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# Establishment of Reciprocal Micrografting of Tomato (*Solanum lycopersicum* L.) and Eggplant (*Solanum melongena* L.)

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## Abstract

Micrografting can be used as a key tool to investigate gene-function, long-distance signal transduction, or metabolite movement in different developmental and physiological stages. In plant production, plant grafting can be applied to improve productivity and/or increase the tolerance of plants to stresses. Here, we describe a simple and high efficiency protocol for reciprocal micrografting of tomato (*Solanum lycopersicum* L.) and eggplant (*Solanum melongena* L.). Tomato and eggplant seeds can be disinfected with 0.5% Presept for 20 min before germinating on MS media. Seedlings of 5-day-old tomatoes and 15-day-old eggplants were suitable for preparation of scions and rootstocks. Scions were cut into 0.5-1 cm lengths for micrografting. Sucrose levels greatly influenced the graft success rate of all graft combinations including of self- and reciprocal micrografting between tomato and eggplant. While self-grafted tomatoes or eggplants required 20 g $L^{-1}$ sucrose to get the highest grafting success rate (72% for tomato and 100% for eggplant), reciprocal micrografting of tomato/eggplant and eggplant/tomato reached the highest success rate (83%) on MS medium supplemented with 30 g $L^{-1}$ sucrose. Grafted plants should be cultured under the illumination conditions of a 16 h light/8 h dark cycle for optimal growth and quality.

## Keyword

Micrografting, grafting, tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.)

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## Introduction

Grafting is a horticultural technique that is used to join parts from two or more plants so that they appear to grow as a single plant. The grafting technique has been widely used for vegetative propagation to improve productivity (Gulati *et al.*, 2001; Grigoriadis *et al.*, 2005; Melnyk and Meyerowitz, 2015; Rehman and Gill, 2015; Gaion *et al.*, 2018), as avalid alternative to traditional

micropropagation in the case of *Pelecyphora aselliformis* Ehrenberg (Badalamenti *et al.*, 2016), or to increase the tolerance of plants to stresses such as the interspecific grafting of eggplant onto tomato for verticillium wilt resistance (Miles *et al.*, 2015). Moreover, grafting can be used to investigate long-distance signaling in *Arabidopsis*, and systemic signaling in *Nicotiana attenuata* in response to herbivory (Turnbull *et al.*, 2002; Li *et al.*, 2016; Regnault *et al.*, 2016; Bozorov *et al.*, 2017; Tsutsui and Notaguchi, 2017). In Vietnam, the protocol for grafting tomato onto eggplant has also been established and applied in practical production (Ha, 2009).

The success of plant grafting largely depends on the connection and formation of vascular tissues at the graft junction. Since the cambium connection between the scion and rootstock will later give rise to phloem and xylem during secondary growth, using similar sized scions and rootstocks are required (Melnyk and Meyerowitz, 2015).

In the *in vivo* grafting technique, the union of the xylem at the graft junction strongly influences the movement of water and nutrients in the xylem and phloem of the vascular system, thereby affecting the growth potential of the grafted plant (Atkinson *et al.*, 2003). In addition, it has been shown that a phloem graft union is a main reason of long-term incompatibility; therefore, plant grafting methods that do not affect plant development should be developed (Goldschmidt, 2014).

In *Nicotiana attenuata*, micrografting plants do not show growth reductions compared to non-grafted plants. Moreover, micrografting *N. attenuata* can be used as a key tool to evaluate gene function, and long-distance signal transduction in different developmental and physiological processes (Fragoso *et al.*, 2011).

Although micrografting efficiency in some plants is high, the success rate largely depends on species (Fragoso *et al.*, 2011). Here, we describe a simple and highly efficient micrografting method for reciprocal micrografting of tomato (*Solanum lycopersicum* L.) and eggplant (*Solanum melongena* L.).

## Materials and Methods

### Materials

Seeds of tomatoes, VNS 585 (F1 hybrid variety), were imported from India and supplied by the Southern Seed Corporation. Seeds of eggplant, PD612 variety, were provided by Phu Dien Trading and Production Company Limited, Hanoi, Vietnam.

### Methods

*Plant cell culture method*

Murashige and Skoog (1962) (MS) culture medium was used to culture the plant cells and was supplemented with 8 g $L^{-1}$ agar and 30 g $L^{-1}$ sucrose (unless otherwise indicated). The pH was adjusted to 5.7-5.8 before being autoclaved. All the experiments followed a completely randomized design with three replications.

*Sterilization method*

Seeds were washed under running water, rinsed with 70% ethanol for 30 seconds, and then treated with either 0.1% $HgCl_2$ or 0.5% Presept solution (Product of Johnson & Johnson, containing sodium dichloroiso cyanurate) supplied with 1-2 drops of Tween 20, and treated with different exposure times. During the sterilization process, the containers were shaken vigorously. Seeds were then rinsed in sterile water five times. Sterilized seeds were placed on MS basal medium for germination. For establishment of the sterilization regime, 100 seeds were used for each treatment.

*Micrografting methods*

Seedlings were cut horizontally across the hypocotyl to prepare the scions or rootstocks. Rootstocks and scions were then placed in contact with each other. A nylon tube was used to wrap each scion and rootstock at the graft junction to keep the scion and rootstock stable. Grafted plants were then placed horizontally on the surface of the MS basal medium and kept in a culture room. Twenty-five grafted plants (self- or reciprocal grafted) were used for each treatment.

To evaluate the illumination conditions, two light regimes were used: dark conditions (for the first five days, the grafted plants were kept in darkness, and then after that they were exposed

to the normal light regime) and normal light conditions (16 h light/8 h dark).

Samples were kept in plant growth room under a light intensity of 2000 lux, 70% humidity, 24 ± 2ºC, and photoperiodic lighting of 16 h light/8 h dark cycles, unless otherwise indicated.

*Statistical Analyses*: All data were analyzed by Excel version 2013. Data shown in Tables 2, 3, 4 and 5, means are presented as averages ± standard errors (SE).

## Results and Discussion

### Effects of the sterilization regime on the establishment of aseptic seedlings

Selecting clean explants is the most important factor for being successful at the initial culture stage of plant tissue culturing. Since the uniformity of samples will greatly affect the interpretation of results, we decided to use seeds of tomato and eggplant instead of shoots or other materials as initial explants. Moreover, seed sterilization is often easier and seeds are considered free from some diseases such as bacteria or even some viruses. Since 0.1% $HgCl_2$ has been used to sterilize tomato seeds for 5 min (Zhang *et al.*, 2012), and sodium dichloroisocyanurate (active component of Presept) is known to be less toxic to explants and therefore can be used at a wide range of concentrations (0.5-2.0%) for different periods of time (from 5-90 min) (Mihaljević *et al.*, 2013; Kendon *et al.*, 2017), we decided to use both 0.1% $HgCl_2$ and 0.5% Presept for sterilization.

The results shown in Table 1 indicated that although seeds were treated differently with two sterilizing agents ($HgCl_2$ or Presept solution) at different durations, all four treatments produced 100% sterilized seeds. These results demonstrated that the tomato and eggplant seeds were of good quality which led to the high efficiency of the sterilizing agents. More importantly, 100% of the tomato seeds and at least 92.5% of the eggplant seeds germinated and all the seedlings grew very well. These results suggest that the tomato and eggplant seeds were slightly or not affected by the disinfectants. Therefore, both $HgCl_2$ and a Presept solution can be used to disinfect tomato and eggplant seeds. However, since $HgCl_2$ is toxic to humans as well as the environment, it is therefore highly recommended to use a 0.5% Presept solution to disinfect tomato and eggplant seeds.

### Effects of plant age after germination on the success rate of self-grafted tomato and eggplant

There are many factors (grafting procedure, grafting position, scion types, and scion length, etc.) that affect the success rate of grafting and plant age is one factor of great importance (Mneney and Mantell, 2001; Khalafalla and Daffalla, 2008; Tanuja *et al.*, 2017). To overcome the incompatibility situation in interspecific micrografting, we decided to work on self-grafted tomatoes or eggplants only. Based on their growth rates, we used tomato plants at the ages of 5, 10, and 15 days after germination, and eggplant plants at the ages of 9, 12, and 15 days after germination.

The age of the tomato plants strongly affected the success rate of the micrograft, and in general, the older plants were, the lower grafting success rate was (Table 2). The highest

**Table 1.** Effects of the sterilization regime on the establishment of an aseptic seedlings 10 days after sterilization

| Sterilizing agent solution | Duration (min) | Tomato seeds | | Eggplant seeds | |
|---|---|---|---|---|---|
| | | Sterilized seeds (%) | Germination rate (%) | Sterilized seeds (%) | Germination rate (%) |
| 0.1% $HgCl_2$ | 5 | 100 | 100 | 100 | 100 |
| 0.1% $HgCl_2$ | 10 | 100 | 100 | 100 | 100 |
| 0.5% Presept | 20 | 100 | 100 | 100 | 92.5 |
| 0.5% Presept | 30 | 100 | 100 | 100 | 95.0 |

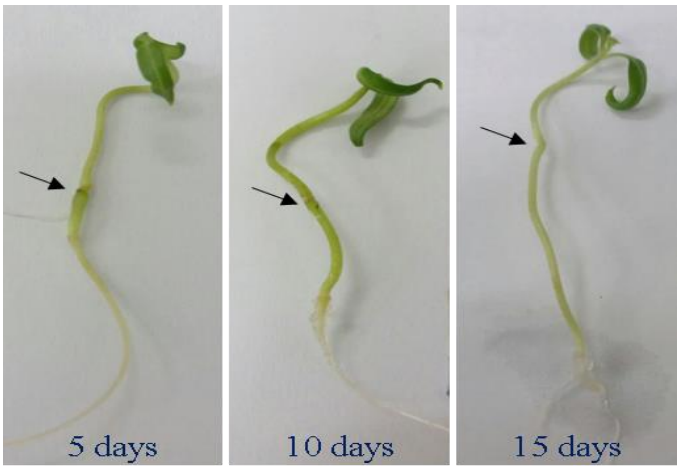
**Table 2.** Effects of plant age on the success rate of self-grafted tomato and self-graft eggplant two weeks after grafting

| | Days after germination (days) | Percentage of successful grafts (%) | Number of leaves (leaves/plant) | Number of roots (roots/plant) | Stalk length (cm) | Growth observation |
|---|---|---|---|---|---|---|
| Tomato | 5 | 53.8 | 2.1 ± 0.14 | 1.1 ± 0.14 | 4.5 ± 0.19 | Very good |
| | 10 | 25.0 | 2.6 ± 0.40 | 1.6 ± 0.24 | 4.9 ± 0.40 | Good |
| | 15 | 15.0 | 2.7 ± 0.33 | 1.7 ± 0.33 | 5.7 ± 0.33 | Good |
| Eggplant | 9 | 60.0 | 2.8 ± 0.14 | 1.1 ± 0.08 | 3.3 ± 0.12 | Very good |
| | 12 | 60.0 | 3.6 ± 0.15 | 2.0 ± 0.25 | 3.8 ± 0.18 | Very good |
| | 15 | 68.0 | 3.8 ± 0.12 | 2.0 ± 0.23 | 4.1 ± 0.22 | Very good |

grafting success rate (53.8%) was achieved when the tomato plants were grafted at the age of 5 days after germination, followed by 10 days after germination (25.0%), and the lowest grafting success rate was only 15.0% when the age of plants was 15 days after germination. The highest success rate of the 5-day-old plants could be explained in that these plants were still at an early stage after germination, so they were younger and therefore better facilitated to the rejoining process at the graft junction. In fact, in *in vivo* sweet pepper (*Capsicum annuum* L.) grafting, the plant age has been shown to influence the results of grafting and older plants had a lower percentage of xylem connections than younger plants (Johkan *et al.*, 2009). Therefore, younger plants showed higher grafting success rates than older plants.

We also collected the growth data of the grafted tomato seedlings in order to evaluate the effects of plant age on their success rate. The plants grafted 5 days after sowing showed the shortest stalk and root lengths, and the lowest leaf number; however, it was obvious that the total growth time (days after sowing) of plants grafted at the ages of 10 and 15 days were 5 or 10 days more than that of the plants grafted 5 days after sowing, respectively (Figure 1). Therefore, it could be concluded that tomato plants at 5 days after sowing are the most suitable for micrografting.

The age of the eggplant plants showed the opposite effect when compared with the results collected from the tomato plants. While the younger tomato plants had higher success rates, the older eggplant plants showed higher success rates than the younger ones. The highest grafting success rate (68%) was achieved when eggplant plants 15 days after sowing were used.




*Note: The dark arrow indicates the graft junction.*

**Figure 1.** Effects of plant age on the success rate of self-grafted tomato two weeks after grafting

The 9 or 12-day-old plants showed the same success rate of 60% after two weeks. These results are in agreement with the micrografting results of *Acacia senegal* (L.) Wild in which 14-day-old rootstocks had higher success rates than 7-day-old rootstocks (Khalafalla and Daffalla, 2008).

It should be noted that two weeks after grafting, the stalk lengths of the tomato plants reached 4.5-5.7 cm; however, the stalk lengths of the eggplants reached only 3.3-4.1 cm after the same culturing time. Moreover, before grafting, eggplants exhibited smaller sizes than tomatoes (data not shown). These results indicated that eggplant plants grew relatively slower than tomato plants which could be one of the reasons why eggplant plants required a longer time after sowing to reach the right stage for grafting. Nevertheless, 68% of the grafts were successful and the grafted plants grew well, therefore, 15 days after sowing is the right stage for eggplant plants to be used for micrografting.

**Effects of scion size on the success rate of reciprocal micrografting**

From the previous experiments, we have known that plant age is one of the factors affecting the success of grafting (Table 2, Figure 1). It has also been reported that the rate of successfully grafted plants is influenced by scion size (Khalafalla and Daffalla, 2008). Therefore, we conducted an experiment to evaluate scion size on grafting. To overcome the incompatibility between scions and rootstocks, we worked only on self-grafted tomatoes or eggplants. Data are presented in Table 3.

In general, different sizes of scions (0.5 and 1.0 cm) did not affect the grafting success rate. Self-grafted tomatoes that had scion sizes of 0.5 cm or 1.0 cm had success rates of 63% and 65%, leaf numbers of 2.7 and 3.1, and root numbers of 1.0 and 1.1 per plant, respectively. In addition, stalk length did not dramatically change (5.5 *vs* 4.6 cm). Self-grafted eggplants showed the same trends in success rates, leaf numbers, and root numbers with the self-grafted tomatoes. In grafting, scion size has been known to affect the success rate of *Acacia senegal* (L.) Wild (Khalafalla and Daffalla, 2008). In *in vivo* mango grafting, the size and age of scions do not affect the grafting success in the spring season; however, from July to September, bigger scions result in higher success rates (Majhail and Singh, 1962). It could be that tomato and eggplant plants are more suitable for micrografting. In addition, *in vitro* plants exhibit higher success rates compared to scions collected from field (Sanjaya *et al.*, 2006). Based on our results, it can be concluded that scion sizes of 0.5-1.0 cm are suitable for micrografting.

**Effects of sucrose concentration on the success rate of reciprocal micrografting of tomato and eggplant**

Sugar positively affects plant growth under *in vitro* conditions. In plant tissue culture, sucrose is the sugar most commonly supplied in media at a concentration of 20-30 g $L^{-1}$ (Khan *et al.*, 2002; Sanjaya *et al.*, 2006). The following experiment was conducted to evaluate the role of sucrose on reciprocal micrografting of tomato and eggplant.

The results in Figure 2 showed that sucrose levels had a great influence on the grafting success rate of all graft combinations between tomato and eggplant.

**Table 3.** Effects of scion size on the success rate of reciprocal micrografting two weeks after grafting

| Scion/rootstock | Scion size (cm) | Grafting success rate (%) | Number of leaves (leaves/plant) | Number of roots (roots/plant) | Stalk length (cm) | Growth observation |
|---|---|---|---|---|---|---|
| Self-grafted tomato | 0.5 | 63 | 2.7 ± 0.13 | 1.0 ± 0.0 | 5.5 ± 0.20 | Very good |
| | 1.0 | 65 | 3.1 ± 0.21 | 1.1 ± 0.09 | 4.6 ± 0.20 | Very good |
| Self-grafted eggplant | 0.5 | 75 | 2.7 ± 0.33 | 1.0 ± 0.0 | 3.7 ± 0.33 | Good |
| | 1.0 | 67 | 2.5 ± 0.29 | 1.0 ± 0.0 | 4.7 ± 0.25 | Very good |

*Note: Scion size was the length from shoot tip to the cut hypocotyl tissue.*

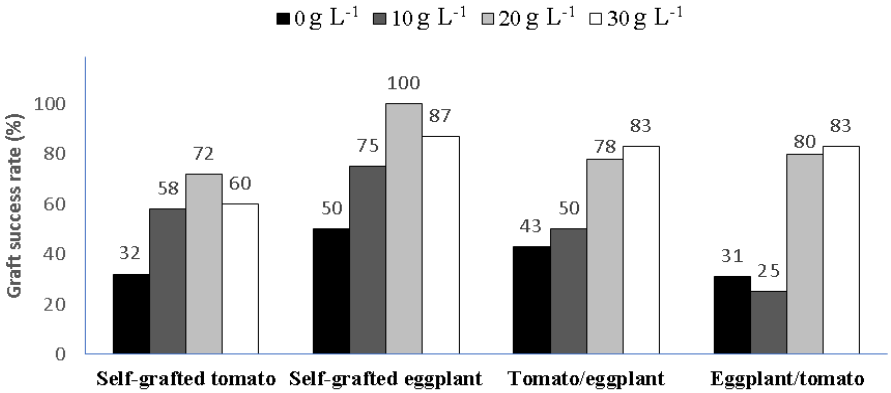


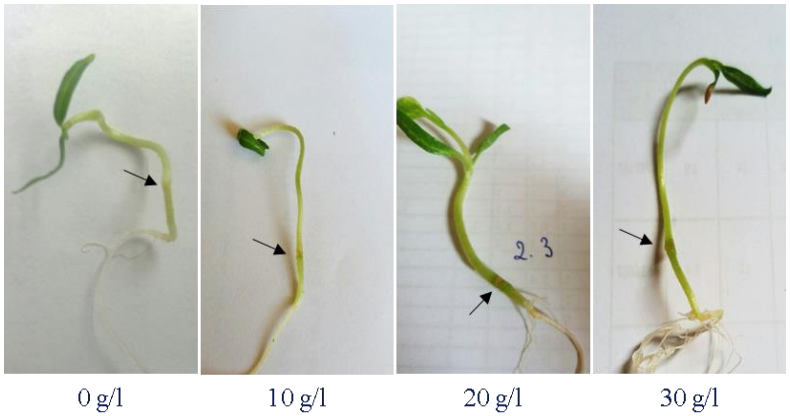

**Figure 2.** Effects of sucrose concentration on success rate of reciprocal micrografting two weeks after grafting

Self-grafted tomato plants reached the highest (72%) success rate on MS medium supplemented with 20 g $L^{-1}$ sucrose and the lowest (32%) in the absence of sucrose. As in the self-grafted tomato plants, self-grafted eggplant also required 20 g $L^{-1}$ sucrose in the medium to get the highest grafting success rate (100%).

Interestingly, while the addition of 30 g $L^{-1}$ sucrose in the self-grafted tomatoes and eggplants caused a reduction in the grafting success rate compared to the medium supplemented with 20 g $L^{-1}$, the addition of 30 g $L^{-1}$ sucrose increased the grafting success rate in micrografting (tomato/eggplant and eggplant/tomato) compared to the medium supplemented with 20 g $L^{-1}$. Tomato/eggplant and eggplant/tomato grafted plants reached the highest success rate (83%) on the medium supplemented with 30 g $L^{-1}$ sucrose, followed by the 20 g $L^{-1}$ sucrose treatments (78 and 80%, respectively). Moreover, low levels of sucrose (without or with the addition of 10 g $L^{-1}$ sucrose) affected the tomato/eggplant success

**Table 4.** Effects of sucrose concentration on the success rate of reciprocal micrografting two weeks after grafting

| Scion/rootstock grafting | Sucrose (g $L^{-1}$) | Number of leaves (leaves/plant) | Number of roots (roots/plant) | Growth observation |
|---|---|---|---|---|
| Self-grafted tomato | 0 | 2.2 ± 0.16 | 1.1 ± 0.13 | Good |
| | 10 | 3.3 ± 0.29 | 1.7 ± 0.18 | Good |
| | 20 | 3.5 ± 0.27 | 2.8 ± 0.31 | Very good |
| | 30 | 3.3 ± 0.33 | 4.0 ± 0.37 | Very good |
| Self-grafted eggplant | 0 | 2.3 ± 0.33 | 1.0 ± 0.00 | Good |
| | 10 | 2.0 ± 0.00 | 1.3 ± 0.33 | Good |
| | 20 | 2.8 ± 0.40 | 2.0 ± 0.37 | Very good |
| | 30 | 3.6 ± 0.30 | 5.6 ± 0.61 | Very good |
| Tomato/eggplant | 0 | 2.7 ± 0.33 | 1.0 ± 0.00 | Good |
| | 10 | 2.0 ± 0.00 | 1.5 ± 0.50 | Good |
| | 20 | 3.1 ± 0.26 | 2.1 ± 0.26 | Very good |
| | 30 | 3.0 ± 0.45 | 2.0 ± 0.49 | Very good |
| Eggplant/tomato | 0 | 3.6 ± 0.24 | 1.2 ± 0.20 | Good |
| | 10 | 3.0 ± 0.00 | 1.0 ± 0.00 | Good |
| | 20 | 4.0 ± 0.41 | 3.3 ± 0.63 | Very good |
| | 30 | 3.2 ± 0.20 | 2.6 ± 0.40 | Very good |




*Note: The dark arrow indicates the graft junction.*

**Figure 3.** Effects of sucrose concentration on success rate of eggplant/tomato micrografting two weeks after grafting

rate slightly, however, they dramatically reduced the success rate of eggplant/tomato grafting when compared to the self-grafted plants.

In addition to the grafting success rate, we evaluated the growth of the grafted plants. The results are presented in Table 4 and Figure 3. While, the levels of sucrose slightly affected leaf numbers on all the graft combinations, they dramatically influenced the root number, stalk length, and growth of the grafted plants. In general, MS media supplemented with 20-30 g $L^{-1}$ sucrose resulted in excellent in growth of the grafted plants (self- and interspecific grafts). For example, the self-grafted tomato plants on the MS medium supplemented with 30 g $L^{-1}$ sucrose resulted in an average of 3.3 leaves, 4.0 roots, and stalk lengths of 6.1 cm, and the growth was very good. While on the MS medium without sucrose, the grafted plants only reached 2.2 leaves, 1.1 roots, and stalk lengths of 4.5 cm. The growth trends were the same for all the other graft combinations as well.

Our data were in agreement with other reports which concluded that sucrose is important for the success of micrografting. In citrus micrografting, an increase of sucrose from 3.0 to 7.0% resulted in an increase in the grafting success rate (Naz *et al.*, 2007). In addition, grapefruit micrografting also improved significantly when cultures were grown on MS medium supplemented with 7.5% sucrose compared to 3.0% (Hamaraie *et al.*, 2005).

**Effects of illumination conditions on the success rate of reciprocal micrografting**

Illumination conditions such as continuous light, continuous dark, or a light dark cycle greatly influence *in vitro* culture results. In general, during *in vitro* culture, a light dark cycle is normally applied. In tomato, exposure to light increases the callus induction efficiency (Rzepka-Plevneö *et al.*, 2006); however, callus induction frequency in *Bixa oreliana* L. is higher in the dark (Khan *et al.*, 2002). In grafting, forming calli at the junction is necessary for the union of the rootstock and scion since calli will later differentiate into phloem and xylem (Melnyk, 2017); therefore, an experiment was conducted to evaluate illumination conditions on the grafting success rate.

Light exposure increased the success rate of all the micrografting combinations, either self- or reciprocal grafting between tomato and eggplant (Figure 4). While culturing under continuous dark conditions gave grafting success rates between 30-50%, exposure to a light regime of 16 h/day resulted in 52-86% graft success rates. Under the light exposure conditions, the self-grafted eggplants had the highest success rate (86%), followed by tomato/eggplant (82%). Interestingly, self-grafted tomatoes had a success rate of only 52% while the reciprocal grafted combinations between eggplant and tomato were 70-82%. These data indicate that eggplant has a higher tissue reunion efficiency than tomato, and thus,

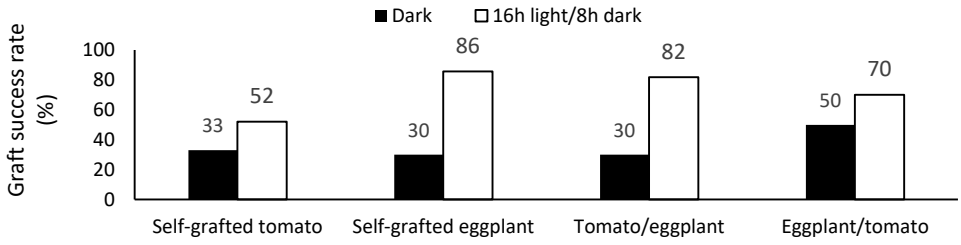



**Figure 4.** Effects of illumination conditions on success rate of reciprocal micrografting two weeks after grafting

**Table 5.** Effects of illumination conditions on the success rate of reciprocal micrografting two weeks after grafting

| Scion/rootstock grafting | Illumination conditions | Leaf number (leaves/plant) | Root number (roots/plant) | Stalk length (cm) | Growth observation |
|---|---|---|---|---|---|
| Self-grafted tomato | Dark | 2.0 ± 0.00 | 1.0 ± 0.00 | 5.7 ± 0.25 | Good |
| | 16 h light/8 h dark | 2.2 ± 0.12 | 1.3 ± 0.13 | 4.8 ± 0.13 | Very good |
| Self-grafted eggplant | Dark | 2.7 ± 0.33 | 1.0 ± 0.00 | 3.3 ± 0.33 | Poor |
| | 16 h light/8 h dark | 3.4 ± 0.15 | 1.1 ± 0.08 | 4.4 ± 0.19 | Very good |
| Tomato/eggplant | Dark | 2.3 ± 0.33 | 1.0 ± 0.00 | 4.7 ± 0.33 | Poor |
| | 16 h light/8 h dark | 2.2 ± 0.15 | 1.1 ± 0.11 | 5.0 ± 0.22 | Very good |
| Eggplant/tomato | Dark | 3.0 ± 0.00 | 1.0 ± 0.00 | 4.5 ± 0.50 | Poor |
| | 16 h light/8 h dark | 2.4 ± 0.20 | 1.4 ± 0.20 | 4.1 ± 0.14 | Very good |

positively affected the grafting success rate. Indeed, self-grafted eggplant always showed the highest success rate (even 100%) among all the graft combination (Figures 2 and 4). In addition to the grafting success rate, we also observed the growth of the grafted plants, and the results are presented in Table 5.

Although leaf number, root number, and stalk length were the same when the grafted plants grew under either dark or light conditions, based on morphology observations, exposure to the light dark regime of 16 h light/8 h dark resulted in a better quality of grafted plants when compared to the continuous dark conditions. All combinations of the grafted plants (self- or reciprocal grafted plant) performed well under the light regime of 16 h light/day while most of the plants grew poorly under the dark conditions. The results from this study were in agreement with previous studies which found improved grafting success and growth of grafted tomatoes under light compared to dark (Vu *et al.*, 2014).

## Conclusions

A simple and high efficiency protocol for the reciprocal micrografting of tomato (*Solanum lycopersicum* L.) and eggplant (*Solanum melongena* L.) was established. Tomato and eggplant seeds can be disinfected with 0.5% Presept for 20 min before germinating on MS media. Seedlings of 5-day-old tomatoes and 15-day-old eggplants were suitable for preparation of scions and rootstocks. Scions cut into 0.5-1.0 cm lengths were suitable for micrografting. Self-grafted tomatoes or eggplants required 20 g $L^{-1}$ sucrose to get the highest grafting success rates (72% for tomato and 100% for eggplant), however, reciprocal micrografting of tomato/eggplant and eggplant/tomato reached highest success rate (83%) on MS medium supplemented with 30 g $L^{-1}$ sucrose. Grafted plants should be cultured under a 16 h light/8 h dark cycle for optimal growth and quality.

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