistical processing was performed using the statistical software R (R Core Team 2014).

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