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Sodium nitroprusside and acetylsalicylic acid provided earliness in peach flower bud phenological stages by triggering xylogenesis

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Abstract

Temperate trees are subjected to a quiescent state during autumn and winter called dormancy. During buds growth, xylogenesis was observed between buds and shoots to maintain water and nutrients. In the present study, the effect of sodium nitroprusside (SNP) and acetylsalicylic acid (ASA) on peach (*Prunus persica* (L.) Batsch, 'J. H. Hale') flower bud phenological stages and xylogenesis between buds and shoots was investigated. Shoots were collected during the leaf fall from peach trees grafted onto the Garnem rootstock (*P. dulcis* × *P. persica*), wrapped in a damp cloth, and stored at 4 ± 1°C temperature for 850 h. After the chilling treatment, the shoots were placed for a month in a controlled chamber with environmental conditions of 21°C temperature, 65% relative humidity, and 16/8 h photoperiod. Five days after the chilling treatment (DACT), the shoots were treated with SNP and ASA, except the control. During the experiment, SNP and ASA treatments provided earliness in the stages of flower bud development. Xylogenesis was observed in all treatments starting with 11 DACT. In this stage, xylem observation was found more clearly in using SNP and ASA compared to the control.
The results of the experiment showed that using SNP and ASA provides earliness in flowering via increasing xylogenesis between buds and shoots.

Keywords: acetylsalicylic acid, bud development, peach, sodium nitroprusside, xylogenesis.

Introduction

The peach (*Prunus persica* (L.) Batsch) is a temperate zone fruit species commonly grown worldwide. A regular development of reproductive buds plays an important role in controlling fruit production. Temperate trees are subjected to a quiescent state during autumn and winter called dormancy. Dormancy refers to the lack of visible growth (Guak, Neilsen, 2013; Fadón et al., 2020). Buds undergo three types of dormancies: endodormancy, ecodormancy, and paradormancy. Winter dormancy in trees is referred to as endodormancy when chilling requirements are accumulated. Once the chilling requirements are fulfilled, endodormancy is released and heat requirements are accumulated to break ecodormancy. Paradormancy is imposed by another part of the plant (Yarur et al., 2016; Lloret et al., 2018; Fadón et al., 2020).

During buds growth, vascular connections are built between buds and shoots to maintain water and nutrients. The formation of xylem (known as xylogenesis) in the junction of buds and shoots takes the pivotal role in water availability (Xie et al., 2018). The xylogenesis process consists of the transition from these meristematic tissues to xylem cells, i.e., dead cells with lignified walls producing an empty conduit through which water flows (Bartolini et al., 2006). Insufficient xylem differentiation prevents the acropetal transport of water and nutrients (Aras et al., 2021; 2022). Thus, xylogenesis seems to be related to the dormancy breaking. Bartolini and Giorgelli (1994) showed that flower bud dormancy release in stone fruits is related to xylogenesis in stage 3 (the first morphological sign of bud growth resumption). Santiago-Mejía et al. (2018) stated that during the dormant stage of peach bud xylogenesis was not observed. Bartolini et al. (2006) reported that xylem was observed in stage 3 up to ¾ of the bud axis in the apricot bud.

Xylem is composed of a phenolic compound, lignin, synthesised in the phenylpropanoid pathway (Boerjan et al., 2003). Many studies show that nitric

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oxide (NO) has been implicated to regulate xylogenesis (Monzón et al., 2014; Wang et al., 2021). NO donors are compounds that could produce NO when applied to the biological systems, and one of the most utilised NO donors is sodium nitroprusside (SNP) (Jian et al., 2016; Aras et al., 2020). The exogenous NO application increased the xylem conduit size in peach leaves (Aras, 2022). Moreover, NO led to xylogenesis through triggering the programmed cell death (Gabaldón et al., 2005). The exogenous NO treatment activated the phenylpropanoid metabolism of peaches (Li et al., 2017) and apples (Ge et al., 2019). Salicylic acid (SA) is also a product of phenylpropanoid pathway using some common precursors with lignin biosynthesis (Lefevere et al., 2020). SA increased lignin accumulation in *Matricaria chamomilla* plants (Kováčik et al., 2009). Acetylsalicylic acid (ASA), a SA derivative, was found to increase the xylem conduit size in peach leaves like NO (Aras, 2022).

Many studies were conducted on xylogenesis observation during bud development. However, as far as is known, no study has been performed to affect the xylogenesis between buds and shoots for the stages of flower bud development. The aim of the current experiment was to evaluate the effect of SNP and ASA on the flower bud phenological stages and the xylogenesis between buds and shoots in the peach tree.

Material and methods

Experimental area and plant material. The experiment was carried out in both 2020 and 2021 seasons in a commercial orchard located in Yerköy, Yozgat, Turkey. In the orchard area, the climate is temperate, and the annual means of precipitation and temperature are 570 mm and 9.2°C, respectively. The plant material used in the experiment consisted of peach (*Prunus persica* (L.) Batsch, 'J. H. Hale') trees grafted onto the Garnem rootstock (*P. dulcis* × *P. persica*). The spacing was 5 × 4 m, and the trees were nine years old.

Chilling treatment. During the leaf fall and before the chilling accumulation, 90 uniform one-year-old shoots were collected from 10 peach trees. To avoid dehydration, the shoots were put into a plastic box and wrapped in a damp cloth. Upon arrival in the laboratory, the shoots were disinfected with a solution of sodium hypochlorite (0.5% v/v). The shoots 20 cm long were cut obtaining 300 shoots for each treatment. Only two leaf buds and three flower buds were kept, while the other buds were removed. The uniform shoots were wrapped in a damp cloth and stored at 4 ± 1°C temperature for 850 h. The 850 h chilling requirement of the peach tree was reported. Regarding the chilling hour model, all hours with temperatures between 0 and 7.2°C were considered chilling hours (Fadón et al., 2020; Hsiang et al., 2021).

Forcing conditions and treatments. Following the 850 h chilling treatment, for heat requirements, the peach shoots were then placed in containers with 250 mL of a sucrose solution (5%) and transferred for a month to a forcing chamber with environmental conditions of 21°C temperature, 65% relative humidity, and 16/8 h photoperiod. Five days after the chilling treatment (DACT), the shoots were treated with sodium nitroprusside (SNP) and acetylsalicylic acid (ASA), except the control. As reported for stone fruits (Bartolini, Giorgelli, 1994), xylogenesis starts with the first morphological sign of buds growth resumption. Thus, the shoots were treated with SNP and ASA, while the buds lacked xylem vessels between S1 and S2 stages. For the experiment, 1 mM SNP and 1 mM ASA doses were chosen according to the results of the previous experiments (Giménez et al., 2014; Aras et al., 2020; 2022). Before the treatments, xylem water flow velocity (V_{XW}) was determined to how many hours are necessary for the treatment imbibition. For this purpose, the shoots were incubated in 1.0% (w/v) aqueous acid fuchsin for 30 min.

V_{XW} was calculated by the equation (Aras, 2021):

V_{XW} = length of the stained part of the shoot (mm) / dye infusion period (h). 15 cm h^{-1} V_{XW} was determined.

Therefore, in the current experiment, the shoots were placed in the 1 mM SNP or 1 mM ASA solutions for 2 h. Following the treatments, the shoots were placed in the sucrose solution including the control. The basal ends of the shoots were cut weekly, and the water was replaced daily (Gariglio et al., 2006).

Evaluation of phenological stages. Phenological observations were carried out every day in the forcing chamber over two (2020 and 2021) growing seasons. The phenological stages were characterised according to Lisandru et al. (2017) and Santiago-Mejía et al. (2018). The stages were computed when the buds of all shoots were overcome when 50% of the buds reached this stage (Richardson et al., 1974; Montazeran et al., 2018) and evaluated among the treatments. Stage 1 (S1): dormant buds – buds closed and covered by dark brown scales (Figure 1A); stage 2 (S2): beginning of bud swelling – buds closed, light brown scales visible (Figure 1B); stage 3 (S3): bud scale open – scales separated (Figure 1C); stage 4 (S4): pink tip visible – pink sepal tips slightly visible (Figure 1D); stage 5 (S5): floral bud swelling – sepal tips fully visible (Figure 1E); stage 6 (S6): flowering (Figure 1F).

Physical properties of buds and shoots were determined at 0, 7, and 14 DACT. The bud weight was measured by a precision scale Seles Thb (Turkey). The width and length of buds were measured by a digital calliper (Mitutoyo). The bud temperature was measured by an infrared thermometer IR-838 (Shenzhen Flus Technology Co, Ltd, China). The water content (WC) of buds and shoots was calculated by the equation:

WC (%) = [(FW − DW) / FW] × 100, where FW is fresh weight, DW – dry weight.

Xylogenesis observation. To determine the effect of the treatments on xylogenesis, the dye infusion method (Xie et al., 2018; Aras, 2021) was used once two

days after the treatments. The shoots were placed in 1.0% (w/v) aqueous acid fuchsin for 3 h. The dye transport into the buds and shoots was photographed.

Experimental design and statistical analysis. The experiment had a randomised plot design with three replicates per treatment. Statistical analysis was performed with the software package SPSS Statistics, version 20.0 (IBM Inc., USA). The average of the data for two consecutive years was analysed. The means were compared by the Duncan multiple range test at the 5% level of significance.

Results

Phenological stages and physical properties. In both experimental years, using of SNP and ASA provided earliness at S3, S4, S5, and S6 compared to the control (Figure 2). Stage S2 was the same among the treatments. The results showed that the lowest number of days to reach flowering was obtained after the ASA treatment (26 days). Furthermore, synchronisation in flowering (greater uniformity in flowering time) was observed at the SNP treatment.

Figure 1. Developmental stages (S) of peach flower buds: S1 – dormant buds (A), S2 – beginning of bud swelling (B), S3 – bud scale open (C), S4 – pink tip visible (D), S5 – floral bud swelling (E), and S6 – flowering (F)

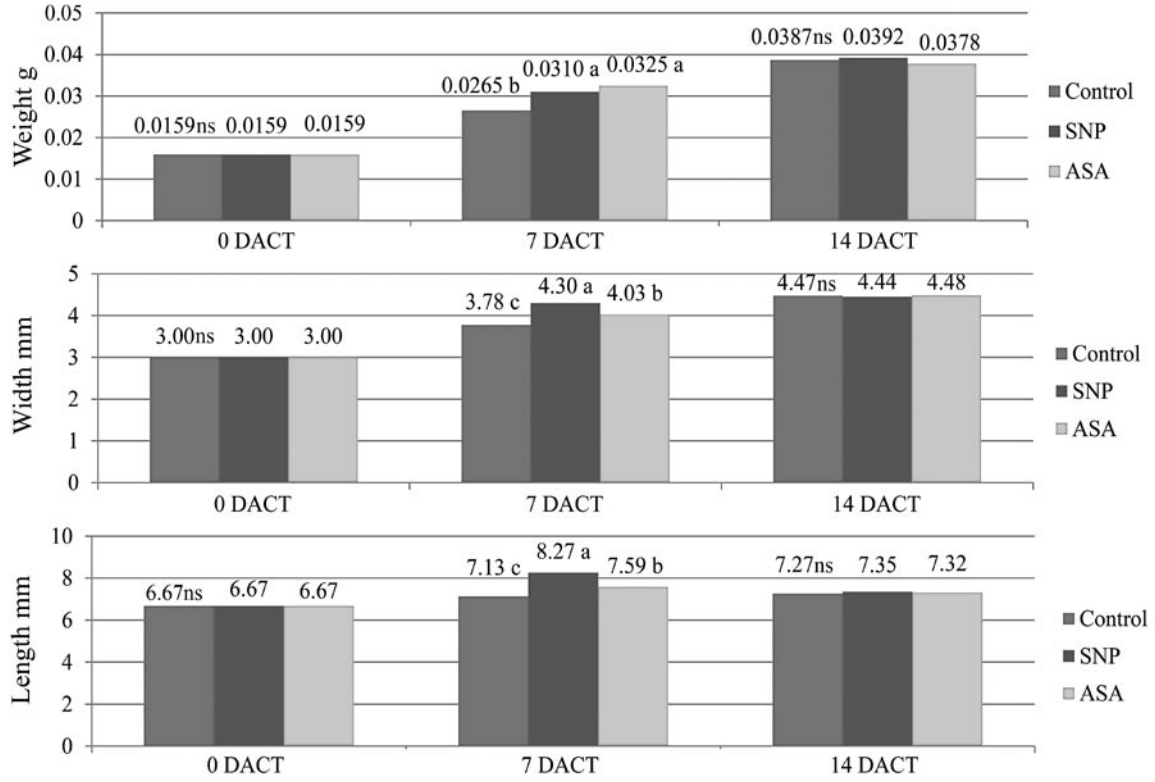
	Days after chilling treatment (DACT)																											
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Control	S1							S2									S3						S4				S5			S6
SNP	S1							S2							S3						S4				S5			S6		
ASA	S1							S2								S3				S4				S5			S6			

Explanation under Figure 1

Figure 2. The effect of sodium nitroprusside (SNP) and acetylsalicylic acid (ASA) on the stages (S) of peach flower bud development

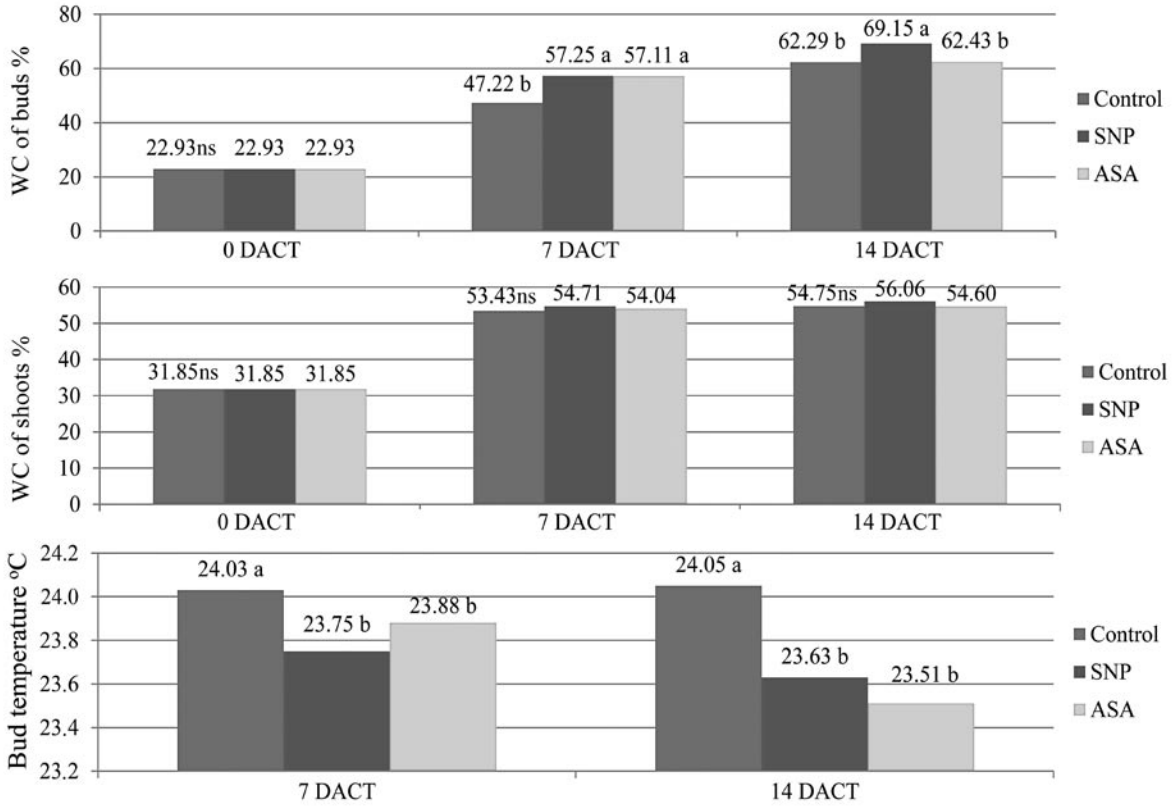
To assess the status of bud swelling, the bud weight, width, and length were measured. The bud weight increased at S2 (7 DACT), and there was no significant difference among the treatments at S3 (14 DACT) (Figure 3). At S2, the SNP and ASA treatments had a higher bud weight compared to the control. The bud width and length also increased at S2 (7 DACT), while there was no significant difference at S3 (14 DACT).

During the bud development, the WC of the buds and shoots changed. At S1, the WC of the buds was 22.93% (Figure 4). The WC was higher in the SNP and ASA treatments compared to the control at S2. The WC



Note. Means separation within column by Duncan's multiple range test at $P < 0.05$; ns – not significant.

Figure 3. The effect of sodium nitroprusside (SNP) and acetylsalicylic acid (ASA) on the peach flower bud weight, width, and length



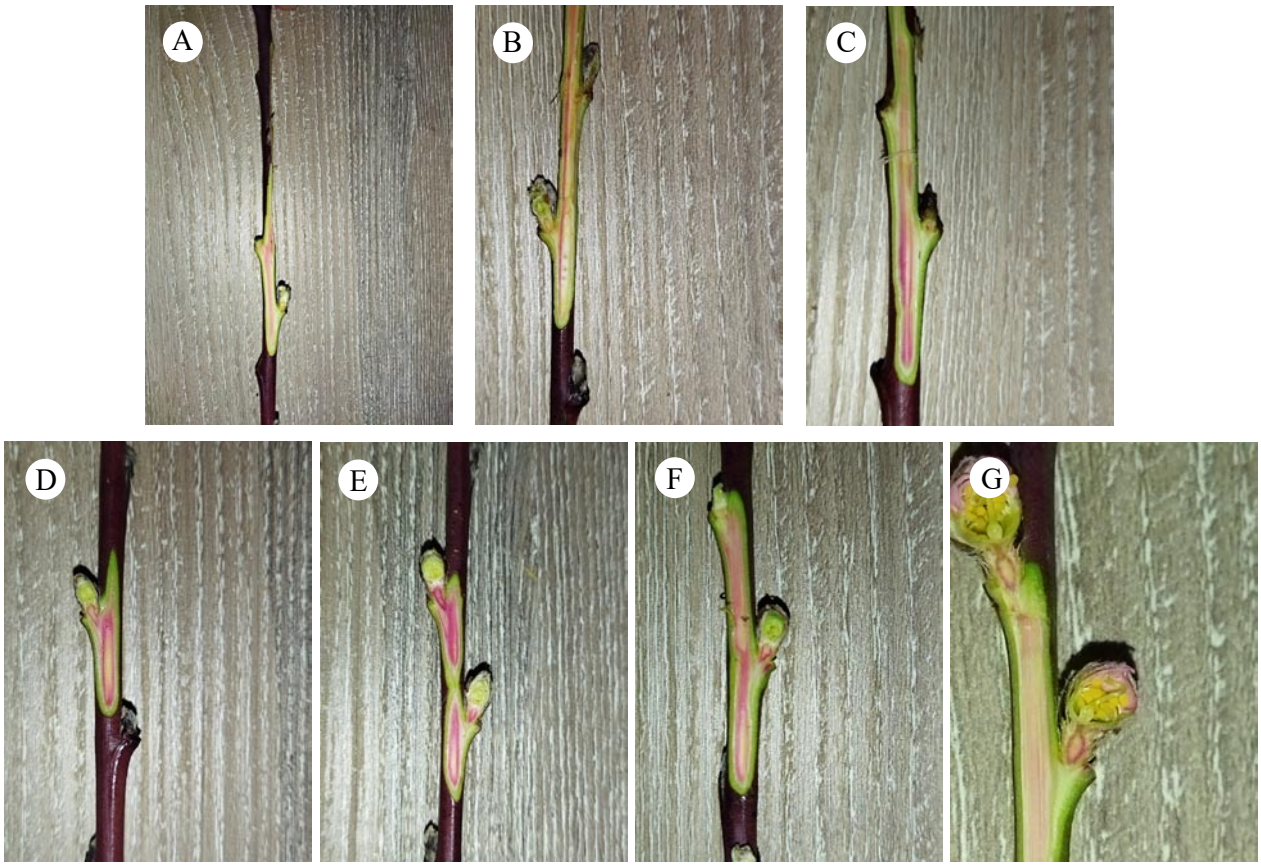
Note. Means separation within column by Duncan's multiple range test at $P < 0.05$; ns – not significant.

Figure 4. The effect of sodium nitroprusside (SNP) and acetylsalicylic acid (ASA) on the water content (WC) of buds and shoots, and the bud temperature of peach flower

of the shoots was 31.85% during the dormant stage, and at S2 and S3, there was no significant difference among the treatments. The bud temperature decreased in the SNP and ASA treatments at S2 and S3.

Xylogenesis observation. To examine the vascular connection between the buds and the shoots during different stages of bud development, the dye infusion method was conducted (Figure 5).

Xylogenesis was observed starting with 11 DACT (between S2 and S3) in all treatments indicating that vascular connections between the buds and the shoots were established between S2 and S3. Between S2 and S3, xylem observation was found more clearly in the SNP and ASA treatments compared to the control. At S3, xylem was seen at the base of the bud. The dye reached the ovary at S4 (Figure 5G).



A – control (9 DACT), B – SNP (9 DACT), C – ASA (9 DACT), D – control (11 DACT), E – SNP (11 DACT), F – ASA (11 DACT), G – general view of xylogenesis at S4

Figure 5. The effect of sodium nitroprusside (SNP) and acetylsalicylic acid (ASA) on peach flower bud xylogenesis observation during different stages

Discussion

Bud development stages are important phenological events related to water and nutrient uptake in the buds (Xie et al., 2018). The xylem observation at S3 in stone fruits in the present study is well known (Bartolini, Giorgelli, 1994; Santiago-Mejía et al., 2018). The current paper noted that some growth regulators can be used to increase xylogenesis during the lack of vascular connections (between S1 and S2, and at S2) to improve water uptake in buds. Moreover, the results of the experiment showed that the lowest number of days to reach flowering was obtained by the ASA treatment followed by the SNP one. As far as we know, it is the first report on the effect of SNP and ASA on xylogenesis in buds at the flowering stage.

In *Prunus* trees, the hydration of buds requires water transport through xylem. Water transport leads to bud swell and development resulting in the WC in buds and shoots (Michailidis et al., 2018). During the bud dormancy (S1), the WC in the buds was 22.93% (Figure 4). At the start of bud swelling, the WC of the buds and shoots increased to reactivate metabolisms in the buds (Xie et al., 2018). At the beginning of the bud swelling (S2), the bud size and weight increased in the SNP and ASA treatments compared to the control. At S3, there was no significant difference in the bud size and weight between the treatments. Thus, the current study may show that exogenous treatments at S2 are important to control bud development. Increase in the WC of the bud was also found at S2. The WC of the buds increased to 62–69% at S3.

The appearance of flower bud xylem vessel elements occurred at the same time among the treatments and xylem developed from the base up to the top of the bud. SNP and ASA did not provide early xylogenesis;

however, xylem was observed more observation was found more clearly in the SNP and ASA treatments. Thus, SNP and ASA could have influenced the process of xylogenesis and increased the xylem vessels.

During the rebuilding of the vascular connection from the shoots to the buds, two important factors influence xylogenesis: environmental temperature and bud hormonal status (Zhang et al., 2018). In a previous study of Aras (2022), it was found that SNP and ASA increased the width of xylem vessels in peach leaves. Xylem is a product of phenylpropanoid pathway (Muro-Villanueva et al., 2019), and some products of phenylpropanoid pathway such as SA or ASA can be used to improve xylogenesis (Lefevere et al., 2020).

Gabaldón et al. (2005) showed that nitric oxide (NO) can improve xylogenesis through triggering the programmed cell death. Della Rovere et al. (2019) reported that xylogenesis is induced by NO in *Arabidopsis*. NO is involved in many processes of plants such as stomatal movement and floral regulation (Sun et al., 2019; Zhang et al., 2019; Aras et al., 2020). In the current experiment, SNP and ASA lead to xylem formation more clearly compared to the control during early stages (between S2 and S3) in bud development (Figure 5). Andreini et al. (2012) reported that the most advanced stage in the progression of xylem differentiation of apricot was stage 3. Santiago-Mejía et al. (2018) stated that there were no signs of vascularisation during the early dormancy in peach buds. In this stage, increase in the bud weight, bud size, and the WC of buds and shoots was found higher in the SNP and ASA treatments compared to the control parallel with the xylogenesis observation during the experiment. Moreover, both treatments provided earliness at S3, S4, S5, and S6 that may affect the fruit harvest time. Triggering earliness in flowering can be also used for an effective pollination period (EPP). SNP or ASA can be treated to a pollinator or a fruit producing cultivar to overlap their EPP.

Conclusion

The paper revealed a new method of analysing the bud development and flowering of a peach tree including chilling and forcing conditions. In the present study, the phenological stages of peach bud development and flowering were determined under forcing conditions. Xylogenesis was not observed in the buds until the bud swelling. After the bud swelling, xylogenesis was observed between buds and shoots in all treatments including the control. The xylem observation was found more clearly in sodium nitroprusside (SNP) and acetylsalicylic acid (ASA) compared to the control. Furthermore, SNP and ASA provided earliness in the stages of flower bud development.

The results of the study suggest that SNP and ASA can be applied to the peach buds during the bud swelling stage to provide earliness in the flowering date.

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Skatindami ksilogenezę natrio nitroprusidas ir acetilsalicilo rūgštis užtikrina persikų žiedų pumpurų fenologinių stadijų ankstyvinimą

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Santrauka

Vidutinio klimato zonos medžiai rudenį ir žiemą patiria ramybės būklę. Pumpurams augant, tarp jų ir ūglių vyksta ksilogenezė; tai padeda išlaikyti vandenį ir maisto medžiagas. Tyrimo metu analizuotas natrio nitroprusido (SNP) ir acetilsalicilo rūgšties (ASA) poveikis paprastojo persiko (*Prunus persica* (L.) Batsch, veislė 'J. H. Hale') žiedų pumpurų fenologinėms stadijoms ir pumpurų bei ūglių ksilogenezei. Ūgliai surinkti krintant lapams nuo persikų medžių su Garnem poskiepiais (*P. dulcis* × *P. persica*), apvynioti drėgnu audeklu ir 850 val. saugoti 4 ± 1° C temperatūroje. Po šaldymo ūgliai mėnesį laikyti reguliuojamoje kameroje tokiomis aplinkos sąlygomis: 21° C temperatūra, 65 % santykinis oro drėgnis ir 16/8 val. fotoperiodas. Penkios dienos po šaldymo procedūros (DACT) ūgliai apdoroti SNP ir ASA, išskyrus kontrolinę grupę. SNP ir ASA paankstino žiedų pumpurų formavimąsi. Ksilogenezė nustatyta visais tarpsniais, pradedant nuo 11 DACT. Šiuo tarpsniu ksilemos formavimasis buvo ryškesnis panaudojus SNP ir ASA, palyginti su kontroliniu variantu.
Eksperimento rezultatai parodė, kad SNP ir ASA panaudojimas užtikrina žydėjimo ankstyvumą skatinant pumpurų ir ūglių ksilogenezę.

Reikšminiai žodžiai: acetilsalicilo rūgštis, pumpurų augimas, persikas, natrio nitroprusidas, ksilogenezė.