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Ascorbic acid promotes graft-take in sweet pepper plants (*Capsicum annuum* L.)

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Abstract

The cause of the low survival rate of sweet pepper plants after grafting was investigated and compared with findings of eggplant and tomato plants, and the promotive effect of ascorbic acid (AA) was determined. Sweet pepper plants formed less callus at the cut surfaces of the stem compared to eggplant and tomato plants. Foliar application with 100 mg L^{-1} AA promoted callus formation at the cut surfaces of the scion stems and improved the survival rate after grafting. We suggest that the low survival rate of grafted sweet pepper plants is caused by their low rate of callus formation, and that AA can be used to promote graft-take through the acceleration of callus formation at the cut surfaces of the stems.

Keywords: Callus; Capsicum; Grafting; Survival rate

1. Introduction

Grafting of vegetable crops is popular in Japan and South Korea (Lee, 1994), however sweet pepper plants are rarely grafted as they tend to be resistant to soil-borne diseases compared with eggplant and tomato plants. Recently, however, new races of the causal pathogen of bacterial wilt have appeared, causing increased occurrence of the disease. The use of methyl bromide, a chemical traditionally used in the control of bacterial wilt, has been prohibited since 2005, and therefore the demand for grafting in the production of sweet peppers has increased. Research on the factors related to successful grafting in sweet pepper plants is extremely scarce; however, it is known that different stem diameters between the scion and rootstock lower the survival rate, and that defoliation and a low survival rate occur as a result of water stress during acclimatization in old seedlings (Shirai and Hagimori, 2004).

Callus formation, and differentiation and connection of vascular bundles at the graft interface are essential for successful grafting (Ogata et al., 2005). Tissue culture of sweet peppers is difficult (Javier et al., 2001), as cell differentiation is late in these plants (De Donato et al., 1989). Thus, neither callus formation nor differentiation and development of vascular bundles are easily achieved in sweet pepper plants.

Despite its advantages, grafting cannot avoid injury stress. Injury stress causes plant cells to generate active oxygen, resulting in various physiological disorders (Berlett and Stadtman, 1997). In tomato plants, the concentration of active oxygen increases after grafting, and activity of antioxidant enzymes such as ascorbic acid peroxidase (APX) and catalase increase to eliminate the active oxygen (Garcia et al., 2004). Ascorbic acid (AA) concentration also increases after grafting in tomato plants (Wadano et al., 1999), and in stored fruits and vegetables. AA is effective in maintaining freshness (Gil et al., 1998).

If active oxygen generated by injury stress could be eliminated using AA as an antioxidant, successful grafting could be achieved by maintaining high cell activity at the graft interface. The objective of this study was to clarify the cause of the low survival rate of sweet pepper plants after grafting in comparison to grafted eggplant and tomato plants. The effect of AA on the survival rate of grafted sweet pepper plants was also investigated.

2. Materials and methods

2.1. Plant material and cultivation

Experiments were conducted from 12 May to 11 October, 2004, at a greenhouse in Osaka Prefecture University. Germinated seeds of sweet pepper (*Capsicum annuum* L. 'New ace'), eggplant (*Solanum melongena* L. 'Daitaro') and tomato (*Lycopersicon esculentum* Mill. 'Momotaro') plants

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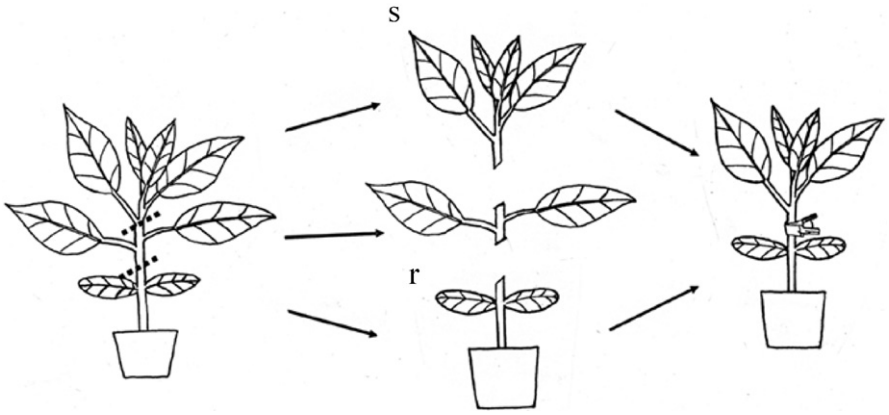

Fig. 1. Method of homo-grafting in this study. (s) Scion, (r) rootstock.

were sown in a 200-cell tray (16 mL cell^{-1}) filled with peat-moss and vermiculite (1:1, v/v). To obtain equal stem diameters at grafting, seeds of eggplant and sweet pepper plants were sown 13 days earlier than those of tomato plants.

The seedlings were grown in a greenhouse with a light transmissivity of 55% and supplied with a nutrient solution containing 4.6 N, 1.3 P, 2.2 K, 1.1 Ca, 0.4 Mg, in μmol L^{-1}. Plants were grown for 34 and 58 days after sowing to obtain plants at young and old stages, respectively. Sweet pepper, eggplant and tomato plants had unfolded the 6th, 3rd, and 5th true leaves at the old stage and the 4th, 2nd, and 3rd true leaves at the young stage, respectively. Young and old stages represent the grafting age for plug seedlings and pot seedlings, respectively.

Stems of the sweet pepper, eggplant and tomato plants were cut under the 4th, 2nd, and 3rd leaves from the top, and homo-grafted at the epicotyl of the rootstock (Fig. 1). The grafted plug seedlings were sprayed with water, placed into containers (73 H cm × 33 H cm × 40 H cm), then covered with a transparent polyethylene film to maintain the relative humidity (RH) at more than 95%. The containers were placed in a growth chamber at 25 °C in the dark. From a day after grafting, the photosynthetic photon flux density (PPFD) was increased from 80 to 400 μmol m^{-2} s^{-1} by increasing the number of white fluorescent lamps (Hitachi Sun Rhine 40 type) every 24 h (Fig. 2). From 5 days after grafting, the plants were hardened by gradual lowering of humidity for 2 days. The grafted plants were then transplanted and grown in the greenhouse.

2.2. Graft-take in solanaceous plants

Sweet pepper, eggplant and tomato plants grafted at young and old stages were used. The numbers of defoliated leaves and surviving scions were counted in 30 grafted plants 14 days after grafting.

2.3. Callus thickness of the scion and rootstock in solanaceous plants

Stems of sweet pepper, eggplant and tomato plants at young and old stages were cut under the 4th, 2nd, and 3rd leaves from the top, respectively. The bottoms of scions were then placed in 1% agar in a transparent plastic container (27 H cm × 21 H cm × 10 H cm) and maintained at more than 95% RH. Rootstocks were cut at the epicotyl, placed in a container, and maintained at more than 95% RH. The containers containing the scions and rootstocks were then placed in a chamber at 25 °C and 22 μmol m^{-2} s^{-1} PPFD. The thickness of the callus formed on the cut surface was measured in six plants 5 days after cutting.

2.4. Effect of AA on callus thickness in sweet pepper plants

Ascorbic acid at 0, 10, 100, and 1000 mg L^{-1} was foliar-applied by hand spray to the leaf surface of old-stage scions and rootstocks. The thickness of the callus formed on the cut surfaces of the scions and rootstocks was then measured in six plants 5 days after cutting.

2.5. Effect of AA on the survival rate of grafted sweet pepper plants

AA at 0 and 100 mg L^{-1} was foliar-applied immediately after grafting of sweet pepper plants at young and old stages. The numbers of defoliated leaves and surviving scions were counted in 30 grafted plants 14 days after grafting.

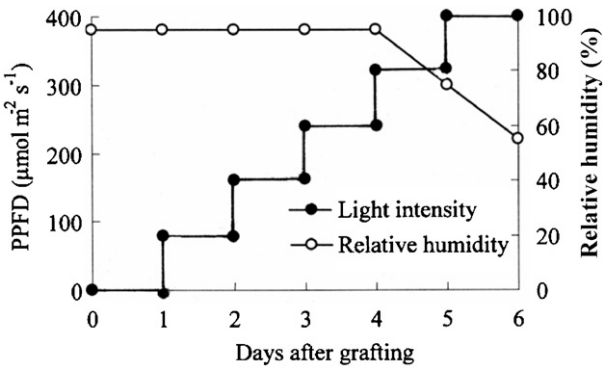

Fig. 2. Acclimatizing conditions of grafted plants. Air temperature was maintained at 25 ± 3 °C.

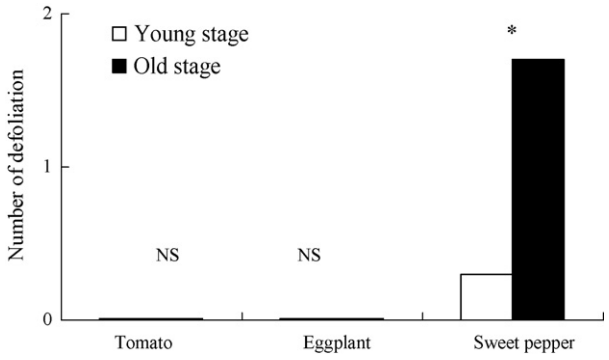

Fig. 3. Defoliation of tomato, eggplant and sweet pepper plants homo-grafted at different ages. Plants at young and old stages were grown for 34 and 58 days after sowing, respectively. NS, nonsignificant; *significantly different (*t*-test, $P = 0.05$).

2.6. Statistical analysis

Mean values were separated by *t*-test at $P < 0.05$ and Tukey's multiple range test at $P < 0.05$ using MEPHAS (Osaka University, Japan).

3. Results

3.1. Graft-take in solanaceous plants

No defoliation was observed in eggplant and tomato plants grafted at young and old stages. In sweet pepper plants, however, an average value of 0.3 and 1.7 leaves fell at young and old stages, respectively (Fig. 3). Survival rates of plants grafted at young and old stages were 100% in both tomato and eggplant plants. In sweet pepper plants, however, only 89 and 44% of grafted plants survived at young and old stages, respectively (Fig. 4).

3.2. Callus thickness of the scion and rootstock in solanaceous plants

The callus formed on the cut surfaces of the scion stems tended to be thickest in the tomato plants, followed by the eggplant and sweet pepper plants (Fig. 5A). Moreover, the callus was thicker on scions from young stage plants than those from old stage plants. The thickness of the callus formed on the cut surfaces of the tomato and eggplant rootstock stems was more than 0.4 mm at young and old stages, but in sweet pepper plants was less than 0.2 mm at both stages (Fig. 5B).

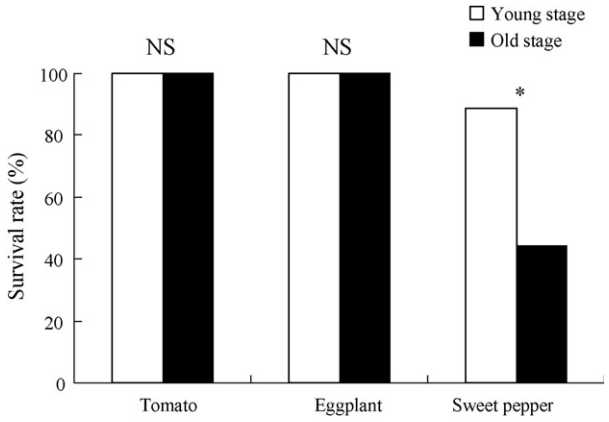

Fig. 4. Survival rates of tomato, eggplant and sweet pepper plants homo-grafted at different ages. Plants at young and old stages were grown for 34 and 58 days after sowing, respectively. NS, nonsignificant; *significantly different (*t*-test, $P = 0.05$).

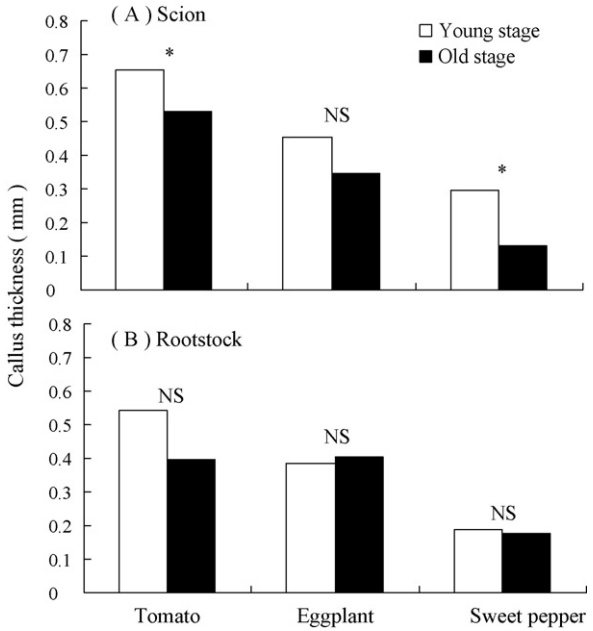

Fig. 5. Thickness of the calli formed at the cut surface of the scion (A) and rootstock (B) stems in tomato, eggplant and sweet pepper plants at different ages. Plants at young and old stages were grown for 34 and 58 days after sowing, respectively. NS, nonsignificant; *significantly different (*t*-test, $P = 0.05$).

3.3. Effect of AA on callus thickness in sweet pepper plants

The thickness of the callus formed at the cut surface of the scion stems increased proportionally with AA concentration, reaching 0.36 mm at 100 mg L^{-1} AA and maintaining a similar thickness at 1000 mg L^{-1} AA. In rootstocks, however, the thickness of the callus was not affected by AA (Fig. 6).

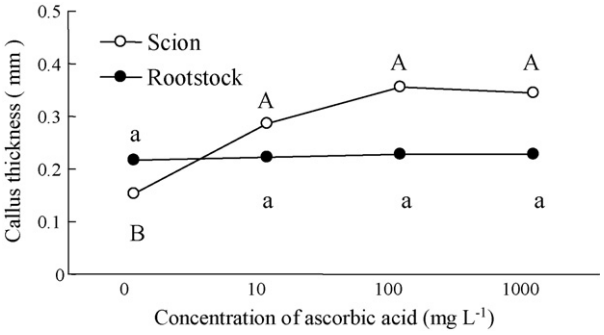

Fig. 6. Effect of foliar-applied ascorbic acid on callus formation at the cut surface of the scion and rootstock stems in sweet pepper plant grafted at an old stage (58 days after sowing). Same letters indicate no statistical difference at $P = 0.05$ (Tukey's multiple range tests).

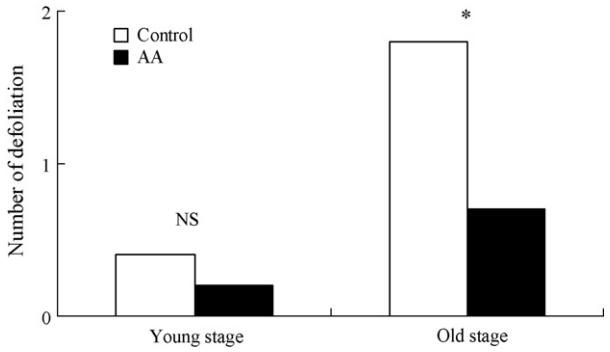

Fig. 7. Effect of 100 mg L^{-1} foliar-applied ascorbic acid on defoliation in sweet pepper plants homo-grafted at different ages. Plants at young and old stages were grown for 34 and 58 days after sowing, respectively. NS, non-significant; *significantly different (*t*-test, $P = 0.05$).

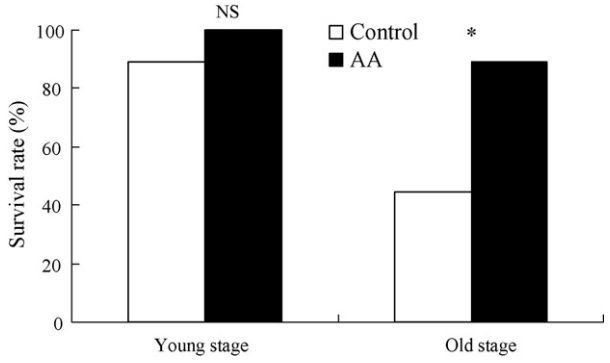

Fig. 8. Effect of 100 mg L^{-1} foliar-applied ascorbic acid on the survival rate of sweet pepper plants homo-grafted at different ages. Plants at young and old stages were grown for 34 and 58 days after sowing, respectively. NS, non-significant; *significantly different (*t*-test, $P = 0.05$).

3.4. Effect of AA on the survival rate of grafted sweet pepper plants

The number of defoliated leaves counted after grafting at the young stage was very low regardless of AA treatment (Fig. 7). However, after grafting at the old stage, the number of defoliated leaves decreased from an average of 1.8 (control) to 0.7 with AA treatment. The survival rate of plants grafted at the young stage was at 89% in the control, but increased to 100% with AA treatment (Fig. 8). After grafting at the old stage, the survival rate was very low in the control (44%), but markedly increased with AA treatment (89%).

4. Discussion

During acclimatization after grafting, no defoliation was observed in the grafted eggplant and tomato plants used in the present study; however, the opposite was true for the sweet pepper plants. This result was most apparent after grafting at an old stage. Sweet pepper plants defoliate as a result of water stress (Nitzsche et al., 1991) and the defoliation was previously observed in sweet pepper plants grafted at an old stage (Shirai and Hagimori, 2004). Although the survival rate of the eggplant and tomato plants was 100% after grafting at both the young and old stages, the survival rate in sweet pepper plants was low, 44%, at the old stage. These results confirmed the low survival rate of grafted sweet pepper plants compared with eggplant and tomato plants.

Callus formation, and the differentiation and connection of vascular bundles in the callus are essential for successful grafting. The differentiation of vascular bundles from the callus influences successful grafting (Moore and Walker, 1981; Moore, 1984), with active callus formation at the cut surface leading to the formation of a callus bridge between the scion and rootstock as well as vascular bundle differentiation (Andrews and Serrano, 1993). Moreover, insufficient connection of vascular bundles between the scion and rootstock decreases the quantity of water flow (Torii et al., 1992). Therefore, efficient callus formation is important for the formation of the graft union and survival of the scion, and it has been suggested that an ability to form callus is positively correlated with grafting success (Ogata, 1995). In our results, the callus was thinner in sweet pepper plants compared with eggplant and tomato plants, and it is therefore thought that the small amount of callus formation at the old stage is the cause of the low survival rate of grafted sweet pepper plants. The number of adventitious buds generated from cotyledons cultured on liquid MS medium was previously shown to be less in sweet pepper plants than tomato plants (Javier et al., 2001). This further supports the hypothesis that low survival rate of sweet pepper plants is attributed to slow callus formation and cell differentiation.

Generally, plant tissue at a younger age has a higher cell division and proliferation ability. In our experiments, the callus was thicker and the survival rate was better in young stage sweet pepper plants compared with old plants. Callus obtained from old tissue of pepper has a low rate of cell differentiation and division (De Donato et al., 1989). In sweet pepper plants grafted at the old stage, the low rates of callus formation might delay the connection of vascular bundles between the scions and rootstocks, cause water stress, and lower the survival rate of grafted plants.

AA acts an antioxidant through a catalytic reaction with APX (Shigeoka et al., 2002). Plants are injured by the cut in the grafting process, and the active oxygen concentration increases as a result of this injury stress (Garcia et al., 2004). The concentration of AA also increases after grafting in tomato (Wadano et al., 1999). Hyper-production of AA is thought to counteract injury stress, because AA has been shown to protect plants from oxidation (Tabata et al., 2001). Therefore, AA treatment after grafting was considered an effective way to protect plants from injury stress in grafted plants.

Although in the present study callus formation was promoted with AA treatments in the scions, there was no effect in the rootstocks. Since the rootstocks had a smaller leaf area—only the cotyledon in this experiment—it seems that little AA would have been absorbed by the plant. Therefore, improvement in the AA treatment of rootstocks may promote the survival rate of grafted sweet pepper plants.

Many physiological functions of AA have been reported besides protection of plants from active oxygen. AA is known to control cell differentiation (Arrigoni, 1994) and to promote

callus division and growth (Tabata et al., 2001). Moreover, ascorbate oxidase, the substrate of which is AA, is also thought to have roles in cell division and hypertrophy (Kato and Esaka, 2000). These results indicate that AA treatment may result in callus formation at the cut surface of the scion via AA's promotion of cell division. Since AA also promoted shoot formation from callus in tobacco tissue culture (Joy et al., 1988), it is thought that AA promotes not only callus formation but also differentiation of vascular bundles.

This study showed that sweet pepper plants form smaller amounts of callus at the cut surface of the stem than eggplant and tomato plants, and that the low survival rate of grafted sweet pepper plants was caused by the low rate of callus formation. It was also found that AA promotes callus formation at the cut surface of the scion and increases the survival rate of grafted sweet pepper plants.

References

Andrews, P.K., Serrano, M.A., 1993. Graft incompatibility. Hort. Rev. 15, 183–232.

Arrigoni, O., 1994. Ascorbate system in plant development. J. Bioenerg. Biomembr. 26, 407–419.

Berlett, B.S., Stadtman, E.R., 1997. Protein oxidation in aging, disease, and oxidative stress. J. Biol. Chem. 272, 20313–20316.

De Donato, M., Perucco, E., Chiavazza, P., Panei, E., 1989. Activation of nuclear DNA replication and mitosis in excised cotyledons of *Capsicum annuum*. Plant Sci. 63, 217–225.

Garcia, N.F., Carvajal, M., Olmos, E., 2004. Graft union formation in tomato plants: Peroxidase and catalase involvement. Ann. Bot. 93, 53–60.

Gil, M.I., Gorny, J.R., Kader, A.A., 1998. Responses of 'Fuji' apple slices to ascorbic acid treatments and low-oxygen atmospheres. HortScience 33, 305–309.

Kato, N., Esaka, M., 2000. Expansion of transgenic tobacco protoplasts expressing pumpkin ascorbate oxidase is more rapid than that of wild-type protoplasts. Planta 210, 1018–1022.

Javier, P.R., Guy, H., Luis, C., Rodolphhe, S., Jesus, C., 2001. Enhanced regeneration of tomato and pepper seedling explants for Agrobacterium-mediated transformation. Plant Cell Tiss. Org. Cult. 67, 173–180.

Joy, R.W., Patel, K.R., Thorpe, T.A., 1988. Ascorbic acid enhancement of organogenesis in tobacco callus. Plant Cell Tiss. Org. Cult. 13, 219–228.

Lee, J.M., 1994. Cultivation of grafted vegetables I. Current status, grafting methods, and benefits. HortScience 29, 235–239.

Moore, R., Walker, D.B., 1981. Studies of vegetative compatibility-incompatibility in higher plants. 1. A structural study on an incompatible autograft in *Sedum telephoides* (Crassulaceae). 2. A structural study of incompatible heterograft between *Sedum telephoides* (Crassulaceae) and *Solanum pennelli* (Solanaceae). Am. J. Bot. 68, 820–842.

Moore, R., 1984. A model for graft compatibility-incompatibility in higher plants. Am. J. Bot. 71, 752–758.

Nitzsche, P., Berkowitz, G.A., Rabin, J., 1991. Development of a seedling-applied antitranspirant formulation to enhance water status, growth, and yield of transplanted bell pepper. J. Am. Soc. Hort. Sci. 116, 405–411.

Ogata, T., 1995. Physiology of graft union formation. In: Kawase, K. (Ed.), Characteristics and Application of Rootstocks in Fruit Trees. Nobunkyou, Tokyo, (in Japanese), pp. 33–40.

Ogata, T., Kabashima, Y., Shiozaki, S., Horiuchi, S., 2005. Regeneration of the vascular bundle at the graft interface in auto- and heterografts with juvenile nucellar seedlings of satsuma mandarin, yuzu and trifoliate orange. J. Jpn. Soc. Hort. Sci. 74, 214–220.

Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y., Yoshimura, K., 2002. Regulation and function of ascorbate peroxidase isoenzymes. J. Exp. Bot. 53, 1305–1319.

Shirai, T., Hagimori, M., 2004. Studies in establishment of transplant production methods of sweet pepper (*Capsicum annuum* L.) by grafting shoots harvested from mother plants: effect of healing conditions of grafts on the rate and quality of successful union. J. Jpn. Soc. Hort. Sci. 73, 380–385.

Tabata, K., Oba, K., Suzuki, K., Esaka, M., 2001. Generation and properties of ascorbic acid-deficient transgenic tobacco cells expressing antisense RNA for L-galactono-1,4-lactone dehydrogenase. Plant J. 27, 139–148.

Torii, T., Kawazaki, M., Okamoto, T., Kitani, O., 1992. Evaluation of graft-take using a thermal camera. Acta Hort. 319, 631–634.

Wadano, A., Azeta, M., Itotani, S., Kanda, A., Iwaki, T., Taira, T., Fujii, Y., Nishiura, Y., Murase, H., Honami, N., 1999. Change of ascorbic acid level after grafting of tomato seedlings. Z. Naturforsch. 54, 830–833.