



Research Article (Araştırma Makalesi)

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Cortical cells, xylem vessels, and chlorophyll biosynthesis improved by acetylsalicylic acid and sodium nitroprusside in peach leaves

Şeftali yaprağındaki kortikal hücrelerin, ksilem damarlarının ve klorofil biyosentezin asetilsalisilik asit ve sodyum nitroprussid ile iyileştirilmesi

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ABSTRACT

Objective: Water and nutrients are required for plant growth and development. Transport of water and nutrients from the roots to the shoots occurs in the xylem vessel. Acetylsalicylic acid (ASA) and sodium nitroprusside (SNP) play important roles in plant growth regulation. However, limited information is known about the relationship between SNP and ASA and leaf anatomy. Therefore, the current study was performed to evaluate the hypothesis that ASA and SNP improve leaf cortex and xylem anatomy and chlorophyll biosynthesis in peach.

Material and Methods: In the study, the roots of two-year-old peach (*Prunus persica* (L.) Batsch) cv. Rich May grafted onto GF 677 were treated with 1 mM SNP and 1 mM ASA (except control) through irrigation. One month after the treatments, many leaf histological responses and chlorophyll biosynthesis were evaluated.

Results: Both treatments increased stomatal conductance compared to control. Chlorophyll biosynthesis was influenced by the treatments. SNP and ASA increased the concentrations of the chlorophyll precursors compared to control. ASA increased cortex thickness by increasing the number of cortex cell layers. Thus, ASA can affect leaf cell division. Furthermore, SNP and ASA can enhance xylem conduits width.

Conclusion: Improvement in xylem conduits may help plants under stress conditions. Therefore, SNP and ASA may be used to improve nutrient and water uptake.

ÖZ

Amaç: Bitki büyümesi ve gelişmesi için su ve besin maddeleri gereklidir. Su ve besin maddelerinin köklerden sürgünlere taşınması ksilem demetinde gerçekleşir. Asetilsalisilik asit (ASA) ve sodyum nitroprussid (SNP), bitki büyüme düzenlenmesinde önemli roller oynamaktadır. Ancak SNP ile ASA ve yaprak anatomisi arasındaki ilişki hakkında sınırlı bilgi bilinmektedir. Bu nedenle, bu çalışma, ASA ve SNP' nin şeftalide yaprak korteks ve ksilem anatomisini ve klorofil biyosentezini iyileştirme üzerine olan hipotezi değerlendirmek için yapılmıştır.

Materyal ve Yöntem: Çalışmada iki yaşında olan ve GF 677 üzerine aşılanmış Rich May şeftalinin (*Prunus persica* (L.) Batsch) kökleri sulama yoluyla 1 mM SNP ve 1 mM ASA (kontrol hariç) ile uygulanmıştır. Uygulamalardan bir ay sonra, yapraklarda birçok histolojik tepki ve klorofil biyosentezi değerlendirilmiştir.

Araştırma Bulguları: Her iki uygulama da kontrole kıyasla stoma iletkenliğini yükseltmiştir. Klorofil biyosentezi uygulamalardan etkilenmiştir. SNP ve ASA, kontrole kıyasla klorofil öncülerinin konsantrasyonlarını artırmıştır. ASA, korteks hücre tabakasının sayısını artırarak korteks kalınlığını artırmıştır. Böylece, ASA yaprak hücre bölünmesini etkileyebilir. Ayrıca, SNP ve ASA ksilem demetini genişletmiştir.

Sonuç: Ksilem demetindeki iyileşme, stres koşulları altındaki bitkilere yardımcı olabilir. Bu nedenle, besin ve su alımını iyileştirmek için SNP ve ASA kullanılabilir.

Keywords: Acetylsalicylic acid, peach, sodium nitroprusside

Anahtar sözcükler: Asetilsalisilik asit, şeftali, sodyum nitroprussit

INTRODUCTION

Plants must acquire water and nutrients to survive. Long-distance transport of water and nutrients from the roots to the shoots occurs in the xylem vessel and xylem transport is driven by the stomatal movement and transpiration rate (White et al., 2012; Brodersen et al., 2019). Maintaining functional xylem is so important that affects plant survival and fruit yield and quality. During plant development, the loss of xylem functionality may occur. This xylem dysfunctionality may be a result of stress factors such as drought (Bauerle et al., 2011) and mineral deficiency (Aras et al., 2021). A decrease in xylem functionality was found in the apple that caused a decline in calcium uptake and consequently bitter pit incidence (Miqueloto et al., 2014). Physical rupture of the xylem in developing fruits was also reported in sweet cherry (Grimm et al., 2017) and grape (Bondada et al., 2005). Cortical cells also take an important role in water and nutrient uptake. The localization and transport of nutrients were found in the cortex layer and cortical cells (Orłowska et al., 2013). Furthermore, larger cortex cells had greater chlorophyll biosynthesis (Aras et al., 2021).

Xylem is composed of lignin that allows mechanical support and water transport (Goicoechea et al., 2005). Lignin is a phenolic compound and its biosynthesis involves the phenylpropanoid pathway, which converts phenylalanine to p-coumaroyl coenzyme A (CoA) (Boerjan et al., 2003). Some studies showed that nitric oxide (NO) promoted lignin biosynthesis in many plants (Monzón et al., 2014; Wang et al., 2021). Gabaldón et al. (2005) reported that NO led to differentiation of xylem by triggering programmed cell death. NO is involved in cell death and photosynthesis (Pedroso & Durzan, 2000; Takahashi & Yamasaki, 2002). Moreover, NO can be used to improve plant tolerance under stress conditions (Liu et al., 2014). Many studies showed that NO is involved in many processes of plants such as induction in cell death, stomatal movement, photosynthesis regulation and floral regulation (Aras et al., 2020). Among the NO donors, SNP is one of the most used ones (Filippou et al., 2012). Salicylic acid is also a product of the phenylpropanoid pathway and uses some common precursors with lignin biosynthesis (Lefevre et al., 2020). Salicylic acid elevated lignin accumulation in *Matricaria chamomilla* plants (Kováčik et al., 2009). Some studies demonstrated that SA can improve fruit quality, increase antioxidant systems and plant tolerance against stress conditions (Giménez et al., 2014; Aras & Eşitken, 2019a).

Increases in cortex cells and xylem functions are so important for plants. Wang et al. (2017) applied gibberellin to carrot to enhance xylem development. Furthermore, melatonin was applied to rapeseed plants to improve xylem vessels traits in a previous experiment (Mohamed et al., 2020). Considering the importance of the xylem in water and nutrient uptake, the studies should focus on the improvement of xylem anatomy. Therefore, the current study was performed to evaluate the hypothesis that acetylsalicylic acid (ASA), a salicylic acid derivative, and sodium nitroprusside (SNP), as a NO donor, improves leaf cortex and xylem anatomy and chlorophyll biosynthesis in peach.

MATERIAL and METHOD

The experiment was carried out on two-year-old peach (*Prunus persica* (L.) Batsch) cv. Rich May grafted onto GF 677. The saplings were planted in 10 L pots containing substrate and perlite (4:1) in the previous year in the greenhouse. The experiment was designed following a randomized plot design involving three replications, with four plants per replication. Plants were irrigated with Hoagland's nutrient solution fortnightly (Hoagland & Arnon, 1950); 5 mM Ca(NO₃)₂, 5 mM KNO₃, 2 mM MgSO₄, 1 mM KH₂PO₄, 25 μM H₃BO₃; 2 μM MnSO₄; 2 μM ZnSO₄; 0.5 μM CuSO₄; 0.4 μM (NH₄)₆Mo₇O₂₄ and 20 μM Fe-ethylenediamine-di-o-hydroxy-phenylacetic acid (Fe-EDDHA). End of the May, 1 mM SNP (Aras et al., 2020) and 1 mM ASA (Giménez et al., 2014) were treated to the plants (except control) through irrigation to the soil. One month after the treatments, many parameters were evaluated.

The concentrations of chlorophyll a, b, and a + b were determined according to Porra et al. (1989). The concentrations of chlorophyll precursors include protoporphyrin IX (Proto IX), Mg-protoporphyrin IX

(Mg-Proto IX), and protochlorophyllide (Pchlde) were determined according to the method of Hodgins & Van Huystee (1986) and calculated by the corresponding formulas (Liu et al., 2015).

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), acid phloroglucin (for xylem), or Sudan III (for epicuticular wax layer) dyes. The samples were placed on a slide after staining and visualized with a light microscope (Olympus CX21) coupled to a digital camera (Kameram 5). Diameters of the cortex, xylem, and midrib, and epicuticular wax depth 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). Stomatal conductance was measured with a leaf porometer (Li-COR).

Statistical analyses were performed with the statistical software package SPSS, version 20.0. The means were compared by the Duncan's test at 5%.

RESULTS

The effects of SNP and ASA treatments on chlorophyll biosynthesis and leaf histology were evaluated in the current experiment. Stomatal properties were significantly affected by SNP and ASA (Table 1). Both treatments increased stomatal conductance compared to control. SNP led increments in stomatal density and stomatal area compared to control, whereas ASA enhanced stomatal area.

Table 1. Effects of SNP and ASA treatments on stomatal properties

Çizelge 1. SNP ve ASA uygulamalarının stoma özelliklerine etkileri

Treatments	Stomatal conductance (mmol m ⁻² s ⁻¹)	Stomatal density (no. mm ⁻²)	Stomatal area (µm ²)
Control	142.5 b	487.3 b	413.4 b
SNP	188.1 a	604.3 a	555.7 a
ASA	184.5 a	526.0 b	539.4 a

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

Chlorophyll biosynthesis was influenced by the treatments (Table 2 and 3). Chlorophyll a was not significantly affected, however chlorophyll b and a+b increased by SNP and ASA (Table 2). The concentrations of chlorophyll precursors including Proto IX, Mg-Proto IX, and Pchlde were determined. SNP and ASA increased the concentrations of the chlorophyll precursors compared to control (Table 3).

Table 2. Effects of SNP and ASA treatments on chlorophyll a, b and a + b

Çizelge 2. SNP ve ASA uygulamalarının klorofil a, b and a + b' ye etkileri

Treatments	Chlorophyll a (µg g ⁻¹ FW)	Chlorophyll b (µg g ⁻¹ FW)	Chlorophyll a + b (µg g ⁻¹ FW)
Control	1.35 ^{NS}	17.61 b	18.97 b
SNP	1.50	20.33 a	21.84 a
ASA	1.35	20.87 a	22.23 a

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

Table 3. Effects of SNP and ASA treatments on the chlorophyll precursors**Çizelge 3.** SNP ve ASA uygulamalarının klorofil öncüllerine etkileri

Treatments	Proto IX ($\mu\text{g g}^{-1}$ FW)	Mg-Proto IX ($\mu\text{g g}^{-1}$ FW)	Pchlde ($\mu\text{g g}^{-1}$ FW)
Control	0.1008 c	0.0602 c	0.0420 b
SNP	0.1715 a	0.0918 a	0.0535 a
ASA	0.1610 b	0.0846 b	0.0522 a

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

After SNP and ASA treatments, the transverse sections of peach leaf midribs were obtained and stained with Toluidine Blue O and acid phloroglucin to highlight the basic anatomical structure (Figure 1). SNP and ASA did not affect cortical cell diameter (Table 4). ASA increased cortex thickness by increasing the number of the cortex cell layers. However, SNP did not have an influence on cortical cell division and expansion. Xylem anatomy was also observed in the present study. SNP and ASA treatments enhanced xylem thickness by increasing xylem conduits width (Table 5). Furthermore, midrib thickness and epicuticular wax depth were evaluated. Both applications increased the parameters compared to control. A positive reaction with Sudan III was observed in the cortex area (Figure 1, E).

Table 4. Effects of SNP and ASA treatments on cortical cell diameter, cortex thickness, and number of cortex cells**Çizelge 4.** SNP ve ASA uygulamalarının korteks hücre çapı, korteks kalınlığı ve korteks hücre sayısına etkileri

Treatments	Cortical cell diameter (μm)	Cortex thickness (μm)	Number of cortex cells
Control	26.7 ^{NS}	274.3 b	10.3 b
SNP	27.9	291.0 b	10.4 b
ASA	26.8	350.3 a	13.1 a

Means separation within the column by Duncan's multiple range test. $P < 0.05$, NS=Non significant.

Table 5. Effects of SNP and ASA treatments on xylem thickness, xylem conduits width, number of xylem conduits, midrib thickness and wax depth**Çizelge 5.** SNP ve ASA uygulamalarının ksilem kalınlığı, ksilem demeti genişliği, ksilem demeti sayısı, orta damar kalınlığı ve waks derinliğine etkileri

Treatments	Xylem thickness (μm)	Xylem conduits width (μm)	Number of xylem conduits	Midrib thickness (μm)	Wax depth (μm)
Control	80.5 b	16.1 b	5.07 ^{NS}	808,6 c	3,69 b
SNP	106.0 a	21.3 a	5.0	848,6 b	5,53 a
ASA	97.4 a	20.3 a	4.8	901,6 a	5,46 a

Means separation within the column by Duncan's multiple range test. $P < 0.05$, NS=Non significant.

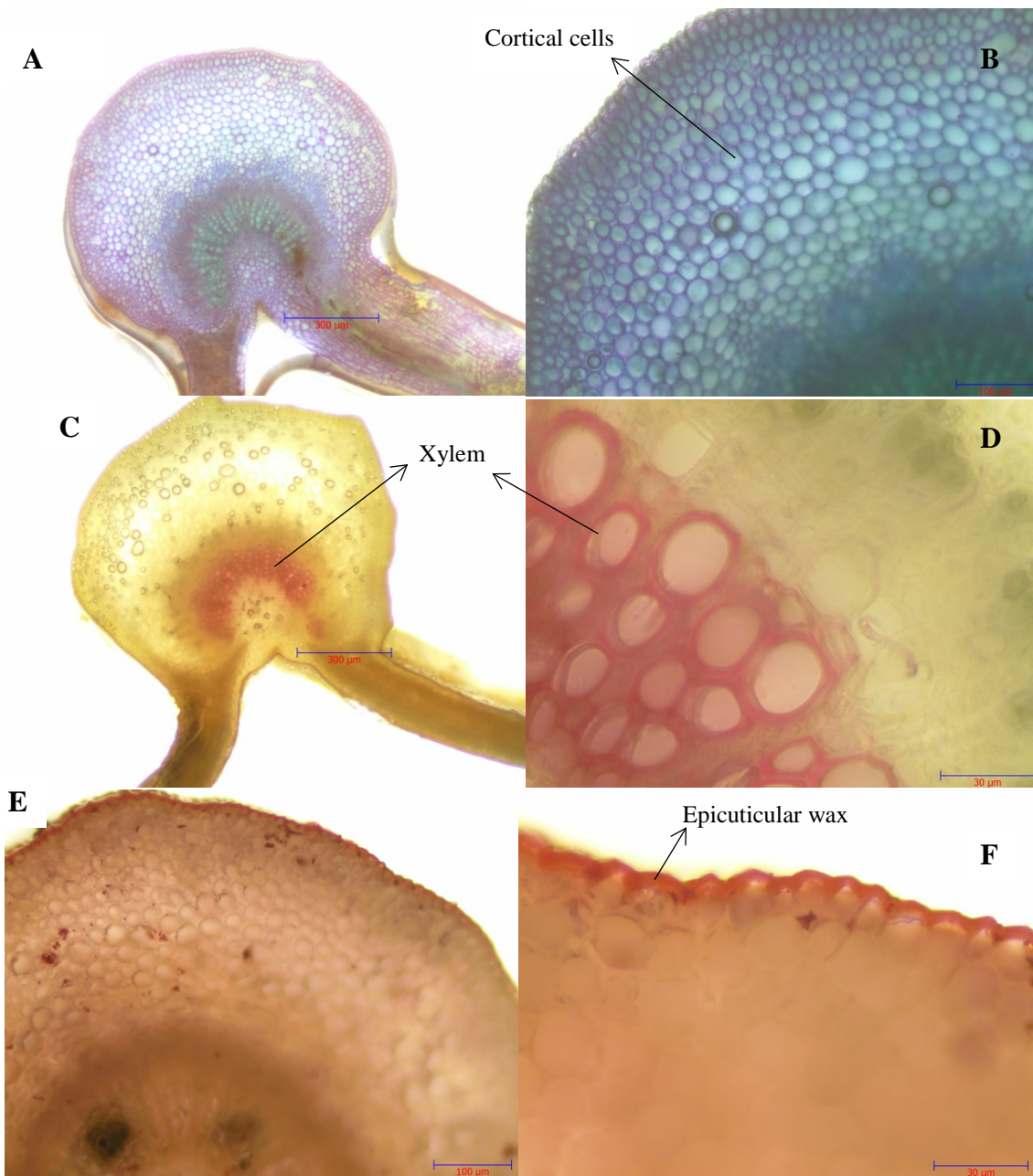


Figure 1. Histochemical characterization of peach leaf midrib cross-sectional images; (A) Toluidine blue dye showing the midrib (at 4x), (B) Toluidine blue dye showing cortical cells (at 10x), (C-D) Phloroglucinol dye showing xylem (at 4x and 40x, respectively), (E) positive reactions for lipids by Sudan III dye (at 10x), (F) Sudan III dye showing epicuticular wax (at 40x). Bars: 300 µm (at 4x), 100 µm (at 10x), 30 µm (at 40x).

Şekil 1. Şeftali yaprağı orta damarının enine kesiminin histokimyasal karakterizasyonu; (A) Orta damarın Toluidine blue boyası ile boyanması (4x), (B) Korteks hücrelerin Toluidine blue boyası ile boyanması (10x), (C-D) Ksilemin Phloroglucinol boyası ile boyanması (sırasıyla 4x ve 40x), (E) Sudan III boyası ile lipidlerin pozitif reaksiyonu (10x), (F) Epikutikular waksın Sudan III boyası ile boyanması (40x). Çizgiler: 300 µm (4x), 100 µm (10x), 30 µm (40x).

DISCUSSION

Xylem and cortical cell development is an essential process in plants. However, limited information is available for improvement in xylem and cortex anatomy. In the current experiment, the effects of SNP and ASA on the leaf anatomy, stomatal movement, and chlorophyll biosynthesis were assessed.

Leaf stomata and pigments take important roles in photosynthesis (Aras & Eşitken, 2019b; Candar et al., 2020). In the study, SNP and ASA elevated stomatal conductance by increasing stomatal density and area. Many studies showed that SNP and ASA increase stomatal gas exchange (Aras et al., 2020; Lotfi et al., 2020). Chlorophyll biosynthesis is also affected by the treatments. SNP and ASA increased chlorophyll concentration. Chlorophyll is a tetrapyrrole that serves a pivotal role in light energy transferring in photosynthesis. The chlorophyll biosynthesis starts with the formation of 5-aminolevulinic acid (ALA) and continues with the formation of other porphyrins including Proto IX, Mg-Proto IX and Pchl_{ide} (Tanaka & Tanaka, 2007). SNP and ASA treated plants had higher concentrations of the chlorophyll precursors compared to control. A decline in the precursors was reported in many plants under stress conditions (Guo et al., 2020; Aras et al., 2021). As far as we know, it is the first report on the effects of SNP and ASA on chlorophyll metabolism. The protective effects of SNP and ASA on chlorophyll were stated under stress conditions (Canakci & Munzuroğlu, 2007; Aras et al., 2020).

Leaf is the main source of photosynthesis and the energy factory of plants. The conversion of a young leaf to a mature leaf consists of two partially phases. In the first phase, cell division occurs. In the second phase, cell expansion initiates (Gonzalez et al., 2012). Thus, leaf development requires success in both cell division and expansion. In the current experiment, we evaluated cortical cells of leaf midrib. Cortical cell diameter was not significantly affected by SNP and ASA treatments, whereas cortex thickness and the number of cortical cell layers increased by ASA. Thus, the study demonstrated that ASA increased cell division in peach leaves. Shakirova et al. (2003) reported that SA induced cell division in roots of wheat. Chen et al. (1997) stated that SA induced synthesis of protein kinases playing an important role in regulating cell division. Furthermore, increase in cell division by SA was found in wheat seedlings (Dolatabadian et al., 2009).

Xylem anatomy of peach leaf midrib was evaluated in the study. Xylem takes a pivotal role in water and nutrient transport (Aras, 2021). SNP and ASA enhanced xylem thickness by increasing xylem conduits width. Xylem is composed of non-living lignified cells and maturation of xylem elements involves programmed cell death (Robert & McCann, 2000). One of the signal molecules is NO involved in programmed cell death (Pedroso & Durzan, 2000) and differentiation of xylem was triggered by NO reported in a previous study (Gabaldón et al., 2005). In the current study, SNP a donor NO increased xylem thickness compared to control. ASA also leded a remarkable increment in xylem thickness. ASA is a product of phenylpropanoid pathway use some common precursors with lignin biosynthesis (Lefevre et al., 2020) and xylem is composed of lignins (Whetten et al., 1998). Thus, we consider that ASA increased xylem thickness by triggering lignin biosynthesis. Improvement in xylem conduits may help plants under stress conditions (Aras et al., 2021).

Leaf midrib is composed of specialized tissues (phloem and xylem) and cortical cells playing pivotal roles in water, mineral and solute transport in leaves (da Silva et al., 2015; Lechthaler et al., 2019; Aras et al., 2021). ASA and SNP applications increased midrib thickness in peach leaves. Furthermore, ASA and SNP increased epicuticular wax depth of peach leaves in the current experiment. Leaves of higher plants are covered by cuticle lipids and cuticles carry a thin film of "epicuticular waxes" (Jetter & Schaffer, 2001). Epicuticular wax is a protective barrier against environment and limits transpirational water loss (Zeisler & Schreiber, 2016). An increment in the wax depth leded by SNP and ASA may increase tolerance against some stress factors such as drought and excess UV-radiation. Lipids similarly observed in epicuticular wax were also histochemically detected in cortical cells in all applications including control plants (Figure 1, E).

CONCLUSION

The results of the study revealed that SNP and ASA can improve chlorophyll biosynthesis and stomatal gas exchange. ASA can increase leaf cell division. Furthermore, SNP and ASA can enhance xylem conduits width. The results provide new insights into the role of SNP and ASA promoting leaf cell physiology and xylem anatomy. Therefore, SNP and ASA may be used to improve nutrient and water uptake under deficient conditions.

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