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Functional vascular connections and light quality effects on tomato grafted unions

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ABSTRACT

In the present study, the functions of vascular connections (graft unions) in the grafted tomato seedlings (*Solanum lycopersicum* cv. Super Doterang grafted on cv. B-Blocking) has been monitored with some indicating factors such as transpiration, water uptake rate, sugar content, flower dye distributions between scion and rootstock, and hardness of the grafted unions. In addition, various light qualities have been tested to evaluate the effects on functions of vascular connections particularly on xylem and phloem. Our results depicted that light had a positive effect on the development of grafted unions as well as their functions. The functions of stomata and vascular connections developed approximately 50% particularly in the first five days function monitoring. Moreover, the reduction of sugar content in the scion parts was inhibited instead of the dark condition. The use of the pure red and blue light sources in the acclimating chamber showed difficulties to keep the water balance of the grafted seedlings and the sudden abiotic stress condition (high light intensity) was observed. At day 4 after grafting, the gap between transpirational water loss and water uptake in the FL (fluorescent light) and LED_{W1R2B1} (light emitting diode white/red/blue) supplying treatment was found to be the smallest, the gap. Our results therefore indicated that light sources from light emitting diodes (LEDs) combined with wave lengths might be beneficial contributions for developing vascular bundles and stomatal behaviors of the grafted seedlings during the wound healing period.

1. Introduction

Grafting, an asexual plant propagation method, is the act of joining two plants together. The upper part of the graft (the scion) becomes the top of the plant; the lower portion (the rootstock) becomes the root system, which are grafted together to stimulate growth, improve agronomic traits and increase plant tolerance to biotic/abiotic (Louws et al., 2010; Colla et al., 2010; Savvas et al., 2010). Grafting is an important technique in horticultural crop production to achieve such beneficial traits mentioned above with relatively less costs. This technique has been extensively used, mainly in tree crops such as grapevines (Clemente Moreno et al., 2014), plums (Ravelonandro et al., 2002), apples (Atkinson et al., 2003), and roses (Van der Salm et al., 1998); however, it is also popular in some vegetable crops such as tomato (Goto et al., 2013), pepper (Penella et al., 2013), watermelon (Muneer et al., 2015), melon (Colla et al., 2006), cucumber (Marsic and Jakse, 2010), artichoke (Temperini et al., 2013) and eggplant (Moncada et al., 2013). Successful grafting, which has a complex process that occurs between two grafted partners, consisting of callus formation followed by the establishment of a functional vascular system (Fernández-García et al., 2004; Pina and Errea, 2005). During the contact of callus cells, the cell walls of the graft unions undergo a termination process, with pores appearing in the cell walls, plasma membranes coming into contact with each other, and appearance of plasmodesmata (Jeffree and Yeoman, 1983).

Abbreviations: $A_{1,2}$, area vessel on balance 1 and 2, respectively; $\Delta(T-U)$, the difference between rate of transpiration and water uptake; FL, fluorescent tube; LED_B, blue light emitting diode; LED_R, red light emitting diode; LEDs, light emitting diodes; LED_W, white light emitting diode; LED_{W1R2B1}, combined light emitting diodes consisted of chips with 1 white, 2 reds and 1 blue; PPFD, photosynthetic photon flux density; T, rate of transpiration; t, time; U, rate of water uptake; V_f, vessel factor; $W_{1,2}$, weight on balance 1 and 2, respectively.

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Graft union development is a complex process during which histological and physiological variations occur, such as the simultaneous events of organ regeneration and development. Further, genetic materials can even be exchanged between the rootstock and scion in graft unions (Stegemann and Bock, 2009; Van der Salm et al., 1998). Graft compatibility–incompatibility between the scion and rootstock is not fully understood (Aloni et al., 2010). Therefore, grafting is not only a useful method but also an interesting research field. A number of research reports have documented the results of graft union studies, especially with respect to histological, physiological and molecular methods (Muneer et al., 2015; Cookson et al., 2014; Cookson and Ollat, 2013; Yan et al., 2012; Flaishman et al., 2008; Olmstead et al., 2006; Fernández-García et al., 2003, 2004). In their reports they have extensively studied changes in graft unions morphologically and physiologically. Besides their studies have also provided a genome of graft unions and proteins involved between graft unions.

The environmental factors such as light, temperature and humidity are important aspects for effective graft union formation and acclimatization. Among several environmental factors, light serves as an energy source for photosynthesis, hormone regulation and several other signaling pathways (Muneer et al., 2015; Velez-Ramirez et al., 2014). The spectral qualities of a light source affect the growth and development of plants in terms of their physiological, morphological, and anatomical plasticity (Schuerger et al., 1997). Dicotyledonous plants appear to be more sensitive to spectral quality compared with monocotyledonous plants (Deutch and Rasmusse, 1974). To achieve a high survival rate, the functional connectivity of the vascular bundles between the scion and rootstock is an important factor for successful grafting and acclimation (Oda, 1995). Most of the studies examining the vascular pathway between the scion and rootstock have been performed on woody plant species such as sweet cherries, apples, and so on (Olmstead et al., 2006). However, the effect of light and various light sources on the functioning of the vascular pathway between the scion and rootstock of greenhouse vegetable crops has rarely been studied.

Light-emitting diodes (LEDs) are an efficient source of light; LEDs have a small mass and provide greater safety and durability for better growth and development of plants (Kim et al., 2013; Yano and Fujiwara, 2012; Yorio et al., 2001). LEDs are commonly used in closed plant growth chambers and they may be valuable for successful graft union formation and further growth and development. Moreover, it has been observed that LEDs, particularly red, blue and white LEDs, have special characteristics and a spectral width that can match the light quality needed for successful grafting (Lee et al., 2010; Vu et al., 2014). Nonetheless, a major challenge for successful grafting is the supply of a sufficient quantity and quality of light. It might be suggested that the combination of different LEDs, particularly the combination of red, blue, and white LEDs, can provide an optimal and suitable light intensity for effective grafting.

The present study was developed to investigate the effect of light qualities (light emitting diodes) on graft union (vascular connections) functionality during healing period. To follow our objective we studied several vascular connection factors such as the transpiration, water uptake rate, sugar content, flower dye distributions between the scion and rootstock, and the hardness of the graft unions during the healing period after grafting. Besides, short- and long-term responses were tested to determine whether the unions were successful. Moreover, the developing functional xylem and phloem connections were examined and directly and indirectly quantified under various light sources. Incomplete vascular bundle connections in the graft unions indicated a physical weakness of the unions as well as physiological disorders in the grafted seedlings, such as stomatal malfunctioning, unbalanced water status and limited sugar transportation to the rootstock.

2. Materials and methods

2.1. Plant materials and environmental conditions

Tomato (*Solanum lycopersicum*) cultivars Super Doterang (the scion) and B-Blocking (the rootstock) were germinated for 4–5 days; the seeds were covered with vermiculite in a moistened peat moss-based substrate in a germination chamber with a temperature of 23 °C and a relative humidity of 85%. After germination, the seedlings were grown on tables with net benches in a plastic film house at the Chojeon Plant Nursery Co., Jinju, Korea (35 N°) for one month.

2.2. Transpiration and water uptake of intact and half-cut stems (Experiment 1)

To evaluate the effects of complete and incomplete vascular connections on various functions, transpiration and water uptake were measured in intact and half-cut stems of the tomato seedlings (*S. lycopersicum* cv. B-Blocking) using two electronic balances (CAS MW-II, CAS Co., Korea) that were part of a synchronized monitoring tool described by Van Ieperen and Madery (1994) (Supplementary Fig. 1). Two vessels containing 250 mL of double distilled water were connected by means of a silicon tube with an inside diameter of 0.4 cm and a length of 16 cm. This silicon tube was connected at the lowest point of each vessel. Each vessel, which was completely covered with aluminum foil to create a stable environment, was placed on an electronic balance. The seedlings grown in plug cells were pre-treated; the substrates were washed off to ensure the roots were clean. Then, the seedlings were carefully inserted into one of the two vessels through a hole. A strong light with a photosynthetic photon flux (PPF) of approximately 1000 μmol m^{-2} s^{-1} was positioned on top of the seedling for half an hour and then turned off for the next half an hour; this process was repeated, during which half of the stem was cut using a sharp razor blade. The weight of each vessel on each balance was recorded every 5 min. The *T* and *U* of each sample were derived from the simultaneous weighing data using Eqs. (1)–(3).

$$T = \frac{\Delta (W_1 + W_2)}{\Delta t} \quad (1)$$

$$U = \frac{\Delta W_2 \times V_f}{\Delta t} \quad (2)$$

$$V_f = \frac{A_1}{A_2} + 1 \quad (3)$$

in which:

T: rate of transpiration [mg 5 min^{-1}]
U: rate of water uptake [mg 5 min^{-1}]
$W_{1,2}$: weight on balance 1 and 2, respectively [mg]
t: time [5 min]
V_f: vessel factor (=1.9995 ± 0.00302) [−]
$A_{1,2}$: vessel area on balance 1 and 2, respectively [cm^2]

Here, we assumed that the main driving force of water flow in the plants was transpiration stimulated by the strong light, i.e., ca. 1000 μmol m^{-2} s^{-1} PPFD.

2.3. Grafting procedure and climate in the acclimating chamber (Experiment 2)

Thirty days after germination, tomato cultivars Super Doterang and Super Sunload (the scions) were grafted onto cultivar B-Blocking (the rootstock) using the 'splice grafting' procedure described by Lee et al. (2010). Grafted plants were placed in a controlled acclimation chamber at a day/night temperature of 23/20 °C and a day/night relative humidity of 90/95% for 1 day under dark