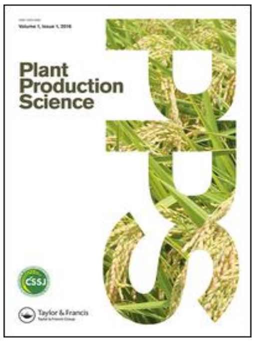

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Evaluation of Cultivar Differences in Preharvest Sprouting of Common Buckwheat (*Fagopyrum esculentum* Moench)

Takahiro Hara, Takahisa Tetsuka, Katsuhiro Matsui, Hiroki Ikoma and Akira Sugimoto

(National Agricultural Research Center for Kyushu Okinawa Region, National Agriculture and Food Research Organization, Koshi, Kumamoto 861-1192, Japan)

Abstract : Preharvest sprouting of buckwheat (*Fagopyrum esculentum* Moench) is an important problem, but cultivar differences in preharvest sprouting and their causes have not been investigated. We detected cultivar differences under natural field conditions. Preharvest sprouting of three cultivars was significantly lower than that of the current main cultivar. Seeds collected before rainfall were threshed and incubated on a wet filter paper in a petri dish for 10 days at 10, 20, 30 and 40°C in the dark, or at an alternating light and temperature condition of 8 h light at 30°C and 16 h darkness at 20°C. Germination was promoted by a higher temperature except for 40°C, suggesting that the risk of preharvest sprouting in buckwheat is higher at a relatively higher temperature. The risk of preharvest sprouting in the field was highly correlated with germination at 20°C (r = 0.98***) and 30°C (r = 0.99***) in the dark, suggesting that germination test can be used to predict preharvest sprouting in the field. Preharvest sprouting was significantly correlated (r = −0.77**) with main stem length, suggesting that ecotype is partly responsible for this phenomenon.

Key words : Agroecotype, Buckwheat flour quality, Differentiation, Germination test, Seed dormancy, Selection, Summer cultivation, Temperature.

Common buckwheat (*Fagopyrum esculentum* Moench) is widely cultivated around the world (Campbell, 1997). Buckwheat flour has high nutrient value and is processed into various foods (Vinning, 2001; Ikeda, 2002).

Buckwheat can be cultivated twice a year in most parts of Japan, in summer and in autumn, although the latter is currently far more popular. Summer cultivation is becoming more important, because the demand for buckwheat soars in summer in the Japanese market (Shibata, 1981; Vinning, 2001), and stabilizes the domestic production by hedging the risk of loss due to bad weather. With the practical studies on sowing time and fertilizer application (Sugimoto and Sato, 1999; Sugimoto et al., 2000; Sugimoto, 2004), summer cultivation has increased especially in western Japan.

Rain after grain maturity but before harvest induces germination of seeds on plants, as reported in Australia, Korea, and Japan (Choi et al., 1992; Bluett, 2001; Morishita and Tetsuka, 2001; Hara et al., 2007). Preharvest sprouting decreases the pasting viscosity of buckwheat flour, thereby affecting its quality (Hara et al., 2007). It occurs especially in summer cultivation (Choi et al., 1992; Morishita and Tetsuka, 2001; Hara et al., 2007). Cultivars with preharvest-sprouting resistance are thus necessary in order to ensure buckwheat flour quality.

Preharvest sprouting in wheat is closely associated with seed dormancy (Reddy et al., 1985; Paterson et al., 1989), so a germination test can be used as a simple evaluation method in programs for breeding of preharvest sprouting resistance in wheat (Paterson and Sorrells, 1990; McCaig and DePauw, 1992; Wu and Carver, 1999). Buckwheat seeds have dormancy shortly after maturity (Samimy, 1994; Wang and Campbell, 2000; Horbowicz and Obendorf, 2005), and the seed dormancy is altered by temperature (Samimy, 1994; Horbowicz and Obendorf, 2005). The relationship between preharvest sprouting and seed dormancy has not been investigated yet.

Common buckwheat cultivars are roughly classified into three ecotypes — summer, intermediate, and late-summer (Onda and Takeuchi, 1942) — although the distribution is continuous (Tetsuka and Uchino, 2002). Michiyama et al. (2005) proposed that morphological characters such as main stem elongation could be used to classify buckwheat ecotypes, but the relationship between preharvest sprouting and ecotype has not been investigated yet.

In this study, we investigated (1) cultivar differences in preharvest sprouting, (2) the relationship between preharvest sprouting and seed dormancy, (3) the use of a germination test to evaluate preharvest resistance in buckwheat breeding programs, and (4) the relationship between preharvest sprouting and

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Abbreviations: ALT, alternating light and temperature of 30°C in the light for 8 hr and 20°C in the dark for 16 hr.

Table 1. Preharvest sprouting (%) under natural rainfall.

Cultivars	Sowing date		Average
	19 Apr	27 Apr	
Kitawasesoba	81	65	73 a
Banshozairai	71	71	71 a
Shinanonatsusoba	75	66	70 a
Hashikamiwase	72	58	65 a b
Iwatewase	67	51	59 a b c
Yaitazairai	58	48	53 a b c
Mogamiwase	53	48	51 a b c
Shinano 1	51	48	50 a b c
Harusoba	49	40	45 b c d
Hitachiakisoba	40	40	40 c d
Kanoyazairai	29	28	29 d

Values with the same letter are not significantly different at the 5% level by the Holm test. The interaction of cultivar×sowing date is not significant.

Table 2. Germination of buckwheat seeds (%) after incubation for ten days at the different temperatures.

(a) Seeds were sampled on 21 June

	Incubation temperature				
	10°C	20°C	ALT	30°C	40°C
Shinanonatsusoba	50	85	100	98	40
Banshozairai	10	78	85	93	53
Hashikamiwase	15	65	83	90	40
Yaitazairai	3	35	53	68	23
Mogamiwase	0	38	50	68	10
Shinano 1	0	33	50	63	8
Harusoba	0	25	30	48	5
Hitachiakisoba	0	15	25	43	0
Kanoyazairai	0	3	8	23	0
ANOVA					
Cultivar (A)			***		
Temperature (B)			***		
A×B			n.s.		

(b) Seeds were sampled on 1 July

	Incubation temperature				
	10°C	20°C	ALT	30°C	40°C
Shinanonatsusoba	80	93	100	100	5
Banshozairai	40	98	100	100	0
Hashikamiwase	45	98	100	98	0
Yaitazairai	18	88	90	100	3
Mogamiwase	28	85	93	98	5
Shinano 1	35	78	85	98	3
Harusoba	10	63	70	83	3
Hitachiakisoba	18	40	58	83	0
Kanoyazairai	13	40	48	63	0
ANOVA					
Cultivar (A)			***		
Temperature (B)			***		
A×B			***		

***: Significant at the 0.1 % level.

ecotype.

Materials and Methods

1. Plant materials

We used 11 common buckwheat cultivars (Table 1). Plants were grown at the National Agricultural Research Center for Kyushu Okinawa Region, Koshi, Kumamoto, Japan. Seeds were sown at 90 seeds m^{-2} in rows 70 cm apart on 19 and 27 April 2005. Experimental plots were arranged in a split-plot design with duplication, in which sowing dates were assigned to the main plots and cultivars were assigned to the subplots.

Meteorological data were obtained from the weather station at National Agricultural Research Center for Kyushu Okinawa Region.

2. Determination of preharvest sprouting under natural rainfall

Twenty plants from each subplot of each cultivar (Table 1) were harvested on 11 July, after a week of continuous rain. All plants were threshed by hand. Preharvest sprouting was determined by counting germinating seeds per 60 sample seeds. Results are expressed as percentage values of germinating seeds. Arcsine transformation was applied to the germination percentage data prior to significance test using analysis of variance.

3. Germination tests in petri dishes

Plants of 9 cultivars (Table 2) sown on 19 April 2005 were used for the germination tests. Twenty plants from each plot were harvested on 21 June and 1 July. All plants were threshed by hand, and the resulting seed lot was divided in half. Only discolored seeds, which were considered mature (Funatsuki et al., 2000), were used in the germination tests. Twenty threshed seeds were immediately placed on filter paper saturated with 8 mL distilled water in a petri dish, 9 cm in diameter (immediate germination test). The dishes were covered and incubated in a germination cabinet at 4 constant temperatures of 10, 20, 30 and 40°C in the dark and at an alternating light and temperature condition; 30°C in the light for 8 h and 20°C in the dark for 16 h (ALT). Petri dishes were checked once a day for 10 d, and germinating seeds were counted and removed. When the filter paper became dry, 2 mL distilled water was added. Seeds were exposed to diffuse light during counting. Petri dishes in

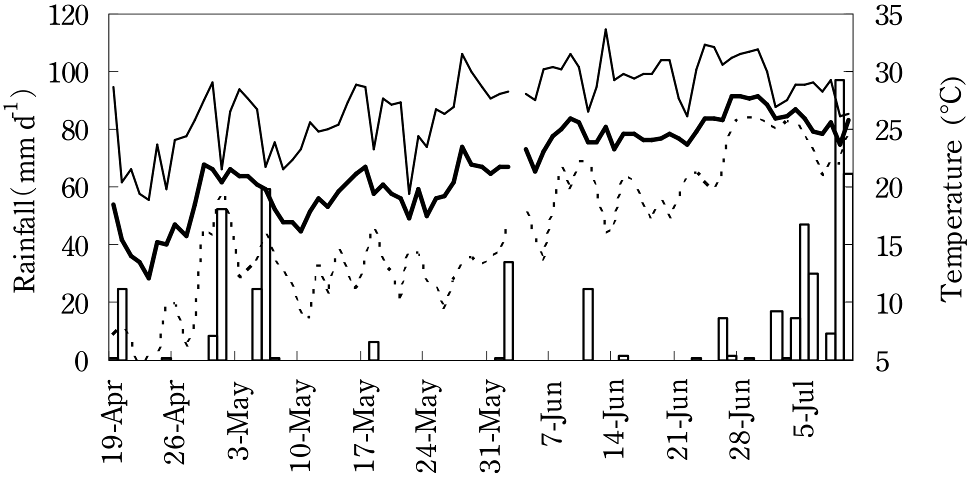


Fig. 1. Rainfall and temperature in the field experiment.
A thin solid line, a thick solid line and a broken line indicate maximum, average and minimum temperatures, respectively. Bars indicate rainfall. Data on 3 June were not recorded.

the ALT treatment were checked during the light period. Results are expressed as percentage values of germinating seeds.

The other 20 threshed seeds were stored for 6 months at 10°C, and then used for a germination test at a constant 20°C in the dark as described above.

4. Morphological characters

In order to study the relationship between preharvest sprouting and ecotype, six plants from each plot sown on 19 April were harvested on 11 July and main stem length was recorded.

Results

1. Preharvest sprouting under natural rainfall

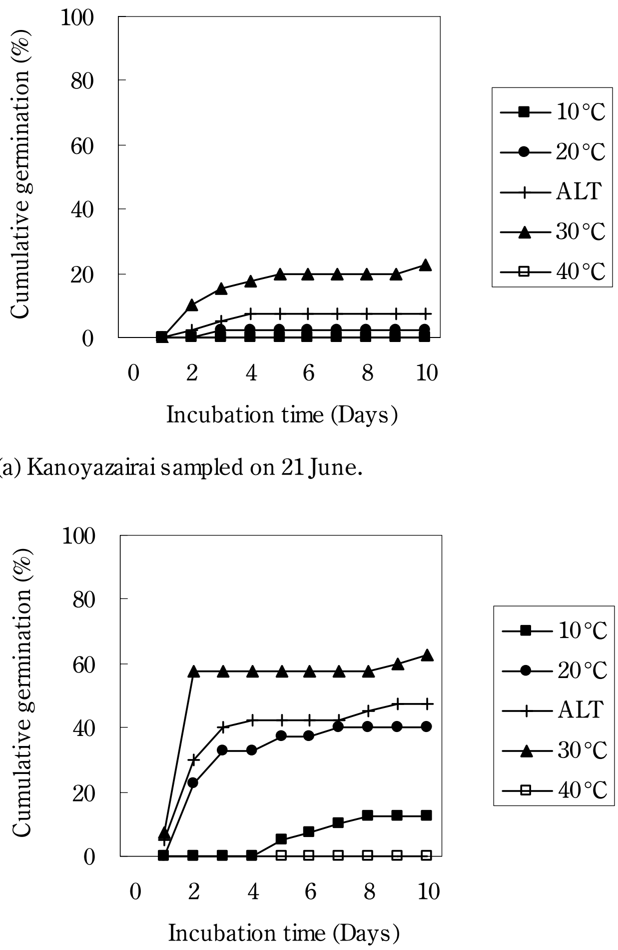
Preharvest sprouting was not observed in the field before 2 July, but was observed after continuous rainfall from 2 to 11 July (Fig. 1). The daily average temperature from 2 to 11 July was 23.7°C to 26.8°C, and the daily minimum and maximum temperatures were 21°C to 25.6°C and 26.2°C to 29.4°C, respectively.

Table 1 shows the percentage of preharvest sprouting of 11 buckwheat cultivars. Significant cultivar differences were detected. Preharvest sprouting in Kanoyazairai was the lowest among the cultivars and was close to 1/3 of that in Kitawasesoba.

2. Germination tests in petri dishes

Fig. 2 shows two examples of cumulative germination as a function of incubation time. Cumulative germination increased rapidly initially, but only slightly later.

Table 2 shows the results of germination tests conducted immediately after threshing. The effects of cultivar and temperature were significant on both sampling dates. Mold was often observed at a



(a) Kanoyazairai sampled on 21 June.

(b) Kanoyazairai sampled on 1 July.

Fig. 2. Patterns of cumulative germination in the germination test.

Table 3. Correlation between preharvest sprouting in the field and germination in petri dish at different incubation temperatures.

Incubation temperature	Incubation time (Days)									
	1	2	3	4	5	6	7	8	9	10
(a) Seeds were sampled on 21 June										
10°C	—	0.34	0.34	0.61	0.55	0.57	0.65	0.65	0.65	0.68
20°C	—	0.89	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
ALT	0.77	0.96	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.99
30°C	0.83	0.97	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
40°C	0.47	0.91	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
(b) Seeds were sampled on 1 July										
10°C	—	—	—	0.74	0.78	0.77	0.74	0.75	0.79	0.79
20°C	0.91	0.92	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
ALT	0.87	0.96	0.93	0.92	0.92	0.92	0.92	0.93	0.93	0.93
30°C	0.97	0.90	0.88	0.86	0.88	0.88	0.87	0.86	0.85	0.85
40°C	−0.21	0.30	0.30	0.21	0.23	0.23	0.23	0.23	0.23	0.23

The correlation coefficients, between the average values of cultivars in Table 1 and the data obtained by the germination test in petri dish in Table 2, are indicated. The nine cultivars listed in Table 2 were used for the calculation. $|r| \geqq 0.63$ is significant at the 5% level. $|r| \geqq 0.77$ is significant at the 1% level (n=9). The hyphens indicate that correlation coefficients were not be calculated, since no germinations were detected in the petri dish experiments.

constant 40°C. Up to 30°C, germination increased with temperature (Table 2). Analysis of variance showed significant interaction of cultivar×temperature on 1 July (Table 2).

After storage at 10°C for 6 months, over 97% seeds germinated (data not shown).

3. Correlation between preharvest sprouting under natural rainfall and germination in petri dish experiments

Table 3 shows the coefficients of correlation between the percentage of preharvest sprouting in the field (Table 1) and germination in petri dish experiments at different incubation temperatures and periods. The correlation coefficients were especially high at 20, 30°C and ALT treatments. In these three conditions, the correlation was significant as early as 2 d of incubation. Correlation coefficients were constant after 3 d of incubation.

4. Correlation between preharvest sprouting under natural rainfall and morphological characters

Preharvest sprouting was significantly correlated with main stem length (Fig. 3).

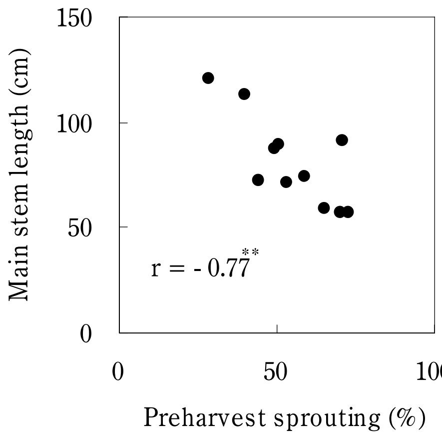


Fig. 3. Relationship between preharvest sprouting and main stem length.
**: Significant at the 1 % level.

Discussion

We demonstrated the high correlation between preharvest sprouting under natural rainfall and germination in petri dish experiments (Table 3). Cultivar differences in preharvest sprouting of wheat have been explained by seed dormancy and awn structure relevant to water uptake (King and Richards, 1984). In our experiment, nearly all the seeds sampled from the field, germinated after a 6-month storage. This suggests that some seeds that did not germinate immediately after sampling were dormant. These findings suggest that the cultivar differences in buckwheat are explained at least partly by seed dormancy.

The germination test in a petri dish can be used in evaluating preharvest sprouting of buckwheat cultivars. It would allow selection in buckwheat breeding programs even when preharvest sprouting is not induced naturally. A significant effect of cultivar ×temperature interaction on seed germination was observed in part of the germination test (Table 2). A similar effect of the interaction was reported in wheat, suggesting that temperature affects cultivar differences (Reddy et al., 1985; Ichinose et al., 2002; Nyachiro et al., 2002). Therefore, it would be safer to use an incubation temperature that is similar to that of the natural environment in a germination test for preharvest sprouting evaluation. In summer cultivation preharvest sprouting occurs when the natural temperature range is 20 to 30°C (Fig. 1). In our experiment, preharvest sprouting closely correlated with germination in a petri dish within this temperature range. Therefore, we recommend the use of an incubation temperature of 20 to 30ºC in the germination test for evaluating preharvest sprouting in summer buckwheat cultivation in western Japan. A temperature of 30ºC and 20ºC will give severer and milder evaluating conditions. Alternating temperature and light (ALT) did not improve the correlation coefficient. We consider incubation for 3 days or longer to be suitable, since the coefficients of correlation between germination and preharvest sprouting remain high.

In western Japan, Kitawasesoba is currently recommended for summer cultivation (Sugimoto and Sato, 1999), and Kanoyazairai is the leading cultivar in autumn cultivation. Kanoyazairai is not generally recommended for summer cultivation because of its low grain yield, but preharvest sprouting was close to 1/3 of that of Kitawasesoba (Table 1). In breeding for preharvest sprouting resistance, Kanoyazairai will be a useful material.

Preharvest sprouting in buckwheat occurs especially in summer cultivation. One reason is that harvest time corresponds with the rainy season. Our experiment showed that buckwheat germination shortly after maturation is promoted by higher temperature (Table 2), consistent with previous reports (Singh and Mall, 1977; Samimy, 1994). This reaction to temperature is opposite that of wheat, in which lower temperature induces preharvest sprouting (Reddy et al., 1985; Ichinose et al., 2002; Nyachiro et al., 2002). The promotion of germination by higher temperature explains why preharvest sprouting in buckwheat occurs frequently in summer cultivation when the temperature in the harvesting season is higher than in autumn cultivation. Cultivating buckwheat in such environment that temperature is relatively low at harvesting season may lower the risk of preharvest sprouting even when rain falls.

Morphological characters were proposed as a basis for classifying cultivar ecotypes in buckwheat (Michiyama et al., 2005). The main stem length was longer in the late-summer ecotype cultivars than in the summer ecotype cultivars (Michiyama and Hayashi, 1998; Michiyama et al., 2005). In our experiment, preharvest sprouting and main stem length were negatively correlated. This suggests that late-summer ecotype cultivars tend to be resistant to preharvest sprouting.

Summer ecotype cultivars of buckwheat are considered to have differentiated from the late-summer ecotype (Minami and Namai, 1986; Iwata et al., 2005). Cultivated species are generally under automatic selection pressure to lose dormancy (Harlan et al., 1973). Double cropping using an identical buckwheat cultivar was conducted in Japan (Onda and Takeuchi, 1942; Sotokawa et al., 1988). Double cropping of summer ecotype cultivars could be superior to that of late-summer ecotype cultivars in grain yield, because long-day condition drastically reduce grain yield of late-summer ecotype cultivars (Yamazaki, 1947; Michiyama and Hayashi, 1998). Many years of double cropping using summer ecotype cultivars could put selection pressure on the generations inducing genetically lower seed dormancy, because the duration between harvesting and the next sowing in double cropping should be shorter than that in single cropping.

Traits such as seed dormancy and photosensitivity are included in domestication-related traits, because these traits will be subjected to selection pressure by cultivated environment (Koinange et al., 1996; Gu et al., 2005; Hancock, 2005). There is accumulating evidence that QTL of domestication-related traits are clustered close together on the same linkage group (Koinange et al., 1996; Takeuchi et al., 2003; Gu et al., 2005; Hancock, 2005). In buckwheat, summer ecotype cultivars are considered to have differentiated from the late-summer ecotype cultivars as a result of adaptation to the long-day condition of summer cropping in high latitude regions (Minami and Namai, 1986). Therefore, selection pressure on photosensitivity may have induced loss of seed dormancy of summer ecotype buckwheat cultivars, due to genetic linkage between photosensitivity and seed dormancy.

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