Scientia Horticulturae 212 (2016) 29–34

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effect of low night temperature on graft union formation in watermelon grafted onto bottle gourd rootstock

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ARTICLE INFO

Article history:
Received 10 June 2016
Received in revised form 30 August 2016
Accepted 8 September 2016
Available online 24 September 2016

Keywords:
Watermelon
Graft union
Low night temperature
Anatomy

ABSTRACT

To explore the effects of low night temperatures on graft union formation in watermelon grafted onto bottle gourd rootstock, an anatomical study during the healing stage was performed. In the present study, we compared the morphological anatomy structures during the healing process in grafted watermelon seedlings under different night temperature treatments. The results showed that concrescence occurs fastest at the night temperature of 18 °C, at which vascular bridges were connected to vascular bundles at 5 days post-grafting (dpg). For night temperatures of 15 °C and 12 °C, vascular bridges were connected to vascular bundles at 7 dpg and 10 dpg, respectively. In general, healing rate slowed as night temperature decreased. Low temperatures delayed the differentiation of vascular tissue, which was not conducive to the connection of vascular bundles. The wider gully between the rootstock and scion cut surface caused the callus formation on both sides to fail to link, which turns to the formation of graft union unsuccessful. These results suggest that night temperatures are implicated in graft development in watermelon plants. A minimum temperature of 18 °C is indicated during the graft union formation stage.

1. Introduction

As a staple fruit of summer, watermelon plays an important role in the global horticulture industry. Because of an increase in consumer demand, the cultivation area of watermelon has also been expanding. However, large-scale production has been restricted in continuous cropping, as soil-borne disease and soil salinization seriously affect the sustainable development of facility production. Grafting is a preferred technology for the realization of continuous cropping through the avoidance of soil pathogens and by increasing tolerance of low temperatures, high salinity, and drought (Davis et al., 2008; Shen et al., 2009; Hao et al., 2010). High temperatures, high humidity and shaded conditions are optimal for the formation of the graft union during the healing stage. Few data are available on the formation of graft unions under low temperature stress, which is the primary factor that influencing plant growth in early spring.

The development of grafted watermelon seedling includes initial adhesion, production of the callus, and formation of secondary plasmodesmata and differentiation of vascular bundles. Establishment of vascular bundle in the graft interface is considered as a successful graft union (Turquois and Malone, 1996; Kester et al., 1997; Fernández-garcía et al., 2004). At the beginning of grafting, injured cells of the rootstock and scion at the graft interface formed isolation layers and became separated from the external environment, which was conducive to wound healing and resisting bacterial infection (Ermel et al., 1997). Vascular bundle cells were formed by differentiation of three cell types: the original residual vascular bundle cells close to the rootstock-scion incision, parenchyma and callus cells of the isolation layer and the original vascular bundle cells of the rootstock-scion. The differentiation direction of these new tracheary elements turned towards the graft union surface. These results correspond with those from histological and cytological research on graft union formation of grafted watermelon seedlings with different scion ages (Zhang et al., 2012).

Anatomical studies of graft union formation have been conducted in various crops, such as apricot (Errea et al., 1994a, b; Pina et al., 2012), apple (Soumelidou et al., 1994), pear (Ermel et al., 1997), tomato (Fernández-garcía et al., 2004), peach (Zarrouk et al., 2010), and grape (Milien et al., 2012). The formation of the graft union in these taxa is a complex process that includes adhesion between grafted partners, callus formation, establishment of new

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http://dx.doi.org/10.1016/j.scienta.2016.09.010
0304-4238/

vascular tissue, and the formation of a functional vascular system across the graft (Pina et al., 2012). Comparatively, studies in watermelon have focused on the rootstocks effect on the yield, fruit quality and tolerance to biotic and abiotic stresses of scions. Little information is available regarding the healing process in graft union formation, especially under low temperature stress. In the present study, we describe the effects of low night temperature stress on the structural development of the graft union formation in watermelon through morphological anatomy structure analysis.

2. Materials and methods

2.1. Plant material and culture conditions

Watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) cultivar 'Sumi 8' and bottle gourd (*Lagenaria siceraria* (Molina) Standl) rootstock 'Hengxi rootstock No. 1' were used as scion and rootstock, respectively. Both cultivars were obtained from the Institute of Vegetable Crops, Jiangsu Academy of Agricultural Sciences, China. The watermelon scions were grafted onto bottle gourd rootstock via the hole insertion grafting technique (Lee and Oda, 2003) when the first true leaf of the rootstock and two cotyledons of the scion had expanded. The grafted watermelon seedlings were divided into three groups and placed in artificial climate boxes with night temperatures of 12 °C, 15 °C, and 18 °C (control) for three different night temperature treatments and a day temperature of 28 °C for 10 h per day. Sixty grafted watermelon seedlings were established for each temperature conditions. The grafted seedlings were wrapped in plastic film with no light source and a constant humidity of 95–100% for the first three days. They were then cultivated in growth chambers at an illuminance of 9600 lux and a humidity of 90%.

2.2. Microscopic examination of anatomy structure

Graft unions were sampled at 3, 5, 7, 9 and 11 days post-grafting (dpg) for the observation of morphological structure. Ten grafted seedlings were taken for each time point. Samples were fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2), placed under vacuum to remove any remaining air and were cut into semithin resin sections and stained with 1% toluidine blue (O'Brien et al., 1964). Each sample was replicated three times.

To observe the formation of vascular bundles, graft unions were sampled at 1 to 12 dpg, washed with distilled water and then soaked in a 6% NaOH solution until samples were transparent. The samples were then transferred into 10 mL 1% safranin staining solution (Ruiz-Sifre et al., 1997). After the vascular bundles were stained red, samples were freehand sectioned and observed under light microscopy. Five grafted seedlings were taken for each time point.

3. Results and discussion

3.1. Observation of the histological differences between the rootstock and scion after grafting

3.1.1. Anatomical structure of the graft union at 3 dpg

A thin and deep-staining isolation layer (Fig. 1A3, B3 and C3) could be observed at 3 dpg. The isolation layer was formed by dead cells and protoplasm of destructed parenchyma cells on the wound surface. Generation of parenchymatous wound callus tissue was also observed on both the rootstock and scion sides around the isolation layer. No callus formation was observed near the isolation layers under 12 °C night temperature treatment (Fig. 1A3). Under the 15 °C night temperature treatment, the isolation layers on the rootstock side produced 1–2 layers of small-volume of callus cells (Fig. 1B3). Under the 18 °C temperature treatment, the rootstock side produced 2–3 layers of small-volume of callus cells, and the scion side also induced the production of 1–2 layers of small-volume of callus cells. These results indicate that the ability to perform cell division was quickly restored at higher night temperatures, thus promoting the generation of irregular callus cells. Comparing the graft union development process among the three different night temperature treatments, we found that cells around the isolation layer on the rootstock side possess higher restorability of cell division than cells on the scion side.

3.1.2. Anatomical structure of the graft union at 5 dpg

At 5 dpg, the callus could be clearly seen. At this time, callus cells continued dividing and proliferating on both sides of the isolation layers. In addition, formation of a continuous cambial connection between rootstock and scion and an increase in cell layers and swelling of the volume of cambial cells occurred. After the isolation layer produced the callus, the parenchyma cells and callus cells can directly differentiate into vessel elements or form sieve tubes and companion cells after one or more rounds of division. After the original vascular bundles are cut off, residual cambial cells or parenchyma cells differentiate into new vessel elements and sieve tubes, which function as the central vessel elements and sieve tubes of the transport organization systems connecting the rootstock and scion. As shown in Fig. 1A5, B5 and C5, the isolation layers could be seen clearly. Under the 12 °C night temperature treatment, cells on the rootstock side of the isolation layer experienced a noticeable enlargement in volume compared to the cells on the scion side. The original vascular bundles began to differentiate into new vessel elements and sieve tubes (Fig. 1A5). Under the 15 °C night temperature treatment, expansion of the callus cells on the rootstock side was irregular, and the original vascular bundles in the scion incision differentiated into vessel elements and sieve tubes that began to break through the isolation layers (Fig. 1B5). With increasing temperature, callus cells on the side of scions had almost the same volume as those on the other side under the 18 °C night temperature treatment (Fig. 1C5). Callus cells on both sides of the isolation layers induced much differentiation and proliferation, and some had broken through the isolation layers to initiate mutual contact. Vessel elements and sieve tubes that had differentiated from the original vascular bundles in the scion incision had begun to communicate with those from the callus cells and parenchyma of rootstocks.

3.1.3. Anatomical structure of the graft union at 7 dpg

It is well-established that callus tissues play central roles in the formation of a successful union. They act as bridge and enable the water and nutrients to by-pass the vascular system (Barnett and Weatherhead, 1988; Moore, 1991; Hartmann et al., 2002; Pina et al., 2012). When the rootstock-scion callus begins communication, the isolation layers gradually disappear over time. At 7 dpg under the 12 °C night temperature treatment, cambial layers are obviously activated, and callus cells continued to divide and increase in volume. Vessel elements and sieve tubes that differentiated from some of the callus cells on the scion side formed active cells with a certain divisive direction, showing signs of the formation of vascular bundles, and had a tendency to link with the original vascular bundles of the scions (Fig. 1A7). Under the 15 °C night temperature treatment, direct vessel element organogenesis by scion callus cells had extended to the initial scion vascular bundles. The original vascular bundles of the rootstock that were not in direct proximity to the graft interface, but were still close in distance, differentiated into a large number of vessel elements extending to the interface (Fig. 1B7). Living cells from the graft interface divided and proliferated in most directions, continuing to produce a large number of vessel elements and sieve tubes to interconnect the rootstock-scion transfusion tissue. Along with the formation of the rootstock-scion