Effect of Bridge Grafting the M9 Self-rooted Rootstock in Trunk-wounded Apple Trees on Vegetative Growth, Yield, and Fruit Characteristics

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Abstract. Bridge grafting is widely applied in trunk-wounded apple trees. In this study, we carried out semigirdling and ring girdling on the trunk of ‘Nagafu 2’ Malus baccata (L.) Borkh apple trees to simulate trunk injury. We then bridge grafted a M9 self-rooted rootstock on the injured trunks to study the effects of bridge grafting on flowering, fruit-set, tree vigor, and fruit characteristics in ‘Nagafu 2’ apple. The results showed that both semigirdling and ring girdling due to the large wounded area caused significant decrease in flowering, fruit-set, and tree vigor (estimated by measuring leaf area, leaf gas exchange, tree height, and shoot growth); in addition, ring girdling increased flesh and peel firmness. However, bridge grafting of M9 self-rooted rootstock on semigirdling and girdling apple trees resulted in partial recovery of tree vigor (leaf area and photosynthesis) and maintaining the reduction of vegetative growth, thereby increasing flowering, fruit-set, yield, fruit weight, and peel firmness.

In China, apple is an important economic fruit crop that is threatened by trunk wounding, which can seriously restrict the development of the apple fruit industry (Wang et al., 2014). In general, trunk injury caused by Valsa canker, mechanical wounding, and other factors, such as winter cold and animal biting, limits the growth and fruit yield of apple trees (Goren et al., 2004; Tan et al., 2017). Valsa canker is a major cause of severe injury to the trunks of apple trees in China, Japan, and Korea (Abe et al., 2007; Hu et al., 2014; Lee et al., 2006; Wang et al., 2005). In 2008, the incidence rate of Valsa canker was as high as 52.7% in the investigated orchard and the disease tended to spread in an unchecked manner (Cao et al., 2009). According to Ma et al. (2007), ≥65% of 13- to 17-year-old ‘Nagafu 2’ apple trees were infected by Valsa canker disease in Shaanxi Province, China.

Severe injury to the trunk of apple trees might affect water, nutrient, and photosynthesis flow from the source to sink organs. This damage can retard growth, reduce photosynthesis, cause leaf chlorosis and abscission, and affect fruit characteristics. It might even cause the death of trees (Fanwoua et al., 2014; Poirier-Pocovi and Buck-Sorlin, 2016; Singh et al., 2015).

Injury to the trunk of fruit trees causes the accumulation of insoluble carbohydrates, such as starch, above the trunk wounds, whereas soluble carbohydrate content decreases, thereby activating glycolysis to match the increased energy requirement (Beruter et al., 1997; Jordan and Habib, 1996; Rivas et al., 2006). In addition, trunk wounding reduces the concentration of cytokinins (CTK), auxin, and abscisic acid (ABA), while significantly increasing the gibberellin acid (GA) content in the shoots (Dann et al., 1985; Skogerbo, 1992). This hormone imbalance caused by trunk girdling might be associated with the altered carbohydrate metabolism, root growth, and vegetative growth (An et al., 2017).

Bridge grafting has been widely used to treat trunk wounds in apple trees. The application of bridge-grafting technology has been shown to alleviate low apple yield and improve fruit characteristics in trunk-wounded apple trees. However, very little is known about the effect of bridge grafting on vegetative growth, yield, and fruit characteristics in Valsa canker–induced trunk-wounded ‘Nagafu 2’ (Red Fuji) apple trees, with regard to the severity of Valsa canker in the region of Chinese apple cultivation, especially in the western part of China. In the present study, to simulate apple tree trunk injury, which is caused by scraping of the infected trunk bark that is commonly used to prevent the spread of Valsa canker disease in apple orchards, we used the method of artificial debarking of trunk in 5-year-old ‘Nagafu 2’ Malus baccata (L.) Borkh apple trees. Next, we planted a M9–T337 (M9) self-rooted rootstock adjacent to every tree to bridge graft the wounded trunks. Subsequently, we investigated flowering, fruit-set, plant vigor, fruit characteristics, leaf photosynthesis, shoot hormone, and carbohydrate content in the bridge-grafted, girdled apple trees to better understand the effect of M9 bridge grafting on trunk-wounded apple trees and to provide empirical guidance for addressing the problem of severe Valsa canker disease requiring the digging of apple trees.

Materials and Methods

Plant materials. This experiment was performed at the Apple Experimental Station, Luochuan, Shaanxi, China (lat. 35°47′N, long. 109°22′E), from Apr. 2016 to Oct. 2017. We used 5-year-old ‘Nagafu 2’ (Red Fuji) on apple rootstocks M. baccata (L.) Borkh, planted at a spacing of 5.0 × 3.0 m in loess soil. The trees were maintained according to local commercial standards throughout the study.

Experimental design. The experiment was performed as described by Samad et al. (1999b) and Tang et al. (2015), with some modifications. The experiment was a two by two factorial design with no girdling and girdling (semigirdling and ring girdling) by two bridge grafting of M9 treatments (treated and untreated). Forty trees were used in a completely randomized design. Experiments were performed using 3–30 replications. The following five treatments were used in this study: 1) no girdling or bridge grafting (control), 2) semicircle girdling of the trunk bark (1/2G), 3) bridge grafting of 1-year-old M9 self-rooted rootstock on 1/2G (1/2G+M9), 4) full-circle girdling of the trunk bark (G), and 5) bridge grafting an M9 on G (G+M9) (Fig. 1).

On 5 Apr. 2016, an 0.2-cm width bark strip was removed from the trunk, 30 cm above the ground, for the 1/2G and G apple trees treatments. In addition, an M9 rootstock was planted adjacent to each experimental apple tree for bridge grafting after survival (1 week). A 1.0-cm-wide split M9 was inserted, with cambial matching at ≈10 cm intervals up to the wound on the 1/2G and G apple trees, which were labeled as 1/2G+M9 and G+M9, respectively. Subsequently, the graft was tightly wrapped with plastic and rope to prevent the entry of moisture and insect pests. Untreated trees were used as controls. Three replications were used per treatment, and one tree per replication.

Data collection and leaf photosynthesis measurements. The leaf area was determined...
on 20 Aug. 2017, by using 10 leaves from each tree. On 20 Dec. 2016, selected 10 annual shoot length per tree crown, tree height, total floral bud number, number of spurs, and medium-long shoots from six main branch per tree were investigated. The number of flowers at full blossom (18 Apr. 2017) and the number of fruit at 30 d after full blossom per tree were recorded. The number of spring shoots was measured when spring shoots stopped growing, on 10 July 2017. Every month, from 10 June to 10 Sept. in 2016, shoot terminals with 7–10 leaves were collected and 1-cm long shoot tips from each shoot terminal were cut, lyophilized, frozen in liquid nitrogen, and stored at −80 °C until extraction for the determination of hormones, carbohydrates, and soluble protein. Nine replications were used per treatment (one shoot per replication and three shoots per tree).

Determination of content of endogenous hormones, carbohydrates, and soluble protein. Endogenous hormones content in shoot tips was determined using an HPLC system (Waters, Milford, MA) according to Zhang et al. (2015). Standard protocols were used for hormone identification and quantification: ABA, GA, indole acetic acid (IAA), and zeatin riboside (ZR) were separated and quantified using an Intertsil C18 ODS-3 chromatographic column (150 mm × 4.6 mm, 5 µm; Sigma Aldrich, St. Louis, MO) and a Waters 2487 detector (λ = 280 nm; Waters). The mobile phase consisted of methanol and 0.5% acetic acid (60:40, v/v), the flow rate was 1 mL·min⁻¹, and the column temperature was 30 °C. Solutions of 0.1 g·L⁻¹ ZR, 20.0 g·L⁻¹ GA, 1.0 g·L⁻¹ IAA, and 0.1 g·L⁻¹ ABA were used as standard samples.

Table 1. P-values of main effects of tree vigor, leaf photosynthesis, the numbers of flower, and fruit-set in apple trees produced using a two-way factorial analysis of variance.

| Treatment | Flowering rate | Fruit No. | No. spring shoot | Length of annual shoot | Increment of plant ht | Proportion of shoot types | Leaf area | Net photosynthetic rate | Stomatal conductance | Transpiration rate | SPAD |
|-----------|----------------|-----------|------------------|-----------------------|------------------------|--------------------------|-----------|------------------------|---------------------|-------------------|-------|
| Girdling  | 0.003          | <0.001    | 0.009            | <0.001                | <0.001                 | <0.001                   | <0.001   | 0.009                  | <0.001              | 0.003             | <0.001|
| Bridge grafting | 0.008          | 0.001     | <0.001           | <0.001                | <0.001                 | <0.001                   | <0.001   | <0.001                | 0.018               | <0.001            | <0.001|
| Girdling × bridge grafting | 0.059          | 0.582     | 0.38             | 0.011                 | 0.988                  | 0.094                    | <0.001   | <0.001                | 0.282               | 0.45              | <0.001|

Effects of girdling and bridge grafting with P < 0.05 are highlighted in bold.
Determination of fruit characteristics. Fruit production per apple tree and single fruit weight were measured on 19 Oct. 2017, by using an electronic scale. Ten fruits from every tree were sampled for the determination of fruit characteristics (one apple fruit per replication and 10 fruits per tree). Fruit length and fruit diameter of the apples were measured using a vernier caliper; chromaticity of the fruit peel was determined using a Reflectance Tintometer (Lovibond RT Series, Dortmund, Germany). Soluble-solid content in the fruit flesh was measured using a pocket refractometer (Atago, Tokyo, Japan); the fruit maturity index was calculated as described by Blanpied and Silsby (2010). Fruit firmness was estimated by the force required for puncturing the fruit in the middle using a TA XT Express Texture Analyzer (Stable Micro Systems, Hamilton, South Lanarkshire, UK) and the data were analyzed using the exponent system (Stable Micro Systems).

Statistical analysis. Correlation coefficients were calculated using the Pearson correlation analysis procedure and means were separated using the least significant difference test at \( P < 0.05 \). Flowering, fruit-set, plant height, shoot number, photosynthesis data, yield, and fruit characteristics were tested using two-way analysis of variance (ANOVA). Hormones, carbohydrates, and soluble protein were tested using three-way ANOVA. All data were analyzed using IBM SPSS software (IBM Corporation, New York).

Results

Effect of bridge grafting on flowering and fruiting. In 2016 and 2017, the results of bridge-grafted M9 rootstocks on trunk-wounded ‘Nagafu 2’ trees were successful, but the trunk wounds were healing faultily (Fig. 1; Supplemental Fig. 1). Bridge-grafted M9 significantly affected flowering in both semigirdled and girdled ‘Nagafu 2’ trees. We counted the number of flowers in the apple trees during bloom and found that the flower number of 1/2G and G apple trees was lower than that in the controls (\( P = 0.003 \)). Furthermore, the number of flowers in 1/2G+M9 and G+M9 apple trees was 40.5% and 85% greater (\( P = 0.008 \)) than that in 1/2G and G apple trees, respectively. Finally, neither of the two treatments was significantly different from the control trees (Fig. 2A; Table 1). In the treatments, \( \approx 30\% \) floral buds blossomed into flowers (\( P = 0.014 \)); however, in the control and bridge-grafted ‘Nagafu 2’ trees, 60% floral buds blossomed (\( P = 0.001 \); Supplemental Fig. 2).

Fruit-set was increased by bridge grafting in 1/2G and G treatment apple trees. The G apple trees showed the lowest number of fruits among all treatments, whereas the fruit number of 1/2G trees was not significantly different from that of the other four treatments (\( P = 0.008 \); Fig. 2B; Table 1). Bridge-grafted M9 led to an increase of 21.5 (33.3%) and 39.7 (55.4%) fruits/tree in 1/2G and G apple trees, respectively (\( P = 0.009 \)).

Table 2. Correlation coefficients between tree vigor and the number of flower and fruit-set.

| Correlation coefficients | Fruit-set | Shoot No. | Increment of plant ht | Ratio of spurs |
|--------------------------|-----------|-----------|-----------------------|---------------|
| Flower number            | 0.685**   | 0.396     | -0.198                | 0.225         |
| Fruit-set                | 0.674**   | 0.045     | 0.538*                | 0.304         |
| Shoot number             |           | 0.516*    |                       | 0.043         |
| Increment of plant height|           |           |                       |               |

\( *P < 0.05; **P < 0.01. \)
Effects of girdling and bridge grafting with G+M9 and G apple trees.

Data within a row represent mean ± SE, n = 30, different letters following the data are significantly different at P < 0.05.

Effects on photosynthetic parameters. Bridge grafting of G+M9 self-rooted rootstocks onto 1/2G and G apple trees increased the Pn, gs, and Tr in leaves (P < 0.001; Fig. 4A–C; Table 1). Leaf Pn was significantly higher in 1/2G+M9 and G+M9 apple trees than in the control trees. Conversely, Pn in 1/2G+M9 and G+M9 was higher than that in control, and a significant difference was noted between G and control (Fig. 4A). Bridge grafting of M9 self-rooted rootstocks increased the leaf gs of G apple trees, which was lower than that in controls. Phloem girdling of apple tree trunk significantly affected the leaf Tr; however, bridge grafting of M9 recovered the Tr.

Similarly, girdling obviously declined leaf SPAD, which was significantly lower than that in G+M9 trees (P = 0.018). No significant difference in SPAD values was noted between 1/2G and 1/2G+M9 trees (Fig. 4D).

Effects on fruit characteristics. The fruit numbers in the mature stage of control, 1/2G, 1/2G+M9, G, and G+M9 trees were lower by 9.2%, 35.9%, 16.3%, 46.8%, and 18.4% than those in full blossom, respectively. Fruit yield, weight, diameter, and length in the 1/2G and G apple trees were significantly lower than those in the 1/2G+M9 and G+M9 treatments (P < 0.001). The color of the fruit peel in G apple trees was inferior to that observed in the other treatments (P < 0.001), but the fruit soluble-solid content in G apple trees was higher than that in the other treatments. Fruit shape index and maturity index were not significantly different among the treatments (Table 6).

The peel firmness (P < 0.001) and peel toughness (P < 0.001) were increased under 1/2G and G treatments and were higher in G than in 1/2G trees (Table 3). Nevertheless, bridge grafting of M9 decreased the flesh firmness in girdled apple trees (P = 0.023). The flesh firmness was significantly and negatively correlated with fruit yield and fruit length (P < 0.05), whereas the fruit soluble-solid content was significantly and negatively correlated with fruit diameter (P < 0.05; Table 4).

Content of endogenous hormones, carbohydrates, and soluble protein. The ZR concentration in shoot tips of apple trees decreased gradually from June to September.
controls and G apple trees. Soluble sugar ($P = 0.023$) and starch ($P < 0.001$) contents were significantly affected by the bridge-grafting treatment; however, the former was unaffected by the trunk-girdling treatment and the latter was increased by girdling ($P = 0.006$; Table 2).

From June to September, soluble protein content in the shoot terminals of apple trees decreased gradually ($P < 0.001$). In June, the soluble protein content in 1/2G+M9 and G+M9 apple trees was higher than that in 1/2G and G apple trees ($P < 0.001$). In July, the soluble protein content in G+M9 was higher than that in the other treatments. No significant difference was noted among the five treatments from August to September (Fig. 6C; Table 5).

**Discussion**

Bridge grafting of M9 restored partial tree vigor in trunk-wounded apple trees. Trunk wounding in fruit trees hinders the synthesis and transport of endogenous hormones; limits the growth of trees trunks, shoots, and roots; and restricts nutrient absorption (Choi et al., 2010). Overall, these changes lead to the loss of tree vigor (Greene and Lord, 1978; Henretty and Forshney, 1971). Trunk girdling, a measure undertaken to counteract these adverse effects, which restricts the flow of carbohydrates down to the root, might have had varying effects on root development (Samad et al., 1999b). In the present study, girdling reduced the annual shoot length, plant height, and spring shoot number; however, bridge grafting of M9 did not have a significant effect (Figs. 2 and 3). This result suggested that the root status has a greater effect on vegetative growth than shoot status, likely via hormonal influences (Fig. 5).

In fruit trees, excessively low IAA content inhibits lateral branch formation and ABA accumulates in the axillary buds and shoot tips, thereby reducing lateral emergence (Kim et al., 1984; Robinson, 2003). In the present study, from June to September in 2016, girdling decreased the IAA content and increased the ABA content in apple trees shoots, thereby reducing the shoot growth of G and G+M9 apple trees and hindering shoot emergence (Figs. 2D, 3A, 5B and D; Bangarth, 1989; Brewer et al., 2009; Wang et al., 1994; Zhang et al., 2015). In addition, during rapid shoot growth, the starch and soluble protein contents in semigirdling and ring girdling apple trees were lower than those of the control providing insufficient energy for shoot development (Goren et al., 2004; Thorpe and Murashige, 1968). Previous studies showed that GA$_4$, GA$_3$, and GA$_5$ can decrease the proportion of spurs and increase shoot length in fruit trees (Little and Macdonald, 2003). From May to July in 2016, during the shoot bud formation in apple trees, high concentration of GA in the G apple trees caused by girdling for wound healing increased the medium-long shoots (Zhang et al., 2015). However, bridge grafting of M9 increased the CTK content and decreased the GA content to promote more spurs. Furthermore, IAA promoted the growth and development of trees shoots, whereas ABA played an opposite role (Tworkoski and Fazio, 2016). In the present study, from June to September, trunk wounding decreased the IAA content owing to the reduction of vegetative growth and increased the ABA content, leading to the inhibition of tree growth and shoot formation in 1/2G, G, and G+M9 apple trees (Bangarth, 1989; Brewer et al., 2009; Wang et al., 1994). Because the girdling area of the trunk in 1/2G apple trees was smaller than that in G apple trees, the effect of bridge grafting M9 on shoot growth and branching ability was more prominent in the former.

Trunk wounding affects the assimilation function and development of trees leaves, including photosynthesis and leaf area (Tang et al., 2015). Girdling decreases leaf $g_c$, $Pn$, and $Tr$ in most species (Schechter and Proctor, 1994). A decrease in leaf $Pn$ of apple trees under wound stress is mainly caused by the closing of the stomatal aperture, decline of RuBPcase activity, and accumulation of soluble sugar because of sink feedback inhibition of photosynthesis (Cheng et al., 2008; Quentin et al., 2013). In the present study, the leaf $Pn$, $Tr$, and $g_c$ and SPAD were the lowest in G apple trees, after bridge-grafted M9 rootstock, which was transferred assimilates from

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### Table 4. Correlation coefficients among fruit characteristics, fruit size, and yield.

| Correlation coefficients | Fruit wt | Soluble solids | Fruit diam | Fruit length | Yield |
|--------------------------|----------|----------------|------------|--------------|-------|
| Flesh firmness           | -0.200   | 0.011          | -0.211     | -0.255       | -0.611* |
| Fruit weight             | -0.288   | 0.921**        | -0.879**   | 0.072        |       |
| Soluble solids           | -0.397*  | -0.241         | -0.123     |              |       |
| Fruit diameter           | 0.760**  | -0.123         | 0.229      |              |       |
| Fruit length             |          |                |            |              | 0.229 |

*P < 0.05; **P < 0.01.

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### Table 5. P values of the contents of endogenous hormones, carbohydrates, and soluble protein (2016) of shoot terminals in apple trees among girdling treatments, bridge grafting, and growth time produced using a three-way factorial analysis of variance.

| P values                | Zeatin riboside content | Indole acetic acid content | Gibberellic acid content | Absicic acid content | Soluble sugar content | Starch content | Soluble protein content |
|-------------------------|-------------------------|---------------------------|--------------------------|----------------------|-----------------------|-----------------|------------------------|
| Girdling                | $<0.001^*$              | $<0.001$                  | $<0.001$                 | $<0.001$             | 0.573                 | 0.076           | 0.012                  |
| Bridge grafting         | $<0.001$                | 0.627                     | $<0.001$                 | 0.048                | 0.023                 | 0.006           | $<0.001$               |
| Girdling × bridge grafting | 0.022                | 0.894                     | 0.011                   | 0.312                | 0.366                 | 0.925           | 0.047                  |
| Time                    | $<0.001$                | $<0.001$                  | $<0.001$                 | $<0.001$             | $<0.001$              | $<0.001$       | $<0.001$               |
| Girdling × time          | 0.004                  | 0.369                     | $<0.001$                 | $<0.001$             | 0.117                 | $<0.001$       | 0.023                  |
| Bridge grafting × time   | 0.014                  | 0.718                     | $<0.001$                 | $<0.001$             | 0.282                 | 0.053           | 0.279                  |
| Girdling × bridge grafting × time | 0.586   | 0.777                     | $<0.001$                 | $<0.001$             | 0.282                 | 0.053           | 0.279                  |

*Effects of girdling and bridge grafting with $P < 0.05$ are highlighted in bold.
upon girdling to M9 rootstock to reduce the inhibition of sink feedback (Neales and Incoll, 1968). Second, in 1/2G apple trees, although they were recovered by bridge grafting of M9, the Tr increased after gs increased in apple trees (Williams et al., 2000). In addition, bridge-grafted M9 increased the leaf area and SPAD values, followed by an increase in \( Pn \) (Peng and Rabe, 1996).

Bridge grafting of M9 increased flowering and fruiting in trunk-wounded apple trees. The aforementioned results indicated that bridge grafting of M9 supplemented certain nutritional factors or hormones for leaf growth, facilitated the partial recovery of the lost vigor, and maintained the reduction of vegetative growth. M9 rootstocks absorbed the deposited carbohydrates in the overground part to accelerate the leaf photosynthesis rate in trunk-wounded apple trees (Table 2) (Goren and Monselise, 1971; Noel, 1970).

**Table 6.** Apple fruit characteristic and production at different treatments and \( P \) values of main effects between girdling and bridge grafting produced using a two-way factorial analysis of variance.

| Treatments | Yield (kg/tree) | Fruit wt (g) | Fruit diam (mm) | Fruit length (mm) | Fruit shape index | Peel redness (\( \Delta a^* \)) | Soluble-solid content (%) | Maturity index |
|------------|----------------|-------------|-----------------|-------------------|------------------|-------------------|------------------------|-------------|
| Control    | 23.80 ± 2.66 a | 265.41 ± 36.47 a | 85.99 ± 3.89 a | 71.38 ± 4.48 a | 0.83 ± 0.05 a | 20.31 ± 1.76 a | 14.67 ± 1.91 b | 7.17 ± 0.41 a |
| 1/2G       | 8.69 ± 0.74 c  | 210.18 ± 46.92 b | 79.10 ± 5.13 b | 64.68 ± 4.62 b | 0.82 ± 0.05 a | 20.68 ± 3.49 a | 14.30 ± 1.04 b | 6.67 ± 1.03 a |
| 1/2G+M9    | 19.21 ± 3.14 ab| 268.04 ± 41.77 a | 86.13 ± 5.48 a | 72.11 ± 5.73 a | 0.84 ± 0.07 a | 18.67 ± 3.22 a | 15.50 ± 1.33 b | 6.67 ± 0.52 a |
| G          | 4.76 ± 2.04 c  | 192.85 ± 28.53 b | 76.25 ± 4.26 b | 63.56 ± 3.95 b | 0.83 ± 0.03 a | 12.98 ± 4.20 b | 17.97 ± 2.27 a | 7.17 ± 0.98 a |
| G+M9       | 18.00 ± 3.71 b | 254.75 ± 42.95 a | 83.47 ± 5.27 a | 71.49 ± 3.93 a | 0.86 ± 0.04 a | 20.25 ± 4.27 a | 14.07 ± 1.24 b | 7.00 ± 0.63 a |

\( P \) values

| Girdling    | <0.001* | <0.001 | <0.001 | <0.001 | 0.428 | <0.001 | 0.064 | 0.34 |
|------------|---------|--------|--------|--------|------|--------|-------|-----|
| Bridge grafting | <0.001 | <0.001 | <0.001 | <0.001 | 0.101 | 0.015 | 0.024 | 0.79 |
| Girdling × bridge grafting | 0.397 | 0.853 | 0.944 | 0.8 | 0.873 | <0.001 | 0.002 | 0.79 |

*The larger the value represents, the deeper the red color of the apple peel.

*Data within a row represent mean ± se, \( n = 30 \), different letters following the data are significantly different at \( P < 0.05 \).

*Effects of girdling and bridge grafting with \( P < 0.05 \) are highlighted in bold.

1/2G = semigirdling apple trees; 1/2G+M9 = bridge-grafted M9 on semigirdling apple trees; G = ring girdling apple trees; G+M9 = bridge-grafted M9 on ring girdling apple trees.
Trunk wounding was found to weaken tree vigor, lead to poor and stunted floral buds, and affect flowering and fruit-set in 2017 (Hartmann and Kester, 1975; Je˛drzejuk et al., 2016; Li et al., 2012, 2017; Sun et al., 2004). The results of correlation coefficient analysis between flowering and proportion of spurs and branching ability confirmed that they have a significantly positive correlation (Table 2). Hence, trunk girdling led to the reduction of flowering and fruit-set in 1/2G and G ‘Nagafu 2’ trees and both were increased by bridge grafting M9 to renew partial tree vigor (Table 1; Supplemental Fig. 2).

Furthermore, high CTK content and low GA content were found to be beneficial for flower bud formation (Sanyal and Bangerth, 1998; Zhang et al., 2015). McLaughlin and Greene (1991) indicated that spraying exogenous CTK facilitates the formation of flower bud in apple trees. In general, CTK is synthesized by plant roots; girdling inhibits the growth of fruit tree roots and, hence, decreases the synthesis of CTK (Cutting and Lyne, 1993; Dann et al., 1984). The period from May to September is the peak growth period of apple trees, whereas girdling hinders the downward transport of photosynthates, thereby limiting root growth and CTK synthesis (An et al., 2017; Havelange et al., 2000). In the present study, the ZR content in the G+M9 treatment was higher than that in the G treatment (Fig. 5A). During flower bud formation, bridge grafting of M9 has been found to promote tree body ZR content and nutrients to stimulate flower bud differentiation. However, high levels of GA1, GA3, GA4, and iso-GA7 inhibit flower bud formation, although they play an important role in the physiological differentiation of flower buds (Ramirez et al., 2004). In addition, high GA content in G treatment inhibited flower bud formation from June to July (Figs. 2A and 5C; Supplemental Fig. 2).

The content of metabolic substrates (such as soluble proteins, amino acids, and carbohydrates) showed a close relationship with floral formation in the tree body (Sun et al., 2004). In the present study, soluble protein content (P = 0.012, Table 5), but not carbohydrate content, was the most important metabolite to affect flower differentiation (P > 0.05) in trunk-wounded apple tree body. Thus, bridge grafting of M9 recovered the vitality of the tree body to increase the accumulation of soluble protein in the shoot of trunk-wounded apple trees for aiding floral induction (Tables 1 and 5; Fig. 6C; Liu et al., 2011).

Bridge grafting of M9 restored yield and fruit quality in trunk-wounded apple trees. Girdling has been widely confirmed to affect fruit characteristics and yield, but different results have been obtained in various fruit trees. For example, Peng and Rabe (1996) showed that girdling on Citrus unshiu Marc significantly decreased yield and tree vigor tended to produce smaller fruit, but Rivas et al. (2006) suggested that girdling could improve fruit-set. Some researchers believed that ring girdling at an appropriate time and such methods could enhance fruit size and color, but they obtained the opposite consequence with excessive and early girdling (Agusti et al., 1998; Morandi et al., 2011). However, little is known about the effect of bridge grafting on fruit quality in trunk-wounded ‘Red Fuji’ trees. Our study indicated that the wider the girdling trunk bark area, the lower the yield. This attributed to not only lower flowering and fruit-setting but also high fruit dropping before fruit

![Fig. 6. Effects of semigirdling, ring girdling, and bridge grafting of M9 on dynamic changes in soluble sugars (A), starch (B), and soluble protein (C) contents in shoot terminals of ‘Nagafu 2’ trees from June to September in 2016. Control = no girdling or no bridge grafting apple trees; 1/2G = semigirdling apple trees; 1/2G+M9 = bridge-grafted M9 on semigirdling apple trees; G = ring girdling apple trees; G+M9 = bridge-grafted M9 on ring girdling apple trees. Dots represent mean ± SE, n = 9. Different letters above the bars indicate a significant difference at P < 0.05 at different times.](http://example.com/fig6.png)
maturation (Fig. 2A and B; Table 6) because weakened tree vigor in girdled apple tree reduced the leaf photosynthesis and leaf area, leading to the decline of the capacity of fruit load and growth (Wargo et al., 2004). However, bridge-grafted M9 on trunk-wounded apple trees promoted nutrient absorption and increased vigor and viability, as well as increased of the leaf photosynthesis rate and leaf area (Figs. 3B and 4), to enhance fruit load and size (Viao et al., 2009; Wilton, 2000). Large apple fruits are softer than small (Blanpied et al., 1978; Samad et al., 1999a). Flesh firmness had a negative correlation with yield and fruit length (Table 4), which indicates that not only fruit size but also fruit load affects flesh firmness (Stopar et al., 2002). However, fruit firmness in 1/2G+M9 and G+M9 apple trees was higher than in control in similar fruit size and load, which displayed that bridge grafting of M9 affects the fruit firmness, but the basic regulatory mechanism is still unknown. Moreover, in the present study, assimilates accumulated on ring girdling that increased fruit soluble-solid content, which corresponded with the finding of Arakawa et al. (1997, 1998). The declined fruit-setting in G apple trees may lead to the increasing of fruit soluble-solid content, compared with G+M9 apple trees (Fig. 2B; Table 4). Bridge grafting of M9 on trunk-wounded apple trees led to partial recovery of remarkably decreased fruit color to control levels (Table 6). Meanwhile, the fruit maturity index in the five treatments had no significant difference (Table 6). We speculated that fruit color might be related to certain nutrients and substances from the roots (DeEll et al., 2001; Ji et al., 2015; Liu et al., 2017).

**Conclusion**

Bridge grafting of M9-T337 self-rooted rootstock on trunk-wounded apple trees effectively recovered partial tree vigor and, importantly, restored yield and fruit characteristics to control levels. The vegetative growth under bridge-grafted M9 treatment was not significantly different from that after ring girdling of ‘Nagafu 2’ trees; this could be attributed to the extraordinary function of M9 dwarfing rootstock. The bridge grafting of M9 treatments was successful in maintaining the reduction of vegetative growth but increased reproductive growth in the trunk-wounded ‘Nagafu 2’ trees and could thus represent an effective strategy to combat Valsa-induced trunk wounding in apple trees.

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Supplemental Fig. 1. Photographs of different treatments in ‘Nagafu 2’ trees in 2017. Control = no girdling or no bridge grafting apple trees; 1/2G = semigirdling apple trees; 1/2G+M9 = bridge-grafted M9 on semigirdling apple trees; G = ring girdling apple trees; G+M9 = bridge-grafted M9 on ring girdling apple trees.

Supplemental Fig. 2. Effects of semigirdling, ring girdling, and bridge grafting of M9 on flowering rate in ‘Nagafu 2’ trees. Control = no girdling or no bridge grafting apple trees; 1/2G = semigirdling apple trees; 1/2G+M9 = bridge-grafted M9 on semigirdling apple trees; G = ring girdling apple trees; G+M9 = bridge-grafted M9 on ring girdling apple trees. Bars represent mean ± SE, n = 3. Different letters above the bars indicate a significant difference at P < 0.05.