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THE EFFECTS OF PHLOEM GIRDLING ON LEAF CELL PHYSIOLOGY AND CHLOROPHYLL BIOSYNTHESIS IN PEACH TREE

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Abstract: Phloem girdling is used for many fruit trees to promote fruit set and quality. Although many studies showed the pomological and biochemical effects of girdling in fruit trees, there is very little information on how girdling affects cell physiology. The current study aimed to characterize the leaf xylem structure, cortex cell division, and expansion affected by trunk girdling during phloem healing in peach. The experiment was carried out on a two-year-old peach cv. Rich May grafted onto Garnem grown in 10 L pots in greenhouse. The girdling was performed on the trunk end of the May. The leaf samples were collected 1, 2, 3 and 4 weeks after treatment (WAT). The study showed that the girdling decreased tree growth, stomatal conductance and stomatal density. Girdling decreased chlorophyll biosynthesis in peach leaves. Furthermore, girdling treatment increased leaf cell expansion, xylem thickness, and xylem conduit number during two weeks. The results have demonstrated that leaf anatomy changed by girdling during phloem healing.

Keywords: Cell division, Cell expansion, Girdling, Phloem, Xylem

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1. Introduction

Carbohydrates are mostly produced in leaves (called sources) and exported to the sink organs such as fruits, and roots (Chai et al., 2021). Fruit quality is closely related to source-sink relationship and photoassimilate distribution between source and sink affects fruit size, color, and soluble solid content (Lo Piccolo et al., 2021). Source-sink balance can be manipulated by girdling treatments. Girdling is defined as surgically removal of a ring of phloem leading accumulation of photoassimilates above the girdle by blocking the phloem transport pathway (Michailidis et al., 2020). Following the girdling, excess sugar accumulates in the leaves leading inhibition of photosynthesis and severe root starvation occurs (Tyagi et al., 2020). This technique is commonly utilized in fruit trees including sweet cherry, peach and mandarin to increasefruit quality (Day and DeJong 1990; Mataa et al., 1998; Michailidis et al., 2020).

Girdling treatment was studied in many fruit trees for many purposes. Lo Piccolo et al. (2021) reported that girdling stimulated anthocyanin accumulation and promoted sugar in red plums. Girdling treatment was performed on 'Aztec Fuji' apple trees and the fruit size, color, and firmness increased (Fallahi et al., 2018). One week after girdling, abscisic acid and gibberellin content was found further in vines (Tyagi et al., 2020). Girdling affected carbohydrates, gas exchanges, and antioxidant activities in olive (Annabi et al., 2019). Furthermore, girdling influenced anatomy of many species (Inoue et al., 1991). Hamada et al. (2009) reported that at ten days after girdling in persimmon, callus formation started in the phloem tissue. The selection of appropriate girdling time is important for fruits. Some researchers stated that girdling at cell expansion period may increase fruit size (Day and DeJong 1990; Chai et al., 2021).

Most studies on girdling have been focused on enhancing fruit quality, anthocyanin accumulation, and assimilate distribution. Although pomological and biochemical effects of girdling in fruit trees have been widely studied, there is very little information on how girdling affects leaf xylem structure, cortex cell division, and expansion. The current experiment aimed to characterize the leaf cell physiology affected by trunk girdling during phloem healing. Furthermore, chlorophyll biosynthesis was evaluated in the present study.

2. Materials and Methods

The experiment was carried out on a two-year-old peach (*Prunus persica* (L.) Batsch) cv. Rich May grafted onto Garnem (*P. dulcis* × *P. persica*) in 2021. The plants were grown in 10 L pots containing substrate and perlite (4:1) in greenhouse. The girdling was performed using a knife at 0.5 m from the ground on the trunk and approximately 10 mm of the bark and phloem were removed from the trunk end of the May (Figure 1.a). The leaf samples were collected 1, 2, 3, and 4 weeks after treatment (WAT). The

experiment was arranged in a randomized plot design with three replicates of four plants per replication.

Rootstock and scion diameters were recorded at 0 and 4 WAT and relative growth rates of rootstock and scion diameters were calculated according to Del Amor and Marcelis (2003) and Aras (2020). Stomatal conductance was measured with a leaf porometer (Li-COR).

The concentrations of chlorophyll a, b and a + b were determined according to Porra et al. (1989). The concentrations of chlorophyll precursors including protoporphyrin IX (Proto IX), Mg-protoporphyrin IX (Mg-Proto IX), and protochlorophyllide (Pchlide) were determined according to the method of Hodgins and Van Huystee (1986) and calculated by the corresponding formulas (Liu et al., 2015). Chlorophyll yield was estimated by chlorophyll a + b/Proto IX (Aras et al., 2021).

For the histological evaluations, the leaves were stored in ethanol 70% and cross sections of the leaf midribs were stained with Toluidine Blue O (for cortical cells) or acid phloroglucin (for xylem) dyes. The samples placed on a slide after staining and visualized with a light microscope (Olympus CX21) coupled to a digital camera (Kameram 5). The cortex, epidermis, and xylem were measured (Aras et al., 2021; 2022). The number of the cortex cell layer was calculated from cortex thickness divided by cortical cell diameter. Cell division was interpreted in terms of the number of the cortex cell layer and cell expansion was explained on behalf of the cortical cell diameter. The stomatal characteristics were measured on the abaxial surface of the leaves. Stomatal length, width, and stomata density were measured. Stomatal area was calculated with the equation of Zhu et al. (2019).

For starch staining, the leaves were boiled in 95% (v/v) ethanol. The depigmented leaves were immersed in Lugol's iodine solution for 15 min. Then leaves were photographed (Wood et al., 1986).

The statistical analyses were performed with the statistical software package SPSS, version 20.0. Data were subjected to two-way ANOVA and were separated by the Duncan's test at a significance level of $P<0.05$.

Figure 1. Girdling treatment (a) and gummosis observation at 2 WAT (b).

3. Results

Girdling treatment affected peach leaves. Gummosis observation was observed in girdling ring at 2 WAT (Figure 1.b). Morphological responses were significantly changed (Figure 2). Relative growth rate of rootstock and scion diameters reduced by 39 and 51%, respectively by girdling treatment. Stomatal movement was influenced by girdling (Figure 3). Stomatal conductance, stomatal density and area significantly increased by girdling at 1 WAT, while stomatal conductance and stomatal density decreased at 2, 3 and 4 WAT.

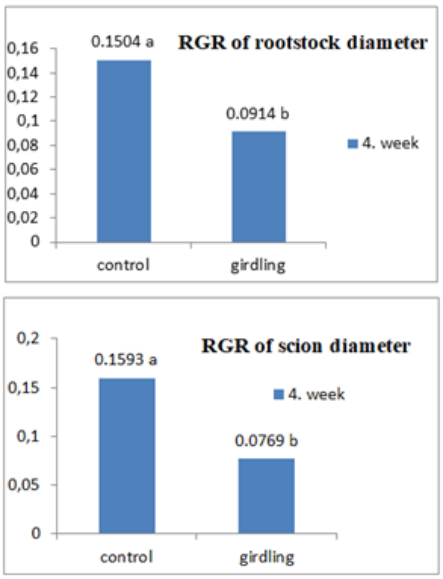

Figure 2. Effect of girdling on relative growth rates of rootstock diameter and scion diameter at 4 WAT.

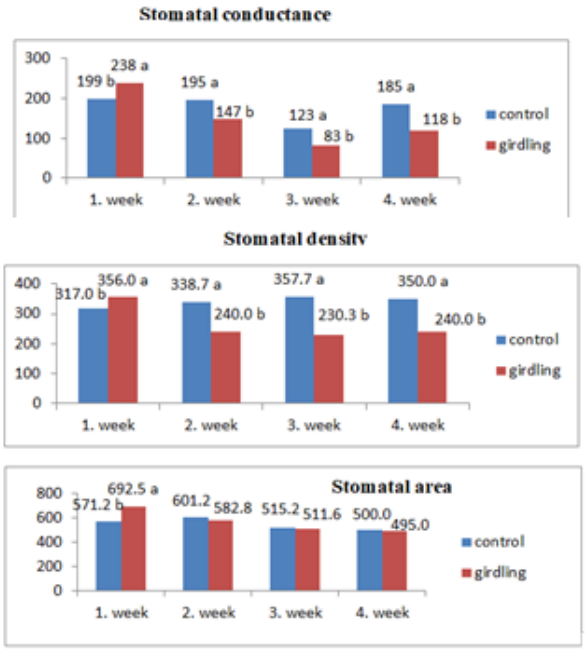

Figure 3. Effect of girdling on stomatal conductance, stomatal density and area.

Girdling affected chlorophyll biosynthesis in peach leaves (Figures 4 and 5). Chlorophyll a, b and a+b did not statistically affect at 1, 2, and 3 WAT, whereas the parameters decreased at 4 WAT. Decline in the concentrations of chlorophyll precursors including Proto

IX, Mg-Proto IX, and Pchlide was found in girdling treatment. Chlorophyll yield significantly decreased by girdling at 4 WAT. Histological responses of the peach leaves affected by girdling treatment were also affected. Cortex thickness of the leaf midrib increased by girdling at 1 and 2 WAT and the increment was found as a result of increase in cortical cell diameter (Figure 6). The results showed that girdling treatment increased cell expansion during 2 weeks. Girdling treatment increased xylem thickness and xylem conduit number during first two weeks (Figure 7). Xylem conduits length was not affected by girdling.

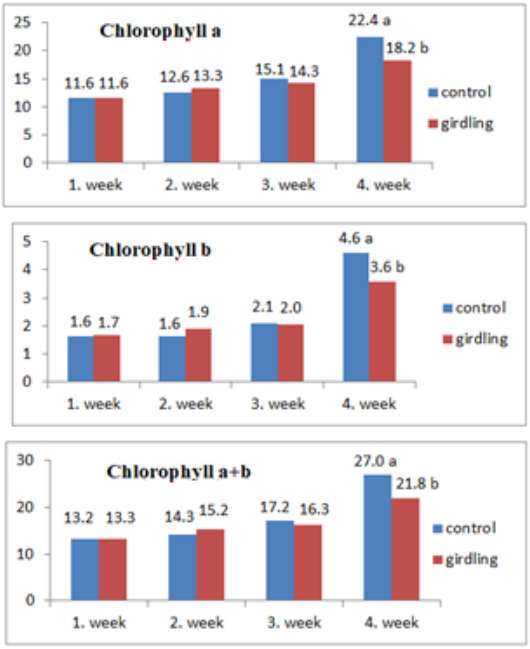

Figure 4. Effect of girdling on chl a, b, a+b concentrations.

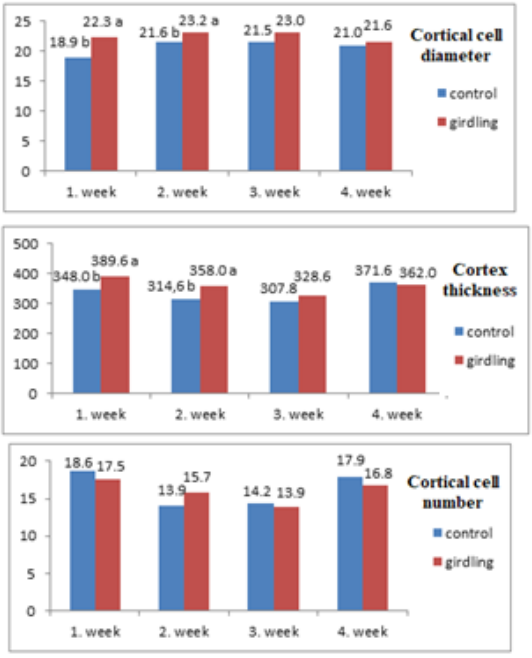

Figure 5. Effect of girdling on Proto IX, Mg- Proto IX and Pchlide concentrations and chlorophyll yield.

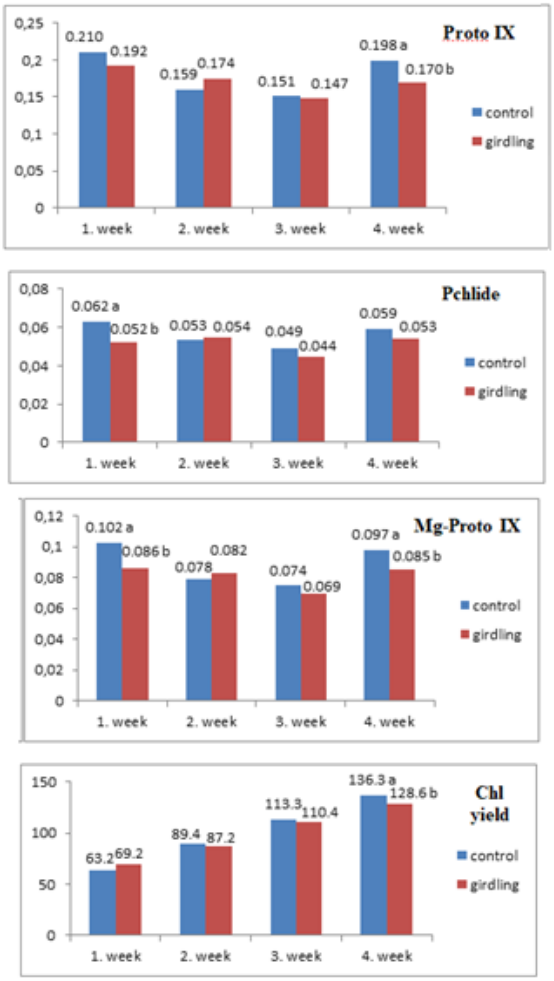

Figure 6. Effect of girdling on cortical cell diameter, cortex thickness and cortical cell number.

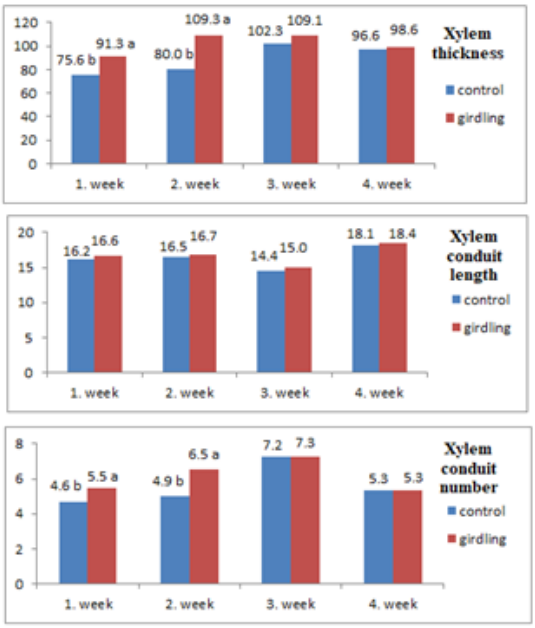

Figure 7. Effect of girdling on xylem thickness, xylem conduits length and xylem conduit number.

The intensity of starch accumulation in leaves was observed by in situ staining with Lugol dye (Figure 8). Girdling at 1 and 2 WAT leaded starch accumulation in the leaves. Starch accumulation was more pronounced in 2 WAT. In 3 and 4 WAT, starch accumulation was not observed.

Figure 8. Effect of girdling on starch accumulation of peach leaves

4. Discussion

The regulation of photosynthesis is linked to the source-sink balance (Matsuda et al., 2011). Girdling leads to photoassimilate accumulation in the leaves by blocking photosynthetic exported products (Lo Piccolo et al., 2021). In the current experiment, the healing response of girdling was observed in leaf midrib cortex and xylem shown in Figure 9.

Girdling treatment decreased tree growth, determined by a logarithmic calculation. Relative growth rates (RGRs) of rootstock and scion diameter decreased by girdling. The parameters were measured under the girdling ring and the decreases in the parameters may show that photoassimilate distribution into roots was hampered by girdling. The decline in plant growth may be linked to loss in photosynthesis and the decrease in photosynthesis is a common phenomenon induced by girdling (Lo Piccolo et al., 2021). Chlorophyll is an important pigment taking pivotal role in photosynthesis. In the current experiment, chlorophyll decreased at 4 WAT by girdling. Reduction in chlorophyll was reported in many studies and the decline may be due to decline in chlorophyll biosynthesis (Guo et al., 2020; Aras et al., 2021; 2022). Chlorophyll is a tetrapyrrole containing Mg and chlorophyll biosynthesis requires the participation of some precursors including Proto IX, Mg-Proto IX, and Pchl (Tanaka, Tanaka 2007). Girdling decreased chlorophyll biosynthesis at 4 WAT by decreasing the concentrations of Proto IX, Mg-Proto IX and Pchl. Chlorophyll yield also decreased at 4 WAT that shows the level of Proto IX driven by chlorophyll (Aras et al., 2021).

Photosynthesis is linked to stomatal conductance (Ainsworth and Rogers, 2007) and girdling may have hampered photosynthesis by decreasing stomatal gas exchange. At 1 WAT, stomatal conductance, density, and area increased by girdling. 2 weeks after girdling, stomatal conductance decreased and the decline was found as a result of decrease in stomatal density. Stomatal area was not affected except at 1 WAT. Girdling led to severe reduction in stomatal formation starting with 2 weeks after treatment. Reduction in stomatal density was reported in barley (Hughes et al., 2017) that improved drought tolerance. Furthermore, decrease in stomatal density promoted water use efficiency in wheat by decreasing water loss (Dunn et al., 2019). Thus, reduction in stomatal density may be a response to girdling in order to uptake further water.

Phloem is a living tissue protects xylem against embolism formation (Hacke and Sperry, 2001) and phloem girdling may induce a direct wounding effect on the xylem (Zwieniecki et al., 2004). Girdling caused gummosis formation leaking from the girdled ring in peach. Gummosis formation is induced by endogenous ethylene hormone (Saniewski et al., 2006) that may show wounding stress effect. Gummosis was observed at 2 WAT (Figure 1.b). Van de Wal et al. (2017) reported that girdling did not hamper xylem functionality in tomatoes. Xylem functionality is very important for water and mineral uptake depending on many factors including tree age, rootstock, and nutritions (Meinzer et al., 2001; Aras, 2021). In the present study, xylem thickness increased at 1 and 2 WAT and increment in number of xylem conduits were attributed to increase in the xylem thickness. The highest increase in the number of xylem conduits was observed at 2 WAT. Xylem consists of lignin (Whetten et al., 1998) and lignin is a phenolic compound (Kleinert, Barth 2008). Tyagi et al. (2020) found that girdling increased phenolics by induction of the phenylpropanoid pathway one week after girdling in grape. The highest increment in xylem thickness was found at 2 WAT and statistical difference was not found in xylem thickness at 3 and 4 WAT between control and girdled peach trees. We consider that phenolic compound formation was induced in 2 weeks after girdling. Girdling can be treated to trees in order to increase xylem functionality.

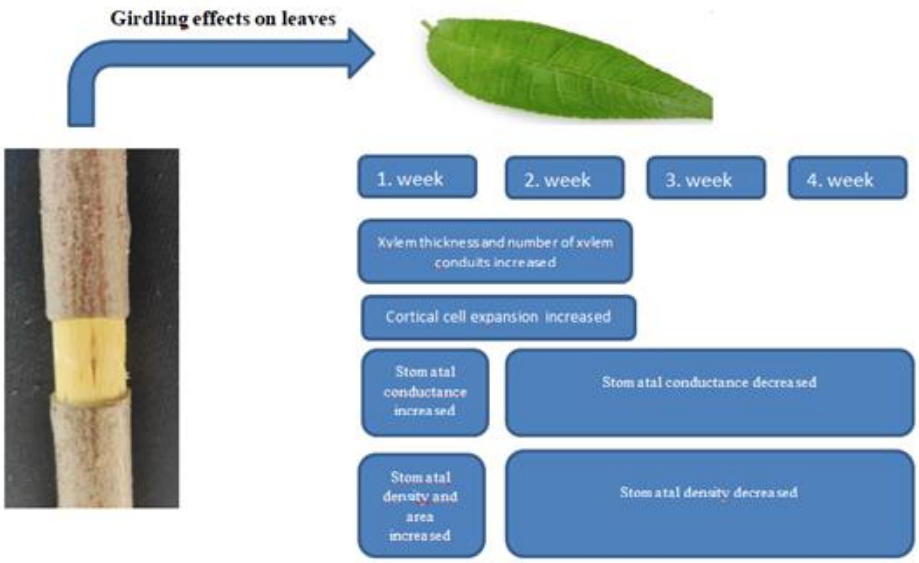

Figure 9. Effects of girdling on peach leaves.

Cortex of leaf midrib was also examined in the current experiment. Cortex plays an important role in mineral acquisition (Malta et al., 2016) and increase in cortex promotes growth of the tissue. Cortex thickness increased at 1 and 2 WAT and the increment was found a result of cell expansion rather than cell division. Leaf expansion consists of two overlapping phases: cell division and expansion (Gonzalez et al., 2012; Boron and Vissenberg 2014). Girdling leaded increments in cortical cell diameter and cortex thickness and cortex cell number did not change. Thus, girdling increased leaf cell expansion and did not alter cell division. Many studies demonstrated that girdling increased fruit growth by driven photoassimilates into fruits (Mataa et al., 1998; Michailidis et al., 2020). In the present study, we did not examine fruit cell physiology, however, we consider that fruit growth improvement is not only a result of the assimilate accumulation but also an increase in cell expansion triggered by girdling. Therefore, we suggest that girdling should be treated to trees during cell expansion period. Day and DeJong (1990) studied effect of different girdling timings in nectarine tree and reported that girdling at the beginning of Stage II was the most effective. Expansion of stone fruits possesses three Stages; cell division (Stage I), cell division+cell expansion (Stage II), and cell expansion (Stage III) (Costa and Vizzotto, 2000). Mataa et al. (1998) found that girdling at early fruit set period may be the best for tree productivity. Thus, the studies prove the results of the current experiment. The present study showed that the healing of the girdling wound was established in 2 WAT. Gummosis formation observed in 2 WAT may be a result of wound healing. Wounded peach shoots induced gummosis formation five days after wounding in a previous study (Li et al., 2015).

In the present study, starch accumulation in the leaves was also evaluated. We suggest that the excess accumulation of starch in leaves may be due to ongoing sucrose production without sucrose export from the leaves due to phloem dysfunctionality. Girdling at 2 WAT induced the starch accumulation in leaves by blocking phloem. Reducing the accumulation of excessive starch is necessary for plants to recover leaf photosynthesis (Lo Piccolo et al., 2020). Starch accumulation was not observed in the leaves of girdling 3 and 4 WAT that may show that phloem healed two weeks after girdling and sugar export from the leaves was succeed. Increase in starch accumulation by girdling was reported in many studies (Onguso et al., 2004; Denaxa et al., 2021).

5. Conclusion

The study showed that the effects of girdling are time dependent. As a result, girdling decreased tree growth, stomatal conductance, and stomatal density. Girdling declined the chlorophyll biosynthesis at 4 WAT by decreasing the concentrations of Proto IX, Mg-Proto IX, and Pchl. Girdling did not cause physical damage to the xylem. At 1 and 2 WAT, girdling increased xylem thickness by increasing the number of xylem conduits. Furthermore, leaf cortical cell expansion increased in two weeks after girdling. Anatomical alterations showed that the healing of the girdling wound was established after two weeks following girdling. The understanding of the process of healing process would contribute to the growers.

Author Contributions

All tasks were done by the single author: S.A. (100%). The author reviewed and approved final version of the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this

study because of there was no study on animals or humans.

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