Vitis **49** (3), 107–111 (2010)

Effects of tape covering and vine vigor on development of surface callus in girdle of grapevine

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Summary

The effects of vine vigor (shoot growth) and covering the girdle surface with plastic tape on the development of a surface callus (SC) in the girdle of grapevine were studied by histological observation. The SC was formed in a tape-covering treatment but was not formed unless the girdle surface was covered with plastic tape (exposing treatment). Histological observation revealed that in the tape-covering treatment, callus cells developed mainly from the ray parenchyma cells on the girdle surface 2 days after girdling (DAG), leading to the formation of the SC, which grew and filled the girdle portion by 7 DAG. When 16 vines were divided into three categories based on scaffold branch length, vines with 7.5 m scaffold branches developed shorter shoots with smaller internode diameters than did vines with 4.5 and 6.0 m scaffold branches. In vines with 7.5 m scaffold branches, the SC covered a smaller area of the girdle surface than in vines with 4.5 and 6.0 m scaffold branches. The length and diameter of the shoot were significantly correlated ($r^2 = 0.75^{**}$ and 0.70^{**}, respectively) with the ratio of the girdle area covered by the SC to the whole girdle area (SC covering ratio). These results show that the SC originates mainly from the ray parenchyma cells and that SC development is strongly affected by vine vigor. Consequently, to ensure SC development, girdling should be done by tape covering in vines with shoot diameters larger than 8 mm.

K e y w o r d s : histological observation, vine vigor, wounding.

Introduction

In grape, girdling before and after fruit set improves the berry set (Brown *et al*. 1988) and increases berry size (Carreño *et al*. 1998), respectively, whereas girdling before the beginning of the ripening phase enhances skin color and berry ripening (Peacock *et al*. 1977, Carreño *et al*. 1998, Yamane and Shibayama 2006 a, Yamane *et al*. 2007). The anthocyanin concentration in the skin of grape berry decreased at high temperatures (Kliewer and Lider 1970, Kataoka *et al*. 1984, Yamane *et al*. 2006, Yamane and Shibayama 2006 b, Mori *et al*. 2007). Because poor skin coloration of grapes in warm regions in Japan is one of the most serious problems in the production of red and purple table grapes, girdling is sometimes practiced in commercial vineyards to enhance skin coloration (Yamane *et al*. 2007).

Girdling sometimes has a debilitating effect on vine growth, however. Yamane and Shibayama (2006 a) indicated that girdling inhibited root elongation but that root growth was restored after the girdle healed. No differences in the subsequent year's vine growth were found if the healing was complete, but incomplete healing led to weakened vine vigor.

Bark removal in girdles increases berry size and sugar content and stimulates skin coloration (Jensen *et al*. 1976, Yamane *et al.* 2008), while complete removal of the bark sometimes has a detrimental effect on the healing of the girdle. Covering the girdle with plastic tape induced callus development on the surface of the exposed xylem, enhancing girdle healing (Yamane *et al*. 2008). Therefore, Yamane *et al*. (2008) recommended covering the girdle with plastic tape to ensure rapid healing.

As for the callus arising from the wound surface, Stobbe *et al*. (2002) summarized previous studies and distinguished the surface callus (SC) from the lateral callus (LC) in the wound area. The LC is formed from the lateral cambium in the wound area, whereas the SC develops directly from the exposed surface in the wound area. The formation of the SC, a botanical peculiarity, is a tree's response to superficial wounding (Stobbe *et al*. 2002). In China, the bark of *Eucommia ulmoides* Oliv. was harvested for medicine by large-scale girdling (1-2 m in length). After harvest, the wound surface was wrapped with a plastic sheet to prevent desiccation, thus stimulating the regeneration of new bark (Zhengli and Keming 1988). However, there have been few reports on SC development in grape. In the present study, a histological observation was conducted to clarify the initiation of the SC at girdled stems in grape by checking the distribution of the newly divided cells.

According to our observation, the extent of SC development after girdling differed among vines in some vineyards. Because the relationship between SC development and vine vigor has not been studied, we tried to clarify it.

Material and Methods

E x p e r i m e n t 1 . H i s t o l o g i c a l o b s e r v a t i o n o f S C d e v e l o p m e n t : Lateral shoots in a 16-year-old 'Aki Queen' (*Vitis labrusca* L. x *V. vinifera* L., tetraploid) grapevine grafted on SO4 root-

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stocks in Higashi-Hiroshima, Hiroshima, were used in this study. Girdling applied at the lateral shoots was used for histological observation to obtain a microsection, although it was also applied at the base of the scaffold branch, as in Experiment 2. The vine was trained to a flat-top trellis and was short-cane-pruned with four secondary scaffold branches with a total length of 16 m. Chemical fertilizers containing 288, 144, and 288 g of N, P, and K, respectively, were applied before bud break. To avoid water stress, 96 L (20 $mm \cdot m^{-2}$) of water was automatically supplied to a vine when the soil water potential dropped to -9.8 kPa (IMAI *et al*. 1991).

Girdling was applied at internodes of lateral shoots 5.5 mm in width by a knife on 11 July 2007. Girdles were either covered with plastic tape immediately after girdling (tape-covering treatment) or exposed without tape covering (exposing treatment). Five samples of girdles from each treatment were collected 1, 2, 3, 5, and 7 DAG.

The samples were fixed in FAA and embedded in paraffin after dehydration with ethanol and butyl alcohol. The tissue embedded in paraffin was cut at 10 μm using a sliding microtome and stained with Schiff reagent for 100 min and fast green for 40 min after the substitution of paraffin with xylene. Histological observation was conducted by light microscope.

The mitotic index (the percentage of newly divided cells) was measured for three replications of 1000 cells in outermost xylem portions consisting fibers and ray, as well as in the newly developed callus cells, on each sampling date (Fig. 1). Callus cells were distinguished from fibers and ray based on cell wall thickness; callus cells had apparently thinner walls than fibers and ray. Indistinguishable cells were omitted.

Experiment 2. Relationship between vine vigor and callus development: Eighteen vines were divided into three groups based on the scaffold branch lengths (4.5, 6.0, and 7.5 m). Vine vigor was estimated by the average length and diameter of newly developed shoots from these scaffold branches. All vines were seven-year-old 'Aki Queen' grapevines grafted on 5BB rootstocks in a vinyl greenhouse. Vines were trained to a flat-top trellis, short-cane-pruned, and planted in boxes (0.5 m in width, 0.25 m in height). The length of each box was adjusted to 90 % of the length of each scaffold branch. Buds on scaffold branches were allowed to grow at intervals of 0.16 m about three weeks after bud break. Each primary shoot was pinched before blooming, leaving 12 nodes; thereafter, each lateral shoot was pinched once a week to leave one leaf. Girdling was applied at the base of scaffold branches 5.5 mm in width on 4 July 2007, 35 days after blooming. After complete removal of the bark tissue, the girdles were covered with transparent plastic tape that was 50 mm in width and 0.16 mm in thickness.

The SC covering ratio was scored in the area of the SC relative to the girdle area at 7 DAG. No LC had developed at 7 DAG. The amount of LC was measured with a caliper on the apex end of the girdle portion at 15 DAG because the LC developed mainly from the apex side of the girdle.

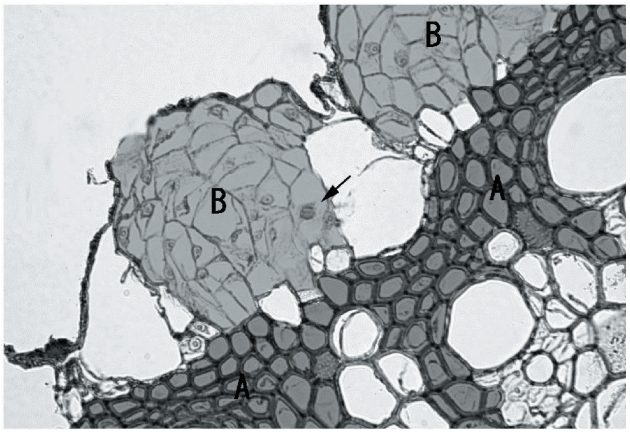

Fig. 1: Areas where newly divided cells were counted. Taping treatment, 3 DAG. A, outermost xylem portions consisting fibers and ray cells; B, callus cells; arrow, newly divided cell.

Results and Discussion

In Experiment 1, the SC developed in all tape-covering treatments but not in the exposing treatment (Fig. 2). In Fig. 3 a, mature fibers, vessels, and parenchyma cells in the ray were observed on the surface of girdle. Because cambium and immature xylem were not observed, they were considered to be removed with outer bark and phloem. Callus like cells on the wound surface (SC) arose mainly from parencymatous cells in the ray at 2 DAG (Fig. 3 b). Then the SC grew and filled the girdle until 7 DAG (Fig. 3 c, d and e). No callus developed in the exposing treatment (Fig. 3 f). These results are consistent with those previously reported for *Hibiscus rosa-sinensis* L., in which the SC developed from the end cells of the ray and meristematic cells destined to form xylem 2 or 3 d after bark stripping (SHARPLES and GUNNERY 1933).

Newly divided cells were observed in the ray parenchyma cells at 1 DAG in the tape-covering treatment (Fig. 4 a), suggesting that proliferation of ray parenchyma cells mainly contributes to the formation of SC. However, a possibility cannot be ruled out that axial parenchyma cells and some immature xylem cells around cambium may contribute to the callus formation because we did not check the radial and tangential sections. The division plane of the

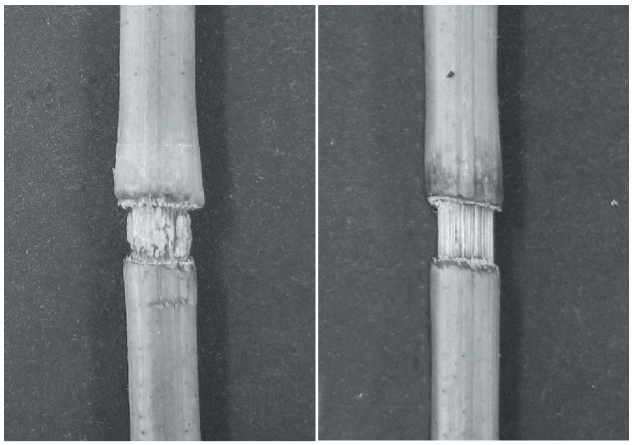

Fig. 2: Girdles at 7 DAG. Left, tape-covering treatment; right, exposing treatment.

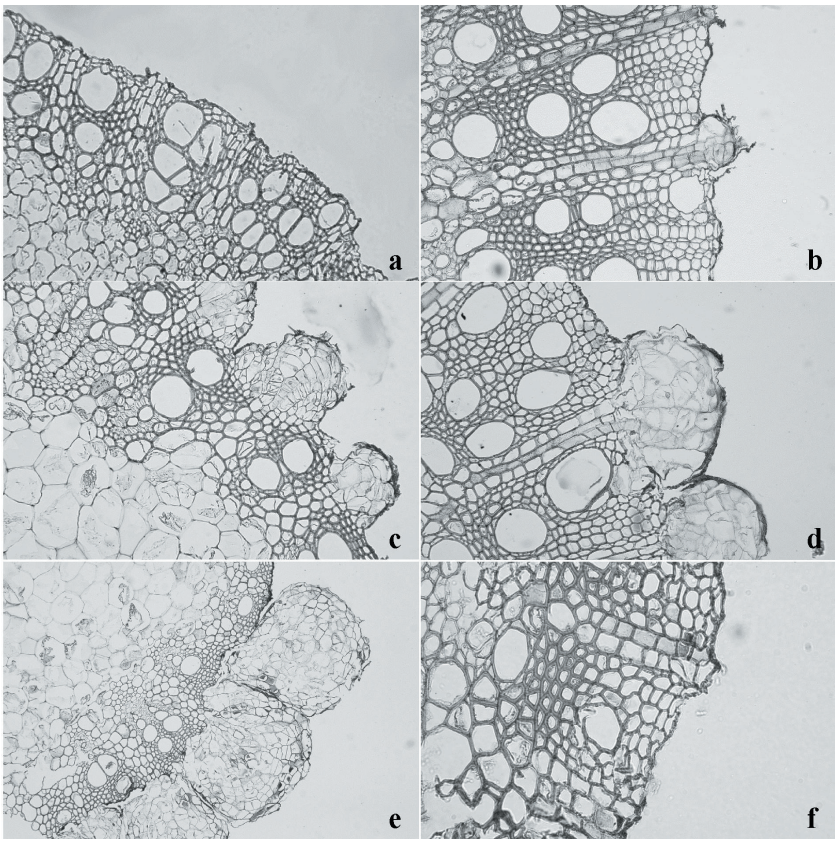

Fig. 3: Traverse section of girdle. **a**-**e**: tape-covering treatment; **f**: exposing treatment. **a**, **b**, **c**, **d**, **e**, and **f** are 1, 2, 3, 5, 7, and 7 DAG, respectively.

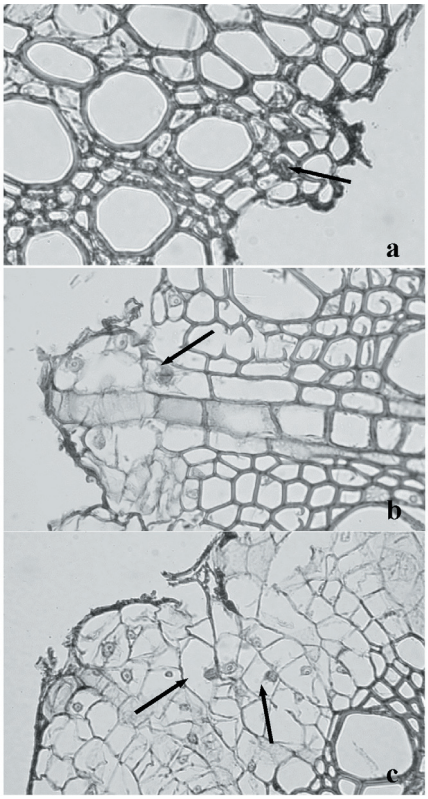

Fig. 4: Early stage callus cells in tape-covering treatment. **a**, **b**, and **c** are 1, 2, and 3 DAG, respectively. **a**: arrow indicates a newly divided cell on the wound surface; **b**: arrow indicates a ray parenchyma cell which will develop into callus cells; **c**: arrows indicate thin walled cells which are distorted in shape.

newly divided cells was anticlinal against the exposed surface of the girdle at 1 DAG. At 2 DAG, newly divided cells were observed in the ray near the surface of the girdle, and their division plane was periclinal against the exposed surface of the girdle (Fig. 4 b). At 3 DAG, newly divided cells were observed in the callus, and the division plane was a mixture of anticlinal and periclinal (Fig. 4 c). The direction of the division plane of the newly divided cells changed from 1 to 2 DAG.

The mitotic index was around 0.1 % in both the fibers and the ray throughout the sampling days, and there were no significant differences between sampling days (data not shown). In callus cells, the mitotic index was 0.7 % at 2 DAG, increased to 1.0 % at 3 DAG, and gradually decreased to 0.5 % at 7 DAG (Fig. 5). The decrease in the mitotic index at 7 DAG may indicate that SC development was almost complete. The SC covered most of the girdled surface at 7 DAG.

McDougall and Blanchette (1996) reported that wrapping fresh wounds with plastic sheeting enhanced LC development by preventing desiccation of the exposed cambium and the subsequent death of cambial cells. It is possible that covering the girdle with plastic tape enhanced SC development as well as LC development through the protection against desiccation in the exposed cambium. In the present study, however, SC development began at 2 DAG, whereas LC development had not yet begun at 7 DAG. Thus, the timing of the callus development is different between SC and LC although callus development was common healing response to wounding.

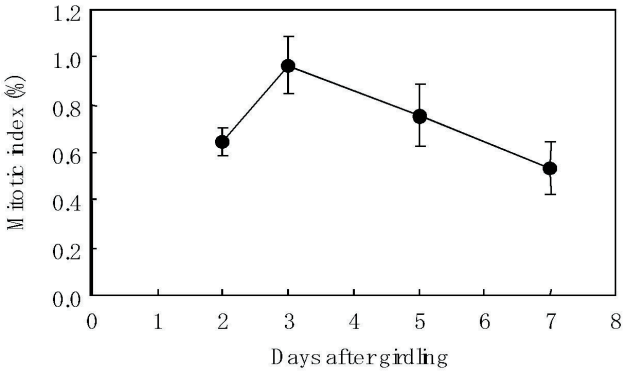

Fig. 5: Changes in the mitotic index of the SC in the tape-covering treatment. 1000 cells each were measured to estimate the mitotic index for three replications. Bars represent standard error (n = 3).

KURODA (1986) reported that the number of ray parenchyma cells increased when the xylem was wounded by a needle. This response indicates that the ray parenchyma cells are ready to repair the wounded xylem. In a comparison of girdling and wounding, LEV-YADUN and ALONI (1992) concluded that the effects of girdling are essentially the same as the wound effects, based on the histological observation of the ray. These reports support the idea that SC development in the girdle is a reaction to wounding.

Although girdling was applied to different areas in Experiment 1 and 2, no apparent differences in the developmental pattern of the SC were observed at 7 DAG.

In Experiment 2, vines with a scaffold branch length of 7.5 m produced shorter and thinner shoots than did vines with scaffold branch lengths of 4.5 and 6.0 m. Vines with 4.5 and 6.0 m scaffold branches produced shoots of almost the same sizes. Shoot length and diameter were significantly correlated (r^2 = 0.75** and 0.70**, respectively) with the SC covering ratio (Fig. 6). However, LC development did not differ with the scaffold branch length (Fig. 7).

Contrary to the results of our study, the results reported by NEELY (1970) showed that LC development was correlated with the annual radial growth rate of the trunk. This disagreement may be ascribed to the difference in the healing period of the wound. NEELY (1970) measured trunk growth and callus development for 3 years, while we measured short-term LC development. In Experiment 1, the SC started developing at 2 DAG, but the LC started developing at 7 DAG. Thus, it is possible that SC development is strongly affected by vine vigor even if the girdles are covered. For successful girdling, vine vigor should be checked before girdling is applied. The results of the present study show that vine vigor can be estimated by two indices: shoot length and diameter. In vineyards, shoots are usually pinched once before blooming and sometimes after blooming. Because the optimal time for girdling to increase coloration and sugar content in berries is between 30 and 35 d after blooming (YAMANE and SHIBAYAMA 2007), shoots are sometimes pinched before girdling. Therefore, shoot length may not be an optimal indicator, but shoot diameter should be a stable, practical indicator of vine vigor.

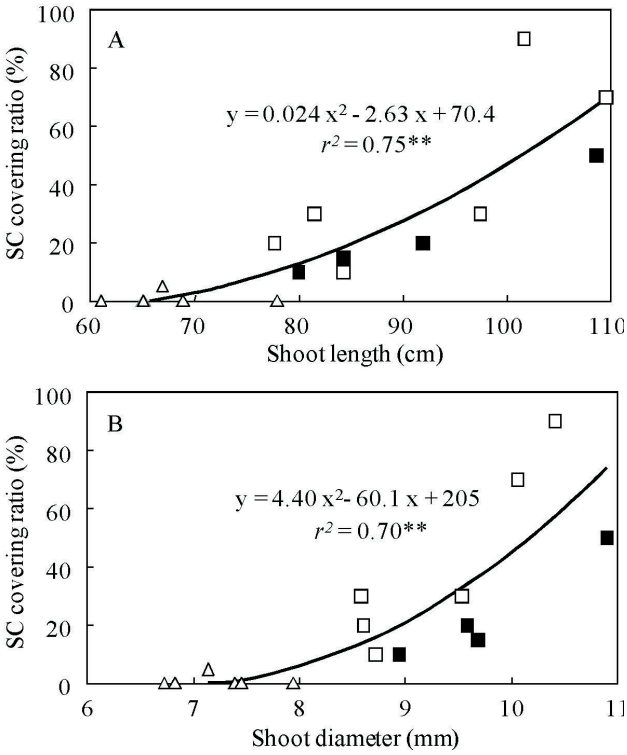

Fig. 6: Relationship between surface callus (SC) covering ratio (%) in girdles and shoot length (**A**) and shoot diameter (**B**) in vines with different scaffold branch lengths. Closed square (■), open square (□), and triangle (Δ) indicate scaffold branch lengths of 4.5, 6.0, and 7.5 m, respectively.

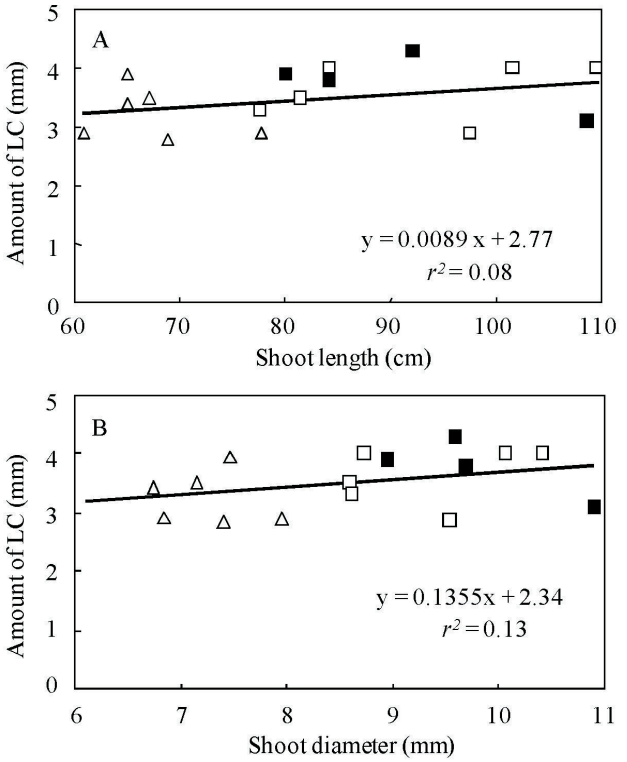

Fig. 7: Relationship between amount of lateral callus (LC) in girdles and shoot length (**A**) and shoot diameter (**B**) in vines with different scaffold branch lengths. Closed square (■), open square (□), and triangle (Δ) indicate scaffold branch lengths of 4.5, 6.0, and 7.5 m, respectively.

In conclusion, SC development is important for girdle healing, and tape covering is essential for SC development from parenchymatous cells at the ray near the girdle surface. Furthermore, girdling should not be done on weak vines that have shoots with a diameter of less than 8 mm. Most table grape cultivars grown in Japan are tetraploids, the shoot growth of which is greater than that of diploid cultivars. Therefore, further study is necessary for practical application in diploid cultivars.

Acknowledgements

A part of this work was supported by the NARO Research Project No. 166, "Establishment of Agricultural Production Technologies Responding to Global Warming."

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Received January 29, 2010