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Stimulation of Rooting by Exposing Cuttings of Runner Plants to Low Temperatures to Allow the Raising of Strawberry Seedlings during Summer

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The effect of exposing runner plants cut from strawberry (*Fragaria* × *ananassa* Duch. 'Tochiotome') to low temperature to stimulate rooting was examined for its usefulness as a practical strategy for raising seedlings from cuttings taken from runner plants dropping in the air to force elevated bed strawberry culture during the summer. The rooting of cuttings cultured at various temperatures for 7 days was good at 15°C to 25°C but was reduced at 5°C and 30°C; however, cuttings excised from ten different cultivars showed varying responses for the most suitable temperature for rooting. The rooting of cuttings from 'Tochiotome' cultured at 30°C for 5 days after pretreatment at 5°C for 2 days was almost the same as for cuttings cultured at a constant 15°C for 7 days, but rooting of cuttings cultured at 30°C for 7 days was retarded. Interestingly, rooting was observed by storing only the crown at 5°C for 2 days even if both foliage and shoot tip were detached. Rooting was better in dark conditions than with 16-h day length, and was best for the subsequent development stage of runner plants with 2.5 leaves attached. Our results showed that rooting occurred during summer by culturing cut runner plants with 2.5 leaves at 30°C for 5 days after pretreatment at 5°C in the refrigerator for 2 days in the dark.

Key Words: cutting, low temperature, rooting, runner plant, strawberry (*Fragaria* × *ananassa* Duch.).

Introduction

Strawberry culture in Japan starts with nursery plant production, usually by vegetative propagation using runner plants. Recently, to avoid soil-borne diseases to ensure high quality fruit year-round and to save labor, a raising seedling method using cuttings taken from runner plants dropping in the air to start seldom rooting was used (Fukuda et al., 1984; Ishihara et al., 1994; Mitsui and Fushihara, 1998; Yabe et al., 1999). Adventitious roots originating from the basement of the crown of strawberry runner plants have thick primary roots and thin lateral roots. Primary roots appear in the pericycle and grow by breaking the cortex (Waisel et al., 2002). The primary roots of runner plants develop as a contractile root that draws the crown under the ground soon after it attaches to the soil. Under natural conditions, the crown drawn into the soil can easily produce roots if the soil moisture and temperature are suitable for rooting. If the primary root is not so active, the growth of runner plants is restricted.

Cuttings of rootstocks of fruit trees, flowering trees and shrubs or trees used for landscape and architecture can not root without suitable environmental conditions, even if the root primordia have already developed (Morishita and Oyama, 1972; Westwood, 1978). Therefore, by picking nursery plants in the most favorable season and by controlling soil moisture, humidity, light or temperature, growers can increase the proportion of established plants (Hansen, 1988; Loach, 1988; Nakayama and Harada, 1962; Ooishi et al., 1978). In strawberry culture, the quality of runner plants and prevention of wilting by shading or mist culture have been investigated to increase rooting (Kimura, 1994). However, in forcing culture, which is the main cropping type of strawberry, the identified problems are delayed rooting of cuttings, missing plants and poorly established plants caused by slow or failed growth of roots, particularly when nursery plants are prepared during the summer. For stem cutting chrysanthemum, rooting is stimulated by low temperature storage for about one week even if the cuttings are grown at higher temperatures (Yonekura et al., 1999). At present, growers

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pick runner plants and transplant them after storage in a refrigerator at 5°C for 2 days during the summer; however, the relationships between rooting and temperature or light are unclear. In this study, we investigated various factors that may influence rooting from runner plants cut from elevated bed-cultured strawberry.

Materials and Methods

Plant materials

Strawberry (*Fragaria* × *ananassa* Duch. 'Tochiotome') plants were planted in an elevated bed system in an expanded polystyrene container every April, 2002–2004. All runners appearing by the end of April were removed to enrich the strawberry plants. Runner plants appearing in July and August were collected and used as cuttings. They were put in a container and mist was provided (each experiment was conducted in the same year). The cuttings were transferred to mesh set 5 cm above the bottom of a plastic box (W22 cm × D32 cm × H23 cm) with 10 mm deep water. The box was covered with thin vinyl film (5 layers of polyethylene/polypropylene, Nippon Paper-Pak Co., Japan) to allow air permeability but maintain sufficiently high humidity.

Root primordia initiation and root development from runner plants in each developmental stage

Strawberry plants were cultured in a ventilated greenhouse at 25°C and their runner plants were cut at each developmental stage of 0, 0.5 (one folded leaf on the shoot tip), 1 (one developed leaf), 1.5, 2, 2.5, and 3 leaves. Root primordia and roots in the basement of the crown cut horizontally and runner plants with 2.5 leaves ventilated at 30°C were observed using a stereoscopic microscope. Rooting was defined as root growth from root primordia without the formation of root primordia.

Relationship between number of leaves attached to runner plants and rooting

Runner samples with 1.5, 2.5, or 3.5 leaves were set in a plastic box and kept at 25°C in the dark. Rooting was observed after 7 days. Two replicates were performed with 12 cuttings in each replicate.

Effects of light and temperature on rooting

Ten cuttings, with 2.5 leaves each, set in a plastic box, were either retained for rooting with 16-h day length or kept in the dark at 25°C. Rooting was observed after 7 days. Two replicates were performed. Ten cuttings, with 2.5 leaves each, set in a plastic box, were cultured for 5 days at various temperatures, 5, 10, 15, 20, 25, and 30°C. Rooting was observed after 5 days and two replicates were performed. To evaluate the difference in sensitivity to temperature among 10 cultivars of 'Fukuba' (raising 1899), 'Donner' (1945), 'Hokowase' (1957), 'Reiko' (1978), 'Toyonoka' (1984), 'Nyoho' (1985), 'Akihime' (1992), 'Akanekko' (1994), 'Tochiotome' (1996), and 'Aisutoro' (2001), each cultivar was cultured in a plastic box at 17°C, 25°C, or 30°C in the dark and their rooting was observed after 7 days.

Stimulation of rooting by pretreatment at low temperature

Ten cuttings, with 2.5 leaves each, set in a plastic box, were pre-stored under different temperature regimes: 1) 15°C for 2 days followed by culture for 15°C for 5 days (15–15°C), 2) 30°C for 2 days followed by culture for 30°C for 5 days (30–30°C), 3) 5°C for 2 days followed by culture for 30°C for 5 days (5–30°C), and 4) 5°C for 2 days followed by culture for 5°C for 5 days (5–5°C). Rooting was observed 7 days after the beginning of pretreatment, which was performed with two replicates. To determine the storage period to best stimulate rooting at low temperature, ten cuttings, with 2.5 leaves each, were kept for 1 to 8 days at 5°C, and then cultured at 30°C for 7 days. Rooting was checked after 7 days. Ten cuttings were used in each treatment, with 2 replications.

Effect of removing foliage and shoot tip on rooting

Three types of crowns were prepared: 1) crown with intact foliage and shoot tip, 2) crown with shoot tip only, and 3) crown only. These 3 types of crown were set for cutting in a plastic box. They were pre-stored for 2 days at either 5°C or 30°C, and then cultured for 5 days at 30°C. Rooting was recorded after 7 days with 20 cuttings for each crown type.

Results

Appearance of root primordia and roots from runner plants at each developmental stage

Adventitious root primordia were already formed during the early developmental stage (0, 0.5, 1.5 leaves) of runner plants cultured at 25°C (Fig. 1a–c). At 25°C, roots appeared slightly on the epidermis at the stages with 2 and 2.5 leaves (Fig. 1d and 1e, respectively) and several roots appeared at the stage with 3 leaves (Fig. 1f). At 30°C, roots were not observed at the stage with 2.5 leaves, but root primordia were formed to the same degree as at 25°C (Fig. 1g).

Cuttings are easily etiolated when the number of leaves attached to the runner exceeds the optimum. As shown in Figure 1g, the best stage of runners was determined according to the number of leaves attached to the runner. The frequency of rooting of cuttings with 2.5 leaves (2 developed leaves and one folded leaf on shoot tip) was significantly higher than that with 1.5 leaves, but no significant differences were recognized between cuttings with 2.5 and 3.5 leaves (Fig. 2). Runner plants with 2.5 leaves were used for subsequent experiments.

Effect of light or dark on rooting

Cuttings in this study were placed on mesh set on the bottom of a plastic box, not in the dark. Thus, we

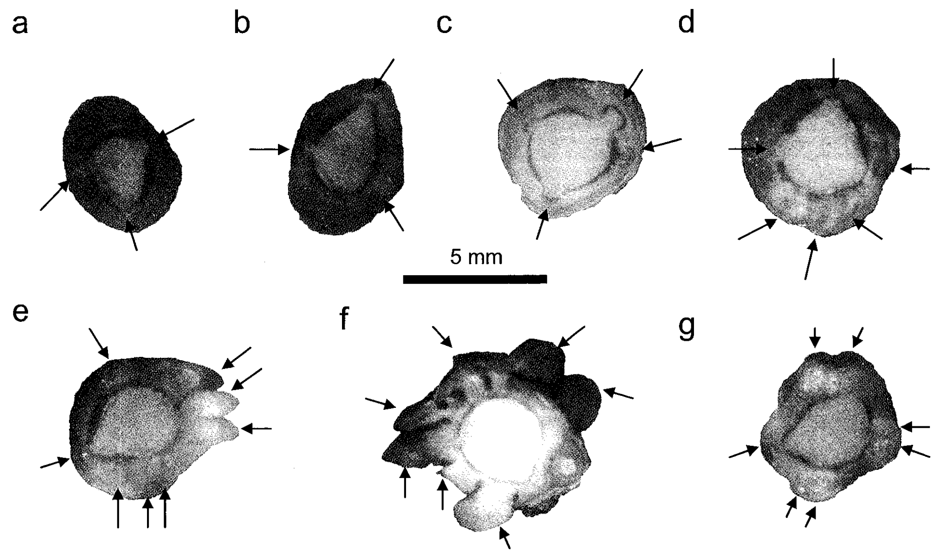

Fig. 1. Appearance of root primordia and roots in each developmental stage of runner plants. Microscopic images (a–g) show a horizontal section of crown basement, a–f are sections prepared at the developmental stages of runner plants with 0, 0.5, 1.5, 2.0, 2.5, and 3.0 leaves, respectively, from runner plants cultured at 25°C; g is a section prepared at the 2.5-leaf stage from runner plants cultured at 30°C. Arrows indicate both root primordia and root.

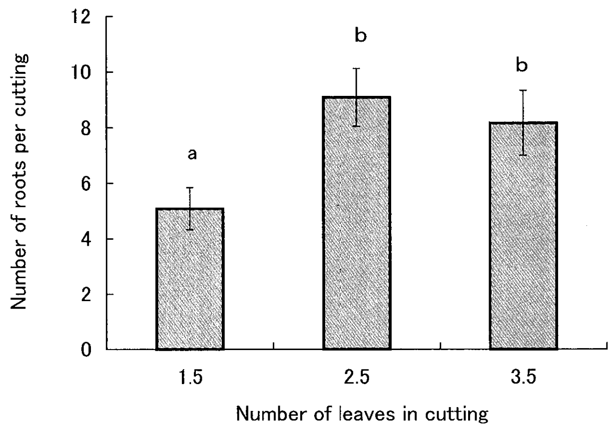

Fig. 2. Number of rootings of cuttings with varying numbers of intact leaves at 25°C for 7 days. The half number of leaves (0.5 number) represents the folded leaf on the shoot tip. Each bar shows SE (n = 12). Means followed by the same letter were not significantly different by Tukey's test at $P < 0.05$ (n = 12).

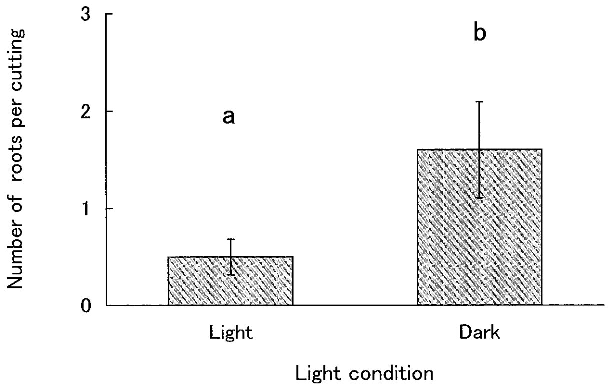

Fig. 3. Number of rootings of cuttings at 25°C for 7 days of culture under light or dark conditions. Each bar represents SE (n = 10). Means followed by the same letter were not significantly different by Tukey's test at $P < 0.05$ (n = 10).

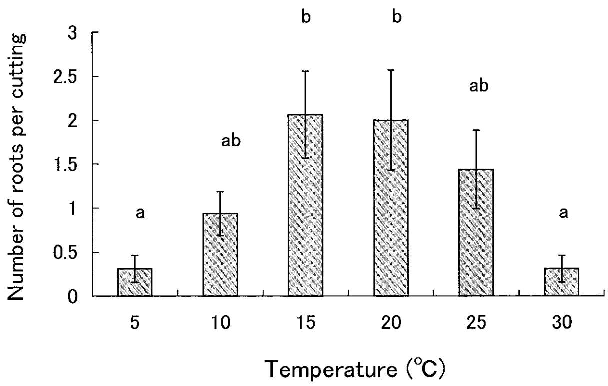

Fig. 4. Number of rootings of cuttings at various culture temperatures for 5 days. Each bar represents SE (n = 16). Means followed by the same letter were not significantly different by Tukey's test at $P < 0.05$ (n = 16).

compared the frequency of rooting between dark and 16-h light conditions. The frequency of rooting was higher in the dark than in the light (Fig. 3). The dark condition was used for subsequent experiments.

Effect of storage temperatures on rooting and comparison among strawberry cultivars

Rooting at 15°C and 20°C was better than at the other temperatures (Fig. 4). Rooting apparently decreased at high temperature (30°C) and low temperature (5°C) compared with 15°C and 20°C. Rooting at 25°C increased compared with that at 30°C (Fig. 4). When suitable temperatures for rooting among strawberry cultivars were compared, rooting of all cultivars decreased at 30°C (Fig. 5). The most suitable temperature

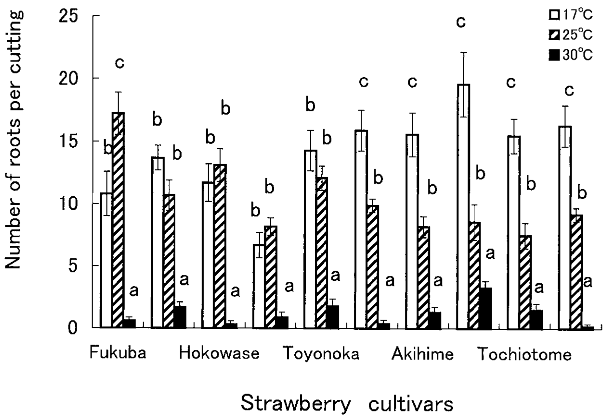

Fig. 5. Comparison of temperatures suitable for rooting among various strawberry cultivars. Cuttings were cultured at 17°C (□), 25°C (▨), or 30°C (■) for 5 days. Each bar represents SE (n = 10). Means followed by the same letter were not significantly different by Tukey's test at $P < 0.05$ (n = 20).

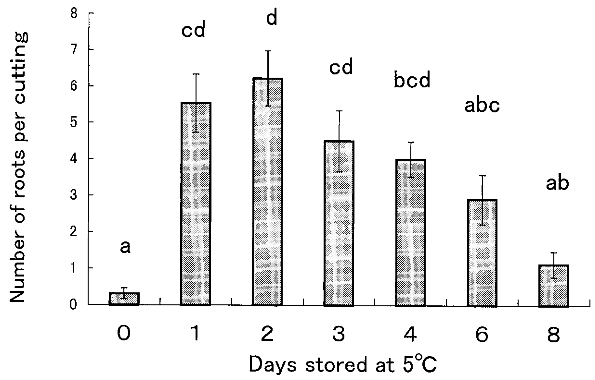

Fig. 6. Effect of pretreatment (5°C) period on rooting. Cuttings were stored at 5°C for several days, then transferred at 30°C for 5 days. Each bar represents SE (n = 10). Means followed by the same letter were not significantly different by Tukey's test at $P < 0.05$ (n = 10).

was 17°C for 'Nyoho', 'Akihime', 'Akanekko', 'Tochiotome', and 'Aisutoro', 17°C and 25°C for 'Donner', 'Hokowase', 'Reiko', and 'Toyonoka', and 25°C for 'Fukuba'.

Effect of pretreatment period on rooting

As shown in Figure 6, the most suitable pretreatment period at 5°C for rooting of cuttings was determined. Pretreatment at 5°C for 2 days was best for rooting but only one day of pretreatment was possible to initiate rooting (Fig. 6). Pretreatment of more than 3 days at 5°C reduced rooting.

It was then examined if rooting with this low temperature treatment is more or less comparable with rooting at the most suitable culture temperatures. As shown in Figure 7, rooting of cuttings cultured at 30°C for 5 days after pre-storing at 5°C for 2 days was almost the same as cuttings cultured at 15°C for 7 days; however, rooting of cuttings cultured at 30°C for 7 days was significantly restricted, similar to the rooting shown in Figure 4.

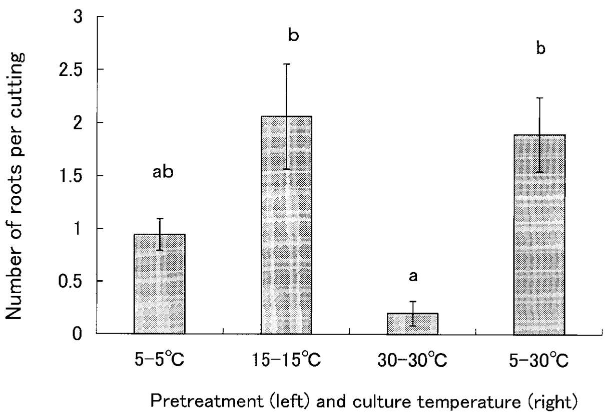

Fig. 7. Effect of combination of pretreatment and culture temperature. 5–5°C (pretreatment at 5°C for 2 days followed by 5°C for 5 days), 15–15°C (pretreatment at 15°C for 2 days followed by 15°C for 5 days), 30–30°C (pretreatment at 30°C for 2 days followed by 30°C for 5 days), and 5–30°C (pretreatment at 5°C for 2 days followed by 30°C for 5 days). Each bar represents SE (n = 20). Means followed by the same letter were not significantly different by Tukey's test at $P < 0.05$ (n = 10).

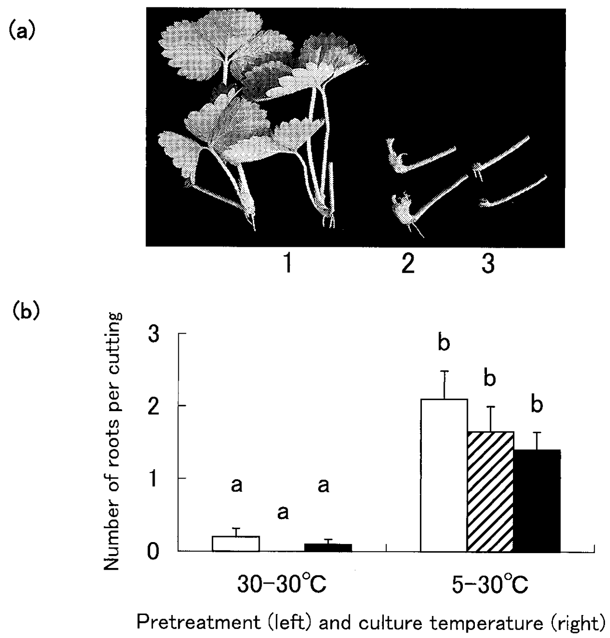

Fig. 8. Effect of removing foliar and shoot tip on rooting of cuttings at 5°C. (a) 1: cuttings with intact foliar and shoot tip, 2: cuttings with shoot tip only, and 3: cuttings devoid of both foliar and shoot tip. (b) Frequency of rooting in 1 (□), in 2 (▨), in 3 (■) stored at 5°C or 30°C for 2 days, then cultured at 30°C for 5 days. Each bar is SE (n = 20). Means followed by the same letter were not significantly different by Tukey's test at $P < 0.05$ (n = 20).

Effect of removal of foliage and shoot tip on rooting of cuttings

Whether the rooting of cuttings needs foliage or the shoot tip was investigated. Rooting was still possible at 30°C even if both foliage and shoot tip were detached before storage at 5°C (Fig. 8).

Discussion

In this study, runner plants cut from strawberry produced roots easily by culturing at 15°C to 20°C for 7 days, but they rooted only slightly at 30°C (Fig. 4). Thus, root production from cuttings is impossible in an ordinary greenhouse during the summer. By pre-storing cuttings at 5°C for only 2 days, then culturing them at 30°C for 5 days, rooting increased similar to cuttings at a constant 15°C for 7 days, but rooting did not increase in cuttings stored continuously at low and high temperatures. Metabolic changes of substances that initiate rooting may occur during pretreatment of cuttings even for only 2 days at low temperature or during storage at 15°C to 25°C for several days (Fig. 7). Rooting of stem cuttings of chrysanthemum and carnation increased by 7-day storage at 15°C with indolebutyric acid (IBA) treatment and by 12-day storage at 14°C with naphthaleneacetic acid (NAA) treatment, respectively (Yonekura et al., 1999; Van de Pol and Vogelegang, 1983). In both stem cuttings, rooting needed for the application of auxin under low temperature treatment. On the other hand, rooting of strawberry runner cuttings of was stimulated by low temperature treatment alone.

The tendency for rooting to be restricted at 30°C, but stimulated below 25°C was recognized in all cultivars used in the present examination. However, the cultivars showed varying responses for the optimum rooting temperature. Rooting of recent cultivars (raising 1985–2001) that bear fruit early, such as 'Nyoho', 'Akihime', 'Akanekko', 'Tochiotome', and 'Aisutoro' was best at 17°C, but comparatively old cultivars (raising 1945–1984), such as 'Donner', 'Hokowase', 'Reiko', and 'Toyonoka' was suitable at 17°C and 25°C. Rooting of the oldest cultivar of 'Fukuba' (1899) was best at 25°C (Fig. 5); however, it is unclear why recent cultivars need lower temperature than old cultivars for rooting.

The adventitious root primordia around the crown were already initiated at the stage of the runner plants used in this study (Fig. 1); therefore, rooting during the experimental period of 7 days may have been mainly due to the growth and elongation of the primordia that had already started, but no rooting from newly formed primordia. Low temperatures initiate root growth from the primordia, but high temperatures may either retard or damage the initiation of root growth from the primordia. The importance of temperature as a major determinant in adventitious root growth was made clear in this study. As shown in Figure 8, the crown is a special organ that can perceive signals of low temperatures for rooting, because rooting occurred even when foliage and the shoot tip were removed; however, the mechanism of why crowns treated with low temperatures could initiate rooting needs further clarification. Hormonal changes in the crown of runner plants treated with low temperature should be investigated because some phytohormones may have an important role in adventitious root formation (Hansen, 1988).

Recently, strawberry farmers in Aichi prefecture who culture strawberry in greenhouses during summer propagated plants by planting runner plants pre-stored at 5°C for only 2 days. The data of this study showed that a broad temperature, ranging 15°C to 25°C, was suitable for rooting of cuttings and keeping cuttings in the greenhouse for rooting and subsequent propagation in all seasons except summer was possible. During the summer, maintaining the temperature in a greenhouse from 15°C to 25°C is hard without an expensive cooling system. From these results, we showed that pretreatment for 2 days at 5°C initiated the rooting of cuttings similar to the rooting frequency at temperatures of 15°C to 25°C. Thus, this study presents a practical and economically viable method for strawberry production in greenhouses during the summer.

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