

Impact of Light with Different Spectra on the Photosynthetic Activity of Cucumber Plants under Fusarium Wilt

Liudmila Kabashnikova^{1,*}, Irina Domanskaya¹, Olga Molchan², Lyubov Pashkevich¹, Irina Dremuk¹, Hanna Martysiuk¹ and Tatsiana Viks¹

¹*Institute of Biophysics and Cell Engineering, of National Academy of Science of Belarus, Akademicheskaya str., 27. 220072, Minsk, Belarus*

²*V.F. Kuprevich Institute of Experimental Botany of the National Academy of Sciences of Belarus, Minsk, 220072, Belarus*

Abstract: The photosynthetic activity of 28-day-old cucumber plants of the Kustovy variety formed under LED illumination with the predominance of red light (RL) or far red light (FRL) and infected with the fungus *Fusarium oxysporum* sp. (*F.ox.*) was studied. The predominance of RL or FRL contributed to an increase in the content of chlorophyll and carotenoids per dry leaf weight compared to the plants grown under white light (WL). In the infected plants grown under WL and RL regimes, an increase in the total content of chlorophyll and carotenoids was observed relative to healthy plants, and a decrease in the same parameters for FRL was noted. In healthy cucumber leaves grown under RL and FRL regimes, an increase in the activity of photosystem (PS)1 and PS2 in comparison with WL was observed. Infection of cucumber plants grown under WL did not cause any changes in the functional parameters of PS2 against the background of a slight reduction in PS1 complexes. Under RL and FRL regimes, a decrease in the photochemical activity of PS 1 and PS 2 in infected leaves was noted. The activity of ascorbate peroxidase – one of antioxidant enzymes in chloroplasts decreased in healthy cucumber leaves under RL and FRL compared to WL and increased in infected leaves compared to the healthy ones under all light regimes, especially strongly under RL. The results obtained demonstrate different response mechanisms for cucumber chloroplasts to the infection with a fungal pathogen, depending on the light conditions of growing.

Keywords: Carotenoids, Chlorophyll, White light, Red light, Far red light, Photosystem 1 and 2, *Cucumis sativus* L. *Fusarium oxysporum* sp.

INTRODUCTION

Light is an important inducer of plant immunity. It acts as a central environmental signal that triggers various adaptive and photomorphogenic reactions in plant organs [1]. Plants have developed several photoreceptors that perceive changes in light intensity, direction, wavelength, as well as its daily and seasonal rhythmicity. Among them, phytochromes (Phy), photoreceptors absorbing RL and FRL have been characterized in more detail [2]. Currently, it is believed that light is closely related to the reaction of plants to abiotic and biotic factors due to common signaling cascades [3]. In this regard, it is important to clarify the mechanisms of the influence of light on the adaptation of chloroplasts in the pathological process. Signaling pathways that mediate the reaction of plants to light and temperature are best characterized. The PhyB photoreceptor acts as a thermal sensor in the process

of plant thermomorphogenesis, a certain set of morphogenesis events and structural changes occurring at high ambient temperatures [4].

Pathogens pose a devastating threat to the survival and reproduction of plants. In the process of evolution, plants have developed universal protective mechanisms to cope with these biotic stressors [5]. Many genes and signaling molecules induced by pathogens and herbivorous insects have been identified in various plant species, and among them two stress phytohormones, salicylic acid (SA) and jasmonic acid (JA), are best studied. It is noteworthy that light and temperature strongly influence the path of the induction of the protective reactions of plants. The coordinated relationship between plant protection and light cycles has been extensively studied in recent years [6, 7]. It is shown that the onset and development of protective reactions change under the influence of the intensity and wavelength of light, as well as light-dark transitions [8, 9]. The light regulation of plant protection is illustrated by two main aspects. It is known that the behavioral and physiological patterns of pests and pathogens are synchronized with the daytime cycles of light, and thus plants are able to predict the

*Address correspondence to this author at the Institute of Biophysics and Cell Engineering, of National Academy of Science of Belarus, Akademicheskaya str., 27. 220072, Minsk, Belarus; Tel: +375 (17) 342 28 88; Fax: +375 (17) 378 23 59; E-mail: kabashnikova@mail.ru

occurrence of their attacks. Another factor to consider is the trade-off between growth and protection that plants have to face [10]. Since protective reactions require the expenditure of metabolic resources and energy, plants have very limited capabilities. Consequently, the excessive release of metabolites and energy for protective reactions may lead to a slowdown in their growth [3]. It is assumed that plants should accurately track information about ambient lighting in order to balance the benefits and costs of energy supply [3].

Recent studies have shown that based on the synchronization of pathogen attacks with light cycles and trade-offs between growth and protection, light signals play an important role in modulating of protective reactions [7, 8]. In the conditions of low RL/FRL, the content of JA decreases, and plants show low sensitivity to SA or JA treatments during the induction of protective reactions [9]. Accordingly, mutants with a deficiency of PhyB having a constitutive phenotype of shadow avoidance exhibit hypersensitivity to pathogens [9]. At the same time, in the conditions of darkness, the biosynthesis of SA and sensitivity of plants decrease, and protective reactions are disrupted [6, 8]. Double mutants of PhyA and PhyB also demonstrate a significant decrease in sensitivity to SA and systemic acquired resistance, which indicates that the Phy-mediated transmission of light signals is important for SA-associated protective reactions. On the contrary, under conditions of a high RL/FRL ratio, the light stabilization of *FHY3* and *FAR1*, two homologous transcription factors important for the transmission of PhyA signals, negatively regulates the biosynthesis of SA and its signal transmission [7]. It is likely that the PhyA-mediated light regulation of biosynthesis and the transmission of SA signals contribute to the fine-tuning of protective reactions associated with SA regulation.

Moreover, it has been shown that PhyB regulates the biosynthesis and sensitivity of JA depending on the ratio of RL/FRL [11, 12]. Under conditions of a low RL/FRL ratio, the expression of the *SULFOTRANSFERASE 2A* (*ST2A*) gene, which is involved in the metabolism of JA, increases in a way dependent on PhyB [12]. The induction of *ST2A* is associated with a decrease in the accumulation of JA. Meanwhile, a low RL/FRL ratio and a mutation of PhyB cause the rapid degradation of proteins well known as repressors of the JA function [13]. It is obvious that

PhyB and PhyA are crucial for enhancement of protective reactions in pathogenesis.

The effect of light on plant immunity when attacked by different types of pathogens (fungi, bacteria, and viruses) has been studied for a long time, but more often in the dark [14] than under the light. In addition, such studies have been performed mainly on *Arabidopsis* mutants and very rarely on vegetable crops. In particular, the studies on tomatoes have demonstrated that under conditions of a low RL/FRL ratio, the mutant *phyB1phyB2*, which avoids shade, has an increased sensitivity to herbivorous insects, such as the thrips and caterpillars of *Spodoptera eridania* [15]. In addition, resistance to the fungal pathogen *B. cinerea* decreases in tomatoes when PhyB is genetically or physiologically inactivated [16]. Similar to *Arabidopsis*, this effect of PhyB inactivation partially depends on JA. It turned out that in tomato, the inhibition of a JA response at a low level of RL/FRL leads to an increase in the content of soluble sugars in the leaves, which probably contributes to the growth of fungi and possibly to their pathogenicity [16].

In many regions of the world, vegetable crops, including cucumber, are grown in greenhouses or other protected areas. LED technology offers significantly improved lighting energy efficiency, as well as control over the spectral composition of light. In this regard, it is very important to choose the optimal ratio of the spectral composition of light. It is known that growing under the RL regime stimulates an increase in dry weight, the growth of roots and stems and other processes [17]. This increases the content of chlorophyll (Chl) and the efficiency of PS2 functioning in *A. thaliana* [18]. It has been established that FRL and RL/FRL ratios are very important for most photoreceptor processes, among which is the process of the formation of the photosynthetic apparatus. It has been shown that an addition of blue light (BL) also has a positive effect on plant development, and the use of RL and BL in the light culture enhances the synergistic effect and the optimal ratio of RL, FRL and BL for each culture individually [19].

Thus, the aim of this work was to study the effect of the spectral composition of light addressed to Phy on the pigment composition, structural and functional state of photosynthetic membranes and antioxidant activity of cucumber plants under *Fusarium* wilt.

MATERIALS AND METHODS

Plant Material and Growth Condition

We used green cucumber plants of the Kustavi variety of the Belarusian selection. Cucumber seeds have been planted in soil based on Universal peat (Terra Vita, Belarus). Plants were grown under fluorescent lamps (housing 28-400-001 XXLI, 220V, 400 W power, IP23 /IP53) (variant "White light", WL) at 22-23 °C and 65% humidity in the mode of 14 h of light and 10 h of darkness before the appearance of the rudiment of the first leaf (10 days). Then the plants were transferred to LED lighting. In the spectral composition of LED lighting, which includes all ranges of physiologically active radiation (from 400 to 800 nm), the ratios of photon flux density levels varied in different ratios. The RL variant has an increased proportion of RL in the ratio of RL/FRL = 5.0 and RL/BL = 4.0. The FRL variant is characterized by an increased proportion of FRL (RL/FRL = 2.0), while maintaining a constant level of photosynthetically active radiation of RL/BL = 4.0. In such conditions of LED lighting, cucumber plants were grown for 25 days. The light intensity was 100 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$ for all lighting options. Then part of the plants was treated with a suspension of spores of the fungus *F.ox.* (10^6 spores ml^{-1}). The analysis was performed 72 h after inoculation with the pathogen. Plants treated with distilled water served as the control.

Determination of Pigment Content

At the 28 days age after 72 h of fungus inoculation, cucumber leaves were fixed with liquid nitrogen and then pigments and enzyme activity were determined. Chl and carotenoids (Car) pigments were extracted with acetone and analyzed spectrophotometrically on the spectrophotometer "Shimadzu, UV-2401PC" (Shimadzu, Japan) [20].

Determination of Photochemical Activity

Photochemical PS2 activity, the most sensitive component of the photosynthetic apparatus, was assessed using the pulse amplitude modulated (PAM) fluorometry applying the Dual-PAM 100 fluorometer (Walz, Germany) [21]. The measurement was carried out in intact 28-day-old cucumber leaves. The obtained values of F_o , F_o' , F_m , F_m' and F_v were used to calculate the maximum (potential) quantum yield of photochemical reactions PS2 – (F_v/F_m) ; the value of the effective quantum yield PS2 – $Y(PS2)$; the quantum yields of unregulated – $Y(NO)$ and regulated – $Y(NPQ)$

non-photochemical fluorescence quenching; the coefficients of the non-photochemical quenching of Chl fluorescence – q_N and NPQ; the coefficient of the photochemical quenching of fluorescence Chl – q_P ; the index of the proportion of open reaction centers – q_L . The efficiency of the functioning of the electron transport chain or the electron transport rate (ETR) was also calculated according to [22]. Measurements of the photochemical activity of PS1 were carried out on the same device with the two-wave (830/875 nm) fluorescence detection module P700 according to [21, 22]. The parameters of the photochemical activity of PS1 such as the efficiency of the electron transport chain or the electron transport rate (ETR) of PS1; the value of P_m , characterizing the maximum fluorescence of P700; the effective quantum yield of PS1 – $Y(1)$ and the parameters of the quantum outputs of the non-photochemical quenching of PS1 – $Y(ND)$ and $Y(NA)$ were calculated.

Antioxidant Activity Analysis

Ascorbate peroxidase (APX, EC 1.11.1.11) catalyzes the decomposition of hydrogen peroxide in the presence of ascorbic acid towards water and monodehydroascorbate [23]. The optical density of ascorbic acid was recorded at 290 nm on the spectrophotometer "Shimadzu, UV-2401PC" (Shimadzu, Japan) using the coefficient of extinction equal to $2.8 \text{ mM}^{-1}\text{cm}^{-1}$.

Statistical Analysis

All studies were carried out in 3-5-fold biological replicates in each experiment. The reliability of differences in average values was determined using the computer programs Statistica 10.0 (StatSoft) and Excel 2010. Statistically significant differences between the parameters at $p < 0.05$ are marked in tables and graphs with an asterisk.

RESULTS

Figure 1 exemplarily illustrates a change in the content of photosynthetic pigments in healthy leaves of cucumber under conditions of WL, RL and FRL illumination. The contents of Chl and Car per dry leaf weight increased by 15 and 60%, respectively, under the predominance of RL and FRL illumination compared with the plants grown under the WL regime. The effect of pathogenic infection on WL was expressed in an increase in the total content of Chl

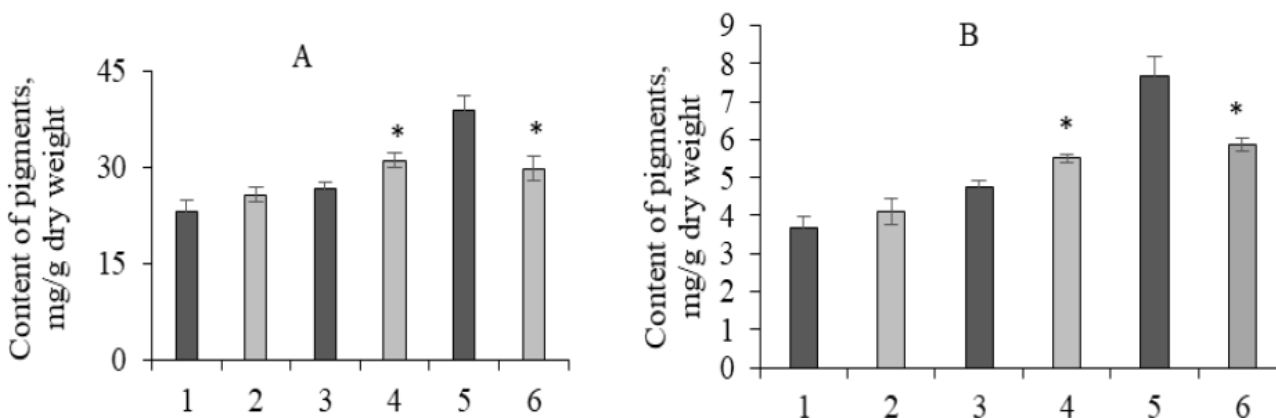


Figure 1: Changes in the total content of photosynthetic pigments in the leaves of healthy and infected with the fungus *F. ox.* cucumber plants grown under different light spectra: Chl (*a+b*) – A, carotenoids – B; 1 – control, WL; 2 – WL + *F. ox.*; 3 – control, RL; 4 – RL + *F. ox.*; 5 control, FRL; 6 – FRL+ *F. ox.*

*–Differences are significant between the control and *F.ox.* (t-test, $P \leq 0.05$).

(*a+b*), the fraction of Chl *a* and the ratio of Chl *a*/Chl *b* by 12-16% compared with healthy leaves. RL-conditioned infection with the fungus stimulated the accumulation of all fractions of photosynthetic pigments (Chl *a* – by 18%, Chl *b* – by 12%, Car – by 16%) compared with the control with a constant ratio of Chl *a*/Chl *b* and Chl (*a+b*)/Car. Infection under the FRL condition led to a decrease in the content of chlorophyll pigments, their fractions and Car by 22-25% in relation to healthy plants.

It was found that infection with the *F.ox.* fungus of cucumber plants grown under the WL regime does not cause any significant changes in the functional and structural parameters of PS2, except for a slight reduction of PS1 complexes (Table 1, 2).

A decrease in the activity of PS2 (ETR(II) and Y(II) parameters) after the fusarium invasion may be associated with stress exposure. Fusarium wilt leads to a decrease in the photochemical activity and electron transport of PS1 (by 20%) in chloroplasts upon illumination with RL and FRL. A decrease in the functional efficiency of PS2 was also observed, most likely due to irreversible changes in pigment-protein complexes under two types of LED lighting. The revealed changes in the photochemical activity of PS2 and PS1 are most clearly manifested in cucumber leaves grown under the RL condition and infected with the fungus *F.ox.*

By the method of PAM-fluorimetry, a significant increase in the electron transport rate in PS2 (ETR II) under LED lighting in healthy cucumber leaves was

found: by 85% – under RL and by 45% – under FRL compared to WL (Figure 2, A). For PS1, this parameter significantly increased by 47% only in the case of RL (Figure 2, B) and practically did not differ with respect to FRL in comparison with WL. A significant increase in the photochemical quenching constant (*qP*) of the fluorescence of Chl PS 2 in the RL variant was recorded by 74%, and in the FRL variant – by 46% compared to WL (Table 1), which indicates an increase in the activity of PS2 not only due to the functioning of the electron transport chain, but also due to an improvement in light harvesting. The parameter of the potential quantum yield of PS2 photochemistry (*Fv/Fm*) in the control plants grown under WL, RL and FRL regimes did not differ significantly (Table 1).

Infection with the fungus *F.ox.* leads to a decrease in both the effective quantum yield of photochemistry PS2 (Y(II)) and the efficiency of electron transfer in the leaves (ETR II) grown under RL and FRL regimes by 13 and 30% respectively relative to the corresponding controls (Table 1). In the literature, a decrease in the effective quantum yield photochemistry of PS2, as well as ETR(II) in PS2, is associated with an increase in the relative amount of non- Q_B -reducing centers of PS2 [24]. A possible reason for a decrease in the efficiency of electron transfer to PS2 and Y(II) under the fungus *F. ox.* action in the conditions of RL and FRL, respectively, may be due to the changes occurring in the protein complexes of the reaction center of PS2, since ETR(II) and Y(II) parameters are not associated with the activation of additional dissociation mechanisms. Indirect evidence is the fact that in these experiments *qP* and *qN* fluorescence quenching

Table 1: Pulse Amplitude Modulated Fluorimetry Parameters of PS2 in the Cucumber Leaves Grown Under White Light, Red Light and Far Red Light Regimes Before (control) and After Infection with *F. ox.*

Parameter	Variants					
	White light, Control	White light + <i>F.ox.</i>	Red light, Control	Red light + <i>F.ox.</i>	Far red light, Control	Far red light + <i>F.ox.</i>
Maximum fluorescence, Fm	4.687±0.031	4.098±0.001*	5.436±0.241	5.277±0.249	5.127±0.323	5.346±0.100
Maximum quantum yield of photochemical reactions PS2, Fv/Fm	0.775±0.009	0.783±0.003	0.802±0.006	0.797±0.005	0.793±0.007	0.788±0.001
Effective quantum yield PS2, Y(II)	0.242±0.023	0.288±0.023*	0.450±0.064	0.392±0.085*	0.348±0.057	0.239±0.026*
Quantum yields of unregulated – non-photochemical fluorescence quenching, Y(NO)	0.530±0.003	0.484±0.011	0.415±0.040	0.405±0.054	0.383±0.042	0.442±0.020
Quantum yields of regulated – non-photochemical fluorescence quenching, Y(NPQ)	0.228±0.020	0.228±0.012	0.136±0.024	0.204±0.030*	0.269±0.020	0.319±0.006
Coefficients of non-photochemical fluorescence quenching, NPQ	0.431±0.036	0.470±0.014	0.320±0.028	0.500±0.008*	0.701±0.045	0.734±0.016
Coefficient of the photochemical quenching of Chl fluorescence, qP	0.341±0.025	0.406±0.030*	0.593±0.076	0.538±0.112	0.499±0.078	0.350±0.039*
Efficiency of the functioning of the electron transport chain PS2, ETR(II)	13.30±1.27	15.87±1.287	24.72±3.53	21.52±4.67*	19.12±3.14	13.15±1.41*
Coefficients of the non-photochemical quenching of Chl fluorescence, qN	0.361±0.023	0.383±0.008	0.287±0.020	0.395±0.005*	0.487±0.020	0.499±0.006
Index of the proportion of open reaction centers, qL	0.131±0.007	0.166±0.014*	0.280±0.055	0.274±0.083	0.243±0.052	0.152±0.021*

*–Differences are significant between the control and *F.ox.* (t-test, P≤0.05) for each light condition.

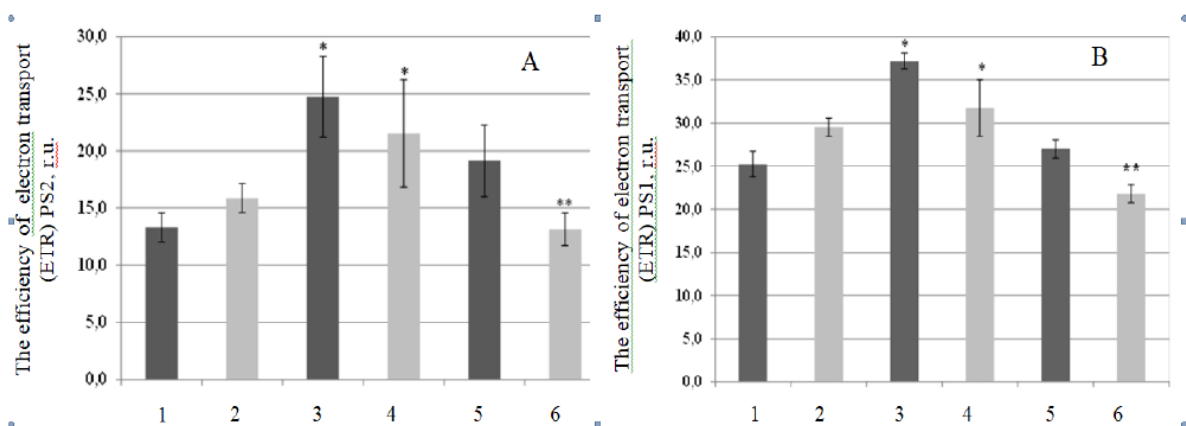


Figure 2: The efficiency of electron transport in the cucumber leaves grown under different light spectra, before and after *F.ox.* treatment: ETR PS2 – A; ETR PS1– B; 1 – control, WL; 2 – WL + *F.ox.*; 3 – control, RL; 4 – RL + *F.ox.*; 5 control, FRL; 6 – FRL+ *F.ox.*

* – Differences are significant between the control and *F.ox.* (t-test, P≤0.05).

coefficients and the quantum outputs of unregulated (Y(NO)) energy dissipation in PS2 undergo only slight changes.

The measurement of P700 absorption using pulse-modulated fluorescence spectroscopy carries similar information about PS1 (the state of the donor and acceptor sides, quantum yields, etc.). Changes

compared to the control were registered for the parameter Pm characterizing the maximum Chl fluorescence of P700, which decreased by 20% in the leaves under WL after infection with a pathogen (Table 2). For the cucumber plants under RL and FRL regimes, the levels of the maximum Chl fluorescence of P700 in healthy and infected leaves did not change. Differences in the quantum yields of the non-

Table 2: Pulse Amplitude Modulated Fluorimetry Parameters of PS1 in the Cucumber Leaves Grown Under White Light, Red Light and Far Red Light Regimes before (control) and after Infection with *F. ox.*

Parameter	Variants					
	White light, Control	White light + <i>F.ox.</i>	Red light, Control	Red light + <i>F.ox.</i>	Far red light, Control	Far red light + <i>F.ox.</i>
Maximum fluorescence of P700, Pm	2.05±0.08	1.64±0.01*	1.79±0.05	1.87±0.14	1.50±0.07	1.50±0.07
Effective quantum yield of PS1, Y(I)	0.459±0.026	0.537±0.019	0.676±0.017	0.577±0.060*	0.491±0.019	0.397±0.019*
Efficiency of the functioning of the electron transport chain of PS1, ETR(I)	25.25±1.45	29.52±1.05	37.17±0.92	31.72±3.26*	27.00±1.06	21.80±1.06*
Quantum outputs of the non-photochemical quenching on the donor side of e PS1, Y(ND)	0.358±0.021	0.285±0.002*	0.208±0.017	0.261±0.011	0.321±0.022	0.441±0.053*
Quantum outputs of the non-photochemical quenching on the acceptor side of the PS1, Y(NA)	0.183±0.006	0.178±0.018	0.117±0.002	0.162±0.050*	0.188±0.041	0.163±0.053

* – Differences are significant between the control and *F.ox.* (t-test, $P \leq 0.05$) for each light condition.

photochemical quenching of PS1 in the cucumber leaves grown under the light of different spectra were also revealed. As is known, the parameter Y(ND) is a measure of energy dissipation on the donor side, and Y(NA) is on the acceptor side of PS1 [25]. The study of the quantum photochemistry outputs of PS1 in infected leaves showed a decrease in Y(NA) on the acceptor side of plants grown under FRL (by 13%) and a corresponding increase in the Y(ND) parameter indicating a higher redox state of PS1 on the donor side (Figure 2, B). In the plants grown under RL, fungal infection stimulated the photochemistry of PS1 on both the donor and acceptor sides of this photosystem. At the same time, the values of the quantum yield (Y(I)) and the efficiency of electron transport (ETR(I)) were reduced by 20% under both RL and FRL conditions. An increase in Y(ND) indicates damage at the PS2 site for the plants grown under FRL, and a decrease in Y(NA)

may be a consequence of the activation of the Calvin cycle in such cucumber leaves.

The effect of LED lighting and infection with the pathogenic fungus *F.ox.* on the development of an immune response involving one of the enzymes of the ascorbate-glutathione cycle – chloroplast ascorbate peroxidase (APR) in the first leaves of cucumber plants was studied. The activity of the antioxidant protective enzyme APR decreases by 20% in both the RL and FRL variants compared to WL. Inoculation of cucumber plants with a fungal pathogen stimulated the activity of APR: in the case of WL, an increase was 59%, RL – 83%, FRL – 46% relative to their controls.

DISCUSSION AND CONCLUSION

In healthy plants grown under the LED lighting regime with a different ratio of physiologically

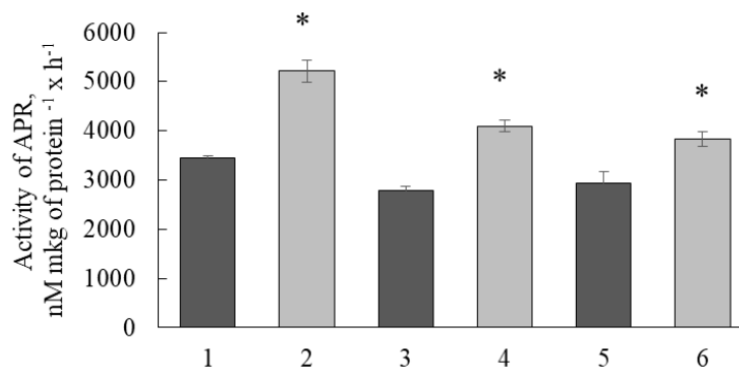


Figure 3: Activity of the ascorbate peroxidase in the healthy and infected leaves of cucumber plants grown under different light spectra: 1 – control, WL; 2 – WL + *F. ox.*; 3 – control, RL; 4 – RL + *F. ox.*; 5 control, FRL; 6 – FRL+ *F. ox.*

* – Differences are significant between the control and *F.ox.* (t-test, $P \leq 0.05$).

significant spectral ranges (RL and FRL), an increase in the content of Chl and Car pigments in terms of dry leaf weight compared with the WL condition was observed. Infection in the conditions of WL and RL regimes caused an increase in the total pigment content of relatively healthy plants, and a reduction in the pigment fund under the FRL regime was noted. The results obtained indicate various mechanisms of the formation of photosynthetic membranes in the cucumber leaves under LED lighting, namely, during RL and FRL, and after infection with the fungal pathogen *F.ox*.

Analysis of the structural and functional state of PS2 and PS1 in the healthy cucumber leaves grown under RL and FRL lighting regimes allowed us to establish an increase in the activity of PS1 and PS2 compared to WL, which particularly clearly manifested themselves when analyzing light curves. The photosynthetic apparatus of plants, perceiving changes in the intensity and spectral composition of light through its pigments (Chl and Car), may act as a site, where signaling mechanisms such as photosynthetic adaptation to lighting conditions are triggered [27]. The adaptation of the photosynthetic apparatus to illumination as one of the mechanisms involves non-photochemical quenching of Chl fluorescence (NQF). The NQF mechanism is the fastest response that allows minimizing the generation of reactive oxygen species (ROS) with an excess of light energy and their damage on photosynthetic membranes. The four parameters of Chl fluorescence induction are associated with NQF: qN, NPQ, Y(NPQ) and Y(NO). In order to determine contributions to the utilization of light energy of regulated NQF, characterizing the adaptation of the photosynthetic apparatus to an excessive amount of energy, and unregulated NQF associated with the non-adaptive dissipation of light energy, the corresponding quantum yields Y(NPQ) and Y(NO) were calculated.

Infection of cucumber plants with the fungus *F.ox* grown under the WL regime does not cause significant changes in the parameters of PS2 functioning. The values of the quantum yield Y(I) and the efficiency of the electronic transport (ETR(I)) of PS1 in such plants were 15% higher than in healthy plants. For the plants grown under RL and FRL regimes, fungal infection led to a decrease in most PS1 and PS2 parameters. A decrease in the effective quantum yield of the photochemistry of PS2, the efficiency of electron transfers to PS2, as well as the number of open reaction centers (qL), especially in the "FRL+*F.ox*." variant, are worth noting. PS2 and PS1 function more

efficiently at RL as evidenced by an increased electron transport rate and the improved light harvesting efficiency. Under the FRL condition, this is typical only for PS2. Thus, the opinion available in the literature that RL as mono-chromatic light enhances the protective functions of plants, possibly through the activation of phytohormone signaling pathways, has been experimentally confirmed on cucumber leaves [28].

The above-described decrease in the activity of PS2 (ETR(II) and Y(II) parameters) after fusarium infection may be associated with stressful effects, including taking into account the large values of the NQF indicator under the conditions of RL lighting. Noteworthy is a significant increase in three of the four parameters of NQF in cucumber leaves: qN, NPQ, and Y(NPQ) – by 40-56% under the RL regime after fungal infection with *F.ox* relative to the healthy control (Table 1). The values of the quantum yield of regulated and unregulated NQF allow us to assert that the excess light energy is effectively utilized by the adaptation mechanism of NQF in the "RL+ *F.ox*." variant, while in the "WL+*F.ox*." and "FRL+ *F.ox*." variants, this mechanism is not effective enough.

The results obtained in this work indicate the participation of the antioxidant enzyme APR in the implementation of the antioxidant protection of cucumber plants formed under the LED lighting regime and infected with the fungal pathogen *F.ox*. The central transformation within the ascorbate-glutathione cycle is aimed at removing the main ROS in chloroplasts, and therefore, the plants grown under RL and treated with *F.ox* cope with this task better than other options.

In conclusion, fungal pathogen *F.ox* infection caused disturbance in the structural and functional state of chloroplasts in cucumber leaves, what is expressed in decrease of chlorophyll and carotenoids content and the lower activity of PS1 and PS2 compared with healthy plants. The LED lighting used in the work with an increased ratio of RL (RL/BL = 4.0, RL/FRL = 5.0) had a positive effect on the functioning of PS and the pigment fund of cucumber plants. Unfortunately, the activity of ascorbate peroxidase, one of antioxidant enzymes in chloroplasts decreased in healthy cucumber leaves under RL and FRL conditions compared to WL and increased in infected leaves under all light regimes, especially strongly under RL. This indicates the involvement of the Pr-form of phytochrome activated by RL and Pfr-form of phytochrome deactivated by FRL in the defense

responses of cucumber plants to the *F.ox.* infection. The phytochrome 'light switch' has been revealed in many of its details over the last decade and much more information is on the way. However, information about the effect of phytochrome on the immune response in higher plants is extremely limited. The obtained results deepen the understanding mechanisms of regulation of the photosynthetic function during infection in the light addressed to the phytochrome and could be used to develop a technique increasing the immunity of cucumber plants based on the light regulation of protective reactions in photosynthetic tissues.

REFERENCES

- [1] Arsovski AA, Galstyan A, Guseman JM, Nemhauser JL. Photomorphogenesis. *Arabidopsis Book*, 2012; 10. <https://doi.org/10.1199/tab.0147>
- [2] Pham VN, Xu X, Huq E. Molecular bases for the constitutive photomorphogenic phenotypes in *Arabidopsis*. *Development*, 2018; 145, 23. <https://doi.org/10.1242/dev.169870>
- [3] Huot B, Yao J, Montgomery BL, He SY. Growth-defense tradeoffs in plants: a balancing act to optimize fitness. *Molecular Plant*, 2014; 7, 1267-1287. <https://doi.org/10.1093/mp/ssu049>
- [4] Casal JJ, Balasubramanian S. Thermomorphogenesis. *Annual Review of Plant Biology*, 2019; 70, 321-346. <https://doi.org/10.1146/annurev-arplant-050718-095919>
- [5] Sowden RG, Watson SJ, Jarvis P. The role of chloroplasts in plant pathology. *Essays in Biochemistry*, 2018; 62(1), 21-39. <https://doi.org/10.1042/EBC20170020>
- [6] Genoud T, Buchala AJ, Chua N-H, Metraux J-P. Phytochrome signalling modulates the SA-perceptive pathway in *Arabidopsis*. *Plant Journal*, 2002; 31, 87-95. <https://doi.org/10.1046/j.1365-313X.2002.01338.x>
- [7] Wang H, Wu G, Zhao B, Wang B, Lang Z, Zhang Ch, et al. Regulatory modules controlling early shade avoidance response in maize seedlings. *BMC Genomics*, 2016; 17, 269. <https://doi.org/10.1186/s12864-016-2593-6>
- [8] Griebel T, Zeier J. Light regulation and daytime dependency of inducible plant defences in *Arabidopsis*: phytochrome signalling controls systemic acquired resistance rather than local defence. *Plant Physiology*, 2008; 147(2), 790-801. <https://doi.org/10.1104/pp.108.119503>
- [9] de Wit M, Spoel SH, Sanchez-Perez GF, Gommers CMM, Pieterse CMJ, Voosenek LA, et al. Perception of low red: far-red ratio compromises both salicylic acid- and jasmonic acid-dependent pathogen defences in *ARABIDOPSIS*. *Plant JOURNAL*, 2013; 75, 90-103. <https://doi.org/10.1111/tpl.12203>
- [10] Ballare C.L. Light regulation of plant defense. *Annual Review of Plant Biology*, 2014; 65, 335-363. <https://doi.org/10.1146/annurev-arplant-050213-040145>
- [11] Cerrudo I, Caliri-Ortiz ME, Keller MM, Degano ME, Demkura PV, Ballare CL. Exploring growth-defence trade-offs in *Arabidopsis*: phytochrome B inactivation requires JAZ10 to suppress plant immunity but not to trigger shade-avoidance responses. *Plant Cell Environment*, 2017; 40, 635-644. <https://doi.org/10.1111/pce.12877>
- [12] Fernandez-Milmanda GL, Crocco CD, Reichelt M, Mazza CF, Köllner TG, Zhang T, et al. A light-dependent molecular link between competition cues and defence responses in plants. *Nature Plants*, 2020; 6(3), 223-230. <https://doi.org/10.1038/s41477-020-0604-8>
- [13] Hou X, Lee LYC, Xia K, Yan Y, Yu H. DELLAs modulate jasmonate signaling via competitive binding to JAZs. *Developmental Cell*, 2010; 19, 884-894. <https://doi.org/10.1016/j.devcel.2010.10.024>
- [14] Roden LC, Ingle RA. Lights, rhythms, infection: the role of light and the circadian clock in determining the outcome of plant-pathogen interactions. *The Plant Cell*, 2009; 21, 2546-2552. <https://doi.org/10.1105/tpc.109.069922>
- [15] Pierik R, Ballare CL. Control of plant growth and defense by photoreceptors: from mechanisms to opportunities in agriculture. *Molecular Plant*, 2021; 14, 61-76. <https://doi.org/10.1016/j.molp.2020.11.021>
- [16] Courbier S, Grevink S, Sluijs E, Bonhomme P-O, Kajala K, Van Wees SCM, et al. Far-red light promotes BOTRYTIS CINEREA disease development in tomato leaves via jasmonate-dependent modulation of soluble sugars. *Plant Cell Environment*, 2020; 40, 2530-2543. <https://doi.org/10.1101/2020.05.25.114439>
- [17] Zakurin AO, Schennikova AV, Kamionskaya AM. Light culture of protected soil crop production: photosynthesis, photomorphogenesis and prospects for the use of LEDs. *Plant Physiology*, 2020; 67(3), 246-258 (in Russ.). <https://doi.org/10.1134/S102144372003022X>
- [18] Kudelina TN, Krivobok AS, Bibikova TN, Molchan OB. Features of morphogenesis of *Arabidopsis thaliana* when using LED lighting of various spectral composition. *Proceedings of the National Academy of Sciences of Belarus. Biological series*, 2021; 66(1), 42-52 (in Russ.). <https://doi.org/10.29235/1029-8940-2021-66-1-42-52>
- [19] Naznin MT, Lefsrud M, Gravel V, Azad OK. Blue light added with red LEDs enhance growth characteristics, pigment content and antioxidant capacity in lettuce, spinach, kale, basil, and sweet pepper in a controlled environment. *Plants Basel*, 2019; 8(4), 93. <https://doi.org/10.3390/plants8040093>
- [20] Shlyk AA. Determination of chlorophyll and carotenoids in extracts of green leaves. *Biochemical methods in plant physiology*. Moscow: Nauka, 1971; pp.154-170 (in Russ.).
- [21] Krause GH, Weis E. Chlorophyll fluorescence and photosynthesis: the basics. *Annual Review of Plant Physiology and Plant Molecular Biology*, 1991; 42, 313-349. <https://doi.org/10.1146/annurev.pp.42.060191.001525>
- [22] Kramer DM, Jonson G, Kiirats O, Edwards GE. New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. *Photosynthesis Research*, 2004; 79, 209-218. <https://doi.org/10.1023/B:PRES.0000015391.99477.0d>
- [23] Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiology*, 1981; 22, 867-880.
- [24] Petsas A, Grammaticopoulos G. Drought resistance and recovery of photosystem II activity in a Mediterranean semi-deciduous shrub at the seedling stage. *Photosynthetica*, 2009; 47, 284-292. <https://doi.org/10.1007/s11099-009-0044-1>
- [25] Makarenko MS., Kozev NV., Usatov AV., Gorbachenko OF, Averina NG. A State of PSI and PSII photochemistry of sunflower yellow-green plastome mutant. *OnLine Journal of Biological Science*, 2016; 16, 193-198. <https://doi.org/10.3844/ojbsci.2016.193.198>
- [26] Klughammer C, Schreiber U. An improved method, using saturating light pulses, for the determination of photosystem I quantum yield via P700+ absorbance changes at 830 nm. *Planta*, 1994; 192(2), 261-268. <https://doi.org/10.1007/BF01089043>
- [27] Dietzel L, Bräutigam K, Pfannschmidt T. Photosynthetic acclimation: state transitions and adjustment of photosystem stoichiometry - functional relationships between short-term and long-term light quality acclimation in plants. *The FEBS Journal*, 2008; 275, 1080-1088.

- [28] Wang D, Dawadi B, Qu J, Ye J. Light-Engineering Technology for Enhancing Plant Disease Resistance.

Frontiers in Plant Science, 2022; 12, 805614.
<https://doi.org/10.3389/fpls.2021.805614>

Received on 28-10-2022

Accepted on 25-11-2022

Published on 21-12-2022

DOI: <https://doi.org/10.12974/2311-858X.2022.10.07>

© 2022 Liudmila Kabashnikova *et al.*

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.