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Modulation of Lettuce (Lactuca sativa L.) Resistance to Botrytis cinerea Under Short-Term Alternating Light/Dark Conditions

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Abstract

This study evaluated the effects of short-term alternating light/dark (AL) cycles on photosynthetic performance and induced resistance of lettuce (*Lactuca sativa* L.) against *Botrytis cinerea*. Plants were exposed to AL regimes (16 min light/8 min dark, 100 µmol photons m^{-2} s^{-1}) for three consecutive days, and physiological and pathological assessments were conducted immediately after treatment (D0), three days (D3), and ten days (D10) post-treatment. Measured variables included tissue water status, dry matter accumulation, chlorophyll *a* fluorescence parameters, OJIP transient-derived indices, and disease severity following fungal inoculation. AL-treated plants displayed significantly higher dry matter content, enhanced PSII photochemical efficiency (Fv/Fm, PI_abs), and a marked reduction in lesion expansion compared with control plants. OJIP analysis revealed increased trapping and electron transport fluxes per reaction center (TR_0/RC, ET_0/RC), accompanied by decreased energy dissipation (DI_0/RC), indicating improved PSII functional integrity. Collectively, these results demonstrate that short-term alternating light/dark cycles act as a priming stimulus, conferring enhanced resistance to *B. cinerea*, likely mediated by improved photosynthetic efficiency and optimized physiological status. This work highlights rhythmic light modulation as a sustainable and innovative strategy for disease management in controlled-environment horticulture.

Introduction

Botrytis cinerea is a widespread necrotrophic fungal pathogen responsible for gray mold disease in a broad range of economically important crops, including lettuce (*Lactuca sativa*). Current management strategies rely predominantly on chemical fungicides; however, their intensive use raises serious environmental, health, and resistance-related concerns. These limitations have driven increasing interest in sustainable, non-chemical approaches aimed at enhancing plant innate resistance to pathogens (Poveda et al., 2020).

Light represents a major environmental cue regulating plant growth, development, and physiological acclimation, and it has emerged as a key modulator of plant immune responses. Accumulating evidence indicates that light quality, intensity, and photoperiod profoundly influence plant susceptibility to pathogens by shaping defense signaling networks and metabolic status (Krishna, 2024). Light-mediated regulation of photosynthesis, redox homeostasis, and hormone signaling has been proposed as a central mechanism linking light perception to plant immunity.

In this context, alternating light/dark regimes have gained attention as a potential priming stimulus. Darwish et al. (2014) demonstrated that short-term exposure of *Nicotiana tabacum* L. seedlings to alternating light/dark cycles (16 min light/8 min dark) over three days significantly enhanced tolerance to the herbicide clomazone. This response was associated with improved photosystem II performance, elevated antioxidant capacity, and activation of defense-related pathways, suggesting that rhythmic light modulation can induce a primed physiological state potentially transferable to biotic stress resistance.

Subsequently, Darwish et al. (2015) demonstrated that comparable alternating light/dark treatments enhanced the tolerance of Nicotiana tabacum to clomazone-induced photooxidative stress. This improved tolerance was associated with more efficient ROS scavenging and a higher accumulation of phenolic compounds. These results further reinforce the hypothesis that light-mediated priming can trigger systemic defense responses, including pathways related to systemic acquired resistance (SAR), ultimately enhancing plant immunity against necrotrophic pathogens.

Beyond alternating light regimes, several studies have highlighted the impact of specific light spectra on plant–*B. cinerea* interactions. Meng et al. (2020) showed that red LED illumination enhanced resistance of strawberry plants to *B. cinerea* through modulation of defense-related gene expression. Conversely, Courbier et al. (2021) demonstrated that far-red light exposure suppressed tomato defense responses, underscoring the complexity and context-dependence of light-mediated regulation of plant immunity.

A deeper understanding of plant light signaling pathways, their integration with immune regulation, and the strategies employed by pathogens to manipulate host light-responsive networks may provide novel opportunities for developing broad-spectrum and environmentally friendly disease control strategies. Moreover, increasing insights into light perception by fungal pathogens themselves suggest that manipulation of light regimes in controlled environments could represent an innovative tool for disease prevention (Breen et al., 2023).

Against this background, the present study aims to assess the effects of short-term alternating light/dark cycles on the resistance of lettuce (*Lactuca sativa*) to *B. cinerea*. Particular emphasis is placed on physiological and photosynthetic parameters, including chlorophyll fluorescence characteristics, water status, and disease progression, to elucidate the mechanisms underlying light-induced priming in this crop species.

Materials and Methods

Plant Material and Growth Conditions

Seeds of lettuce (Lactuca sativa L.) were germinated in plastic containers filled with sterilized potting soil. Germination was carried out under controlled growth chamber conditions at 22/17 °C (day/night), with a 16 h light/8 h dark photoperiod, a photosynthetic photon flux density (PPFD) of 100 µmol photons m^{-2} s^{-1}, and 65 % relative humidity. Growth conditions were consistent with those described by Darwish et al. (2015). Seedlings were maintained under these conditions until the three-leaf stage, at which point experimental treatments were initiated.

Alternating Light/Dark Treatment

Plants were randomly assigned to two experimental batches: a control one (Con) and an alternating light/dark treatment batch (AL). Plants in the AL group were exposed to repeated cycles of 16 min light

followed by 8 min dark at a light intensity of 100 µmol photons m^{-2} s^{-1} for three consecutive days. Control plants were maintained under a standard photoperiod of 16 h light/8 h dark. Sampling and physiological assessments were performed at three time points: immediately after completion of the AL treatment (D0), three days later (D3), and ten days later (D10).

Determination of Water and Dry Matter Contents

Water content (WC, %) and dry matter content (DM, %) were determined gravimetrically. Fresh leaf samples were weighed immediately after harvest and subsequently dried in a forced-air oven at 105 °C until a constant dry weight was achieved. WC and DM percentages were calculated based on the difference between fresh and dry weights, following AOAC (1990) procedures.

Phytopathological Assay and Lesion Area Measurement

Lettuce leaves were inoculated with Botrytis cinerea strain SAS-405 by placing a fragment of actively growing fungal mycelium onto the midrib of each leaf. Following inoculation, leaves were incubated in a growth chamber at 21 °C under a 16 h light photoperiod.
Lesion development was monitored daily for seven days post-inoculation. Lesion areas were quantified using ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA). Disease progression was assessed at successive time points corresponding to D3 (prior to inoculation), D4 (24 h post-inoculation), D5 (48 h), D6 (72 h), and D7 (96 h).

Chlorophyll Fluorescence Measurements

Photosystem II (PSII) performance was evaluated using rapid chlorophyll a fluorescence measurements on dark-adapted lettuce leaves. Measurements were conducted at D0, D3, and D10 on five plants per treatment. Leaves were dark-adapted for 20 min prior to fluorescence acquisition. Polyphasic chlorophyll a fluorescence transients (OJIP curves) were recorded using an IMAGING-PAM fluorometer (WALZ GmbH, Nuremberg, Germany).

The maximum quantum yield of PSII photochemistry ($Fv/Fm = (Fm - F_0)/Fm$), the performance index on an absorption basis (PIabs), and the relative variable fluorescence at the J-step ($1 - V_J$), reflecting electron transport beyond QA, were calculated according to Maxwell and Johnson (2000). OJIP-test parameters describing energy fluxes per reaction center—absorption (ABS), trapping (TR), electron transport (ET), and dissipation (DI)—were derived following the approach of Strasser et al. (2000).

Additional fluorescence parameters were measured under actinic light at an intensity of 320 µmol m^{-2} s^{-1}. Measurements were taken every 20 s between 50 and 290 s, with saturating light pulses of 8 s duration, as specified in the IMAGING-PAM instruction manual (Heinz Walz GmbH). The effective quantum yield of PSII ($\Phi PSII = (Fm' - Fs)/Fm'$), the photochemical quenching coefficient ($qP = (Fm' -$

Fs)/(Fm' − F_o')) (Genty et al., 1990), and non-photochemical quenching (NPQ = (Fm − Fm')/Fm') (Zuo, 2025) were recorded automatically.

ΦPSII images were analyzed using ImagingWin 3D software. Measurements were performed using maximum measuring light intensity and a gain setting of 8. False-color images of ΦPSII were generated, with values ranging from 0 (black) to 1 (purple).

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA). Mean comparisons were performed using Tukey's honestly significant difference (HSD) test at a significance level of $p \leq 0.05$. Results are presented as mean values ± standard error (SE).

Results

Water Content and Dry Matter

Water content (WC) decreased progressively from D0 to D10 in both control and AL-treated plants. Notably, plants subjected to alternating light/dark cycles (AL) exhibited slightly lower WC percentages compared to controls, suggesting a more efficient water-use strategy. In contrast, dry matter (DM) content increased significantly over time, reaching the highest values at D10 in AL-treated plants, indicative of enhanced biomass accumulation and metabolic activity under AL exposure (Figure 1).

Lesion Development Following B. cinerea Inoculation

AL-treated plants showed a significant reduction in lesion development compared to controls (Figure 2). Lesion areas were consistently smaller at 2, 3, and 4 days post-inoculation, reflecting improved resistance to B. cinerea. The difference in lesion size was particularly pronounced at day 4 relative to day 3 in the AL group. Additionally, lesion suppression persisted at day 10, indicating that AL treatment imparts sustained physiological protection beyond the treatment period (Figure 3).

Chlorophyll Fluorescence Parameters

AL treatment markedly enhanced PSII performance (Figure 4). Values of Fv/Fm and Fv/F_o were elevated in AL plants compared to controls, with progressive increases in 1 − Vj and PIabs observed up to D10. Stability of F_o and F□ indicated absence of photoinhibitory damage. The improved PIabs reflects enhanced photochemical energy conversion efficiency and electron transport, consistent with a primed photosynthetic state.

OJIP Test Parameters and PSII Activity

OJIP fluorescence analysis revealed distinct alterations in energy fluxes per PSII reaction center (Figure 5). At D0, ABS/RC, TR_0/RC, DI_0/RC and ET_0/RC values were significantly similar between groups. However, by D3, AL plants exhibited significantly lower ABS/RC, TR_0/RC and DI_0/RC ($p \leq 0.05$) compared to controls. At D10, the values of the four parameters were significantly higher in AL plants compared to controls. These results indicate that AL exposure enhances PSII reaction center stability, increases photochemical energy utilization, and decreases energy dissipation, collectively reflecting improved PSII functionality under alternating light conditions.

Actual PSII Efficiency (ΦPSII), Photochemical Quenching (*q*P), and Non-Photochemical Quenching (NPQ)

Dynamic fluorescence measurements (Figure 6) showed that AL-treated lettuce maintained higher ΦPSII and photochemical quenching (*q*P) than controls, particularly during AL phases 1 and 2. Conversely, non-photochemical quenching (NPQ) was consistently lower under AL treatment, indicating reduced thermal energy dissipation and more efficient use of absorbed light energy. These findings confirm that AL cycles promote enhanced PSII activity and photochemical stability, supporting improved physiological resistance to B. cinerea.

Discussion

This study demonstrates that short-term alternating light/dark (AL) cycles significantly improve resistance of lettuce (Lactuca sativa L.) to Botrytis cinerea. This enhanced resistance was evidenced by reduced lesion development, increased dry matter accumulation, and improved chlorophyll fluorescence parameters relative to control plants. AL treatment promoted more efficient photosystem II (PSII) performance, as reflected by elevated Fv/Fm, PIabs, and ET_0/RC values, alongside reduced non-photochemical quenching (NPQ). Collectively, these data suggest that AL cycles induce a physiological “photochemical priming” state, enhancing energy utilization, photoprotection, and defense readiness in lettuce.

These results align with findings from Darwish et al. (2014, 2015, 2025), who reported that alternating light/dark regimes improve PSII efficiency and antioxidant capacity in Nicotiana tabacum, with concomitant accumulation of phenolic compounds involved in defense metabolism. Similarly, Meng et al. (2020) demonstrated that red LED light exposure increased strawberry resistance to B. cinerea by modulating defense gene expression. Together, these studies highlight that modulation of light quality, intensity, or periodicity can regulate plant defense responses at physiological and molecular levels.

Our data further support the hypothesis that improved PSII efficiency contributes to enhanced resistance by optimizing photochemical energy distribution. Increases in Fv/Fm and PIabs reflect a higher fraction of open PSII reaction centers and more stable electron transport between photosystems, thereby minimizing photoinhibitory stress. This concurs with Zuo (2025), who emphasized the necessity of controlled NPQ levels to maintain PSII integrity under stress. The observed reduction in DI_0/RC also

indicates decreased energy dissipation, consistent with improved photosynthetic efficiency and chloroplast redox homeostasis.

Light-mediated defense regulation is known to intersect with circadian and hormonal signaling pathways. Roden and Ingle (2009) identified the circadian clock as a critical modulator of plant–pathogen interactions, while Zhang et al. (2019) demonstrated that the circadian component LUX ARRHYTHMO coordinates crosstalk between clock genes and immune responses. Thus, the AL cycles applied here may synchronize photosynthetic and defense gene expression, promoting rhythmic priming that enhances stress responsiveness. Moreover, Breen et al. (2023) highlighted that manipulating light wavelengths influences plant immunity through regulation of defense hormones such as salicylic acid (SA) and jasmonic acid (JA), which are key in resistance against necrotrophic pathogens like B. cinerea.

The increase in dry matter following AL treatment suggests that enhanced photosynthetic performance supports greater biomass allocation and metabolic readiness for defense. This aligns with Li and Cheng (2023), who reported similar links between carbon status, energy metabolism, and defense activation. Enhanced carbohydrate availability and secondary metabolite biosynthesis are essential for sustaining energy-demanding immune responses and cell wall reinforcement. Additionally, the reduction in water content under AL may indicate improved water-use efficiency, potentially limiting tissue susceptibility to B. cinerea, a pathogen favored by humid conditions and compromised host tissue (Schumacher, 2017).

Finally, this study reinforces the concept of light-induced priming, whereby prior exposure to specific light regimes enhances subsequent stress responses. This mechanism parallels systemic acquired resistance (SAR) and induced systemic resistance (ISR) described by Poveda et al. (2020), involving long-lasting activation of defense pathways. The persistence of enhanced resistance at D10 post-treatment supports the existence of a durable primed state, potentially mediated by transcriptional memory, redox modulation, and activation of photoreceptors such as phytochromes and cryptochromes implicated in both light perception and immune signaling (Krishna, 2024).

In conclusion, short-term alternating light/dark cycles improve lettuce resistance to B. cinerea by coordinating enhanced PSII efficiency, reduced energy dissipation, and physiological priming. These findings expand the understanding of light-mediated stress tolerance and support the use of light modulation as a sustainable, non-chemical strategy for crop disease management. Future work integrating transcriptomic and hormonal analyses is needed to elucidate the underlying signaling networks, facilitating optimized light management in controlled-environment agriculture to enhance productivity and disease resilience.

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Figures

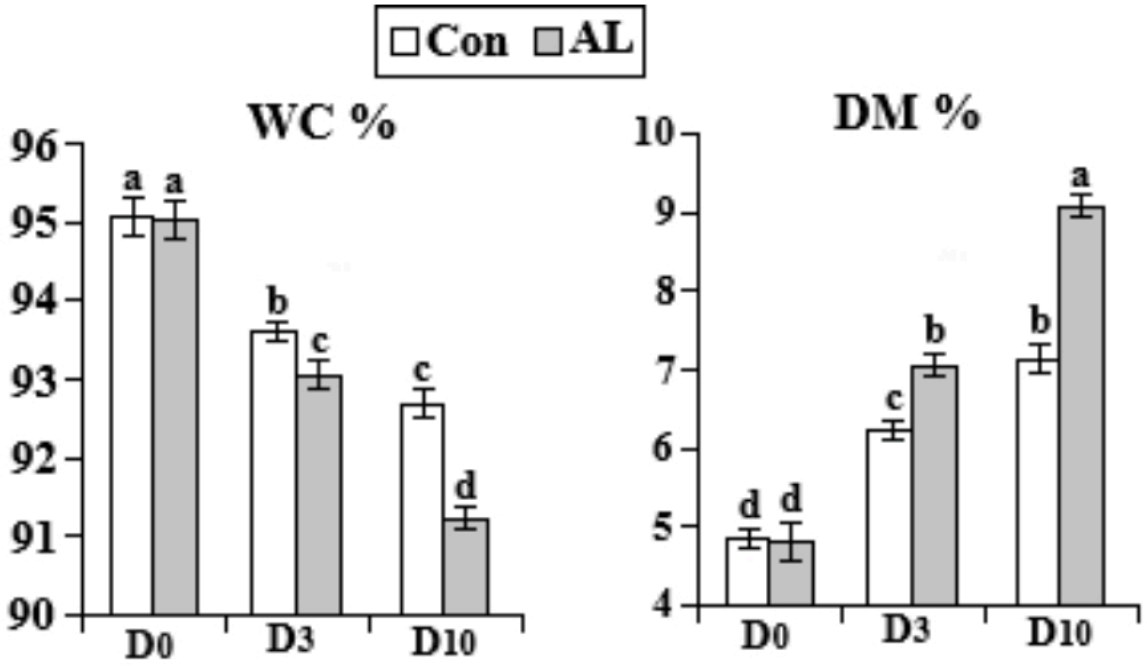


Figure 1

Water content (WC%) and dry matter (DM%) of lettuce leaves under control (Con) and alternating light/dark (AL) treatments. Measurements were performed at 0 (D0), 3 (D3) and 10 (D10) days after AL treatment. Reported data are obtained from five replicates, n = 5. Different letters indicate significant difference within each treatment according to an ANOVA-Tukey test at the 95% confidence level.

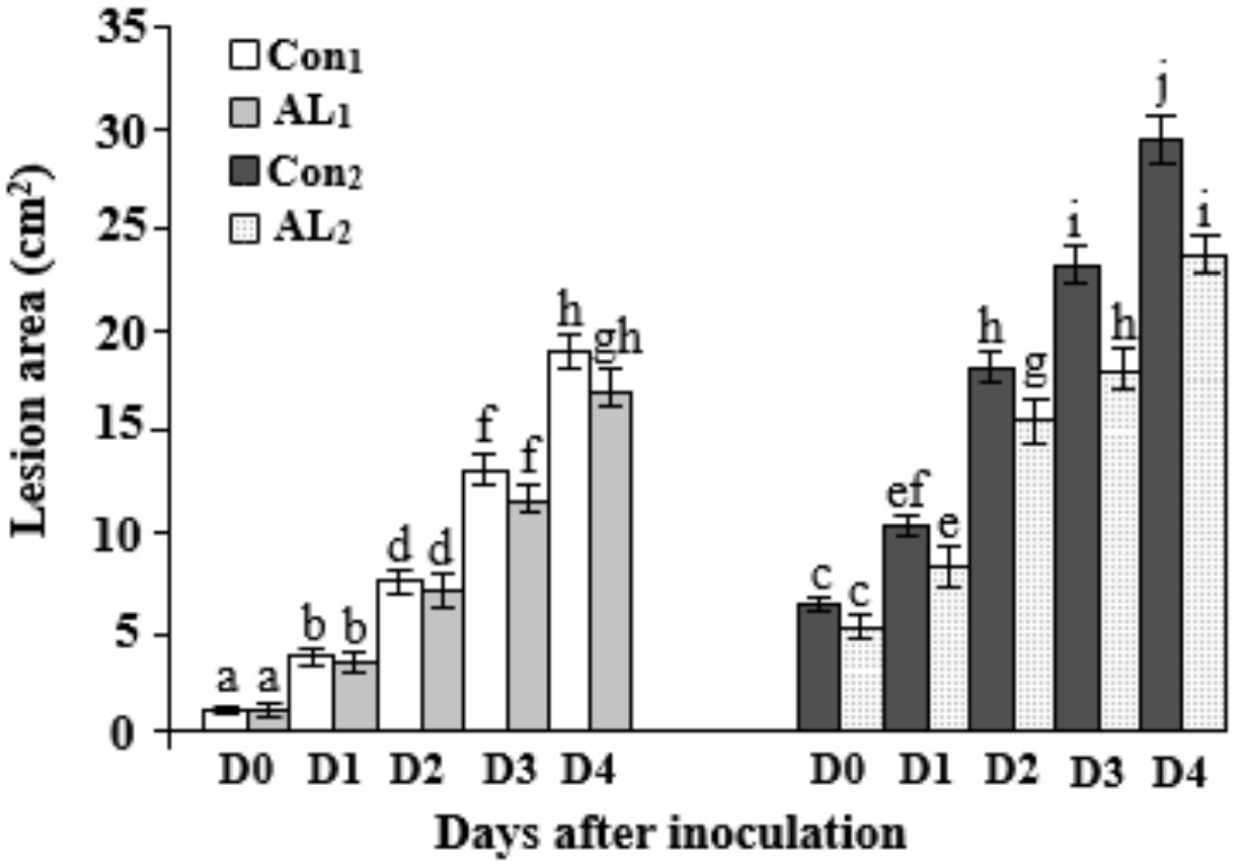


Figure 2

Lesion area progression following B. cinerea inoculation in control (Con_1, Con_2: without alternation of light/dark periods) and AL-treated plants (AL_1, AL_2) at 3 and 7 days of Alternation of light/dark periods. Measurements were performed at 0 (D0), 1 (D1), 2 (D2), 3 (D3) and 4 (D4) days after inoculation with mycelium of B. cinerea. Reported data are obtained from five replicates, n = 5. Different letters indicate significant difference within each treatment according to an ANOVA-Tukey test at the 95% confidence level.

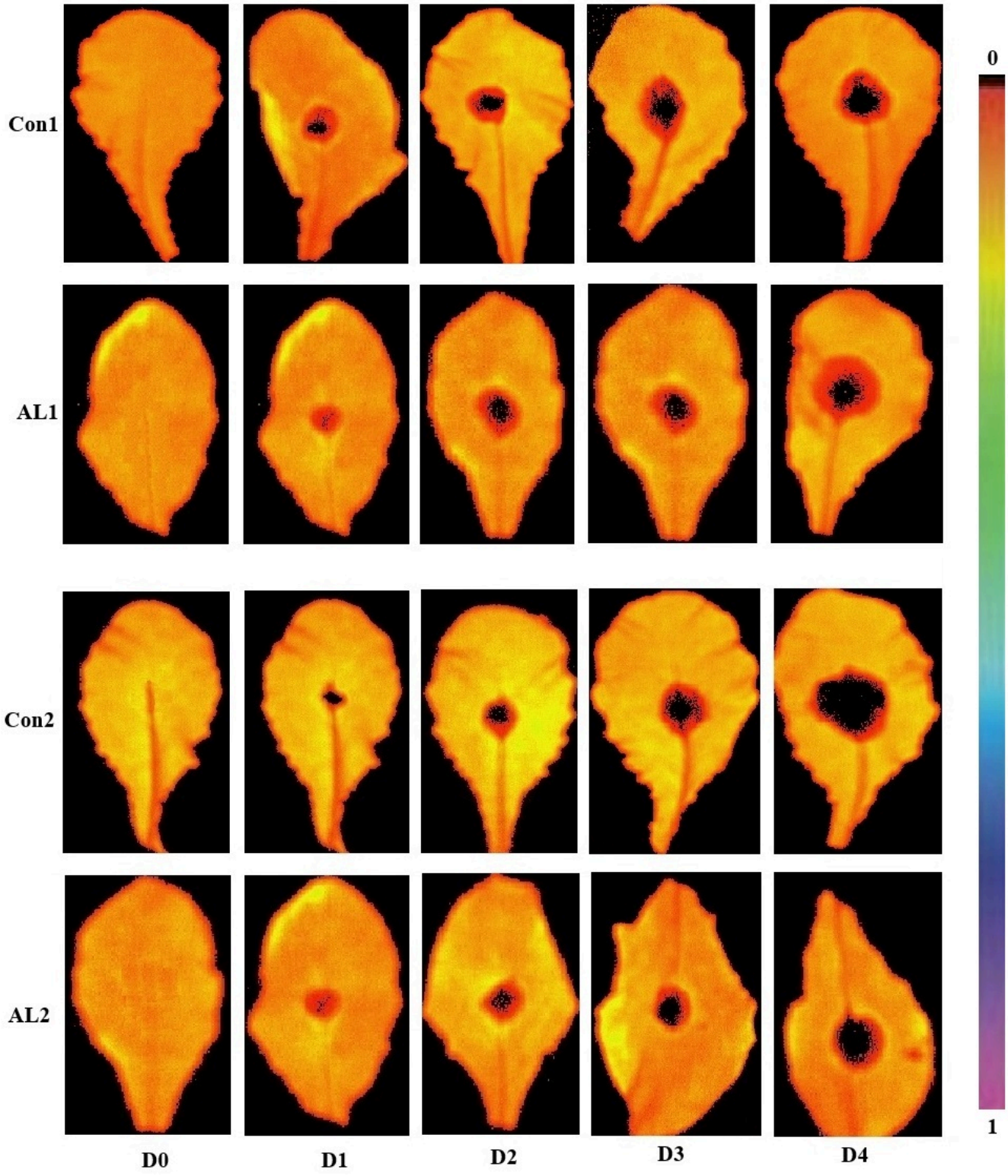


Figure 3

Chlorophyll fluorescence imaging showing changes in Φ_{PSII} in B. cinerea-infected lettuce leaves**.** The measurements were performed with 320 µmol photons m^{-2} s^{-1}, at 0 (D0), 1 (D1), 2 (D2), 3 (D3) and 4 (D4) days after inoculation with B. cinerea in control (Con_1, Con_2: without alternation of light/dark

periods) and AL-treated plants (AL_1, AL_2) at 3 and 7 days of Alternation of light/dark periods. The color code depicted at the bottom ranges from values 0.0 to 1.0.

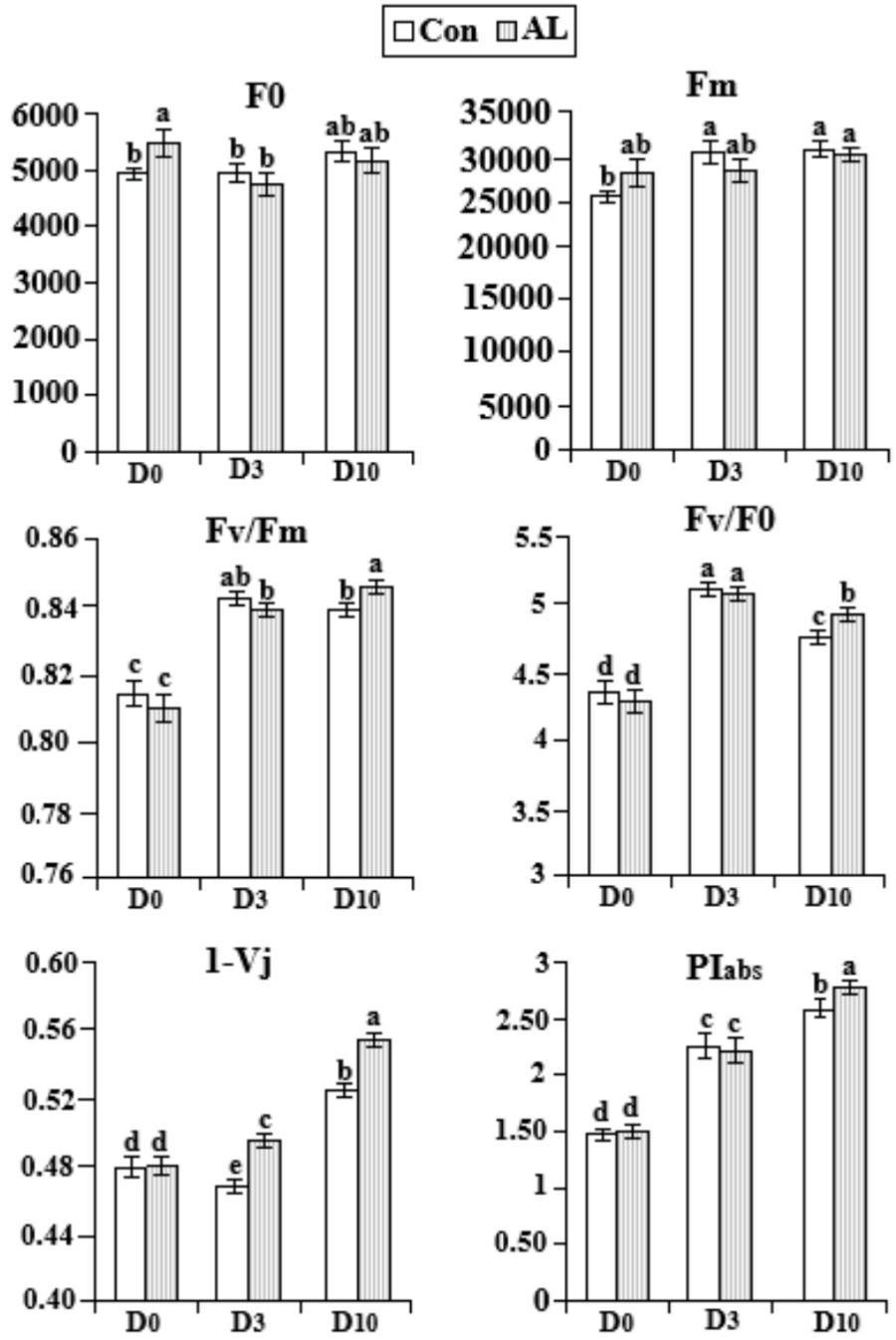


Figure 4

Chlorophyll a fluorescence intensity (Fv/Fm, Fv/F_o, 1-Vj, PIabs) evaluated from the fast chlorophyll fluorescence curves in control and AL-treated lettuce leaves. Measurements were performed at 0 (D0), 3 (D3) and 10 (D10) days after AL treatment. Reported data are obtained from five replicates, n = 5. Different letters indicate significant difference within each treatment according to an ANOVA-Tukey test at the 95% confidence level.

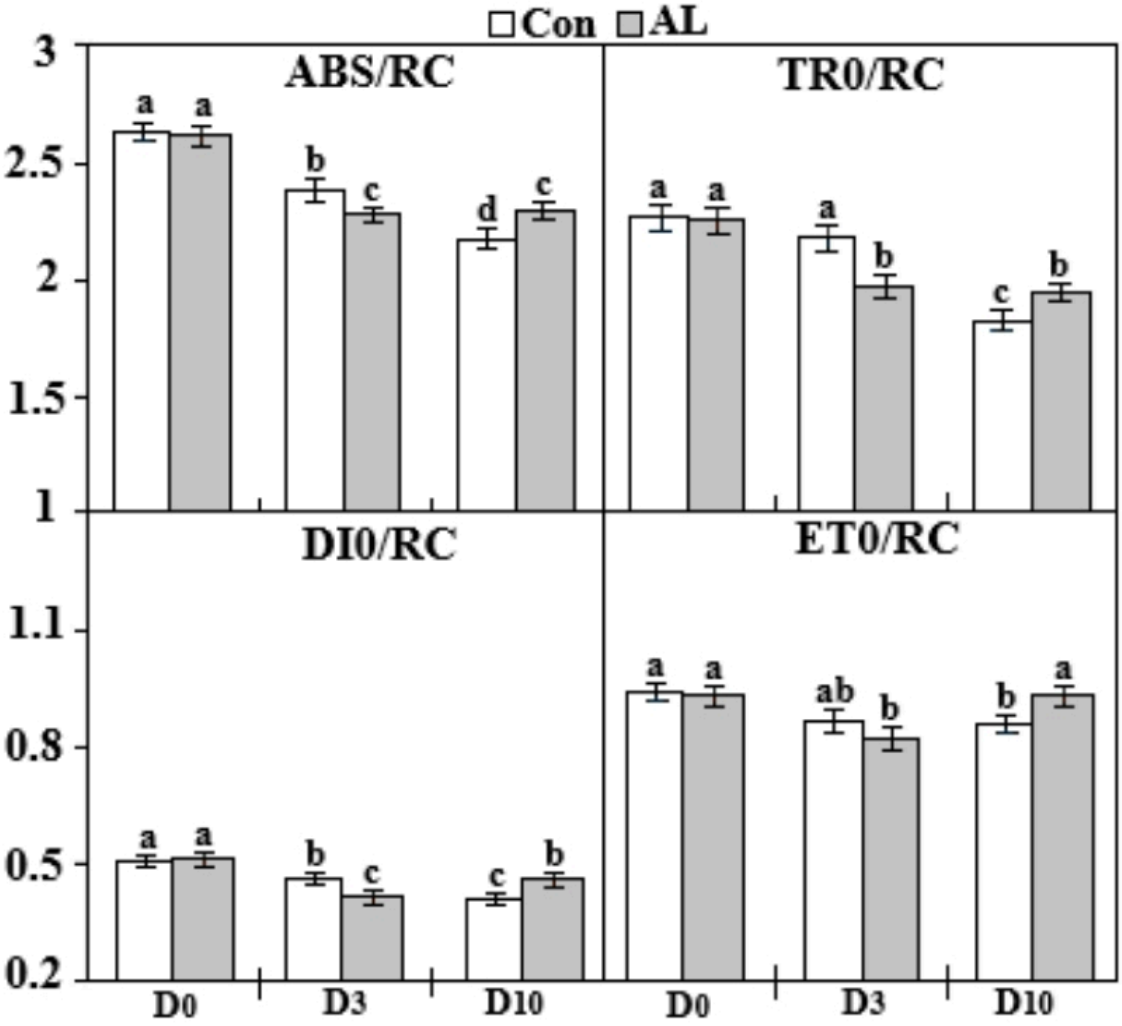


Figure 5

OJIP test parameters (ABS/RC, TR_o/RC, DI_o/RC, ET_o/RC) showing PSII energy flux changes in control (Con) and AL-treated (AL) plants. Measurements were performed at 0 (D0), 3 (D3) and 10 (D10) days after AL treatment. Reported data are obtained from five replicates, n = 5. Different letters indicate

significant difference within each treatment according to an ANOVA-Tukey test at the 95% confidence level.

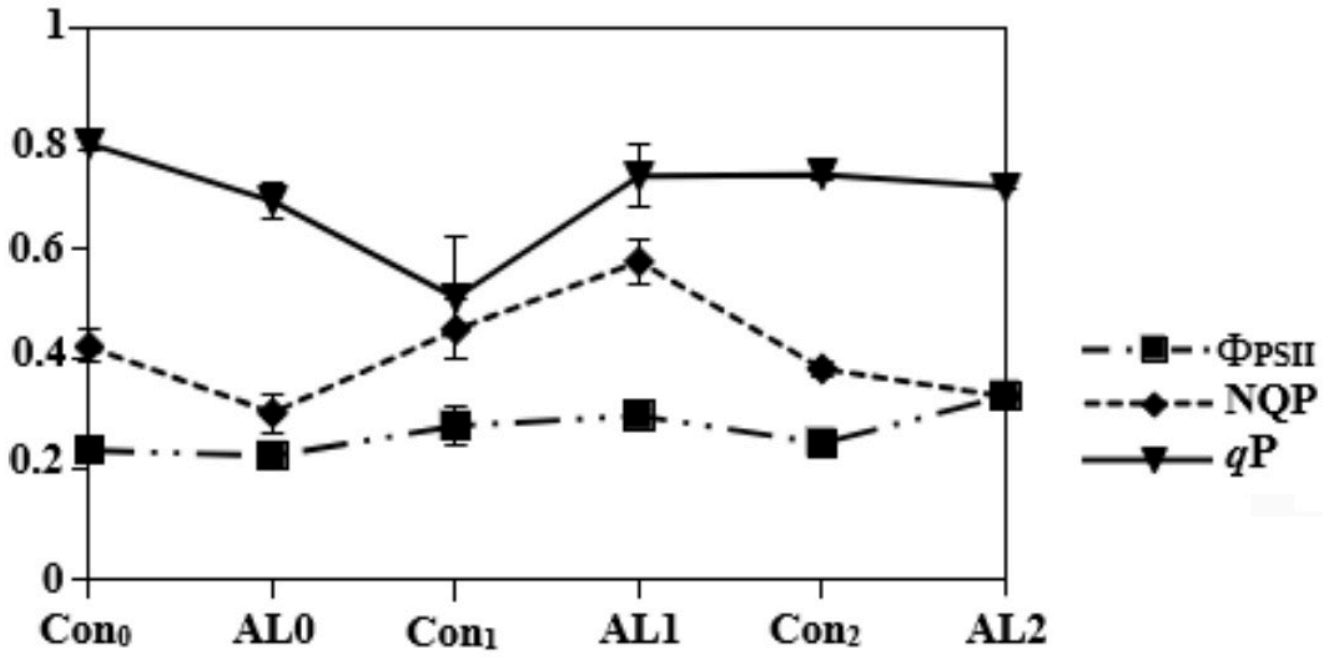


Figure 6

The actual PSII efficiency (Φ_{PSII}), the non-photochemical quenching (NQP) and the photochemical quenching (*q*P) in control (Con_0, Con_1, Con_2: without alternation of light/dark periods) and AL-treated (AL_0, AL_1, AL_2) lettuce plants at 0, 3 and 7 days of Alternation of light/dark periods. Reported data are obtained from five replicates, n = 5. Different letters indicate significant difference within each treatment according to an ANOVA-Tukey test at the 95% confidence level.