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Improvement of Storage Quality of Eggplant Grafted Cuttings by Warming of Graft Union at the Beginning of Low-air-temperature Storage

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Summary

In storage of grafted cuttings, development of graft union at beginning of storage is important, because the scions are easy to be exposed to water stress during storage when the vascular tissues are not connected. Warming treatments were carried out to eggplant grafted cuttings by soaking the cuttings in warmed nutrient solution from the basal cut-end to the graft union at beginning of storage to improve the graft development. The treatment was applied for 4 d at the beginning of the 28 d storage. Values of the chlorophyll fluorescence parameter Φ_{PSII} of the warmed cuttings did not decrease significantly during the 28 d storage, but those of the non-warmed cuttings did. The net photosynthetic rate of scion leaves decreased during storage in warmed and non-warmed cuttings, but values were higher in the warmed cuttings than in the non-warmed. Leaf conductance and water content in the scions of the warmed cuttings was maintained during storage, whereas those of non-warmed decreased. Rooting from the rootstock was not initiated during the 28 d storage. When cuttings were grown for 4 d in a growth chamber following storage, fresh weights of both shoot and root from warmed cuttings were significantly greater than those from non-warmed, control and non-stored cuttings. Those results indicate that the storage quality of the grafted cuttings was improved by warming of the graft union at the beginning of storage, which probably improved the development of the graft union and thereby improved water and nutrient supply to the scion.

Key words. chlorophyll fluorescence – cutting graft – leaf conductance – net photosynthetic rate – localized temperature control – water stress

Introduction

The cutting-graft method is often used in the production of vegetables. Grafted cuttings are obtained by grafting scions onto unrooted rootstock cuttings taken from seedlings, and are then transplanted in growing medium for rooting. The cut rootstocks lack roots and are thus easier to handle in grafting operations. In addition, using the cutting-graft method, we can obtain stronger adventitious roots than the case in unmanipulated seedlings. If grafted cuttings could be stored for several weeks, efficiency of transplant production would be improved through flexibility in grafting, transplanting, and shipping.

Previous studies investigated low temperature storage with lighting which improves storage quality of transplants (Heins et al. 1994; Kubota et al. 1995; Kozai et al. 1996). The low temperature storage under lighting is also effective to cut herbaceous shoots (Fujiwara et al. 1997, 1999). Supply of nutrient solution is essential to improve storage quality of cut shoots with the lighting, because storage quality of cut shoots of chervil is sometime reduced under lighting when nutrient solution is not supplied (Fujiwara et al. 1997). Supplying nutrient solution to cut shoots can improve their storage quality, particularly the appearance of leaves (Fujiwara et al. 1997, 1999). However, in the case of grafted cutting, if nutrient solution is supplied from basal cut-end of the rootstock only, it cannot reach the scion, which is then exposed to water stress, because the vascular tissues are not immediately connected. For that reason, development of graft union at beginning of storage seems be important to improve storage quality of grafted cuttings.

In a previous study (Shibuya et al. 2007b), we reported that warming tomato and eggplant grafted cuttings by supplying warmed water to the graft union at low air temperature improves graft development and consequent growth. However, we did not examine the effects of the warming treatment on long-term storage required for the production adjustment. If graft development can be improved by the warming treatment at the beginning of storage, nutrient solution would reach the scions during the remaining storage period. This would enhance nutrient uptake by scions, and consequently improve the storage quality. In this study, we evaluated the effects of the warming treatment at the beginning of long-term storage on storage quality of grafted cuttings which was determined by the photosynthetic efficiency, water status and early growth of both root and shoot after storage.

Materials and Methods

Eggplant (*Solanum melongena* L.) cultivar 'Senryou-nigou' and *S. torvum* Sw. cultivar 'Torubamu-biga' were used as scion and rootstock, respectively. Seedlings of scion and rootstock were grown in a plastic-film greenhouse at Bergearth Co., Ltd. (Ehime, Japan) for 28 and 49 d, respectively, after seeding. A plastic tray with 200 and 162 cells containing a peat medium was used for growing scion and rootstock, respectively. The cuttings of scion and rootstock were taken from the seedlings, and then splice-grafted. Grafting tubes (inner diameter 1.7 mm, Nasunics Co., Ltd., Japan) were used to hold the graft union. Shoot length of scion and rootstock at the grafting was approximately 20 and 40 mm, respectively. Scion cuttings had two foliage leaves. Leaves of rootstock cuttings were removed at the start of storage. Experiments were begun the next day, at the laboratory at Osaka Prefecture University (Sakai, Japan).

The treatment regimes are shown in Fig. 1. The experiment was conducted with one replication. The grafted cuttings were stored in a cold chamber at an air temperature of 10 °C for 28 d. White fluorescent lamps (FL20SW, Matsushita Electric Industrial Co., Ltd., Japan) gave continuous illumination to the cuttings' canopy at a PPFD of 10 μmol m^{-2} s^{-1}. The relative humidity (RH) was not controlled. The RH near the scion monitored with a humidity data logger (TR-72U, T and D Co. Ltd., Japan) ranged from 86 to 92 % during storage. The warming treatment was applied to the graft union by soaking the cuttings from the basal cut-end of the rootstock to the graft union in warmed nutrient solution (A-type recipe of Otsuka House Solution, diluted to 1/2; Otsuka Chemical Co., Ltd., Japan), which was maintained at 28 °C with an electric heater controlled by thermostat (Fig. 2). The composition of the solution in grams per 1000 L of tap water was, total N, 130; P_2O_5, 60; K_2O, 202.5; CaO, 115; MgO, 30; MnO, 0.75; B_2O_3, 0.75; Fe, 1.35; Cu, 0.015; Zn, 0.045; Mo, 0.015. Electric conductivity was at approximately 1.3 mS/cm. Temperature of the nutrient solution and graft union was measured with thermocouples (type T) of Ø 0.32 mm and Ø 0.01 mm, respectively. The temperature in the graft union was maintained at 26 °C; 2 °C lower than the water temperature. Non-warmed treatment was carried out by soaking cuttings in non-warmed solution (10 °C; almost same as air temperature). The cuttings were supported by insertion up to the graft union in a hole (Ø 6 mm) in a styrene-foam adiabatic board (5 mm thick) floating on the solution. The warming and non-warmed treatments lasted 4 d, as determined from a preliminary experiment in which roots appeared at the basal cut-end of the rootstock at approximately 5 d. After the treatment, the cuttings were pulled from the supporting board and then held in non-warmed solution during the remainder of the storage period, with only approximately 20 mm of the rootstock submerged in the solution, because the storage quality of the rootstock is probably reduced by the anaerobic environment in the water (Shibuya et al. 2007b). During the remainder of the storage period, cuttings were supported by inserting the hypocotyl of the rootstock through a hole (Ø 3 mm) in the supporting board floating on the solution. Control cuttings were stored with only the basal cut-end of the rootstock in non-warmed solution throughout the 28 d storage period.

Six stored cuttings in each treatment were transplanted in plastic pots (Ø 60 mm; 55 mm height) with vermiculite medium (the maximum grain size: 5 mm or smaller) at 28 d after the start of storage (DAS), and then grown in a growth chamber (Ikeya Co., Ltd., Japan) at an

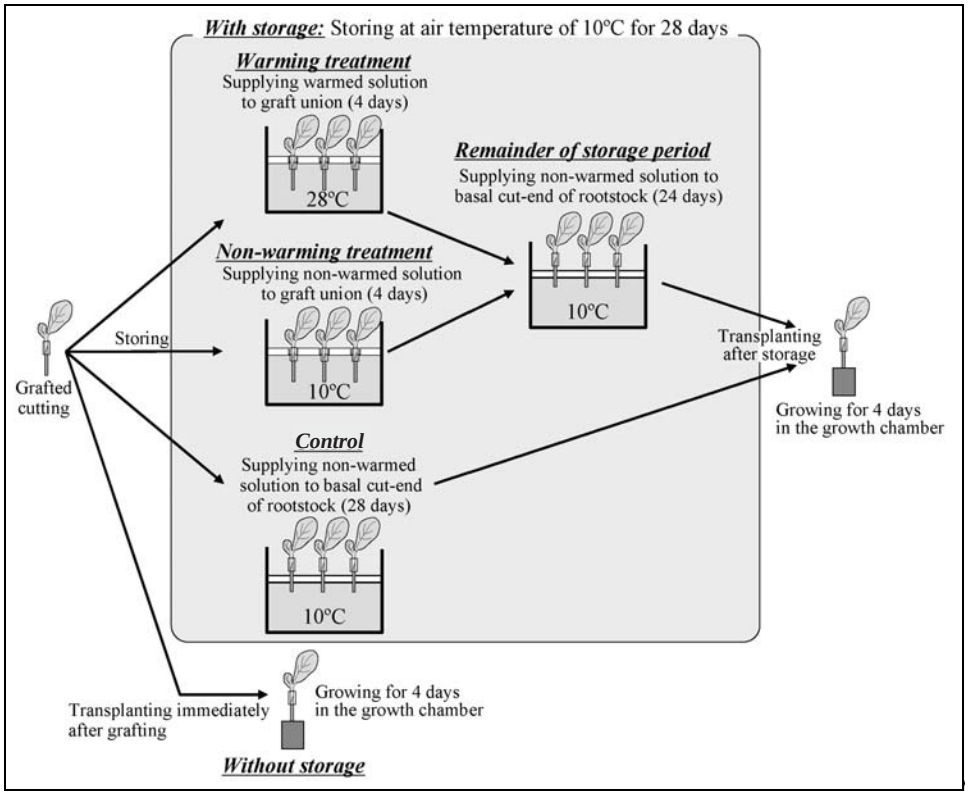


Fig. 1. Diagram of treatment regimes.

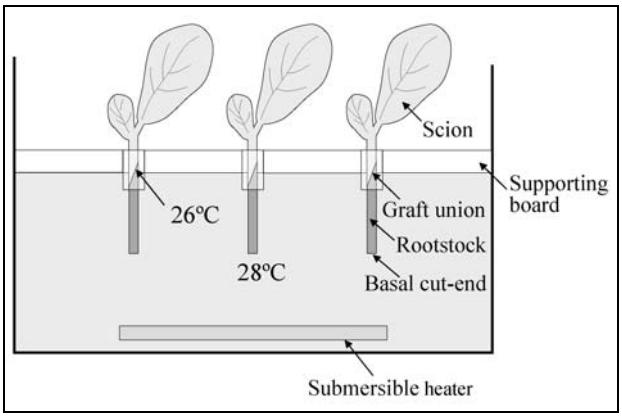


Fig. 2. Experimental setup of the warming treatment.

air temperature of 27 °C, 90 % RH, and a PPFD of 100 μmol m^{-2} s^{-1} at plant canopy level with a 12-h photoperiod. Non-stored cuttings were also transplanted at the same environmental condition immediately after grafting, and then grown in the same way. The nutrient solution (A-type recipe of Otsuka House Solution, diluted by 1/2) was supplied to the medium from the bottom of the pot as necessary. White fluorescent lamps (FPL55EX-N; Matsushita Electric Industrial Co., Ltd., Japan) were used for illumination. The shoot and root fresh weights of grafted plants were measured at 4 d after transplanting (DAT).

Samples of 5 and 6 grafted cuttings in each treatment were taken at 0 and 28 DAS, respectively, for measuring the net photosynthetic rate (P_n), chlorophyll fluorescence parameter Φ_{PSII} and leaf conductance (g_l). The P_n, Φ_{PSII} and g_l of first foliage leaves of scion were measured with a photosynthesis and fluorescence measuring system (LI-6400-40, LI-COR Inc., Lincoln, NE, USA). The Φ_{PSII} indicates quantum yield of photosystem II photochemistry (Maxwell and Johnson 2000). The PPFD of actinic light and saturating light used for measuring Φ_{PSII} was 500 and approximately 7000 μmol m^{-2} s^{-1}, respectively. The saturating light was irradiated, after fluorescence of leaf under actinic light was stabilized. The air temperature, RH and PPFD in the leaf chamber of the measuring system were maintained at 20 °C, 60 % to 70 % and 500 μmol m^{-2} s^{-1}, respectively. Other samples of 5 cuttings in each treatment were taken to measure fresh and dry weight of scion at 0 and 28 DAS. The fresh samples were dried for 72 h in drying oven at 80 °C. The water content of scions was estimated from the fresh and dry weights. The relative chlorophyll content in the scion leaves of 12 grafted cuttings was evaluated with a chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan) at 28 DAS. The means of each treatment were compared with the Tukey-Kramer test at P=0.05. The significance between means at 0 and 28 DAS were determined with the Student t-test at P=0.05 and 0.01.

Results

The values of P_n, g_l , Φ_{PSII} and relative chlorophyll contents of warmed cuttings was higher than those of non-warmed and control cuttings at 28 DAS (Table 1). The P_n values of the warmed, non-warmed and control cuttings at 28 DAS were 0.65, 0.10 and 0.18 times of the respective pre-storage values measured at 0 DAS. The P_n values of the warmed cuttings at 28 DAS were 6.76 and 3.54 times those of the non-warmed and control cuttings, respectively. The Φ_{PSII} value of the warmed cuttings did not decrease significantly during the 28 d storage, whereas those of the non-warmed and control cuttings did. The Φ_{PSII} value of the warmed cuttings at 28 DAS was 1.89 and 1.40 times those of the non-warmed and control cuttings, respectively. The g_l value of the warmed cuttings also did not decrease significantly during the 28 d storage, whereas those of the non-warmed and control cuttings did. The relative chlorophyll contents of the warmed cuttings at 28 DAS were 1.33 and 1.16 times those of the non-warmed and control cuttings, respectively.

At the end of storage, fresh weight and water content of scion of warmed cuttings was greater than those of non-warmed and control cuttings (Table 2). The scion water content of the warmed cuttings remained stable and was not different from the value determined at 0 DAS, whereas water contents of non-warmed and control cuttings decreased during the storage period. The scion dry weight of warmed cuttings at 28 DAS was greater

Table 1. Net photosynthetic rate (P_n), chlorophyll fluorescence parameter Φ_{PSII}, leaf conductance (g_l), and relative chlorophyll content of eggplant grafted cuttings at 0 and 28 d after the start of storage (DAS).

Treatment		P_n (μmol CO_2 m^{-2} s^{-1})	Φ_{PSII}	g_l (mm s^{-1})	Relative chlorophyll content[w]
0 DAS (at the start of storage)		[z]5.61±0.43	0.161±0.009	4.4±1.0	–[v]
28 DAS	Warming treatment	3.65±0.42 a[y]** [x]	0.136±0.009 a[NS]	4.9±1.3 a[NS]	27.2±1.1 a
	Non-warmed treatment	0.54±0.05 b**	0.072±0.004 b**	0.4±0.1 b**	20.5±0.7 b
	Control	1.03±0.44 b**	0.097±0.009 b**	0.6±0.1 b**	23.4±0.6 b

[z]Means ± SE (0 DAS: n=5; 28 DAS: n=6; relative chlorophyll content: n=12).
[y]Means with different letters are significantly different among the different storage treatments at 5 % level according to the Tukey-Kramer test.
[x]NS, non-significant; *, **, significantly different from the value at the start of storage at P=0.05 and P=0.01, respectively, according to t-test.
[w]Shown as chlorophyll meter value (SPAD-502, Minolta Co., Ltd., Japan).
[v]Relative chlorophyll content at 0 DAS was not measured.
Measurements of P_n, Φ_{PSII}, and g_l were taken at PPFD of 500 μmol m^{-2} s^{-1}, air temperature of 20 °C, and RH of 60 %–70 %.

Table 2. Fresh and dry weight and water content of eggplant scion at 0 and 28 d after the start of storage (DAS).

Treatment		Fresh weight (mg)	Dry weight (mg)	Water content (%)
0 DAS (at the start of storage)		[z]143 ± 12	14.5±1.4	89.8±0.7
28 DAS	Warming treatment	194 ± 7 a[y]**[x]	19.5±0.8 a*	89.9±0.4 a[NS]
	Non-warmed treatment	126 ± 7 b[NS]	17.3±0.6 a[NS]	86.2±0.4 b**
	Control	127 ± 9 b[NS]	19.1±0.7 a*	84.6±0.9 b**

[z]Means ± SE (fresh and dry weight, and water content: n=5).
[y]Means with different letters are significantly different among the different storage treatments at 5 % level according to the Tukey-Kramer test.
[x]NS, non-significant; *, ** significantly different from the value at the start of storage at P=0.05 and P=0.01, respectively, according to t-test.

Table 3. Fresh weight of eggplant grafted plants with and without storage 4 d after transplanting.

Treatment		Fresh weight (mg)	
		Shoot	Root
Without storage		[z]310 ± 16 b[y]	1±0 b
With storage	Warming treatment	439 ± 7 a	50±3 a
	Non-warmed treatment	312 ± 18 b	5±2 b
	Control	364 ± 15 b	4±1 b

[z]Means ± SE (n=6)
[y]Means with different letters are significantly different among the different storage treatments at 5 % level according to the Tukey-Kramer test.

than that at 0 DAS, but there was no significant difference among the different storage treatments. Wilting of scion leaves of non-warmed cuttings was observed during storage, whereas warmed cuttings remained turgescent. Emergence of roots from the rootstock was not observed in any treatment until 28 DAS.

When cuttings stored with different treatments were grown for 4 d in a growth chamber, the shoot and root fresh weights of plants grown from the warmed cuttings at 4 DAT were greater than those of plants grown from the non-warmed, control, and non-stored cuttings (Table 3). The shoot fresh weights of the grafted plants from the warmed cuttings were 1.41, 1.21, and 1.42 times those from the non-warmed, control, and non-stored cuttings, respectively. The warmed cuttings rooted well, whereas the others rooted poorly

Discussion

Decreases in Φ_{PSII} indicate the decline of photosynthetic efficiency in storage. The relatively high values of Φ_{PSII} of the warmed cuttings at 28 DAS indicate that the warming treatment reduced the decline. The values of Φ_{PSII} at 0 DAS were relatively low comparing with our previous investigation (Shibuya et al. 2007b) although same measurement method was used. This is probably due to the difference in growing or shipping conditions. The decreases in g_l of the non-warmed and control cuttings at 28 DAS indicate a decline in gas exchange ability as stomata close in response to the decrease in water content. The higher water content of scions of warmed cuttings compared with those of non-warmed and control cuttings at 28 DAS is probably the result of the improved water supply to the scion through improved graft development at the beginning of storage. This probably also allowed for enhanced nutrient supply to the scion which may have contributed to the maintenance of higher chlorophyll levels. The higher P_n of warmed cuttings than those of non-warmed and control cuttings at 28 DAS seems to be a result of the reduced decline of photosynthetic efficiency, gas exchange ability, and chlorophyll content. Ideally, dry weight should be maintained during storage, yet that of warmed cuttings increased. This increase was probably due to a higher light intensity during storage than the light compensation point. If the experiment were repeated with illumination at the light compensation point to maintain the dry weight of the warmed cuttings, that of the non-warmed and control cuttings might be decreased. The higher early growth of warmed cuttings after storage also seems to be a result of the higher photosynthetic ability, in addition to the improved graft development due to warming of the graft union (Shibuya et al. 2007b) and the improved growth stage of the position at which roots developed (Shibuya et al. 2007a; Terakura et al. 2004; Shimizu-Maruo et al. 2008) due to warming of the rootstock's basal cut-end.

The water content of the control cuttings decreased significantly during storage. This decrease was due to the relatively low RH compared with conventional storage of grafted cuttings. This indicates the possibility of maintaining storage quality of grafted cuttings by warming treatment even at relatively low RH. In conventional storage of grafted cuttings, a RH of nearly 100 % is necessary

to maintain water status of scions. To produce such a condition, high-cost storage equipment is necessary. If the grafted cuttings could be stored at lower RH, the storage equipment could be simplified. In addition, storage of grafted cuttings at lower RH, made possible by the development of the graft union at the beginning of storage, would improve storage quality, because storage at high RH causes elongation and promotes the development of diseases (HEINS et al. 1994).

In conclusion, the storage quality of grafted eggplant cuttings, as determined by the photosynthetic efficiency, water status and early growth of both root and shoot after storage, was improved by warming of the graft union at the beginning of long-term storage, which probably improved the development of the graft union and thereby improved water and nutrient supply to the scion. This technique allows for storage of grafted cuttings in low-cost storage equipment that cannot maintain high RH".

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