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# Salicylic Acid Pretreatment Improves the Tolerance of Tobacco Seedlings to Alternation of Light/Dark Periods Stress

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## ABSTRACT

The effect of salicylic acid treatment (SA) on tobacco seedlings tolerance to the alternation of light/dark stress (AL) (16/8 min light/dark cycles and a photosynthetic photon flux density (PPFD) of 50 µmol photons $m^{-2}$ $s^{-1}$ for three days) was studied in this experiment. The photosynthesis efficiency was determined by mean of chlorophyll *a* and JIP-test. The data showed that the AL treatment decreased PSII activity and increased the level of hydrogen peroxide ($H_2O_2$) and lipid peroxidation as evaluated by the accumulation of malonyldialdehyde (MDA). The SA treatment enhanced the growth parameters. The SA pretreatment reduced the inhibitory effect of AL on the PSII activity and photosynthesis, as indicated by the increase in the maximum quantum efficiency of PSII ($F_v/F_m$), the performance index on the absorption basis ($PI_{abs}$), the observed rate of $Q_A$ reduction (TR0/RC), the rate of electron transport beyond $Q_A^-$ (ET0/RC) and the efficiency in which an absorbed photon results in electron transport beyond $Q_A^-$ (ET0/ABS), and as well the decrease in the energy dissipation as heat (DI0/RC) and the $H_2O_2$ and MDA accumulations. Therefore, the observed oxidative damages provoked by AL were noticeably reduced when seedlings were pretreated with SA.

**Keywords**: Alternation of light/dark; Chlorophyll fluorescence transients; Salicylic acid; Stress tolerance; Tobacco.

**Abbreviations:** ABS PSII light absorption flux.- DI0 PSII excitation dissipation flux.- ET0 electron transport flux.- $F_0$, $F_v$, $F_m$ minimal, variable and maximal chlorophyll fluorescence.-$F_v/F_m$ maximum photochemical efficiency of PSII.- $H_2O_2$ hydrogen peroxide.- $LHC_s$ light-harvesting chlorophyll *a/b* binding protein complex of photosystem I(II).- MDA malonyldialdehyde.- PFDD photosynthetic photon flux density.- $PI_{abs}$ performance index on absorption basis.- PSII photosystem II.- $Q_A$ primary quinone electron acceptor of PSII.- RC reaction center.- ROS reactive oxygen species.- TR0 PSII light energy flux trapping.- 1-$V_J$ a measure of the electron flux beyond $Q_A$.

## INTRODUCTION

The variation of intensity and duration of light, under natural conditions, occurs when sun irradiance can be blocked by clouds, trees and buildings. Once happened, leaves, in this state, are exposed to alternate light conditions (Zhu *et al*., 2004). This alternate light produces modulation of the response of plants to biotic and abiotic stress due to the accumulation of ROS (Osmond *et al*., 1997; Asada, 1999; Müller *et al*., 2001). These ROS are considered to be the primary causes of irreversible loss of photosynthetic activity in the plant leaves exposed to light stress or other environmental stresses (Allen, 1995;

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Niyogi, 1999; Kruk and Szymańska, 2012).

Oxidative stress is a common disorder in plants during or following exposure to adverse stressful conditions. This state induces the generation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radicals ($O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxyl radicals ($OH^-$) in plants. In turn, oxidative stress occurs when the generation of reactive oxygen species (ROS), primarily known as by-products of various $O_2$-utilizing processes, exceeds the capacity of ROS scavenging reactions. This increased ROS level in plants causes oxidative damage that inhibits photosynthesis and growth as a result of the destruction of biomolecules such as lipids, proteins and nucleic acids, thus altering the redox homeostasis (Smirnoff, 1993; Mittler, 2002; Torres, 2010; Gill and Tuteja, 2010). Nevertheless, the oxidative stress that accompanies unfavorable environmental conditions should not be viewed as symptom of cellular dysfunction but it can represent a perquisite signal to induce proper acclimation mechanisms (Foyer and Noctor, 2003, 2005, 2009; Miller *et al*., 2010).

The recent reports of Darwish *et al.* (2014, 2015) indicated that an alternation of light/dark periods led to a low accumulation of hydrogen peroxide ($H_2O_2$), which indicates a state of moderate-oxidative stress. This state is thought to be important to activate the ROS detoxification systems.

To protect the photosynthetic apparatus and reduce oxidative damage, plants have developed several mechanisms to maintain the ROS concentration within a sub-lethal range. The enzymatic antioxidant system, including superoxide dismutase (SOD) and catalase (CAT), and the enzymes belonging to Ascorbate-glutathione cycle such as ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and glutathione reductase (GR), is a part of these mechanisms. Some of phenolic compounds that should be included in the category of phytohormones are suggested as part of the non-enzymatic antioxidant system, such as salicylic acid (SA) (Hayat *et al*., 2010).

Several studies support a major role of SA in mediating plant response to tolerate many abiotic stresses including the salinity (Khodary, 2004; Yusuf *et al*., 2008), heat (Larkindale and Knight, 2002; Scott *et al*., 2004), water deficit (Bandurska and stroinski, 2005; Hayat *et al*., 2008), ultraviolet radiation (Ervin *et al*., 2004), heavy metals (Metwally *et al*., 2003; Zhou *et al*., 2009), poor nutrition (Yamada and Takeno, 2014), ozone (Rao and Davis, 1999) and osmotic stress (Borsani *et al*., 2001).

The objective of this study was to investigate whether or not the pretreatment of tobacco seedlings with SA improves their tolerance to the moderate-oxidative stress induced by the alternation of light/dark cycles. To assess this effect, several parameters related to seedling growth, photosynthetic pigments, $H_2O_2$ contents, chlorophyll *a* fluorescence and JIP-test were studied.

## MATERIALS AND METHODS

### Plant material, growth conditions and treatments

Seeds of tobacco (*Nicotiana tabacum* L. *cv.* Virginievk51) were germinated in a plastic container with sterilized soil for two weeks at a 22/17°C day/night temperature, a 16/8-h light/dark cycle and a photosynthetic photon flux density (PPFD) of 50 µmol photons $m^{-2}$ $s^{-1}$. Germination was carried out under sterile conditions and in accordance with the protocol of Darwish *et al*. (2013, 2014, and 2015). The experimental design is shown in Figure 1. In brief, after 5 weeks of germination, seedlings (three-leaf stage) were transferred into a hydroponic system containing Hoagland's nutrient solution (1 M $KNO_3$; 1 M $Ca(NO_3)_2.H_2O$; 1 M $MgSO_4.7H_2O$; 1 M $KH_2PO_4$ and 0.01 M FeEDDHA) under the same conditions as those used during the

germination. Then, salicylic acid (SA) (Sigma-Aldrich, USA) was added at a concentration 0 or 10 µM to the Hoagland's nutrient solution. After 10 days of the pretreatment with SA, the seedlings were exposed or not to an alternation of light/dark periods (AL) (16/8-min light/dark cycle, PPFD of 50 µmol photons $m^{-2}$ $s^{-1}$) in a growth chamber for three days; the other part remained without an AL to be used as a control (16/8-h light/dark cycle, PPFD of 50 µmol photons $m^{-2}$ $s^{-1}$) as described by Darwish *et al*. (2015). Twenty four days after SA treatment, the growth and chlorophyll *a* fluorescence parameters were measured for the leaves of the four treatments: 1) Con (Control, without salicylic acid pretreatment and without an alternation of the light/dark periods), 2) SA (10 µM salicylic acid), 3) AL (alternation of the light/dark periods), and 4) SAAL (pretreatment of salicylic acid with an alternation of the light/dark periods). Additionally, fresh leaves were collected and stored at -80°C for biochemical assays.

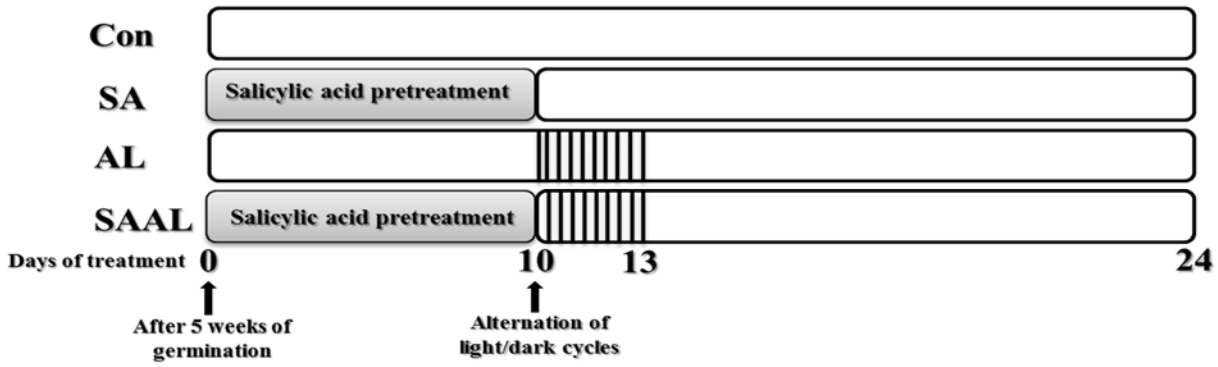


**Fig. 1. Schematic representation of expirmental design and treatments. 0, 10, 13 and 24 refer to days after the treatment with the salicylic acid. Con, without salicylic acid and without alternation of light/dark periods; SA, treatment with 10 µM salicylic acid; AL, alternation of light/dark periods; SAAL, pretreatment of salicylic acid and treatment with alternation of light/dark periods.**

**Plant growth**

Twenty four days after treatment with SA, plant growth was determined by measuring plant height, root length, leaf, and root dry weight.

**Photosynthetic pigments**

The total chlorophyll and carotenoid contents were measured according to Lichtenthaler (1988) using pure acetone as the extraction solvent. The absorbance at 662, 645 and 470 nm was measured immediately after extraction.

**$H_2O_2$ content**

The hydrogen peroxide levels were measured according to a modified method of Darwish *et al*. (2015). Two hundred and fifty milligrams (250 mg) of tobacco leaves from seedlings that were treated or untreated with SA and AL, was homogenized in 1 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 × *g* for 15 min at 4 °C. Aliquots of 100 µL from each tube were placed in 96-well plates, and 50 µL of 10 mM potassium phosphate buffer (pH= 7.0) and 100 µL of 1 M KI were added to each well. Control samples were prepared using water instead of KI to remove the color background of the extract at 390 nm. Each plate also contained commercial $H_2O_2$ to generate a

standard curve. The plate was briefly vortexed, and the absorbance readings were recorded at 390 nm in a microplate reader. The concentrations of $H_2O_2$ were determined using a standard curve and expressed as nmol $g^{-1}$ fresh weight (FW).

**Malonyldialdehyde content (MDA)**

The malonyldialdehyde content as an end product of lipid peroxidation was measured as described by Murshed *et al*. (2013) using thiobarbituric acid (TBA) test with some modifications. Briefly, 250 mg of tobacco leaves from seedlings that were treated or untreated with SA and AL was homogenized in 1 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 × *g* for 15 min at 4°C, and 0.5 mL of the supernatant was added to 1 mL 0.5% TBA in 20% TCA. The reaction was incubated in boiling water for 30 min and then stopped by using an ice bath. Aliquots of 200 µL from each tube were placed in 96-well plates, and the absorbance readings were recorded at 532 nm in a microplate reader. The amount of MDA-TBA complex was calculated from the coefficient 155 $mM^{-1}cm^{-1}$ and expressed as nmol $g^{-1}$ fresh weight (FW).

**Fast chlorophyll *a* fluorescence transients**

The rapid kinetics of chlorophyll *a* fluorescence transients emitted by leaves of seedlings that are adapted to darkness was evaluated. After 24 days of treatment with SA, fast chlorophyll *a* fluorescence transients were measured on 8 leaves of tobacco seedlings. Before taking measurement, the leaves were dark-adapted for 20 min. The polyphasic chlorophyll *a* fluorescence (expressed in relative units) was measured using a portable Handy-PEA (Hansatech, Kings Lynn, UK) under illumination with a light intensity of 3,000 µmol photons $m^{-2}$ $s^{-1}$. The fluorescence parameter $F_v/F_{m=}(F_m-F_0)/F_m$ represents the maximum photochemical quantum yield of PSII. The JIP-test that is based on the energy flux theory in the thylakoid membrane (Strasser *et al*., 2004; Stirbet and Govindjee, 2011) shows the kinetics of the energy absorbed by the PSII antenna pigments (ABS). Part of this energy in turn is dissipated as heat and fluorescence (DI); the other part that is trapped (TR) by the PSII reaction centers (leading to $Q_A$ reduction) proves to be converted into electron transport flux (ET). Appendix describes some JIP-test parameters, and how they are calculated from the original data of the fast chlorophyll *a* fluorescence transients ($F_0$, $F_{300}$, $F_J$ and $F_m$).

**Statistical analysis**

The experiment was carried out using completely randomized design with 8 replications (n=8). Statistical analyses were performed with the R statistical software using an ANOVA with Tukey's test. The results are displayed as means±SE and are considered significant at $P<0.05$

**RESULTS**

**Plant growth**

The SA-treated seedlings showed a significant increase ($P$<0.05) in the dry weight of the roots and plants by approximately 48% and 8%, respectively, compared to the control. However, the AL-treated seedlings were not significantly different ($P$>0.05) in the dry weight of the roots and plants from the control seedlings (Table 1).

In the AL-treated seedlings, the roots length was significantly decreased ($P$<0.05) by approximately 10% compared to that of the control. However, the seedlings length was not significantly different ($P$>0.05) between the AL and control treatments. In the SA-treated seedlings, the root and seedling lengths were increased by approximately 6% and 23%, respectively, compared to those of the control (Table 1).

The SA pretreatment was able to regulate the plant growth. There was no negative effect of AL treatment on dry weight and length of tobacco seedlings (Table 1).

**Table 1: Dry weight and length of tobacco seedlings subjected to four treatments.**

| Treatment | Dry weight (g) | | Length (cm) | |
|---|---|---|---|---|
| | Root | Plant | Root | Plant |
| **Con** | 0.023 ± 0.005 a | 0.012 ± 0.001 a | 11.1 ± 0.3 b | 2.2 ± 0.1 a |
| **SA** | 0.034 ± 0.005 b | 0.013 ± 0.001 b | 11.7 ± 0.2 c | 2.7 ± 0.2 b |
| **AL** | 0.026 ± 0.005 ab | 0.012 ± 0.002 a | 10.1 ± 0.4 a | 2.1 ± 0.1 a |
| **SAAL** | 0.021 ± 0.004 a | 0.012 ± 0.001 a | 10.6 ± 0.3 ab | 2.2 ± 0.1 a |

Con, without salicylic acid and without alternation of light/dark periods; SA, treatment with 10 μM salicylic acid; AL, alternation of light/dark periods; SAAL, pretreatment of salicylic acid and treatment with alternation of light/dark periods. Different letters indicate significant difference within each treatment according to an ANOVA-Tukey test at the 95% confidence level. Data are expressed as means ± SE (n=8).

**Photosynthetic pigments (chlorophyll and carotenoids)**

Total chlorophyll content was significantly decreased ($P$<0.05) in the SA-, AL- and SAAL-seedlings by approximately 17%, 9% and 20%, respectively, compared to those in the control (Table 2).

The level of carotenoids was significantly increased ($P$<0.05) in the SA-, AL- and SAAL-seedlings by approximately 16%, 9% and 13%, respectively, compared to those of the control (Table 2).

The AL and SAAL treatments significantly increased ($P$<0.05) LHC contents (Chl *a/b* ratio) in the thylakoid membrane of tobacco leaves by approximately 55% and 21%, respectively, compared to those of the control (Table 2). However, the SA treatment had no effect on LHC contents as compared with the control (Table 2).

**Table 2: Chlorophyll (Chl a, Chl b, Chl a+b) and carotenoid (Car) contents per fresh weight in tobacco leaves subjected to four treatments.**

| Treatment | Chl *a* (μg $g^{-1}$ FW) | Chl *b* (μg $g^{-1}$ FW) | Chl (*a*+*b*) (μg $g^{-1}$ FW) | Car (μg $g^{-1}$ FW) | Chl *a/b* |
|---|---|---|---|---|---|
| **Con** | 516 ± 35 a | 206 ± 12 b | 722 ± 43 a | 138 ± 4 a | 2.50 ± 0.14 a |
| **SA** | 430 ± 22 b | 170 ± 11 bc | 600 ± 30 bc | 164 ± 5 c | 2.52 ± 0.14 a |
| **AL** | 350 ± 11 d | 308 ± 26 a | 658 ± 32 b | 152 ± 4 b | 1.13 ± 0.18 c |
| **SAAL** | 382 ± 22 c | 192 ± 30 c | 574 ± 25 c | 158 ± 5 bc | 1.98 ± 0.12 b |

Con, without salicylic acid and without alternation of light/dark periods; SA, treatment with 10 μM salicylic acid; AL, alternation of light/dark periods; SAAL, pretreatment of salicylic acid and treatment with alternation of light/dark periods. Different letters denote significant differences for each parameter between means within each traitment ($P$<0.05, ANOVA-Tukey test). Data are expressed as means ± SE (n=8).

**$H_2O_2$ and MDA contents**

The tobacco seedlings treated with AL exhibited a higher $H_2O_2$ level than that of the control (Fig. 2A). The SA pretreatment reduced the accumulation of $H_2O_2$ in tobacco seedlings. The SAAL-treated seedlings showed a significant decrease ($P$<0.05) by approximately 53%

compared to that of the AL-treated seedlings alone.

Similarly, AL-treated seedlings showed an increase in the MDA content as compared to that of the control, while in SAAL-treated seedlings, the MDA content was significantly decreased ($P$<0.05) by approximately 87% compared to that of the AL-treated seedlings alone (Fig. 2B).

**Chlorophyll *a* fluorescence transients**

In comparison with the control, the maximal quantum of the photochemical yield of PSII ($F_v/F_m$) significantly decreased ($P$<0.05) by approximately 9% in the AL-treated seedlings. In contrast, the SAAL-treated seedlings showed a significant increase ($P$<0.05) in $F_v/F_m$ by approximately 11% compared to those seedlings that were treated with AL alone (Table 3).

The performance index on an absorption basis ($PI_{abs}$) of the AL-treated seedlings was considerably decreased ($P$<0.05) by approximately 21% compared to that of the control seedlings. In contrast, the SA pretreatment significantly increased ($P$<0.05) the $PI_{abs}$ value in the SAAL-treated seedlings by approximately 56% compared to that of the AL-treated seedlings (Table 3).

The SA treatment significantly decreased ($P$<0.05) the 1-$V_J$ parameter to that of the control. However, the AL treatment had no significant effect ($P$<0.05) on the 1-$V_J$ parameter as compared with the control (Table 3).

In AL treatment, the flux of photons absorbed by PSII antenna per reaction center (ABS/RC) and the excitonic flux trapped per reaction center (leading to $Q_A$ reduction) (TR0/RC) were significantly decreased ($P$<0.05) compared to those of the control (Fig. 3A, B). Similarly, the electron transporting beyond $Q_A^-$ per the active reaction center (ET0/RC) and the quantum yield of the electron transport beyond $Q_A^-$ (ET0/ABS) were significantly decreased ($P$<0.05) in the AL-treated seedlings (Fig. 3 D, E). The portion of the absorbed energy that was dissipated as heat and fluorescence (DI0/RC) was significantly increased ($P$<0.05) in the AL-treated seedlings. In comparison with AL treatment, the SAAL treatment showed a significant increase ($P$<0.05) in the TR0/RC, ET0/RC and ET0/ABS parameters as well as a significant decrease ($P$<0.05) in the DI0/RC parameter (Fig. 3B, C, D, E).

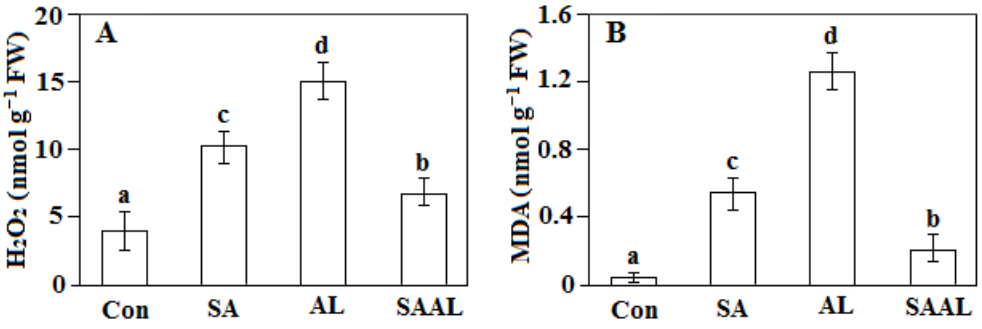


**Fig. 2. Effect of salicylic acid and alternation of light/dark periods treatments on $H_2O_2$ and MDA contents in leaves of tobacco seedlings. Con, without salicylic acid and without alternation of light/dark periods; SA, treatment with 10 µM salicylic acid; AL, alternation of light/dark periods; SAAL, pretreatment of salicylic acid and treatment with alternation of light/dark periods. Different letters indicate significant difference within each treatment according to an ANOVA-Tukey test at the 95% confidence level. Data are expressed as means ± SE (n=8).**

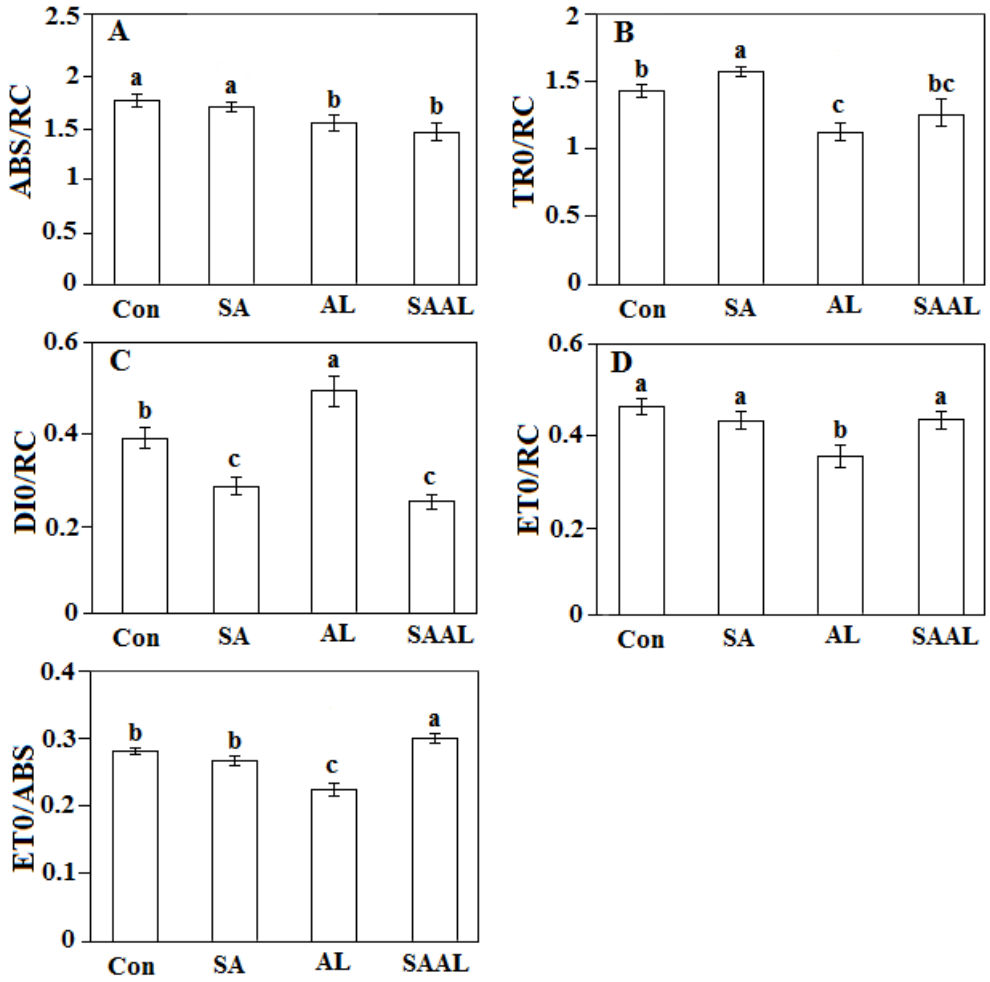


**Fig. 3. Effect of salicylic acid and alternation of light/dark periods treatments on ABS/RC, TR0/RC, DI0/RC, ET0/RC and ET0/ABS contents in leaves of tobacco seedlings. Con, without salicylic acid and without alternation of light/dark periods; SA, treatment with 10 µM salicylic acid; AL, alternation of light/dark periods; SAAL, pretreatment of salicylic acid and treatment with alternation of light/dark periods. Different letters indicate significant difference within each treatment according to an ANOVA-Tukey test at the 95% confidence level. Data are expressed as means ± SE (n=8).**

**Table 3: Chlorophyll *a* fluorescence intensity ($F_0$, minimum fluorescence; $F_m$, maximum fluorescence; $F_v/F_m$, maximum photochemical efficiency of PSII), the performance index ($PI_{abs}$) evaluated from the fast chlorophyll fluorescence curves (i.e., OJIP transients) in tobacco leaves subjected to four treatments.**

| Treatment | $F_0$ | $F_m$ | $F_v/F_m$ | $PI_{abs}$ | 1-$V_J$ |
|---|---|---|---|---|---|
| **Con** | 350 ± 35 b | 1672 ± 51 a | 0.79 ± 0.029 b | 1.15 ± 0.16 a | 0.35 ± 0.01 a |
| **SA** | 277 ± 10 c | 1651 ± 54 a | 0.83 ± 0.003 a | 1.26 ± 0.07 a | 0.31 ± 0.002 b |
| **AL** | 437 ± 43 a | 1598 ± 93 a | 0.72 ± 0.017 c | 0.91 ± 0.11 b | 0.36 ± 0.007 a |
| **SAAL** | 363 ± 41 ab | 1857 ± 98 b | 0.80 ± 0.017 b | 1.42 ± 0.15 a | 0.35 ± 0.01 a |

Con, without salicylic acid and without alternation of light/dark periods; SA, treatment with 10 µM salicylic acid; AL, alternation of light/dark periods; SAAL, pretreatment of salicylic acid and treatment with alternation of light/dark periods. Different letters indicate significant difference within each treatment according to an ANOVA-Tukey test at the 95% confidence level. Data are expressed as means ± SE (n=8).

**DISCUSSION**

The alternation of light/dark periods (AL) treatment, as a moderate stress, can improve tolerance of tobacco seedlings to photo-oxidative stress induced by herbicide (Darwish *et al*., 2014, 2015). Although AL has a positive effect, AL induced a stress condition that is observed by several diminutions in the physiological and biochemical parameters (Darwish *et al*., 2015). In the present study, the objective was to test whether pretreatment of tobacco seedlings with SA can reduce the negative effect of AL treatment.

The results confirmed that treating tobacco seedlings with SA treatment alone enhanced root dry weight and seedling length by 48% and 23%, respectively as compared to control seedlings. Spraying a low concentration of SA promoted seedling growth of *Hordeum vulgare* L., *Glycine max* L., *Sinapis alba* L., and *Zea mays* L., (Pancheva *et al*., 1996; Gutierrez-Coronado *et al*., 1998; Fariduddin *et al*., 2003; and Khodary, 2004). These positive changes in the growth parameters of SA seedlings promoted by SA were partially related to the efficiency of $CO_2$ fixing in the photosynthesis process. It has been confirmed that SA application leads to enhance the net photosynthetic rate, internal $CO_2$ concentration, stomatal conductance and transpiration rate in the plants of *B. Juncea*, corn, and soybean (Fariduddin *et al*., 2003; Khan *et al*., 2003). However, the data revealed that AL had no considerable effect on the growth of tobacco seedlings.

Our experiment showed that the SA and AL treatments reduced total chlorophyll content as well as increased carotenoids content by 16% and 9%, respectively compared to those of the control. The effect of these treatments on LHC content (Chl *a/b* ratio) was more pronounced in AL-treated seedlings (as evidence by the decrease of Chl *a/b* ratio) compared to SA-treated seedlings, where SA treatment had no significant effect ($P<0.05$) on the LHC content (Table 2). Moharekar *et al*. (2003) reported that the SA treatment induced a decrease in total chlorophyll and the intensity of this decrease was related to the concentration of the applied SA. The results in the present study was consistent with other researchers (Moharekar *et al*., 2003) who reported that the SA treatment promoted the carotenoids biosynthesis which have a mainly role to protect the photosynthetic apparatus from ROS generated under stress (Demmig-Adams, 1990).

The results of the present study indicated that AL induced moderate-oxidative stress, as evidenced by the reduction in the $F_v/F_m$ and $PI_{abs}$ (Table 3). This stress induced by AL caused alterations in the structure of

antenna pigments and its functions as well as the function of the reaction centers ($RC_s$) (reflected in changes in ABS/RC, TR0/RC, DI0/RC, ET0/RC and ET0/ABS) (Fig. 3). In this context, Ikeuchi *et al*. (2014) reported that fluctuating light condition by reducing the PsbS protein in LHCII caused an increase in thermal dissipation. This dissipating light energy as heat in turn was associated with photoinhibition process (Horton *et al*., 1996; Niyogi, 1999; Maxwell and Jonson, 2000). The impaired electron transport capacity consequently leads to the formation of $H_2O_2$ (Fig. 2A) at the level of PSI as a result of the decrease in the reduced NADPH (Murata *et al*., 2007; Darwish *et al*., 2014). This higher $H_2O_2$ leads to a considerable accumulation in the MDA content (Fig. 2B). A strong correlation has been reported between the hydrogen peroxide ($H_2O_2$) and lipid peroxidation (MDA) contents under oxidative stress (Roy Chowdhury and Choudhuri, 1985; Velikova *et al*., 2000; Verma and Dubey, 2003).

The SA treatment enhanced the capacity of tobacco seedlings to tolerate the oxidative stress induced by the AL treatment. The capacity of the SAAL-treated seedlings to effectively use the absorbed photons by antenna pigments, leading to the enhancement in the electron flux beyond $Q_A^-$, can be observed by the increase of $F_v/F_m$, $PI_{abs}$ (Table 3), TR0/RC, ET0/RC and ET0/ABS (Fig. 3B, D and E). This RCs efficiency of SAAL-treated seedlings led to the decrease of energy dissipating into heat (DI0/RC) (Fig. 3A).

In regards to $H_2O_2$ and MDA contents, SA treatment contributed to reducing the oxidative stress provoked by the high accumulation of $H_2O_2$ (Fig. 2A and B). In SAAL-treated seedlings, the considerable decreases in $H_2O_2$ and MDA contents confirmed that the seedlings were well protected from the oxidative damages effects induced by the AL treatment. The SA has been reported was found to enhance the activities of antioxidant enzymes, including APX, SOD and POX, which can increase the plant tolerance to environmental stresses (Krantev *et al*., 2008; Shi and Zhu, 2008; Hayat *et al*., 2008).

In summary, SA pretreatment of tobacco seedling increased PSII RCs efficiency, decreased heat dissipation after photons absorption, and decreased the accumulation of ROS which can protect the photosynthetic apparatus from direct effect of ROS induced by AL treatment.

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# تُحسن المعاملة الأولية بحمض الساليسيليك من تحمل بادرات التبغ للإجهاد المتسبب عن تردد فترات الضوء/الظل

**مجد محمد درويش***

## ملخص

درست التجربة تأثير المعاملة بحمض الساليسيليك (SA) في تحمل بادرات التبغ للإجهاد المتسبب عن تردد فترات الضوء/الظل (AL) (دورات من 16 دقيقة ضوء/8 دقيقة ظل ومعدل للإضاءة الفعالة (PPFD) 50 ميكرومول فوتون/مترمربع/ثانية لمدة ثلاثة أيام). تم تقييم كفاءة عملية التمثيل الضوئي باستخدام تقنية التوهج اليخضوري (chlorophyll *a* fluorescence) واختبار جيب (JIP-test). أظهرت النتائج بأن المعاملة AL قد خفضت من فاعلية النظام الضوئي الثاني (PSII activity) كما وأدت لزيادة مستوى الماء الأوكسجيني ($H_2O_2$) واللبيدات المتأكسدة (lipid peroxidation) المحسوبة على أساس قياس مركب المالونيل–دي الدهيد (MDA). هذا وحسنت المعاملة SA من معايير النمو النباتي. كما وخفضت المعاملة الأولية SA من الأثر المثبط للمعاملة AL على فاعلية النظام الضوئي الثاني؛ وكان ذلك ملحوظا من خلال زيادة الفاعلية الكوانتية الكيميائية–الضوئية العظمى للنظام الضوئي الثاني ($F_v/F_m$)، مؤشر الكفاءة الحيوية للنبات على اساس الامتصاص الضوئي ($PI_{abs}$)، معدل طاقة الإثارة التي تؤدي لأكسدة الكينون أ ($Q_A$) (TR0/RC)، معدل الإلكترونات المنقولة من طرف الكينون $Q_A^-$ (ET0/RC) والفاعلية العظمى المعبرة عن مساهمة فوتون ضوئي واحد من الفوتونات الممتصة في انتقال إلكترون طرف الكينون $Q_A^-$ (ET0/ABS). فضلاً عن ذلك، قادت المعاملة الأولية SA لتخفيض كمية الطاقة الضوئية الضائعة على شكل حرارة (DI0/RC) ولتراكم منخفض من الماء الأوكسجيني ($H_2O_2$) ومركب المالونيل–دي الدهيد (MDA). وهكذا، انخفضت الأضرار التأكسدية الملحوظة والمتسببة عن المعاملة AL في البادرات التي تم معاملتها مسبقاً بحمض الساليسيليك.

**الكلمات الدالة:** تردد فترات الضوء/الظل؛ معايير التوهج اليخضوري؛ حمض الساليسيليك؛ تحمل الإجهاد؛ التبغ.

**الاختصارات:** ABS، معدل الضوء الممتص من قبل النظام الضوئي الثاني؛ DI0، طاقة الإثارة المصرفة في النظام الضوئي الثاني؛ ET0، معدل الإلكترونات المنقولة؛ $F_0$، $F_v$، $F_m$، التوهج اليخضوري الأصغري، المتغير والأعظمي؛ $F_v/F_m$، الفاعلية الكيميائية–الضوئية الأعظمية للنظام الضوئي الثاني؛ $H_2O_2$، الماء الأوكسجيني؛ $LHC_s$، معقدات الصبغات كلوروفيل أ/ب الممتصة للضوء للنظام الضوئي الثاني؛ MDA، المالونيل–دي الدهيد؛ PPFD، الأشعة الضوئية الفعالة لعملية التمثيل الضوئي؛ $PI_{abs}$، مؤشر حيوية النبات على اساس الامتصاص الضوئي؛ PSII، النظام الضوئي الثاني؛ $Q_A$، الكينون الأولي المستقبل للإلكترونات في النظام الضوئي الثاني؛ RC، مركز التفاعل؛ ROS، أنواع الأوكسجين التفاعلية؛ TR0، معدل الطاقة الضوئية المكتسبة من قبل مركز التفاعل؛ $V_J$-1، مقياس للإلكترونات المنقولة بدءاً من الكينون $Q_A$.

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