Effets à long terme du prétraitement par une alternance de lumière/obscurité sur la tolérance au clomazone chez le tabac (*Nicotiana tabacum* L.)

Effet à long terme du prétraitement par une alternance de lumière/obscurité sur la tolérance au clomazone chez le tabac (*Nicotiana tabacum* L.)

Ce chapitre est présenté sous la forme d'un article soumis à *Pesticide Physiology and Biochemistry*.

Il a été montré qu'un prétraitement par l'alternance de cycles courts de lumière/obscurité (16 min/8 min) améliore la tolérance au clomazone de la variété *Virginie*, la variété la plus sensible. Les effets de ce prétraitement ont été évalués trois semaines plus tard et après deux semaines de traitement en présence de clomazone. Le prétraitement améliore le fonctionnement de la chaîne de transport des électrons, et a donc un effet de limitation du stress photooxydatif, ceci étant lié directement ou non à une stimulation des activités des enzymes antioxydantes.

L'objectif du travail présenté dans ce chapitre a été connaitre quelle est la durée de l'effet protecteur de ce prétraitement. Pour cela, des plantes de tabac, ayant ou non subi le prétraitement de lumière alternée, ont été cultivées en serre en présence de clomazone, jusqu'au stade de floraison.

Expérimentation

La variété de tabac *Nicotiana tabacum* L. *Virginie vk 51*, sensible au clomazone, a été utilisée. Une partie des plantules au stade trois feuilles ont été exposées pendant 3 jours à des cycles de lumière/obscurité de 16 min/8 min sous une intensité de 50 µmol m^{-2} s^{-1}, l'autre partie placée sous une alternance de 16 h/ 8h est utilisée comme contrôle. Après une semaine de croissance, les plantules ont été transférées dans des pots contenant un mélange de terreau et 100 µM de clomazone. Les plantes ont été placées en conditions de serre (à 28/24 ° C, 16 h/ 8 h de cycle jour/nuit et sous éclairage naturel).

Principaux résultats

Les plantes prétraitées par l'alternance manifestent une plus grande tolérance au clomazone et ceci au cours de toute leur croissance jusqu'au stade floraison. Cela se traduit par une augmentation de la taille, de la surface folaire et de l'accumulation de matière sèche. L'amélioration de la croissance s'accompagne d'une amélioration du taux d'assimilation du CO_2 (P_n et P_{max}) et de l'efficience photochimique du PSII.

Conclusion

Le prétraitement de trois jours au stade plantule a un effet protecteur à long terme vis-à-vis du clomazone qui perdure tout au long de la vie de la plante. Cet effet dit de "priming" pourrait potentiellement être utilisé pour améliorer la production ou la tolérance à d'autres stress chez d'autres espèces végétales.

Publication 5

Priming Effects of Short Alternating Light/Dark Periods Improve the Tolerance of Tobacco (*Nicotiana tabacum* L.) to the Herbicide Clomazone in Soil

Majd Darwish*, Félicie Lopez-Lauri, Huguette Sallanon

Laboratoire Physiologie des Fruits et Légumes (EA 4279), Université d'Avignon et des Pays de Vaucluse, Bât Agrosciences, 301 rue Baruch de Spinoza, BP 21239, F-84916 Avignon cedex 9, France

ABSTRACT

Nicotiana tabacum L. *cv. Virginie vk51* plantlets at the three-leaf stage that were grown in a hydroponic culture system were subjected to a photoperiod priming treatment with alternating light/dark periods (AL) (16 min light/8 min dark cycles and a photosynthetic photon flux density (PPFD) of 50 µmol photons m^{-2} s^{-1}) for 3 days. After 2 weeks, the plantlets were transferred to soil treated with 0 (control) or 100 µM clomazone. Compared with the non-primed plants, the AL-primed plants exhibited greater clomazone tolerance with respect to all of the growth parameters and during all of the growth stages. This AL priming effect was demonstrated by increases in plant leaf area and dry and fresh plant weights, which might have been related to increases in the CO_2 assimilation rate (P_n and P_{max}). Furthermore, the reduced photon absorption (ABS/RC) and heat dissipation (DI0/RC) rates as well as the observed increase in photosystem II (PSII) efficiency (the maximum quantum yield of PSII (F_v/F_m), photochemical quenching (*q*P), actual PSII efficiency (Φ_{PSII})) and electron transport (the light-saturated electron transport rate (ETR) and electron flux beyond the first quinone electron acceptor of PSII (Q_A) evaluated as ($1–V_J$)) provide strong evidence of a higher tolerance to clomazone. Collectively, these results suggest that AL priming treatment could potentially improve the protection or production of other transplanted species.

Keywords: Clomazone; Chlorophyll *a* fluorescence transients; *Nicotiana tabacum* L.; Photosynthesis; Photoperiod priming; Tolerance

Abbreviations used: ABS, PSII light absorption flux; ^{3}Chl*, chlorophyll triplet state; DI0, PSII excitation dissipation flux; DXP, 1-deoxy-d-xylulose 5-phosphate; ET0, electron

Transport flux; ETR, photosynthetic electron transport rate; 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; LHCII, light-harvesting complex II; $^1O_2$, singlet oxygen; $^3O_2$, oxygen triplet state; O_2^-, superoxide radical; PCD, programmed cell death; PI_{abs}, performance index on absorption basis; P_n, net photosynthetic rate; PPFD, photosynthetic photon flux density; PQ, plastoquinone; PSII, photosystem II; Φ_{PSII}, actual PSII efficiency; Q_A, primary quinone electron acceptor of PSII; *q*P, photochemical quenching; RC, reaction center; ROS, reactive oxygen species; RuBP, ribulose-1,5-bisphosphate; TR0, PSII light energy flux trapping; $1\text{-}V_J$, a measure of electron flux beyond Q_A.

1. Introduction

The use of herbicides is a matter of recent concern because only a small fraction of these chemicals reach the target plants [1], with the remaining herbicide potentially impacting non-target plants. Although herbicides are important in agriculture, they act as chemical pollutants in certain circumstances and can deteriorate soil and threaten non-target plants. Herbicide concentrations may vary from a few µg to mg per kg soil because most of the applied chemicals are retained within the top 5 cm of soil [2]. Herbicides in soil, even at very low concentrations, can affect the physiological and biological processes of non-target plants.

One of the herbicides commonly used in agriculture for weed control is clomazone [2-(2-chlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one] [3]. Its recommended use ranges from 280 to 1100 gha^{-1} for the control of many grass species in crop fields (Brown and Masiunas) [4]. The pretransplant application of clomazone at a rate of 720 gha^{-1} causes transient crop injury [5], and when applied pre-emergence, it causes 5%, 4%, and 1% visual injury at 1116 gha^{-1} and 11%, 10%, and 4% visual injury at 2232 gha^{-1} in dry beans at 7, 14, and 28 days after emergence, respectively [6]. Clomazone can remain for up to 130 days in agricultural water and has been detected in 90% of water samples collected from fields near rice cultivation regions [7]. It has been reported that clomazone reduces photosynthesis rates in soybean and barley seedlings as a result of a reduction in photosynthetic pigment biosynthesis [8,9]. Clomazone is converted to 5-OH clomazone and subsequently to 5-keto clomazone in the soybean [10]. This 5-keto clomazone derived from clomazone oxidation has been reported to inhibit 1-deoxy-d-xylulose 5-phosphate (DXP) synthase, which is the enzyme catalyzing the first committed step in the chloroplastic isoprenoid pathway [11]. In this context, the decrease in carotenoid synthase represents the diminished activity of the system that protects the structural and functional components of the thylakoids against photooxidative stress. Darwish et al. [12,13] have reported that clomazone leads to photosystem II (PSII) photooxidative damage, which might be caused by a reduction in light-harvesting complex II (LHCII) concentration, the redox state of the plastoquinone (PQ) pool or a decrease in reactive oxygen species (ROS) detoxification in the leaves of tobacco plants. Yasuor et al. [14] have demonstrated that the mechanism underlying clomazone resistance is part of a complex multifactorial process in which the level of oxidative metabolism in conjunction with enzymatic conjugation and photooxidative damage mitigation endow *Echinochloa phyllopogon*, and likely other organisms, with efficient resistance to clomazone herbicides.

Similarly to other herbicides that affect PSII, clomazone induces the generation of ROS by stimulating charge recombinations in PSII. This process promotes the formation of the chlorophyll triplet state (^{3}Chl*) and generation of ROS, such as $^1O_2$, O_2^- and H_2O_2 [13,15,16]. However, the build-up of H_2O_2 as a result of superoxide dismutase (SOD) activity caused by the dismutation of O_2^- generated at the level of PSI is the primary pathway for ROS accumulation, which was described by Darwish et al. [13]. This accumulation of ROS leads to PSII photodamage, inducing programmed cell death (PCD) [17,18]. The ROS generated by the inhibition of photosynthetic electron transport or energy transfer respond primarily to PSII photodamage by inhibiting its repair [19]. In turn, the photodamaged PSII can be detected by chlorophyll *a* fluorescence and JIP-test parameters [13,20,21]. The JIP test has been developed as a sensitive indicator of stress caused by changes in environmental conditions [12,13, 22-26].

Recently, Darwish et al. [13] revealed that an alternating light/dark period pretreatment (AL) (16 min/8 min of light/dark cycles for 3 days) improves tobacco tolerance *via* the stimulation of antioxidant activity that protects the photosynthetic apparatus from the effects of ROS and relieves excess photon stress. A priming process involving temporally limited exposure to an environmental stimulus that can prepare the plant to more successfully cope with future environmental stimuli has been suggested as a potential strategy to increase plant tolerance to several abiotic stresses, including salt stress in *Lactuca sativa* [27] and temperature stress in *Triticum aestivum* [28,29].

In our work presented here, we hypothesized that priming tobacco plantlets by pre-exposure to AL treatments can have long-term effects that improve the growth and tolerance of tobacco plants cultivated in clomazone-treated soil and field conditions.

In this regard, the experiments conducted in this study were designed to simulate AL-pretreated tobacco plant growth (at the 3-leaf stage under growth chamber conditions) following transplantation to fields, which is where the agricultural use of clomazone occurs. For this purpose, we focused on modifications to the growth parameters, gas exchange, and chlorophyll *a* fluorescence in addition to JIP-test responses to investigate (i) whether the AL priming treatment affected the function of the electron transport chain and CO_2 assimilation, and whether these effects resulted in the improvement of tobacco plant growth; and (ii) whether the effects of the AL priming treatment were sustained between the 3-leaf stage and flowering to demonstrate its efficiency.

2. Materials and methods

2.1. Plant materials, growth conditions and chemical treatment

Nicotiana tabacum L. *cv. Virginie vk51* seeds were used in this study, and they were germinated in a plastic container containing sterilized potting soil for 2 weeks. Germination was conducted under sterile conditions in an environmentally controlled growth chamber at 22/17 °C under a 16 h/8 h light/dark cycle and photosynthetic photon flux density (PPFD) of 50 µmol photons m^{-2} s^{-1}, according to the method of Darwish et al. [12]. After 5 weeks of germination, the plantlets (three-leaf stage) growing in a hydroponic system containing Auckland's nutritive solution were exposed [or not] to an AL cycle (16 min/8 min of light/dark and a PPFD of 50 µmol photons m^{-2} s^{-1}) for 3 days; the other plantlets were not treated with AL and were used as controls as described by Darwish et al. [13]. After a week, the plantlets were transferred into pots containing soil treated with clomazone and grown under greenhouse conditions (at 28/24 °C, 16 h/8 h light/dark cycle and PPFD of 500 µmol photons m^{-2} s^{-1}). Clomazone (CL) (Sigma-Aldrich, St. Louis, MO, USA) was added to the pots at concentrations of 0-100 µM for the following treatments: (i) Con (Control, without alternating light/dark periods or CL); (ii) CL (100 µM CL); (iii) AL (alternating light/dark periods); and (iv) AL+CL (alternating light/dark periods with 100 µM CL). The irrigation and fertilization systems were applied in accordance with the agronomic characteristics of the tobacco plants.

2.2. Determination of growth parameters

Plant growth was determined by the leaf area, length and fresh and dry plant weights. The leaf area and plant length were measured three times during the tobacco growth period: after 15 days (early stage (ES)), 30 days (intermediate stage (IS)) and 60 days (harvesting stage (HS)) of growth in a greenhouse. The fresh and dry weights of the plant were determined at the beginning of flowering (at the end of tobacco vegetative growth (HS)). The leaf area was calculated using the Image J software.

2.3. Determination of chlorophyll *a* fluorescence transients

The PSII efficiency can be evaluated based on the fast and slow kinetics of chlorophyll *a* fluorescence induction. Fast chlorophyll *a* fluorescence transients are determined at the ES and IS of tobacco growth. Tobacco leaves with 8 plantlets were dark-adapted for 20 min, and

then chlorophyll *a* fluorescence (expressed in relative units) was measured using a portable Handy-PEA fluorometer (Hansatech Ltd., Kings Lynn, UK). Illumination at a light intensity of 3,000 µmol m^{-2} s^{-1} was used to determine the chlorophyll *a* fluorescence in leaves. The maximum photochemical quantum yield of PSII or quantum yield of the open centers of the PSII parameter (F_v/F_m= (F_m–F_0)/F_m) and performance index based on absorption (PI_{abs}) were calculated automatically [20,30]. The JIP-test developed by Strasser et al. [31] was used for data analysis. This model describes the manner by which the energy produced by the flux of photons that are absorbed by photosynthetic antenna pigments (ABS) is dissipated as heat and fluorescence (DI) or is transported as trapped flux (TR) by PSII reaction centers (RCs) (leading to Q_A reduction) for its conversion to redox energy by reducing plastoquinone Q_A to Q_A^-. Q_A^- is then reoxidized to Q_A, and the generation of electron transport flux (ET) leads to CO_2 fixation [32]. These fluxes are described in terms of a specific energy flux (per RC) or as proportions of other fluxes (ratios or yields). The calculation of the energy flux and ratios of a specific flux have been fully explained by Strasser and his coworkers [22,33,34], see the appendix. The effective quantum yield of PSII (Φ_{PSII}= (F_m'–F_s)/F_m') and photochemical quenching coefficient of *q*P= (F_m'–F_s)/(F_m'–F_0') [35] were measured using a leaf chamber fluorometer (LI-6400-40 LCF, LI-Cor, Lincoln NE, USA) at a PPFD of 500 or 1500 µmol photons m^{-2} s^{-1}. The light-saturated electron transport rate (ETR) through PSII was calculated as follows: ETR= Φ_{PSII} × PPFD × A × 0.5 [36], where A is the proportion of incident PPFD absorbed by the sample, and the value 0.5 accounts for the presence of two photosystems, assuming their equal involvement in the linear electron flow.

2.4. Determination of gas exchanges

The net and maximum photosynthetic rates (P_n; P_{max}) were measured using a portable photosynthetic analyzer (LI-Cor, Lincoln, NE, USA) equipped with a leaf chamber fluorometer (LI-6400-40 LCF). The sample leaf was placed in a cuvette that was maintained at an ambient temperature and humidity and exposed to a PPFD of 500 µmol photons m^{-2} s^{-1} and ambient CO_2 concentration of 400 µmol mol^{-1} for (P_n) or PPFD of 1500 µmol photons m^{-2} s^{-1} and ambient CO_2 concentration of 2000 µmol mol^{-1} (P_{max}) when using an LI-6400-01 CO_2 injector with a high-pressure liquid CO_2 cartridge source.

2.5. Statistical Analysis

The statistical analysis was performed with the R statistical software using an analysis of variance (ANOVA) and Tukey's test. The results were displayed as the means of 8 replicates ± standard error (SE). The results were considered significant at a value of $P<0.05$.

3. Results

3.1. Plant growth

The negative effect of the CL treatment on the leaf area was shown at all stages of plant growth by significant decreases ($P<0.05$) of approximately 46%, 15% and 26% compared with the leaf area of the control plants. The AL pretreatment was able to improve the plant growth and prevent the negative effects of CL on the plant leaf area in the AL+CL treatment compared to that of the control plants (Fig. 1A).

The CL plant lengths decreased compared to that of the control, and this decrease was greater during the ES (approximately 33%) than the IS and HS of plant growth (approximately 18% and 16%, respectively). The AL pretreatment enhanced the lengths of the AL+CL plants, which were not significantly different ($P>0.05$) compared with the length of the control plants (Fig. 1B).

The dry and fresh weights of the CL plants significantly decreased ($P<0.05$) by approximately 33% and 27%, respectively, compared with those of the control plants. These decreases were not observed in the AL+CL plants, which were not significantly different ($P>0.05$) than the control plants (Fig. 1C and D).

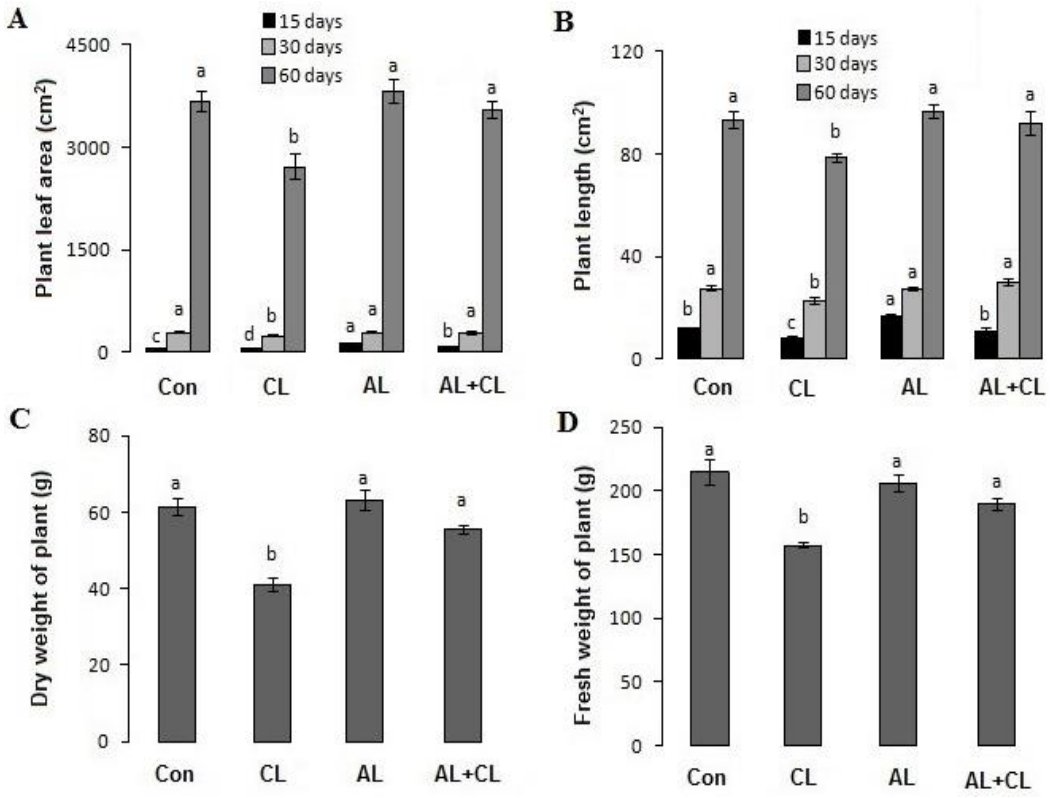


Fig.1. Effect of clomazone and alternation of light/dark periods on the leaf area, length and fresh and dry tobacco plant weights. Measurements were performed at 15, 30 and 60 days of the plant growth. Con, without alternation of light/dark periods, or clomazone; CL treatment with 100 µM clomazone; AL, alternation of light/dark periods; AL+CL, pretreatment with alternation of light/dark periods+ 100 µM clomazone treatment. 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.

3.2. Chlorophyll *a* fluorescence transients

The maximum photochemical quantum yield of PSII (F_v/F_m) significantly decreased (P<0.05) in the CL plants during the ES and IS of plant growth by approximately 3% and 4%, respectively, compared with the yield of the control plants. In contrast, the F_v/F_m parameter of the AL+CL plants showed a significant increase (P<0.05) during these stages of approximately 4% compared with that of the CL plants, and no significant differences (P>0.05) were observed in this parameter between the AL+CL and control plants (Table 1).

Table 1. Effect of clomazone and alternation of light/dark periods treatments on chlorophyll *a* fluorescence intensity (F_0, minimum fluorescence; F_m, maximum fluorescence; F_v/F_m, maximum quantum yield of PSII) and on the performance index (PI_{abs}) evaluated from the fast chlorophyll flourescence curve (i.e., OJIP transients). Measurments were performed at 15 and 30 days of tobacco plant growth. Con, without alternating of light/dark periods, or clomazone; CL, treatment with 100 µM clomazone; AL, alternation of light/dark periods; AL+CL, pretreatment of alternation of light/dark periods+ 100 µM clomazone treatment. 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.

Treatment	Growth (days)	F_0	F_m	F_v/F_m	PI_{abs}	1-V_J
Con	**15**	263 ± 2.7 a	1422 ± 3.1 a	0.81± 0.001 b	1.5 ± 0.02 b	0.34 ± 0.001 a
	30	264 ± 3.6 a	1715 ± 18.6 c	0.85 ± 0.002 a	2.2 ± 0.13 a	0.34 ± 0.006 a
CL	**15**	301 ± 5.9 b	1576 ± 10.2 b	0.78 ± 0.001 c	1.1 ± 0.05 c	0.31 ± 0.005 b
	30	303 ± 8.9 b	1778 ± 47.6 c	0.82 ± 0.003 b	1.6 ± 0.33 b	0.32 ± 0.005 b
AL	**15**	278 ± 5.5 a	1522 ± 22.5 b	0.82 ± 0.001 b	1.5 ± 0.01 b	0.34 ± 0.001 a
	30	270 ± 7.3 a	1789 ± 45.1 c	0.85 ± 0.001 a	2.5 ± 0.06 a	0.35 ± 0.001 a
AL+CL	**15**	320 ± 10.5 b	1702 ± 45.2 bc	0.81 ± 0.001 b	1.4 ± 0.04 b	0.33 ± 0.002 ab
	30	262 ± 4.5 a	1729 ± 21.1 c	0.85 ± 0.001 a	2.1 ± 0.10 a	0.34 ± 0.003 a

Compared with the control plants, the CL treatment lowered the performance index (PI_{abs}) that was calculated based on the energy absorption during the ES and IS of plant growth. The AL pretreatment enhanced the PI_{abs} values by approximately 27%, and this value increased by 33% in the AL+CL plants during the same growth stages; however, these values were not significantly different (P>0.05) compared with the values of the control plants (Table 1).

The 1–V_J parameter was used as an indicator of electron transport at the acceptor side (Q_A) of PSII. The AL pretreatment reduced this negative effect of CL during the ES and IS of plant growth, and the 1–V_J values significantly increased (P<0.05) in the AL+CL plants compared with that of the CL plants (Table 1).

In the CL plants, the total influx of excitation transferred to the reaction center (ABS/RC) and excitonic flux trapped per reaction center (leading to Q_A reduction) (TR0/RC) significantly increased (P<0.05) compared with that of the control plants. These increases were significant (P<0.05) during the ES and IS. Similarly, the portion of absorbed energy that was dissipated as heat and fluorescence (DI0/RC) significantly increased (P<0.05) in the CL plants (Table 2). In contrast, the AL+CL plants showed significant decreases (P<0.05) in the ABS/RC, TR0/RC and DI0/RC parameters during ES and IS compared with that of the CL plants (Table 2). The quantum yield of the electron transport beyond Q_A^- (ET0/ABS) significantly increased (P<0.05) in the AL+CL plants during ES compared with that of the CL plants (Table 2). However, the ABS/RC, TR0/RC, DI0/RC, ET0/RC and ET0/ABS parameters

showed no significant differences (P>0.05) in the AL+CL plants during the same stages of plant growth compared with that of the control plants (Table 2).

Table 2. Effect of clomazone and alternation of light/dark periods treatments on ABS/RC, TR0/RC, DI0/RC, ET0/RC and ET0/ABS. Measurments were performed at 15 and 30 days of tobacco plant growth. Con, without alternating of light/dark periods, or clomazone; AL, alternation of light/dark periods; CL, treatment with 100 µM clomazone; AL+CL, pretreatment of alternation of light/dark periods+ 100 µM clomazone treatment. 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.

Treatment	Growth (days)	ABS/RC	TR0/RC	DI0/RC	ET0/RC	ET0/ABS
Con	**15**	1.6 ± 0.01 b	1.3 ± 0.01 b	0.29 ± 0.002 b	0.44 ± 0.002 b	0.28 ± 0.001 a
	30	1.3 ± 0.05 a	1.1 ± 0.04 a	0.20 ± 0.008 a	0.37 ± 0.007 a	0.29 ± 0.005 a
CL	**15**	1.9 ± 0.03 c	1.5 ± 0.02 c	0.36 ± 0.012 c	0.47 ± 0.003 c	0.25 ± 0.005 c
	30	1.5 ± 0.02 b	1.2 ± 0.02 b	0.25 ± 0.002 b	0.39 ± 0.004 a	0.27 ± 0.003 b
AL	**15**	1.5 ± 0.01 b	1.3 ± 0.01 b	0.28 ± 0.002 b	0.43 ± 0.001 b	0.28 ± 0.005 a
	30	1.2 ± 0.02 a	1 ± 0.02 a	0.17 ± 0.003 a	0.35 ± 0.005 a	0.29 ± 0.001 a
AL+CL	**15**	1.5 ± 0.02 b	1.2 ± 0.02 b	0.29 ± 0.004 b	0.41 ± 0.004 b	0.27 ± 0.002 b
	30	1.3 ± 0.03 a	1.1 ± 0.02 a	0.20 ± 0.006 a	0.37 ± 0.004 a	0.28 ± 0.003 a

At a PPFD of 500 µmol photons m^{-2} s^{-1}, the photosynthetic ETR, photochemical quenching of the variable chlorophyll fluorescence (qP) and actual photochemical efficiency of PSII (Φ_{PSII}) noticeably decreased (P<0.05) in the CL plants during the ES and IS compared with the control plants (Fig. 2A, B and C). In contrast, the ETR, qP and Φ_{PSII} values significantly increased (P<0.05) in the AL+CL plants at the same stages of the plant growth compared with the CL plants (Fig. 2A, B and C). However, the AL and AL+CL plants showed higher values for these parameters during the IS stage compared with the controls (Fig. 2A, B and C). At a PPFD of 1500 µmol photons m^{-2} s^{-1}, the CL plants showed no significant differences (P>0.05) in these parameters during the ES and IS compared with that of the control plants, whereas these parameters increased in the AL plants compared with the control plants at the same stages of growth (Fig. 2A, B and C).

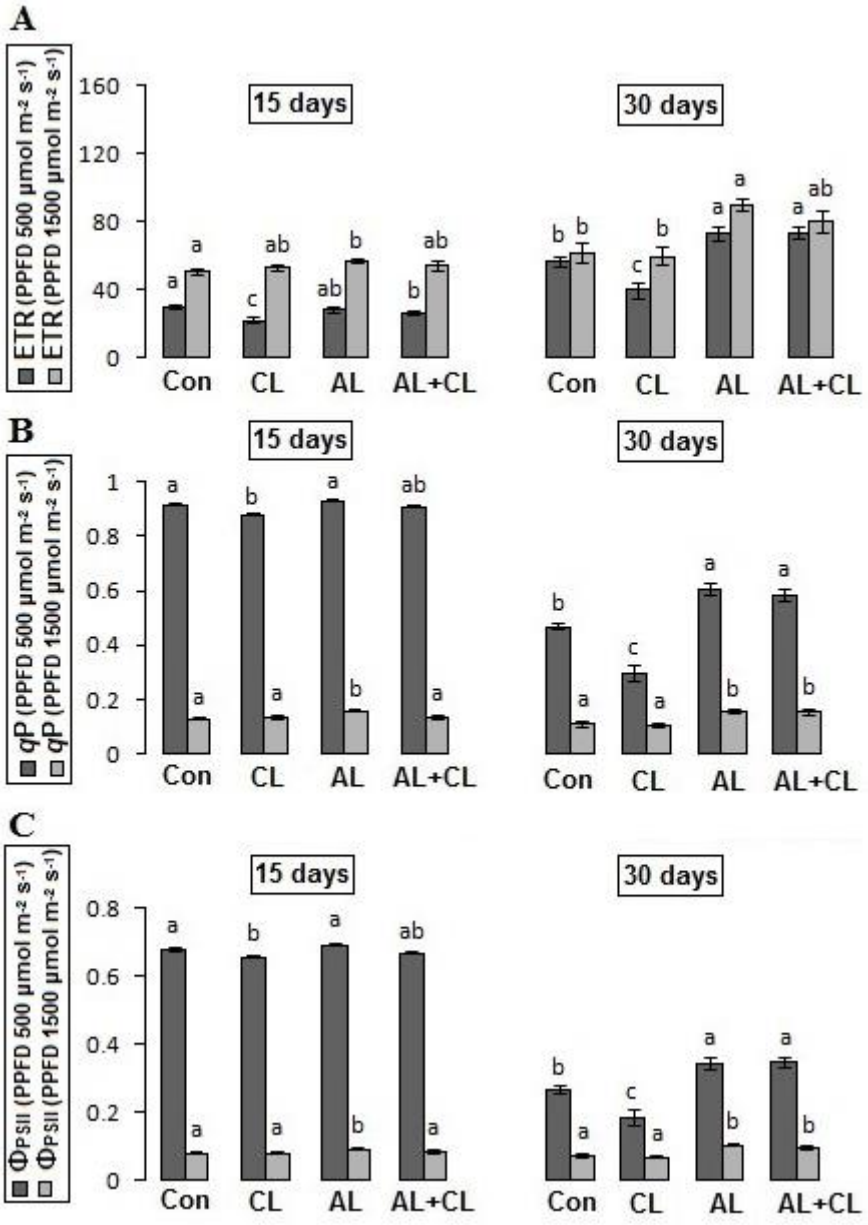


Fig.2. The photosynthetic electron transport rate (ETR), the photochemical quenching coefficient (*q*P) and the actual PSII efficiency (Φ_{PSII}) in the control, alternation of light/dark periods and clomazone treatments of tobacco plant (*Nicotiana tabacum* L. *cv.Virgenie vk51*). Measurements were performed at 15 and 30 days of the plant growth. For measuring, tobacco leaves were exposed to a PPFD of 500 µmol m^{-2} s^{-1} or to a PPFD of 1500 µmol m^{-2} s^{-1}. 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.

3.3. Gas exchanges

The net CO_2 assimilation (P_n) decreased in the CL plants during the ES and IS (by approximately 26% and 21%, respectively, compared with the control plants). In contrast, the

AL pretreatment led to an increase in the net photosynthesis rate (P_n) of approximately 27% and 43%, respectively, in the AL+CL plants during the same stages of growth compared with the CL plants (Fig. 3).

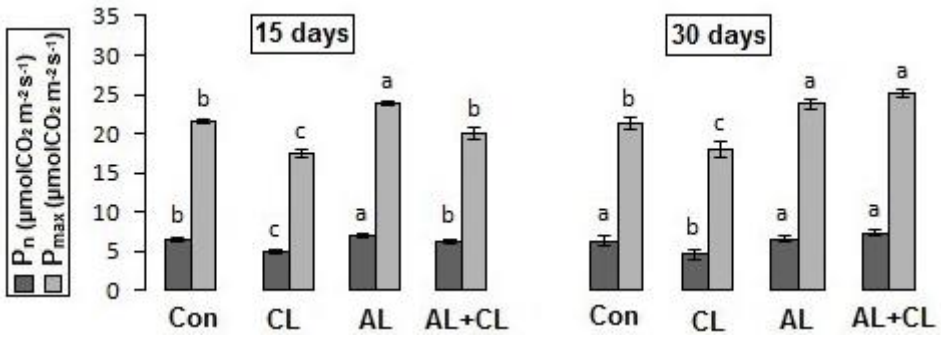


Fig.3. The net and max CO_2 assimilation (P_n; P_{max}) in the control, alternation of light/dark periods and clomazone treatments of tobacco plant (*Nicotiana tabacum* L. *cv.Virgenie vk51*). Measurements were performed at 15 and 30 days of the plant growth. For measuring, tobacco leaves were exposed to a PPFD of 500 µmol m^{-2} s^{-1} and ambient CO_2 concentration of 400 µmol mol^{-1} for (P_n) or to a PPFD of 1500 µmol m^{-2} s^{-1} and ambient CO_2 concentration of 2000 µmol mol^{-1} for (P_{max}). 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.

The max CO_2 assimilation (P_{max}) was observed at low levels in the CL plants during the ES and IS compared with the control plants (Fig. 3). The AL pretreatment enhanced the P_{max} by approximately 15% and 40% in the AL+CL plants during the ES and IS, respectively, compared with the CL plants (Fig. 3). However, the P_{max} of the AL plants increased by approximately 11% and 10% during the ES and IS, respectively, compared with the control plants (Fig. 3).

4. Discussion

The results showed that the CL treatment reduced the growth parameters during all stages of plant growth. This effect was clearly pronounced during the early stage of growth, with CL inducing noticeable decreases of approximately 46% and 33% in the leaf areas and plant lengths, respectively, compared with the control plants (Fig. 1A and B). These data indicate that CL stress is more potent during the early stages of growth. Although this stress has little effect during the intermediate stage of the growth, the resulting deterioration in the dry and fresh plant weights that occurred during the HS were fairly high, with observed decreases of

approximately of 33% and 27%, respectively, compared with the control plants (Fig. 1C and D).

In contrast, the AL pretreatment induced an improvement in plant growth and inhibited the effects of CL. These priming effects of the AL pretreatment were observed under all growth parameters and during all stages of plant growth and may have been caused by the following factors: (i) the efficiency of the protection of tobacco plants from the excess photon stress induced by CL (see ABC/RC, TR0/RC and DI0/RC (Table 2)); and (ii) the efficiency of CO_2 fixation in the photosynthesis apparatus (see *q*P and Φ_{PSII} (Fig. 2B and C), and P_n (Fig. 3)). However, a comparison of the IS and ES of plant growth indicated that the decreases in *q*P and Φ_{PSII} observed for all treatments could have resulted from the effects of the leaf and plant age on the chlorophyll fluorescence kinetics [37], which was not the focus of our work presented here.

The F_v/F_m parameter is used as an indicator of the stress state, and the decrease in F_v/F_m could have resulted from PSII photoinhibition [13,20,38-40]. We believe that the decrease in F_v/F_m in the CL plants (Table 1) was a result of photoinhibitory damage at the acceptor side of PSII [12,13]. In the absence of an active acceptor of electrons further than Q_A^- (see the reduction of $1–V_J$, Table 1) and considering the increases of excitation energy (ABS), the trapped rate (TR0) and electron transport (ET0) by the active (Q_A^- reducing) RC induced the accumulation of Q_A^- and reduction of the PQ pool, consequently increasing the dissipation of energy as heat (DI0/RC) (Table 2). This effect was also demonstrated by the observed decrease in the PI_{abs}, which is considered to be a sensitive indicator of the vitality of plants under stress conditions [12,26,41].

In contrast, the AL pretreatment enhanced the photosynthesis efficiency and systemic capacity of the plants, allowing the plants to avoid the photooxidative stress induced by CL. The results also showed that the AL+CL plants had an increased ability to utilize the absorbed photons, converting them into redox energy and generating the electron transport (ET). This ability in the presence of active electron acceptors in the photosynthetic apparatus leads to increased CO_2 assimilation. This PSII efficiency explains the increases in F_v/F_m, PI_{abs}, *q*P, Φ_{PSII}, and ET0/ABS and decrease in DI0/RC that were observed. Moreover, enhanced electron flux beyond Q_A^-, reduced accumulation of the Q_A^- pool and regulated redox state of the PQ pool were also observed (see the increase in $1–V_J$ (Table 1), ET0/RC (Table 2) and ETR (Fig. 2A)).

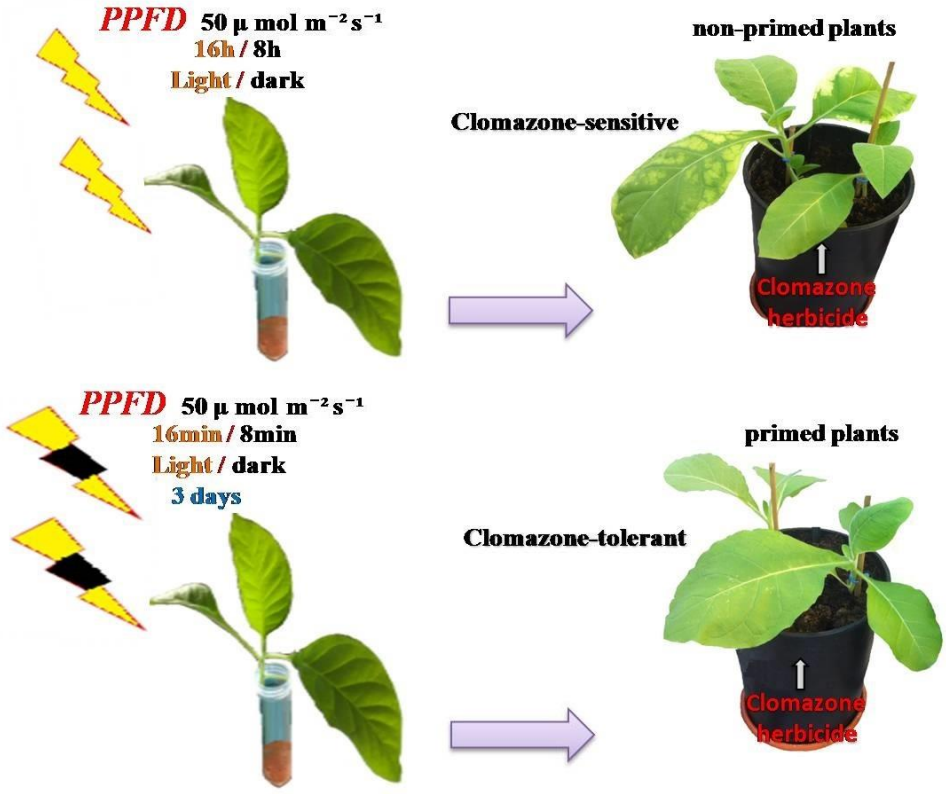
CL induced strong P_n and P_{max} inhibition during the early and intermediate stages of growth (Fig. 3). The decreases in P_n and P_{max} in the CL plants were not linked to stomatal

conductance (g_s), and the CL treatment had no significant effect (P>0.05) on the g_s compared with the control plants (data not shown). During these stages, the P_n and P_{max} rates were strongly limited by photosynthetic ET in the treated plants. Furthermore, the decreases in these values may have been caused by the effects of PSII photoinhibition induced by CL (see PI_{abs}, ABS/RC, TR0/RC and DI0/RC, Tables 1, 2). This photoinhibitory process led to a reduction in photosynthetic ET (see $1–V_J$, Table 1) and ETR (Fig. 2A) that decreased the NADPH and ATP concentrations, RuBP regeneration and CO_2 assimilation rates (see the P_n and P_{max}, Fig. 3) as a result of the inhibition of photodamaged PSII repair [9,42]. A tight correlation between ATP synthesis and PSII repair confirms that the synthesis of ATP might regulate the repair of PSII damage [43].

Considering the positive priming effects of the AL pretreatment on the growth of the CL-treated plants, the improved systemic capacity of the plants to CL tolerance under the AL pretreatment is important. The AL-primed plants may be protected against the CL-induced photooxidative stress through the stimulation of ROS detoxification enzymes, which was reported by Darwish et al. [13]; thus, the damage to the PSII reaction centers is decreased because of the repair process of the photodamaged PSII. Furthermore, the increased rate of CO_2 assimilation might have been caused by an improvement of PSII efficiency (see the increase in ETR and Φ_{PSII}, Fig. 2A and C) as well as RuBP regeneration and CO_2 fixation rate.

Taken together, these results demonstrate that the AL pretreatment effectively improved the long-term tolerance of the tobacco plants to the herbicide CL during plant growth. In addition, these data showed that the AL plants had a high level of physiological vitality, which was revealed by their greater PSII efficiency and photosynthetic capacity.

In conclusion, the use of AL priming to improve CL tolerance in tobacco is a topic of recent research that should be investigated further using other transplanted species and additional pretransplant-applied herbicides.



The priming effect of short alternation light/dark periods to protect of tobacco plants against the photooxidative stress provoked by clomazone.

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6. References

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