Effects of fruit skin and water temperature during soaking before germination on the emergence rates of common buckwheat

Mikio Sekinuma[1], Futoshi Kato[2], Futoshi Sasaki[3], Satomi Takeda[1], Naoto Inoue[1] and Shigemitsu Kasuga[1]

[1]*Faculty of Agriculture, Shinshu University, Minamiminowa, Nagano 399-4598, Japan*

[2]Present address : *College of Bioresource Sciences, Nihon University, Fujisawa, 1866 Kameino, Kanagawa 252-0880, Japan*

[3]Present address : *Aomori Prefectural Industrial Technology Research Center, Kuroishi, Tanaka, Aomori 036-0522, Japan*

Summary

The studies focused effects of the presence of fruit skin (hull or pericarp) and water temperature during water soaking before germination on the emergence rates of common buckwheat (*Fagophyrum esculentum* Moench) after seeding. We aimed to understand the mechanisms underlying the poor emergence rates that have been observed after water flooding before germination. Shinano No. 1 was exposed to water soaking treatments at temperatures of 10, 15, 20, or 25°C for 3 days. After soaking, the seeds were grown at 20°C, and the percentages of emerged seedlings were investigated 7 days after seeding. Dehulled seeds and normal seeds were soaked for 4 days at 25°C, and the contents of dissolved oxygen were measured in the water. The emergence rates of normal intact seeds (fruits) decreased significantly ($p < 0.01$) with an increase in soaking water temperature before germination. The emergence rates of dehulled seeds improved after removing the fruit skin, and it reached 70%, even at 25°C. Soaking treatment for 96 h at 25°C decreased the emergence rates of normal seeds and dehulled seeds. The emergence rate of normal seeds was significantly lower than that of dehulled seeds ($p < 0.001$). Moreover, there was no difference in the emergence rates of dehulled seeds between plots with or without daily water replacement. The contents of dissolved oxygen in the water at 25°C did not differ between normal seeds and dehulled seeds after 50 h. These results suggested that high temperatures affected embryo viability and that the relative mechanical resistance of the fruit skin on the embryo directly affected the seed emergence rate.

Key words : Dehulling, Emergence, *Fagophyrum esculentum*, Fruit skin, Water temperature

Introduction

Flooding after heavy rains drastically reduces seedling establishment rate and early growth in common buckwheat (*Fagophyrum esculentum* Moench). Nishimaki (1983) and Bjorkman (2001) have reported that common buckwheat is more sensitive to waterlogging after sowing. Murayama *et al.* (2004) has reported a varietal difference in flood tolerance before the germination stage among cultivars. In addition, Sakata and Ohsawa (2005, 2006) have described varietal differences in flood tolerance during the germination period. These reports of varietal differences in flood tolerance suggest that morphological, ecological, and physiological mechanisms are related to the different viabilities.

In the fields located in the highlands of Shinshu district, the establishment rates and yields of common buckwheat were decreased drastically after heavy rains in early June, during the seedlings had not yet

Received January 20, 2015
Accepted February 2, 2015

established. Field investigations of seed establishment in the fields of the Education and Reserch Center of Alpine Field Science (AFC) satellite station at Nobeyama, Nagano Pref. have suggested that the soil and air temperature during the developmental stage before germination are very important for establishment and early growth (Inoue *et al*., unpublished data).

However, decreased germination rates have been observed after the traditional treatments of soaking the fruits in the cold river streams of the Shinshu district for maintaining the food quality (Shindoh *et al*., 2001). The water temperature of the stream was usually maintained at 2-4°C during the 1 week of treatment in winter, and the germination rate decreased slightly (Inoue *et al*., unpublished data). However, it decreased significantly during the freeze-drying treatment under the cold winter wind after soaking treatment in the cold-water stream (Inoue *et al*., unpublished data). We observed that the air temperature during the freeze-drying treatment fluctuated from −1°C to 10°C at Chino city, Shinshu, and the germination rate decreased to 40%. The final recorded germination rate was about 50% after the freeze-drying treatment (Inoue *et al*., unpublished data). The results of the traditional cool-water soaking treatment after harvesting and air drying suggested that the viability of the fruit, which is the seed with pericarp, was damaged by the higher air temperature that occurred during freezing and melting.

The fruit skin (hull or pericarp) that covers the fruit plays a major role in the physical and chemical defense systems in most higher plants. The fruit skin of common buckwheat contains many antioxidant compounds that protect the fruit from environmental stresses (Watanabe *et al*., 1997 ; Watanabe, 1998). Watanabe *et al*. (1997) isolated the major compounds, including rutin, quercetin, hyperin, protocatechuic acid, and 3,4-dihydroxybenzaldehyde, in fruit skin, and suggested that the isolated flavonoids in the fruits act as protectants against oxidative damage. The fruit skin of common buckwheat has stronger antioxidant activity under aerobic conditions. However, the effects of fruit skin on the viability of the inner seed is unknown under the anaerobic conditions that occur during the restricted airflow in water.

This study aimed to evaluate the effects of water soaking before germination on the emergence rates after seeding in order to understand the poor seedling emergence and establishment after exposure to flooding by heavy rains before germination. In particular, we examined the effects of the presence of fruit skin and water temperature on seedling emergence rates.

Materials and Methods

Experiment 1 : Effects of fruit skin and soaking water temperature

Shinano No. 1, which is an intermediate agroecotypical variety in Japan, was used to investigate the effects of fruit skin and water temperature on the emergence rate. The seeds harvested in Nagano, Japan in 2010 and 2011, were used for emergence test in 2011-2012. Soaking treatments with distilled water were conducted for 3 days in a transparent plastic box (50×50×100 mm), and the temperature was maintained at 10, 15, 20, or 25°C in incubator. Fifty pretreated seeds were sown in a seedling case (500 × 400 × 50 mm) containing the upland soil from the AFC fields for seedling growth. All experiments were performed in triplicate. After the soaking treatments, the seeds were grown at 20°C in incubator, sufficient soil humidity that was maintained by a clear plastic film placed on the case, and the emerged seedlings with fully developed cotyledons were investigated 7 days after seeding.

The dehulled seeds and the fruit skin were added to the water to determine the effects of the water -soluble materials, eluted from the fruit skin, on the emergence rates of dehulled seeds. Normal seeds, dehulled seeds, and dehulled seeds that were added with fruit skin that was separated by dehulling were soaked at 25°C for 72 h in water. Unless otherwise mentioned, each treatment was performed in triplicate with 100 seeds per replicate and the amount of fruit skin that was separated as byproducts by the dehulling was equivalent to the amount of 100 seeds.

Experiment 2 : Effects of soaking water exchange

Dehulled seeds and normal seeds were soaked in distilled water and incubated for 96 h in a transparent plastic box (50×50×100 mm) in incubator that was maintained at 25°C. Two plots were provided to observe the effects of water quality during the soaking treatment on seed viability and the emergence rate after seeding in the soil beds. The soaking water was not replaced in one plot, while it was replaced every day in the other plot. The water temperature in each plot was maintained at 25°C during the soaking treatment. The 50 pretreated seeds were sown in triplicate in a seedling case (500×400×50 mm), as described in experiment 1.

In the soaking treatment condition in which the water was not changed, the contents of dissolved oxygen in the water at 25°C were measured with a fluorescence sensor module (Model FO-960S, Automatic System Research Co., Ltd., Saitama, Japan) during the 72 h. Each treatment was triplicated with 100 seeds per replicate. Number of fruit skin as by-products from dehulling was 100 seeds. In the present study, seeds with normal or abnormal morphological characteristics were not separated at the time of seeding or during the counting of established seeds.

Results

The emergence rate of normal intact seeds was lower than the treated seeds at both 20°C and 25°C than at 10°C and 15°C, and decreased significantly ($p < 0.01$) with an increase in the soaking water temperature before emergence (Fig. 1). However, the emergence rate of dehulled seeds was improved by removing the pericarp, reaching 70%, even at 25°C (Fig. 2). There were no significant differences among the treatments at 10, 15, and 20°C. The relationship between soaking water temperature and the emergence rate is shown in Fig. 3.

Soaking treatment for 96 h at 25°C decreased the emergence rates from normal seeds and dehulled seeds (Fig. 4). The emergence rate from normal seeds was significantly lower than that of dehulled seeds ($p < 0.001$). However, there was no difference in the emergence rate from the dehulled seeds between the plots with and without daily water replacement. However, the water exchange treatment significantly improved the rate in normal seeds by about 10% ($p < 0.05$).

The contents of dissolved oxygen in the water at 25°C decreased drastically in the first 12 h of the experiment with unchanged soaking water (Fig. 5). There was no significant difference in the contents of dissolved oxygen from 50 h after soaking between normal seeds and dehulled seeds.

The emergence rate of dehulled seeds did not significantly differ from the rate in the plot with removed fruit skins in addition to dehulled seed (Fig. 6). The emergence rate was not decreased by

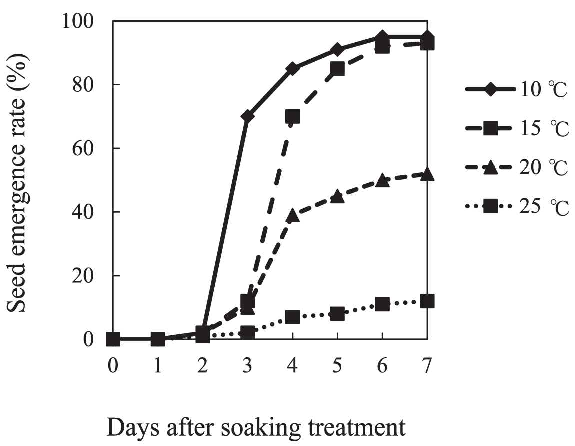

Fig. 1 Rate of emergence of normal seeds according to water temperature for 72 h of soaking before germination.

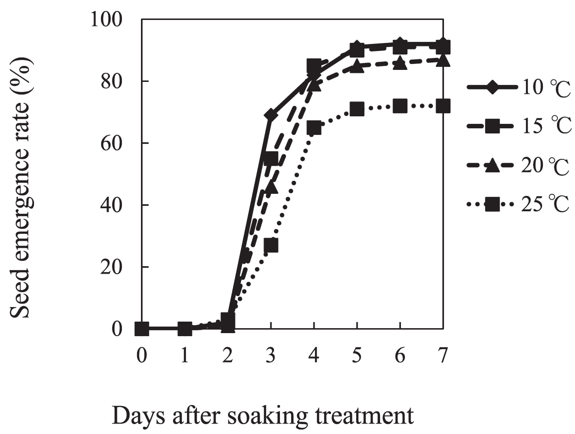

Fig. 2 Rate of emergence of dehulled seeds according to water temperature for 72 h of soaking before germination.

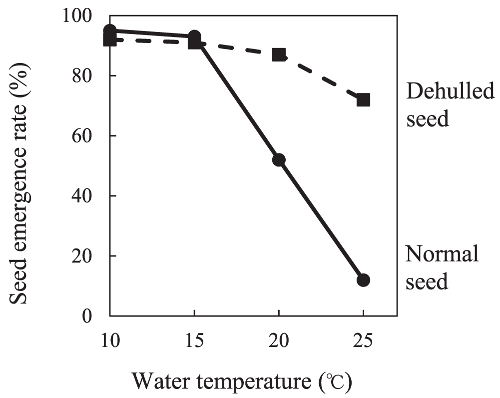

Fig. 3 Effects on the seed emergence rate of water temperature after 72 h of soaking before germination.

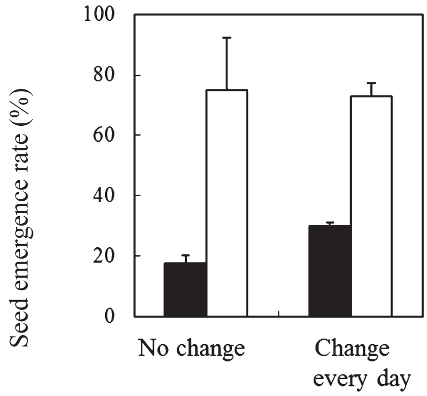

Fig. 4 Effects of fruit skin for 96 h soaking in 25°C water before germination on seed emergence rate.
■ : Normal seed, □ : Dehulled seed
* : The rate of normal seeds was lower significantly than dehulled seeds ($p < 0.001$).

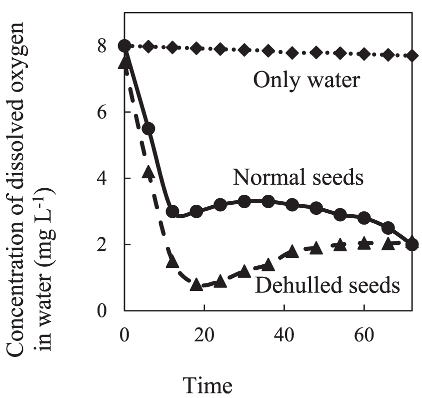

Fig. 5 Change in the contents of dissolved oxygen during soaking in 25°C water before germination .

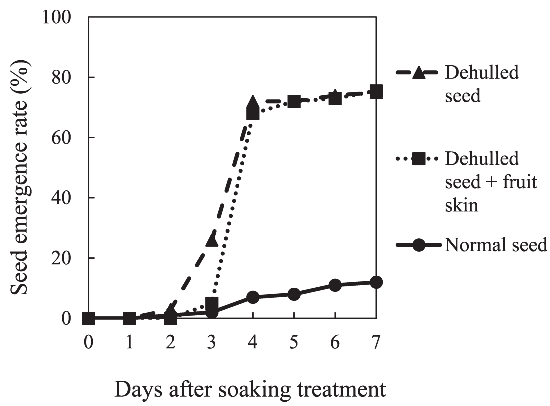

Fig. 6 Effects of dehulling on the emergence rate after soaking for 72 h at 25°C.

removing the fruit skin, and the emergence rate reached a final value of about 75%, even at 25°C. In contrast, the emergence rate of the normal seeds was decreased by only 12%.

Discussion

Dehulling enhanced the emergence rate after water soaking compared to the results of Figs. 2, 3, and 4. In particular, after 96 h of the soaking, the emergence rate did not decrease remarkably in the dehulled seed plots. In the treated seeds, changes in the seed surface color and mold formation were not observed. Iimura and Hosono (1998) have shown that common buckwheat seeds are contaminated by a large number of bacteria. The antifungal activities of the bacteria that are endemic to buckwheat seeds protect the germinating seeds from contaminating fungi. Murata *et al*. (2013) reported that soaking the fruit in cold water is a famous traditional method that is used in Japan to maintain seed viability, which increases the contents of γ-amino-n-butyric acid in seeds without fruit skin, and improve its food quality. It has been inferred that the viability of seeds is not decreased by fungal growth during the soaking conditions. The results of the present study and those of previous reports suggest that the inhibition of seed viability by fungal growth is not a major factor in flooding stress due to heavy rains after seeding.

The emergence of dehulled seeds was not inhibited by the removal of the fruit skins (Fig. 6). Common buckwheat secretes allelopathic materials that inhibit the activity of weeds (Tominaga and Uezu, 1995). Kalinova (2007) has shown that common buckwheat fruit skin contains allelopathic substances that inhibit the growth of *Lactuca sativa*. Gallic acid and (+) -catechin in the buckwheat plant body have also shown strong activity related to the growth (Iqbal *et al*., 2003), and the fruit skin contains a significant amount of catechins and other flavonoids that contribute to antioxidant activity (Watanabe, 1997, 1998). The results of the current study and those of previous reports suggest that the antioxidant constituents of the fruit skin protects the seed against oxidative damage and that these constituents do not limit the germination and growth of the seed itself.

In *Spinacia oleracea* that is the same caryophyllales as buckwheat, a water-soluble germination inhibitor is present in the fruit skin, and it can be readily removed by soaking treatment (Suganuma and Ohono, 1984). Elucidations about germination inhibitors were effective for improving germination. However, our results suggested that there was no water-soluble intensive germination inhibitor in buckwheat fruit skin. Fig. 5 shows that the results of the dissolved oxygen contents during water soaking, which indicates that the embryo's requirement for oxygen did not differ because of the presence of fruit skin from 2 days after soaking.

Elucrdations

Soaking water temperature above 15°C inhibited the rate of emergency in both normal and dehulled seeds, while removing the fruit skin drastically increased the inhibiting effect. These results suggested that the high temperature affected the embryo and that the relative mechanical resistance of the fruit skin on the embryo under the soaking conditions seemed to directly affect seed germination, resulting in poor germination and emergence.

Common buckwheat greatly accumulates water-soluble proteins in the seed coat and cotyledons to increase osmotic pressure, and it appears to be developed a cold tolerance in the fruit for the moist winters in mountainous conditions in Asia. The water-soluble proteins that are eluted under the fruit skin in warm water may decrease the relative mechanical resistance of the seed by the fruit skin. In the future, the effects of water temperature and the materials eluted from the seed on relative mechanical resistance need to be examined.

References

Bjorkman T (2001) Causes of poor stand establishment after heavy rains. *Proceedings of the 8th International Symposium on Buckwheat, Chunchon, Korea*. pp. 134-137.

Iimura K and Hosono A (1998) Antifungal activities of bacteria endemic to buckwheat seeds. Fagopyrum 15 : 42 -54.

Iqbal Z, Hiradate S, Noda A, Isojima S and Fujii Y (2003) Allelopathic activity of buckwheat : isolation and characterization of phenolics. Weed Science 51 : 657-662.

Kalinova J (2007) Allelopathic effect of buckwheat (*Fagophyrum esculentum* Moench). *Advances in Buckwheat Research-Proceedings of the 10th International Symposium on Buckwheat, Yangling, China*. pp. 233-237.

Murata Y, Inoue N, Sasaki F and Sekinuma M (2013) A new method of soba noodle preparation by using dough made from dehulled, water-soaked, and directly kneaded seeds without dry milling. *Fagopyrum* 30 : 57-62.

Murayama S, Suyama Y and Yanokuchi Y (2004) Varietal difference of pre-germination flooding tolerance in buckwheat. *Proceedings of the 9th International Symposium on Buckwheat, Prague, Czech*. pp. 143-145.

Nishimaki, Kiyoshi (1983) Present status of buckwheat cultivation and its technical problems. *Nougyo Oyobi Engei* 58 : 140-146 (In Japanese with English summary).

Sakata K and Ohsawa R (2005) Effect of flooding stress on the seedling emergence and the growth of common buckwheat. *Jpn. J. Crop Sci*. 74 : 23-29 (In Japanese with English summary).

Sakata K and Ohsawa R (2006) Varietal differences of flood tolerance during germination and selection of the tolerant lines in common buckwheat. *Plant Prod. Sci.* 9 : 395-400.

Shindoh K, Yasui A, Ohsawa R, Horita H, Suzuki T, Kaneko K and Suzuki T (2001) Composition change of buckwheat (*Fagopyrum esculentum* Moench) groats after soaking in cold water. Nippon Shokuhin Kogaku Kaishi 48 : 449–452. (In Japanese with English summary)

Suganuma N and Ohno H (1984) Role of pericarp in reducing spinach (*Spinacia oleracea* L.) seed germination at supra-optimal temperatures. *J. Japan Soc. Hort. Sci*. 53 : 38–44.

Tominaga T and Uezu T (1995) Weed suppression by buckwheat. *Curr. Adv. in Buckwheat Res.* 2 : 693–697.

Watanabe M, Ohshita Y and Tsushida T (1997) Antioxidant compounds from buckwheat (*Fagopyrum esculentum* Moench) hulls. *J. Agric. Food Chem*. 45 : 1039–1044.

Watanabe M (1998) Cathechins as antioxidants from buckwheat (*Fagopyrum esculentum* Moench) groats. *J. Agric. Food Chem*. 46 : 839–845.

発芽前に冠水した普通ソバの出芽率に及ぼす果皮と水温の影響

関沼幹夫[1]・加藤　太[2]・佐々木大[3]・武田聡美[1]・井上直人[1]・春日重光[1]

信州大学農学部[1]，日本大学生物資源学部[2]，青森県産業技術センター[3]

要　　旨

発芽前に冠水した普通ソバの出芽率に及ぼす果皮と水温の影響を検討した．普通ソバは，湿害になりやすく，出芽のメカニズムの解明と対策が課題である．果皮付きの種子と丸抜き種子を異なる温度条件で浸水処理し，播種して出芽を観察し，果皮の有無と温度の二つの要因が発芽・出芽に大きく関わっていることが分かった．特に，果皮のない丸抜き種子は，３，４，５日と浸水処理を長くしても出芽率が非常に高く，浸水時 10～25℃という温度条件においても出芽率が下がらなかった．今後，果皮付きの種子と丸抜き種子，両者の有効活用を栽培において検証することが課題である．

キーワード：果皮，普通ソバ，丸抜き，出芽，温度