Short-term Bottom-heat Treatment during Low-air-temperature Storage Improves Rooting in Squash (Cucurbita moschata Duch.) Cuttings Used for Rootstock of Cucumber (Cucumis sativus L.)

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We investigated the effects of bottom-heat treatment (BHT) during low-air-temperature storage on the rooting and growth of squash cuttings after planting following storage. Rooting of cuttings was much improved with BHT temperatures ranging from 27 to 32°C, and the root fresh weight after planting increased linearly with increasing BHT duration from 0 to 24 h. Rooting during storage was observed 30 h after starting BHT, but was not observed at 26 h. BHT should end just before rooting begins to avoid the inhibition of root growth caused by root damage during planting; therefore, the optimum BHT duration is thought to be 26 to 30 h (approximately 1 day). To test the practicality of BHT in transplant production, we used cuttings obtained by grafting cucumber (Cucumis sativus L.) scions onto squash rootstock. The squash cuttings used for the rootstock were either exposed or unexposed to BHT (30°C) during storage at 9°C for 24 h, and were then grafted with the cucumber scions. One portion of the grafted cuttings was planted in growing medium immediately after grafting. Six days after planting, total and root fresh weights of the BHT cuttings were 1.3 and 4.0 times higher compared to those of the non-BHT cuttings, respectively. Another portion of the grafted cuttings was stored at an air temperature of 9°C for 7 days. The root-promoting effects of BHT were maintained during the low-temperature storage.

Key Words: adventitious roots, cutting graft, localized temperature control.

Introduction

The cutting-graft method is often used in the production of fruit-vegetable plants, particularly for Cucurbitaceae and Solanaceae species (Shiraki et al., 1999). In this technique, grafted cuttings are obtained by grafting scions on rootstock cuttings harvested from seedlings, and then they are planted in a growing medium for rooting. The cut rootstocks lack roots and are thus easier to handle in grafting operations (Oda, 1997). In addition, using the cutting-graft method, we can obtain stronger adventitious roots than is the case in unmanipulated seedlings. The grafted cuttings are easily damaged by water stress immediately after planting, however, when the adventitious roots have not yet been produced. Therefore, an acclimatization process to reduce the water stress is necessary (Hartmann et al., 2002).

Previously, we reported that bottom-heat treatment (BHT), which involves soaking the cut end of the cuttings in warmed nutrient solution at a low air temperature, can reduce water stress after planting by improving subsequent rooting, using the cucumber (Cucumis sativus L.) as a model (Terakura et al., 2004). In addition to improved rooting, BHT allows localized temperature control around the cut ends of the cuttings, and we proposed that the treatment can thus be used to maintain the quality of cuttings during low-temperature storage. Our study showed that BHT can improve the storage quality of cuttings by improving the absorption of nutrient solution during storage (Terakura et al., 2004).

Effective storage of transplants is essential for maintaining a sufficient supply to meet the demand in the horticultural industry. As a result, many studies have investigated environmental control technology for maintaining the quality of the transplants during storage.
We propose that storage at low air temperatures, combined with BHT will improve the quality of rooting while keeping overall quality as high as if the whole cutting had been stored at a low air temperature. In transplant production based on the cutting-graft method, supplying almost-rooted rootstock cuttings “just in time” in response to the demand for grafting by taking advantage of the BHT technology would increase the production efficiency by adjusting production processes such as grafting, planting, and shipping and by shortening the rooting stage of the acclimatization process.

In the present study, we investigated the optimum BHT temperature and duration with squash (*Cucurbita moschata* Duch.) cuttings used as rootstocks for cucumber, and then tested the practicality of BHT by applying it in the commercial production of grafted transplants to gather the basic data required to permit the practical application of the technique.

### Materials and Methods

#### Experiment 1. Optimum BHT temperature

Squash (*C. moschata*) ‘Yuuyuuikki’ (black type) seedlings were grown in a growth chamber with artificial light for 7 days at an air temperature of 28°C, relative humidity of 80%, photosynthetic photon flux density (PPFD) of 550 µmol·m⁻²·s⁻¹, and 12 h photoperiod. Cuttings were then harvested by cutting the hypocotyl of seedlings 60 mm below the cotyledons.

Four groups of cuttings (*n* = 10) were exposed to BHT with different temperatures (22, 27, 32, or 38°C) during 1 day of storage at an air temperature of 14°C, a relative humidity of 80%, and PPFD of 10 µmol·m⁻²·s⁻¹ with continuous lighting in a refrigerator. BHT was conducted by soaking 20 to 30 mm beyond the cut end of the hypocotyl in nutrient solution (A-type recipe of Otsuka House Solution, diluted by 1/2; Otsuka Chemical Co., Ltd., Japan) warmed in water baths with electric heaters controlled by thermostats. Control cuttings (*n* = 10) were soaked in nutrient solution maintained at 16°C. During BHT, each cutting was supported by inserting the hypocotyl in a hole (ϕ = 6 mm) in adiabatic board (10-mm thickness) floating on the solution. After the treatments, cuttings were transplanted into glass vessels containing the nutrient solution and grown for 3 days in a growth chamber with an air temperature of 28°C, relative humidity of 60–80%, PPFD of 200 µmol·m⁻²·s⁻¹, with continuous lighting. Root fresh weights of the rooted plants were measured 3 days after planting. In this and the other two experiments, we only included roots longer than 1 mm in our weight measurements and counts. To investigate the effects of air and BHT temperatures on rooting after planting, the experiment was conducted under nine combinations of air temperature (16, 20, and 26°C) and BHT temperature (14–32°C).

#### Experiment 2. Optimum duration of BHT

Squash cuttings harvested following the same procedure described for Experiment 1 were exposed to BHT (31°C) for 8, 12, 18, or 24 h of storage (*n* = 7) at an air temperature of 14°C, relative humidity of 80%, and PPFD of 10 µmol·m⁻²·s⁻¹ with continuous lighting. After the treatments, the cuttings were transplanted into glass vessels containing nutrient solution, and grown for 3 days in a growth chamber with an air temperature of 29°C, relative humidity of 60–75%, PPFD of 200 µmol·m⁻²·s⁻¹, and 12 h photoperiod. An additional 10 cuttings were exposed to BHT up to 66 h to observe the rooting of cuttings during BHT. Changes in the proportion of cuttings that exhibited roots as a function of the time after starting BHT was estimated based on visual observations.

#### Experiment 3. The practicality of BHT in transplant production

Figure 1 illustrates the steps used in this experiment. The growing of seedlings and subsequent grafting were conducted according to the commercial production process used by Bergearth Co., Ltd. (Ehime, Japan). Rootstock seedlings of ‘Yuuyuuikki’ squash (black type) and scion seedlings of ‘Gurinrakkusu II’ cucumber were grown simultaneously until cotyledon expansion occurred in a commercial greenhouse operated by Bergearth Co., Ltd. In one group of squash plants,

![Fig. 1. Diagram of the two treatment regimes used in Experiment 3.](image-url)
rootstock cuttings were harvested by cutting the hypocotyl of each squash seedling 60 mm below the cotyledons, and the rootstock cuttings were exposed to BHT (30°C) for 1 day at an air temperature of 9°C, relative humidity of 95–99%, and PPFD of 10 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \). Control squash seedlings were not exposed to BHT, but were instead grown in the same greenhouse for 1 day longer. Rootstock and scion cuttings were harvested immediately before grafting in both treatments.

Using the cut grafting method, scion cuttings were grafted onto rootstock cuttings exposed to BHT or onto control cuttings. One portion (BHT: \( n = 10 \), control: \( n = 10 \)) of the grafted cuttings was planted in growing medium containing peat as the main component. The cuttings were then grown for 6 days in a plastic-film tunnel at air temperatures of 20–34°C and 17–20°C (day and night) and relative humidities of 75–99% and 85–99% (day and night). These humidity conditions were relatively low compared with those (a minimum of 90%) commonly used in acclimatization. Another portion (BHT: \( n = 10 \), control: \( n = 10 \)) of the grafted cuttings was stored at an air temperature of 9°C for 7 days, during which time nutrient solution was supplied to the cut end of the grafted cuttings. The grafted cuttings were transplanted into peat-based growing medium after storage, and were then grown for 8 days in the plastic-film tunnel. At the end of the growing period, we measured the shoot and root fresh weights and counted the number of roots.

**Results and Discussion**

**Optimum BHT temperature**

Three days after planting, the fresh weight of roots from the cuttings increased with increasing BHT temperature up to 27°C, then decreased at higher temperatures (Fig. 2). Root fresh weights of cuttings exposed to BHT temperatures of 27 and 32°C were 1.7 and 1.6 times the control value (at 16°C), respectively. The root fresh weight of cuttings exposed to a BHT temperature of 38°C was 0.8 and 0.5 times the value in the control and 32°C treatments, respectively. This trend agrees with the results in our previous study that used cucumber cuttings as a model (Terakura et al., 2004).

Root fresh weights of the cuttings 4 days after planting tended to increase with increasing BHT temperature up to 32°C at air temperatures of 16, 20, and 26°C (Fig. 3). However, there was no significant difference among values for a given BHT temperature at different air temperatures. The influence of air temperature on the emergence of adventitious roots is probably small for squash cuttings that root quickly, whereas air temperature should be carefully controlled throughout the rooting period in a general cutting propagation (Hartmann et al., 2002). However, BHT should be conducted under air temperatures high enough to avoid low-temperature injury but low enough to protect the quality of the cuttings during storage, because metabolism increases with increasing temperature (Heins et al., 1994).

Controlling the air temperature at a relatively low level also effectively maintains a low vapor pressure deficit and thus reduces the excessive transpiration of the cuttings due to the high vapor pressure deficits that can arise at higher air temperatures.

**Optimum BHT duration**

The fresh weight of roots from planted cuttings increased linearly with increasing BHT duration from 0 to 24 h (Fig. 4). For cuttings that remained in the BHT solution (i.e., were not planted), rooting was observed in 10% of the cuttings 30 h after starting BHT, whereas no rooting was observed at 26 h. The percentage of rooting increased with time from 30 h after starting BHT and reached 100% by 66 h (Fig. 5). To avoid damage to roots during planting, it is desirable to end BHT just...
before cuttings begin to root; damage to the root system during transplanting may delay the subsequent growth of the root system and may allow the entry of soil fungi that cause root rots (Styer and Koranski, 1997). Based on the results of our experiment, the optimum BHT duration for squash cuttings is 26 to 30 h (approximately 1 day).

The practicality of BHT in transplant production

Hypertrophy of cut ends was observed in all cuttings at the end of BHT. Rooting was not observed during BHT, although white protuberances were observed on the hypertrophied cut ends of several cuttings. The hypertrophy of cut ends probably indicates an improving of the quality in the rooting sites of cuttings. Two days after planting the cuttings, wilting of the rootstock cotyledons was observed in all plants. However, by 4 days after planting, 90% of the BHT cuttings had recovered from the wilting, whereas all of the non-BHT cuttings (i.e., those subject to conventional acclimatization) failed to recover (Fig. 6). The faster recovery of BHT cuttings from wilting seems to have been caused by the earlier development of adventitious roots. As can be seen in Figure 7, BHT cuttings produced a more substantial root system than non-BHT cuttings, thus allowing for greater water absorption. The relatively severe wilting observed in this experiment may be attributed to the relatively low relative humidity after planting compared with the humidity levels used in the more common acclimatization process. Because cuttings exposed to BHT recovered from wilting earlier than those unexposed to BHT even at this relatively low humidity, this suggests that the acclimatization process could be simplified due to the improved rooting after planting that was observed in BHT cuttings.

Six days after planting the grafted cuttings, total and root fresh weights of the BHT plants were 1.3 and 4.0 times the corresponding values for the non-BHT plants, respectively, and the number of roots on the BHT plants was 1.6 times that of the non-BHT plants (Table 1). Eight days after planting following storage at 9°C for 7 days, the total and root fresh weights of the BHT plants

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**Fig. 4.** Effects of the bottom-heat treatment (BHT) duration on root fresh weight 5 days after planting (Experiment 2). Vertical bars indicate SE (n = 7).

**Fig. 5.** Time course of the proportion of squash cuttings that rooted during bottom-heat treatment (BHT; Experiment 2).

**Fig. 6.** Condition of the grafted plants from squash cuttings that were either exposed (left) or unexposed (right) to bottom-heat treatment (BHT). The photograph was taken 4 days after planting.

**Fig. 7.** Degree of root development 6 days after planting in grafted plants from squash cuttings that were either exposed (left) or unexposed (right) to bottom-heat treatment (BHT).
were 1.3 and 2.5 times those of the non-BHT plants, respectively, and the number of roots on the BHT plants was 1.3 times that of the non-BHT plants (Table 2).

These results indicate that the root-promoting effects of BHT can be maintained during subsequent low-air-temperature storage, which confirms the results of our previous report using cucumber cuttings as a model (Terakura et al., 2004).

In conclusion, the application of short-term BHT on the squash cuttings during storage at a low air temperature induced localized growth-enhancement at the cut end, and rooting of the cuttings was improved after planting compared with that of cuttings that were not exposed to BHT. We estimated an optimum BHT temperature of 27 to 32°C and optimum treatment duration of approximately 1 day. In the simulated commercial production of grafted cucumber transplants, BHT during storage at a low air temperature reduced post-planting water stress in the grafted cuttings by improving their rooting significantly compared with the conventional acclimatization process. This technique would thus reduce labor requirements of transplant growers during the acclimatization phase because it would ensure that the cuttings are ready to develop roots quickly, regardless of the weather.

**Table 1.** Fresh weights and number of roots of cuttings obtained by grafting cucumber scion cuttings on squash rootstock cuttings with or without bottom-heat treatment (BHT) for 1 day during storage at a low air temperature (Experiment 3).

| Treatment | Before planting | 6 days after planting |
|-----------|-----------------|-----------------------|
|           | Total FW (g/cutting) | Total FW (g/plant) | Root FW (g/plant) | Number of roots |
| BHT       | 1.90±0.09\(^*\)       | 2.06±0.07           | 0.12±0.01         | 27.0±2.0        |
| Non-BHT   | 1.99±0.09           | 1.54±0.10           | 0.03±0.00         | 17.4±1.8        |

Significance of difference (\(t\)-test)\(^\(\ast\)\):

- NS: non-significant at \(P = 0.05\);
- \(*\): significant at \(P = 0.01\).

\(^\(\ast\)\) Means ± standard errors are shown (before planting: \(n = 5\); 6 days after planting: \(n = 10\)).

**Table 2.** Fresh weights and numbers of roots of grafted cuttings stored for 7 days at 9°C (Experiment 3).

| Treatment | Before planting | 8 days after planting |
|-----------|-----------------|-----------------------|
|           | Total FW (g/cutting) | Total FW (g/plant) | Root FW (g/plant) | Number of roots |
| BHT       | 2.15±0.15\(^*\)       | 2.23±0.07           | 0.15±0.02         | 24.1±2.0        |
| Non-BHT   | 2.36±0.05           | 1.72±0.09           | 0.06±0.01         | 18.6±3.2        |

Significance of difference (\(t\)-test):

- NS: non-significant at \(P = 0.05\);
- \(*\): significant at \(P = 0.01\).

The cuttings were obtained by grafting cucumber scion cuttings on squash rootstock cuttings that were either exposed or unexposed to bottom-heat treatment (BHT) for 1 day during storage at a low air temperature.

\(^\(\ast\)\) Means ± standard errors are shown (before planting: \(n = 5\); 8 days after planting: \(n = 10\)).

\(^*\) NS: non-significant at \(P = 0.05\); \(*\): significant at \(P = 0.01\).

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