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Araştırma Makalesi

Alterations in Leaf Cellular Physiology and Chlorophyll Biosynthesis During Leaf Expansion in Peach Tree

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Abstract: The effects of leaf expansion on leaf cell physiology and chlorophyll biosynthesis were evaluated in peach tree. The expanding leaves (young leaves) and the expanded leaves (old leaves) of Rich May peach cultivar grafted onto Garnem were compared. The parameters related to leaf expansion were evaluated end of May. Stomatal conductance increased in expanded leaves, while leaf temperature decreased. The difference in stomatal conductance appeared to be associated with stomata size rather than stomata number. The concentrations of chlorophyll precursors including Proto IX, Mg-Proto IX and Pchlide increased during leaf expansion. Cortical cell division and expansion increased during leaf expansion. Furthermore, xylem size and number significantly increased during leaf expansion. Cell expansion had a major influence on leaf size in peach.

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1. Introduction

Leaves are the energy factories of plants. Leaf size and age play pivotal roles in photosynthesis and dry matter accumulation (Marcotrigiano et al., 2010). Young, expanding leaves and mature, expanded leaves differently respond to environmental factors. Expanding leaves possess lower photosynthetic activity and are warmer than expanded leaves (Hall et al., 2014) that may be a result of fewer functional stomata and transpiration.

Leaf development relies on the coordinated progression of cell division and expansion. Expansion of leaves consist of two phases. In the first phase, cell division occurs. In the second phase, cell division has ceased and cell expansion initiates (Gonzalez et al. 2012). Numerous factors have been found effective in cell division and expansion. Pantin et al. (2011) stated that leaf expansion is controlled by leaf hydraulics. Jasmonate suppressed leaf growth by repressing cell division (Noir et al., 2013). Calcium deficiency affected leaf cortex cell physiology in peach trees (Aras et al., 2021).

Changes in leaves during expansion have been studied in many plants. Few studies have investigated histological alterations during leaf expansion. Expansion of leaves is a prominent factor in photosynthesis and environmental adaptations. Here, the study reports the alterations in leaf cell physiology and stomatal properties during leaf expansion in peach tree. Furthermore, chlorophyll biosynthesis was evaluated between expanding and expanded leaves.

2. Material and methods

The study was performed on two-year-old peach cultivar Rich May (Prunus persica Batsch) grafted onto Garnem (P. dulcis x P. persica) in a greenhouse. The trees were grown in 10 L pots containing substrate. Nine similar trees and three branches per tree were used for the study. The expanding leaves (young leaves) at the shoot apex and the expanded leaves (old leaves) at the base were compared. The parameters related to leaf expansion were evaluated end of May.

Stomatal conductance and leaf temperature values were estimated with 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 (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) and acid phloroglucin (for xylem) dyes and visualized with a light microscope. The cortex, epidermis and xylem were measured. 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. Stomata area was calculated with the equation of Zhu et al. (2019).

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 $P < 0.05$.

3. Results

The physiological and histological properties of peach leaves were significantly changed during leaf expansion. Stomatal conductance increased in expanded leaves, while leaf temperature decreased (Table 1). The decline in leaf temperature may be a result of the increase in stomatal gas exchange. Similar with stomatal conductance, stomatal area remarkable increased in expanded leaves (Table 2). However, stomatal density decreased during leaf expansion. Stomata size (length and width) was higher in expanded leaves.

Table 1. Stomatal conductance and leaf temperature of expanding and expanded peach leaves

Peach leaves	Stomatal conductance (mmol m^{-2} s^{-1})	Leaf temperature (°C)
Expanding leaves	58.9 b	34.4 a
Expanded leaves	252.3 a	33.9 b

Means separation within column by Duncan's multiple range test. $P<0.05$, NS: Non Significant

Table 2. Stomatal properties of expanding and expanded peach leaves

Peach leaves	Stomatal length (µm)	Stomatal width (µm)	Stomatal density (no. mm^{-2})	Stomatal area (µm^{2})
Expanding leaves	26.5 b	22.8 b	395 a	475 b
Expanded leaves	33.6 a	26.4 a	355 b	699 a

Means separation within column by Duncan's multiple range test. $P<0.05$

Chlorophyll biosynthesis significantly changed during leaf expansion shown in Table 3 and 4. Chlorophyll a, b and a+b increased in expanded leaves (Table 3). The concentrations of chlorophyll enhanced during leaf expansion. However, chlorophyll yield did not significantly change.

The histological changes were significantly affected. Cortical cell division and expansion increased during leaf expansion (Table 5). Furthermore, xylem size and number significantly increased during leaf expansion (Table 6).

Table 3. Chlorophyll a, b, a+b concentrations of expanding and expanded peach leaves

Peach leaves	Chlorophyll a (µg g^{-1} fw)	Chlorophyll b (µg g^{-1} fw)	Chlorophyll a+b (µg g^{-1} fw)
Expanding leaves	1.00 b	12.64 b	13.64 b
Expanded leaves	1.20 a	13.80 a	15.00 a

Means separation within column by Duncan's multiple range test. $P<0.05$.

Table 4. Concentrations of chlorophyll precursors and chlorophyll yield of expanding and expanded peach leaves

Peach leaves	Proto IX (µg g^{-1} fw)	Mg-Proto IX (µg g^{-1} fw)	Pchlide (µg g^{-1} fw)	Chlorophyll yield
Expanding leaves	0.0584 b	0.0292 b	0.0168 b	78.3 ^{NS}
Expanded leaves	0.0663 a	0.0337 a	0.0197 a	75.4

Means separation within column by Duncan's multiple range test. $P<0.05$.

Table 5. Cortical cell diameter, cortex thickness and number of cortex cell layer of expanding and expanded peach leaf midribs

Peach leaves	Cortical cell diamater (µm)	Number of cortex cell layer
Expanding leaves	21.9 b	11.5 b
Expanded leaves	26.6 a	12.5 a

Means separation within column by Duncan's multiple range test. $P<0.05$.

Table 6. Xylem thickness, xylem conduits length and number of xylem conduits of expanding and expanded peach leaf midribs

Peach leaves	Xylem thickness (µm)	Xylem conduits length (µm)	Number of xylem conduits
Expanding leaves	73.7 b	14.2 b	5.2 b
Expanded leaves	108.0 a	18.0 a	6.0 a

Means separation within column by Duncan's multiple range test. $P<0.05$. NS: Non Significant

4. Discussion

Leaf expansion plays an important role in light interception and photosynthesis. Here, for the first time the study shows changes in cell physiology and chlorophyll biosynthesis in peach leaves during expansion. Expanding leaves were warmer and had lower stomatal conductance than expanded leaves. This was similar as stated in cotton (Hall et al., 2014) and Rhus glabra (Snider et al., 2009). In the current study, stomatal density was found higher in expanding leaves, however stomatal area was further in expanded leaves. Therefore, the difference in stomatal conductance appeared to be associated with stomata size rather than stomata number.

Chlorophyll biosynthesis was evaluated during leaf expansion. In expanded leaves, the concentrations of chlorophyll a, b and a+b were found higher. Increase in chlorophyll content was reported in expanded leaves of Rhus glabra (Snider et al., 2009). Chlorophyll a and b are the main light-harvesting pigments in the photosynthesis (Formaggio et al., 2001). Environmental challenges cause reduction in chlorophyll concentration (Aras et al., 2022). Chlorophyll is a tetrapyrrole serves a pivotal role in light energy transferring in photosynthesis. The chlorophyll biosynthesis starts with formation of 5-aminolevulinic acid (ALA) and continues with formation of other porphyrins including protoporphyrin IX (Proto IX), Mg-Proto IX and protochlorophyllide (Pchlide) (Tanaka and Tanaka, 2007). In the present study, the concentrations of chlorophyll precursors in peach leaves increased during leaf expansion. The decrease in the concentrations of chlorophyll precursors were stated in stressed plants (Guo et al., 2020; Aras et al., 2021). We also assessed chlorophyll yield to evaluate the level of Proto IX driven by chlorophyll during leaf expansion. Chlorophyll yield was found similar between expanding and expanded leaves. In a previous experiment, chlorophyll yield decreased in peach leaves under calcium deficiency conditions (Aras et al., 2021).

Leaf expansion possesses two phases: cell division and expansion. Many treatments such as cytokinin, have been used to increase cell division (Werner et al., 2001). In the present study during leaf expansion, leaf cell size and cell layer increased by 21 and 2.5%, respectively. The data revealed that leaf expansion is a result of cell expansion rather than cell division. Leaves expand with water acquisition and water uptake is provided by transpiration, stomatal conductance and xylem vessel (Miqueloto et al., 2014; Aras et al., 2021). Expanded leaves had higher stomatal conductance, stomatal area and larger xylem conduits compared to expanding leaves. Thus cell expansion successfully occurred.

Xylem vessel also evaluated in the study. Xylem consists of lignin that acropetally transports water and minerals (Aras, 2021). Calcium (Ca) is translocated through xylem (Martinez et al., 2020). Loss of xylem functionality may occur during plant growth that causes Ca deficiency (Aras et al., 2021). Xylem functionality may be related with xylem size depending on plant age and use of rootstock in trees (Aras, 2021). Miqueloto et al. (2014) reported that loss of xylem functionality is the main factor of bitter pit incidence in apple caused by Ca deficiency. Another explanation of bitter pit in apple was reported by De Freitas et al. (2013). They found that leaf transpiration has higher rates than fruit transpiration that may drive further calcium into leaves. Thus, leaf transpiration, function of stomata and xylem vessel play a pivotal role in Ca distribution. Stomatal conductance, stomatal functions and xylem size were found higher in expanded leaves. The current study may offer that removal of expanded mature leaves may alter Ca translocation for the benefit of fruit.

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