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# BUCKWHEAT GROWTH AND PHYSIOLOGY AS AFFECTED BY SOWING IMMEDIATELY AFTER BURNING UNDER DROUGHT CONDITION

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## ABSTRACT

Shifting cultivation with sowing buckwheat seed immediately after burning, when the ash on the soil is still hot, is widely practiced in Kyushu mountains, Japan. This study was conducted to investigate the benefit of seed sowing immediately after burning under well-irrigated and drought conditions on buckwheat. The seeds were heat-treated and allowed to grow under well-irrigated and drought condition. Plant height and leaf number of the plants, and physiological parameters were measured. Under drought stress condition, stomatal conductance and photosynthetic rate of the buckwheat plants with heat treatment were increased compared to those of the plants without heat treatment, though those under well-irrigated or light drought stress conditions were not changed. The plant with heat treatment to seed opened its stomata and maintained a high photosynthetic rate even under drought stress condition. Heat treatment to seed did not change plant height and leaf number noticeably. Therefore, in shifting cultivation, where buckwheat is often exposed to drought stress, seed sowing immediately after burning might be effective in maintaining plant growth.

**Keywords**: Cross protection, drought stress, heat stress, photosynthetic rate, slash and burn cultivation, stomatal conductance

## Introduction

The area of shifting cultivation in Kyushu mountains, Japan, was about 2,500 ha in 1950's, and after that the area decreased rapidly (Sasaki, 1970). In Kyushu mountains, crop rotation and/or mix cropping of buckwheat (*Fagopyrum esculentum*), Japanese millet (*Echinochloa esculenta*), foxtail millet (*Setaria italica*), adzuki bean (*Vigna angularis*), soybean (*Glycine max*) and rice bean (*V. umbellata*) was commonly practiced (Kondo and Koga 2021), and this type of shifting cultivation was reported to widely practiced from tropical forest at mainland of Southeast Asia to temperate forest of East Asia (Sasaki 1988). At Shiiba Village, Miyazaki Prefecture, located at the northern part of Kyushu mountains, the shifting cultivation is still practiced. In that Village buckwheat is cultivated as the first crop after burning in early August. Buckwheat seeds are sown immediately after burning, when the ash on the soil is still hot. In a preliminary survey, we observed that the surface temperature of the ash was 50-60 °C on August 3, 2019 when the buckwheat seeds were sown. This cultivation method was found widely practiced in Kyushu mountains, though in the Southeast Asia this method is not considered a standard practice.

From our observations, interviews, and old proverbs (Matsuoka 1977), it was revealed that sowing buckwheat immediately after burning, when the ash on the soil was still hot, had been widely practiced in Kyusyu mountains. The farmers in Shiiba Village informed that buckwheat growth was enhanced due to applying high temperature to seed by sowing immediately after burning, but heat treatment to the buckwheat seed did not improve growth under well irrigated condition in our preliminary experiment (data not shown). Since buckwheat grown in shifting cultivation is often exposed to drought stress, we hypothesized that under drought condition the plant growth may be increased due to exposure of seed to high temperature. Therefore, we initiated this study to evaluate the effect of heat treatment to seeds on the growth performance of buckwheat under drought condition.

## Materials and Methods

The effects of heat treatment to buckwheat seed on plant growth and physiology were examined in three experiments under different climatic conditions.

**Experiment 1**

Buckwheat, local variety in Shiiba Villege, was used for the experiment 1. On June 12, 2020 the seeds were subjected to a heat treatment as described below. A stainless tray filled with quartz sand was placed in an oven at 70 °C. Then, the stainless tray was floated in a water bath set at 60 °C. The seeds were placed on the quartz sand for 90 minutes. The surface temperature of the quartz sand ranged from 56 to 62 °C. The seeds without heat treatment were used as the control. Replication of each treatment was 8. On June 15, the seeds germinated indoors beforehand were sown in 1/5000-a Wagner pots in the greenhouse at the University of Miyazaki (131.4 °E, 31.8 °N). Soil collected from the shifting cultivation field at Shiiba Village used for the experiment following the methods of Kondo and Koga (2021). Magnesium lime of 2.5 g and 2 g of chemical fertilizer (N:P:K=8:8:8) were applied per pot.

The plant was irrigated appropriately for not to be subjected to a drought stress from June 15 to July 21. From July 22 to 24, all the plants were subjected to a drought stress. On July 21 water was applied until water came out through the bottom of the pots at 9:00. For exposing to the stress, on July 22, 55 mL of water was applied at 9:00 and 12:00. On July 23, 55 mL of water was applied at 9:30. On July 24, no water was applied. After July 25, sufficient water was applied. Soil water content (v/v), air temperature, and photosynthetic active radiation (PAR) were measured once every 30 min during the experiment using a soil moisture sensor, a thermometer, and a PAR photon flux sensor (EC-5, VP-4, and QSO-S respectively; METER Group, Inc., USA). These data were recorded on a data logger (EM50, METER Group, Inc., USA). Using the data of the soil moisture sensor and a soil water potential sensor (TERSO-21, METER Group, Inc., USA), the relationship between water content (v/v) and water potential of the soil was determined, and then the soil water potential was calculated.

Stomatal conductance was measured at 6:00, 9:00, 12:00, 15:00, and 18:00 on July 21 and 23 using a leaf porometer (SC-1, METER Group, Inc., USA). The maximum quantum efficiency of photosystem II (Fv/Fm) was measured at 20:00 on the same days, using a portable chlorophyll fluorometer (OS-30P, Opti-Sciences, Inc., USA). Relative leaf chlorophyll content (SPAD value) was also measured using a chlorophyll-meter (SPAD-502, Konica Minolta Sensing, Inc., Japan) on the same days. The fourth to sixth leaves from the top were used for these measurements. Plant height and leaf number were measured on July 10, 17, and 24. On July 27 dry weight of the above-ground part was measured.

**Experiment 2**

The same buckwheat variety as in the experiment 1 was used for the experiment 2. On August 7, 2020 the seeds were subjected to a heat treatment using the same method as of the experiment 1. Replication of each treatment was 15. On August 10, the seeds, germinated indoors beforehand, were sown in 1/5000-a Wagner pots in the greenhouse at the University of Miyazaki. Soil collected from the experimental field in the University of Miyazaki was used. Magnesium lime of 2.5 g and 2 g of chemical fertilizer (N:P:K=8:8:8) was applied per pot.

During the experiment 2, the amount of irrigation was reduced compared to the experiment 1. From August 10 to 31, 110 mL of water was applied twice a day on sunny days and once a day on cloudy and rainy days. From September 1 to 24, 55 mL of water was applied once a day on sunny and cloudy days and no water was applied in rainy days. Soil water content, air temperature, and PAR were measured, and soil water potential was calculated during the experiment using the same method as in the experiment 1.

Photosynthetic rate was measured at 13:00 on September 9 using a photosynthetic rate measurement device (MIC-100, Masa International Co., Ltd., Japan). The measurement conditions were as follows: PAR 1200 $\mu mol/m^2/s$, stabilization time 3 sec, measurement started when $CO_2$ concentration was stable at 400 ppm, and measurement $CO_2$ span was 10 ppm. The stomatal conductance was measured at 15:00 on September 9 and SPAD value was measured on September 12 using the same devices as in the experiment 1. The fourth to sixth leaf from the top was used for these measurements. Plant height and leaf number were measured on August 21, 28, September 5, 13, and 24. On September 24, dry weight of above-ground part was measured.

**Experiment 3**

The same buckwheat variety as in the experiment 1 was used for the experiment 3. On July 6, 2021 the seeds were subjected to a heat treatment using the same method as the experiment 1. Replication of each treatment was 7. On July 9, the seeds, germinated indoors beforehand, were sown in 1/5000-a Wagner pots in the greenhouse at the Kyoto University (135.8 °E, 35.0 °N). Soil mixed with weathered granite soil and bark compost at a ratio of 1:1 by volume was used. Magnesium lime of 2.5 g and 2 g of chemical fertilizer (N:P:K=8:8:8) were applied per pot.

The plant was irrigated appropriately not to be subjected to a drought stress from July 9 to August 6. From August 7 to 11, all the plants were subjected to a drought stress as described below. On August 7, 55mL of water was applied at 12:00. After that no water was applied. Soil water content, air temperature, and PAR were measured, and soil water potential was calculated during the experiment using the same method as in the experiment 1.

Photosynthetic rate was measured on August 6 and 11, from 10:00 to 12:00 using the same device as in the experiment 2. The measurement conditions were the same as in the experiment 2. On August 6 and 11, the stomatal conductance was measured at 6:00, 9:00, 12:00, 15:00 and 18:00, and SPAD value was measured using the same devices as in the experiment 1. On the same days Fv/Fm was measured at 20:00 using the same device as in the experiment 1. Plant height and leaf number were measured on August 1, 6, and 11. On August 13, dry weights of above-ground part and root were measured.

**Statistical analysis**

The data, except for those of stomatal conductance in the experiment 1 and 3, were analyzed using t-test. The data of stomatal conductance in the experiment 1 and 3 were analyzed by a two-way ANOVA (treatment × measurement time). The significance level was adjudged at $p < 0.05$.

## Results

### Experiment 1

Until July 21, soil water potential was higher than -6.8 kPa and ranged from -55 to -80 kPa on July 23 (Figure 1). Maximum and minimum temperature during the measuring time were 41 °C and 25 °C on July 21 and 38 °C and 23 °C on July 23. Maximum PARs were 1256 and 1091 μmol/m$^2$/s on July 21 and 23, respectively.

Interaction between measurement time and heat treatment for stomatal conductance on July 21 and 23 was not observed. On July 21, stomatal conductance under well-irrigated condition was not affected by heat treatment (Figure 2). On July 23, stomatal conductance under drought stress condition was increased by heat treatment.

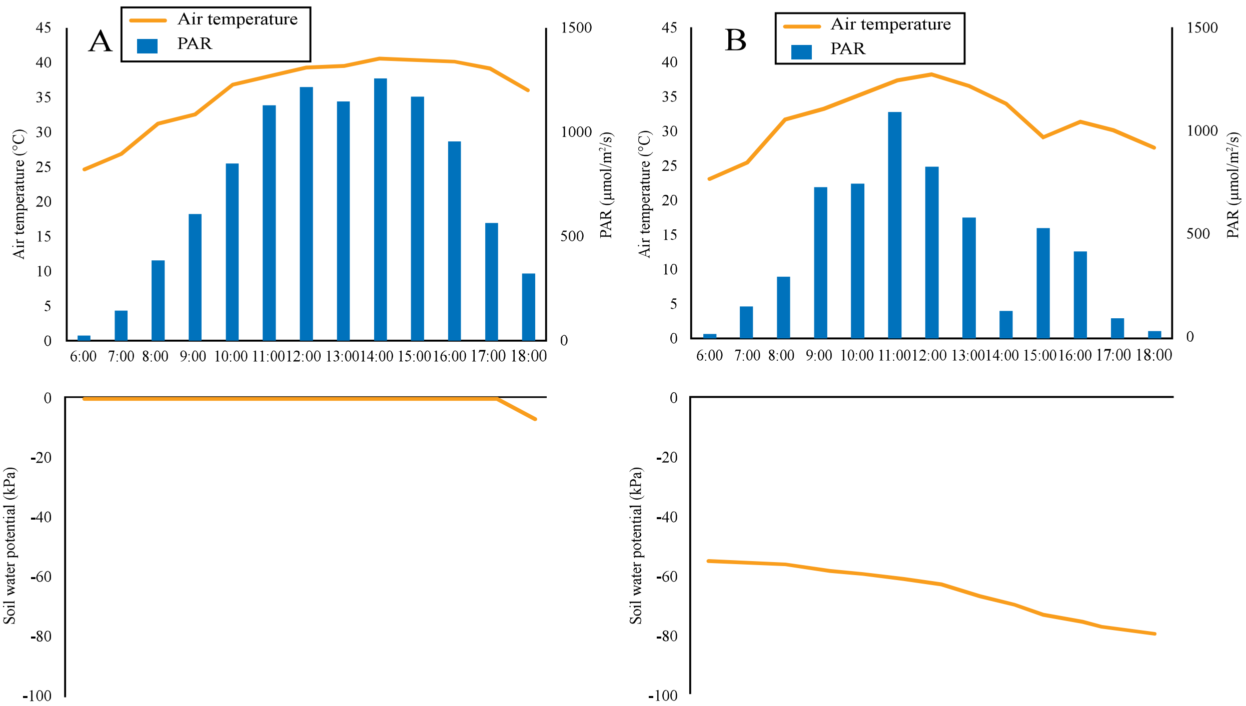


**Figure 1.** Air temperature, PAR and soil water potential on July 21 (A) and 23 (B) in the experiment 1.

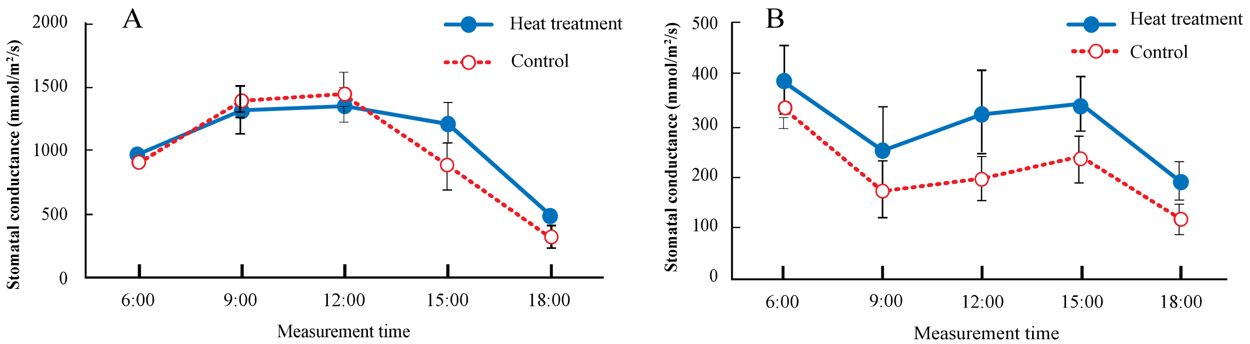


**Figure 2.** Effect of heat treatment to buckwheat seed on stomatal conductance under well-irrigated (A) and drought stress (B) conditions. Bars indicate standard errors (n=8).

Fv/Fm and SPAD value on July 21 and 23 were not affected by heat treatment (Table 1). Heat treatment did not affect plant height during the experiment (Figure 3). On July 24, leaf number was decreased by heat treatment. Dry weight of above-ground part was not affected by heat treatment.

## Experiment 2

Soil water potential decreased gradually from August 28 to lower than -300 kPa (Figure 4). Increase of soil water potential to more than -1.0 kPa on September 7 was due to rainfall from the typhoon that removed the plastic film of the greenhouse. Then again, the soil water potential decreased to lower than -500 kPa. On September 9, during the measuring time, soil water potential ranged from -65 to -75 kPa, air temperature was about 30 °C and maximum PAR was 835 μmol/m$^2$/s (Figure 5).

Under drought stress, photosynthetic rate in heat treatment plant was about 30 % higher than that in control plant, though stomatal conductance was not affected by heat treatment (Table 2). SPAD value on September 12 was not affected by the heat treatment. Dry weight of above-ground part of the plants was not affected by the heat treatment. Heat treatment did not affect plant height and leaf number during the experiment 2 (Figure 6).

## Experiment 3

Until August 6, soil water potential was higher than -1.0 kPa, and that on August 11 ranged from -55 to -75 kPa (Figure 7). On August 6 and 11 temperatures ranged from 25 to 35 °C and 21 to 33 °C and maximum PARs were 923 and 648 μmol/m$^2$/s during the measuring time.

Interaction between measurement time and heat treatment for stomatal conductance was not observed, and stomatal conductance was not affected by heat treatment with and without drought stress (Figure 8). Photosynthetic rate, Fv/Fm, and SPAD value were not affected by heat treatment (Table 3). Dry weight of above-ground part, root, and sum of them were not affected by heat treatment. Root/shoot ratio was not affected by heat treatment. Heat treatment did not affect plant height and leaf number during the experiment 3 (Figure 9).

## Discussion

By heat treatment to seed, stomatal conductance was increased in the experiment 1 and photosynthetic rate was increased in the experiment 2. Photosynthetic rate also

**Table 1. Effects of heat treatment to buckwheat seed on Fv/Fm and SPAD value under irrigated and drought conditions and dry weight of above-ground part**

| | Fv/Fm | | SPAD value | | Dry weight of above-ground part (g) |
|---|---|---|---|---|---|
| | Irrigation (July 21) | Drought (July 23) | Irrigation (July 21) | Drought (July 23) | |
| Control | 0.80 | 0.80 | 41.2 | 43.5 | 41.8 |
| Heat treatment | 0.80 | .081 | 40.2 | 44.8 | 42.1 |
| | ns | ns | ns | ns | ns |

NS indicates not significant by t=test (n=8).

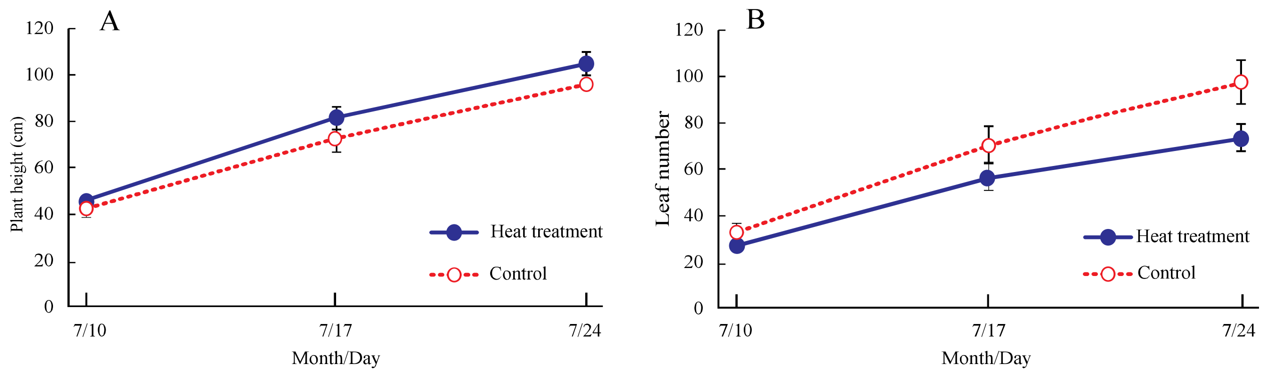


**Figure 3.** Effect of heat treatment to buckwheat seed on plant height (A) and leaf number (B). Bars indicate standard errors (n=8).

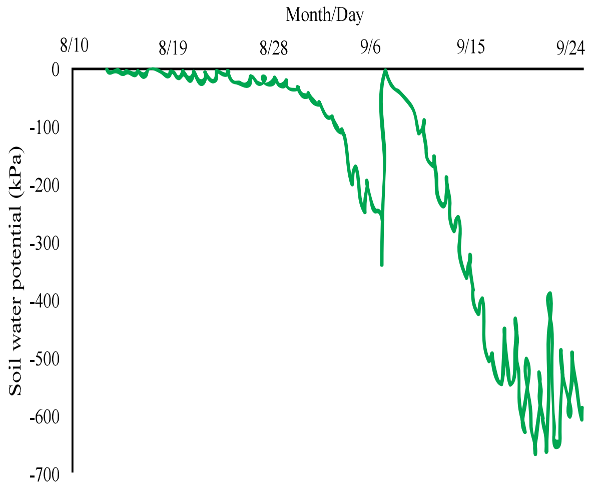


**Figure 4.** Soil water potential during the experiment 2.

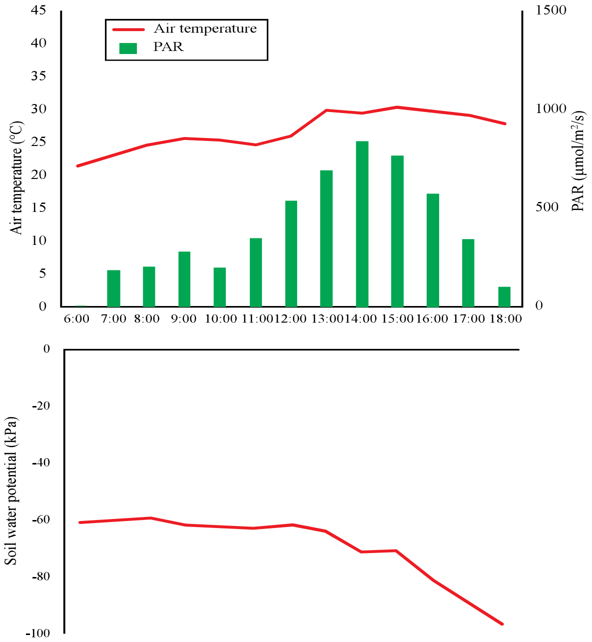


**Figure 5.** Air temperature, PAR, and soil water potential on September 9 in the experiment 2.

**Table 2. Effects of heat treatment to buckwheat seed on photosynthesis rate, stomatal conductance, SPAD value, and dry weight of above-ground part under drought condition**

| | Photosynthetic rate ($\mu mol/m^2s$) | Stomatal conductance ($\mu mol/m^2s$) | SPAD value | Dry weight of above ground part (g) |
|---|---|---|---|---|
| Control | 14.7 | 247.8 | 40.7 | 7.1 |
| Heat treatment | 19.3 | 350.9 | 42.3 | 7.5 |
| | * | ns | ns | ns |

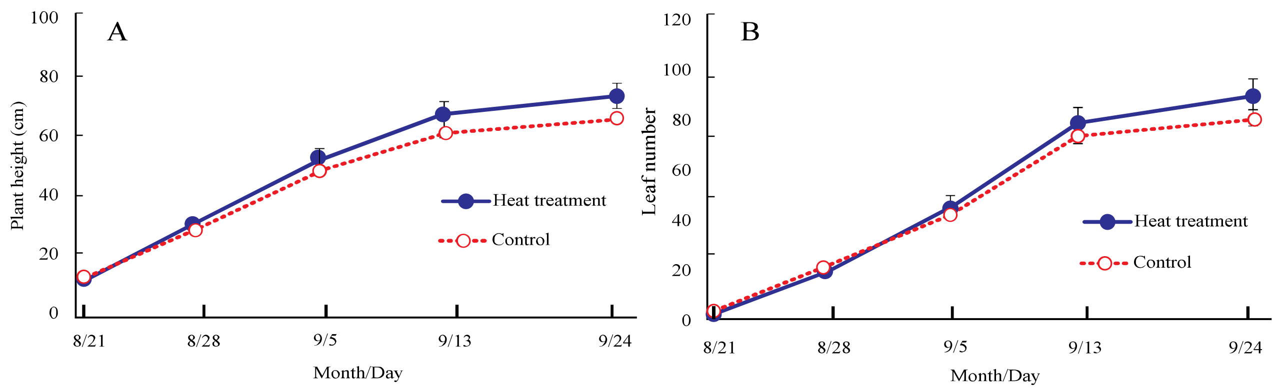


**Figure 6.** Effect of heat treatment of buckwheat seed on plant height (A) and leaf number (B). Bars indicate standard errors (n=15).

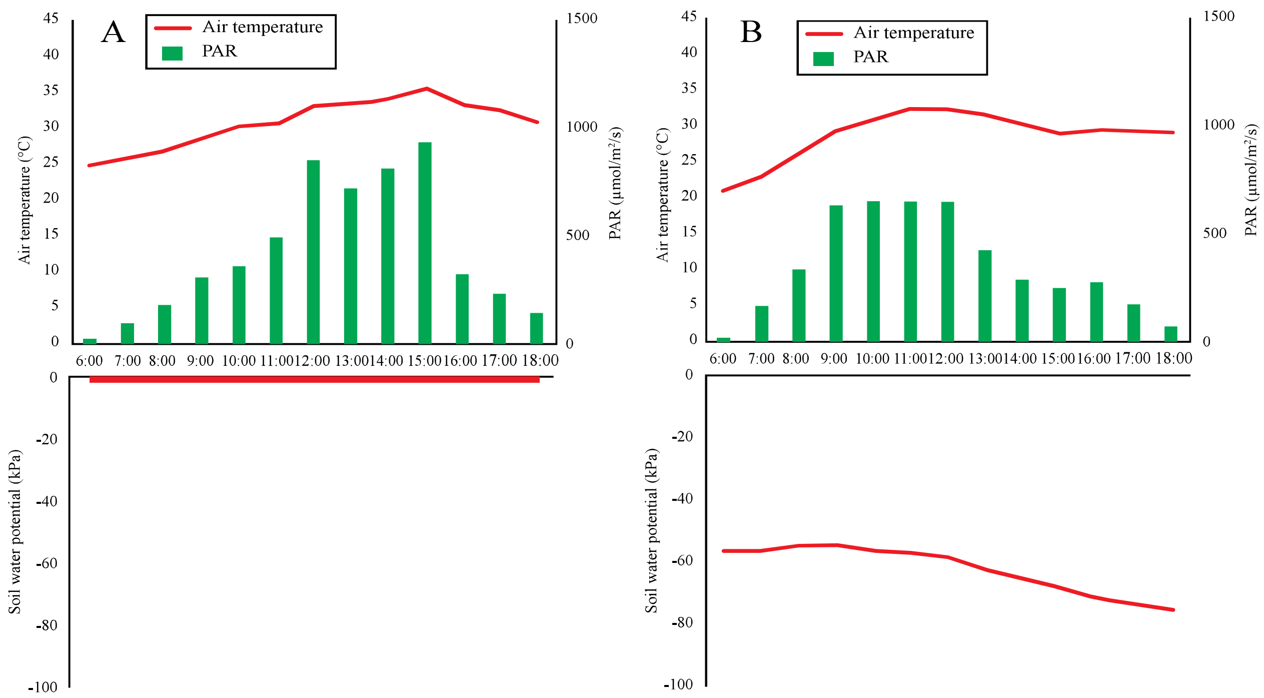


**Figure 7.** Air temperature, PAR, and spo; water potential on August 6 (A) and 11 (B) in the experiment 3.

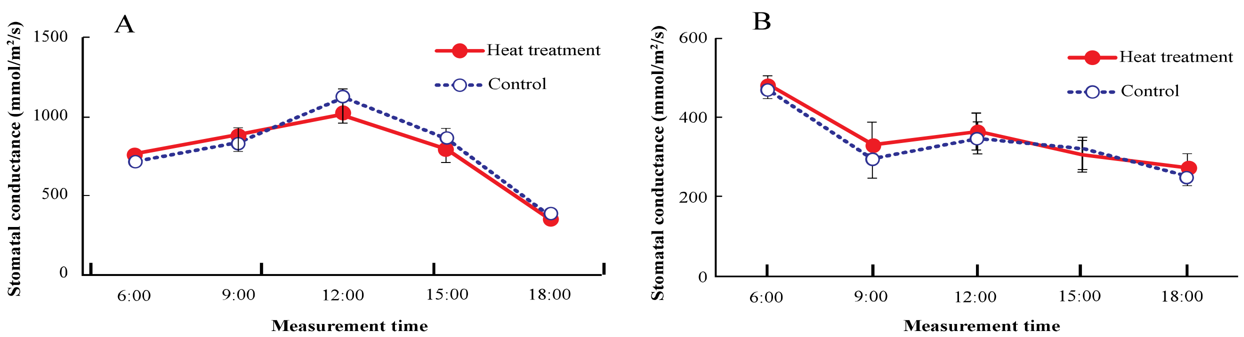


**Figure 8.** Effect of heat treatment to buckwheat seed on stomatal conductance under well-irrigated (A) and drought stress (B) conditions. Bars indicate standard errors (n=7).

**Table 3. Effects of heat treatment to buckwheat seed under irrigated and drought conditions on photosynthesis rate, Fv/Fm, SPAD value, , dry weights and Root/Shoot ratio**

| | Photosynthetic rate | | Fv/Fm | | SPAD value | | Dry weight (g) | | | Root/ Shoot ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| | Irrigation (Aug. 6) | Drought (Aug. 11) | Irrigation (Aug. 6) | Drought (Aug. 11) | Irrigation (Aug. 6) | Drought (Aug. 11) | Sum | Above-ground part | Root | |
| Control | 18.6 | 13.7 | 0.83 | 0.84 | 39.3 | 44.7 | 7.54 | 5.94 | 1.60 | 0.27 |
| Heat treatment | 19.5 | 15.6 | 0.83 | 0.84 | 39.2 | 46.7 | 7.50 | 5.76 | 1.74 | 0.31 |
| | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

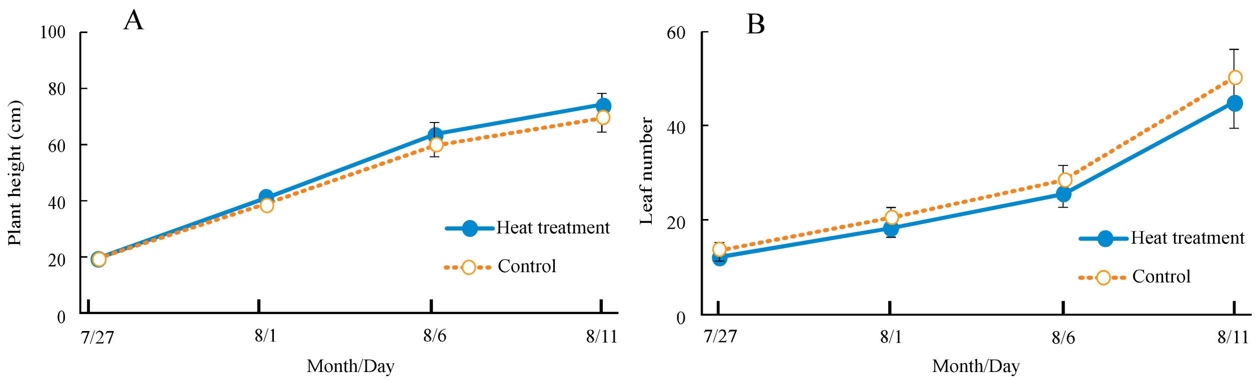


**Figure 9.** Effect of heat treatment of buckwheat seed on plant height (A) and leaf number (B). Bars indicate standard errors (n=7).

might have increased in the experiment 1, due to high stomatal conductance, though we did not measure the rate. Thus, buckwheat plants grown with heat stressed seeds might have kept open its stomata and maintained high photosynthetic rate even under drought condition. Sowing immediately after burning, which has been practiced in shifting cultivation in Kyushu mountains, may have the effect of keeping growth even under drought condition, while in the experiment 2 under long-term drought stress buckwheat growth with heat treatment tended to be increased though the difference was not significant. The reason for such increased of buckwheat growth under drought condition could be due to cross-protection; exposure of plants to moderate stress induces resistance to other stress (Sabehat *et al*. 1998). Heat stress induced tolerance to abiotic stresses, such as drought, salinity, and chilling stress with accumulation of heat-shock proteins, and changing in the synthesis of plant hormones and signaling molecules (Hossain *et al.* 2018). In the present study buckwheat also might have acquired drought stress tolerance induced by cross-protection with heat stress. Drought tolerance induced by cross-protection with sowing immediately after burning practiced in shifting cultivation was not reported earlier, though there are many reports about the benefit of cross-protection on plant growth (Sabehat *et al*. 1998, Hossain *et al.* 2018).

Under well-irrigated condition, heat treatment did not enhance stomatal conductance and photosynthetic rate. Therefore, the positive effect of heat treatment might be appeared under drought condition. However, stomatal conductance and photosynthetic rate were not enhanced by the heat treatment in the experiment 3 even under drought condition. Stomatal conductance of the control plant during the day, 12:00 and 15:00, in the experiment 3 was higher than those at the experiment 1 and 2 (300-350 mmol/m$^2$/s in the experiment 3, 250 mmol/m$^2$/s in the experiment 2, and 200-230 mmol/m$^2$/s in the experiment 1). Thus, drought stress in the experiment 3 might have been lighter, though the soil water potential in the experiment 3 was as low as those in the experiment 1 and 2. Cloudy weather on the measuring day in experiment 3 might lessened the drought stress effect on the plant. Therefore, under severe drought stress, with lower stomatal conductance than 250 mmol/m$^2$/s, heat treatment might enhanced stomatal conductance and photosynthetic rate.

In conclusion, heat treatment to seed improved drought tolerance due to cross-protection. The unique methods of shifting cultivation at Kyushu mountains, i.e. sowing immediately after burning, might be effective to enhance buckwheat growth.

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