

Effect of UV-B radiation on chlorophyll fluorescence, photosynthetic activity and relative chlorophyll content of five different corn hybrids

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ABSTRACT

This research presents an experimental study of the effect of UV-B radiation (7.5 Wm^{-2}) on the change of the total concentration of chlorophyll ΔChl and energy that a plant can store during the process of photosynthesis. The aim was to investigate the effect of UV-B radiation to spectral lines of five genetically different corn hybrids and find the lines with better resistance. Chlorophyll fluorescence from plant leaves was used as experimental method. The plants were exposed to UV-B radiation for 19 days. The following results were obtained: a) there is a significant variation between different corn hybrids regarding the effect of UV-B radiation, b) an indicative element of change in the functioning of the photosynthetic apparatus is represented in variations in the relative composition of photosynthetic pigments, c) regardless of what may be the cause of the change of the plant's ability to deposit a part of absorbed energy in the primary products of photosynthesis, it has been shown that two out of five investigated corn hybrids show great resistance to UV-B radiation, and d) relative change of photosynthesis can be used as a measure of the plant's resistance to the harmful effect of UV-B radiation.

Introduction

It can be said freely that photosynthesis is the most important process in the living world without which there would be no life on the planet Earth. Besides, one of the products of photosynthesis (biomass) is the food basis for a large number of animals and for humans; it is the basis for numerous technologies as well. On the other hand, UV radiation is the byproduct of damage to the ozone layer leading to the increase of UV radiation, which then influences the photosynthetic activity of plants. Likewise, the increase in UV radiation presents a threat to the survival of the plants. Many research found that UV radiation causes various changes in plants; consequently, it is considered as one of the greatest ecological problems. UV radiation induces the uncoupling of the chlorophyll molecules in the light-harvesting system [1], resulting in reduced photosynthesis in many plant species [2]. UV-A radiation is highly damaging for Photosystem II [3]. In many cases, even small doses of UV radiation cause a shift in the ontogenetic sequence of photosynthetic capacity [4]. UV radiation causes dramatic changes in biomass production [5], leaf development [6], stomas [7]. All these changes affect photosynthesis directly. The results of radiation on the photosynthesis process, and thus plants as well, is a topic of great interest to

researchers who aim at a) understanding the effect of UV radiation to photosynthesis, and b) finding the plant species resistant to UV radiation, thus preventing the photosynthesis inhibition. In accordance with the previously said, this research presents a step forward in the investigation of the effect of UV-B radiation on different corn hybrids and finding the ones with better resistance. The presented research is focused on the effects of intense UV radiation on photosynthetic apparatus activity and chlorophyll concentration in several *Zea mays* types using results of leaf fluorescence in accordance with the earlier presented method [8].

Materials and methods

Sample preparation and measuring

The objects of the experiments were five genetically different corn hybrids (*Zea mays* L): ZP-677, ZP-704, ZP-42A, ZP-735 and ZP-434. These hybrids were chosen as the most frequently sold hybrids produced by the Maize Research Institute, and provided with the courtesy of researchers from this institute. Before exposure to the UV-B radiation, the corn plants were grown for 5 weeks in a greenhouse under controlled

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conditions. After 5 weeks, the plants were randomly separated into two groups: a) control group (30 plants) and b) experimental (stressed) group (30 plants). Plants in each group included five subgroups, one for each considered corn hybrid. All plants continued to grow in controlled conditions during the experiment (19 days). Experimental (stressed) plants were also placed in five groups where each group consisted of 30 specimens. The stressed plants were irradiated for 8h for 19 days. The experiment was conducted in a standard greenhouse of approximate dimensions $1.5 \times 2 \times 2$ m, which is a typical setting in the Maize Institute in Zemun Polje, where the hybrids came from. As all considered plants grow at identical environmental conditions, it is clear that: a) growing conditions (humidity, lighting, temperature, nutrition of the soil) can affect both groups (control and stress) in the same way; b) obtained difference in fluorescence spectra for control and stressed group, as a response on environmental conditions, can be only due to the fact that stressed group was exposed to the UV-B radiation. UV-B spectra of the lamp used for laboratory irradiation of the plants and clear-sky solar spectra were obtained with equipment used in this research and explained in the text below Figure 1. shows the spectra of the UV-B fluorescence lamp and sky.

The stressed plants were irradiated with Philips UVB 311 nm Lamp (PL = S 9W/01/2P) fluorescent lamp. The UVB lamp was placed over the plants, at 90° and at 1m distance. The intensity of UV-B light was 0.75 mW/cm^2 , similar to other experiments on plants [6, 9]. The UV-B interference filter (280 nm – 315 nm) was placed in front of the lamp. In this way we eliminated the lines of the UV-B lamps at 550 nm [10,11]. The measurements have been done on the flag leaf. Fluorescence of each leaf was measured for 1.5 minutes.

For the excitation of the leaves and obtaining the fluorescence spectra of the leaf, we used the mentioned UVB light source. The entrance aperture of the fiber was placed 10 mm away from the leaf and directed to the center of the illuminated area at 45° of the leaf axis. Fluorescence emitted radiation from the intact leaf was collected and directed through an optical fiber (N.A. of 0.22 and 1000 μm diameter) that was coupled to the portable 2048-element CCD spectrometer (AVANTES 1000 PC). Data collection and spectrum processing were conducted in real time with microcomputer and commercial software OOI Base (AVANTES Inc.). In the mentioned measurements, we have made a large number of measurements and in this way satisfied the criterion of the central limit condition, as for any physical occurrences which depend on one stochastic argument [12]. Therefore, relative error is less than $\pm 2\%$. For each group of plants, the results are represented as

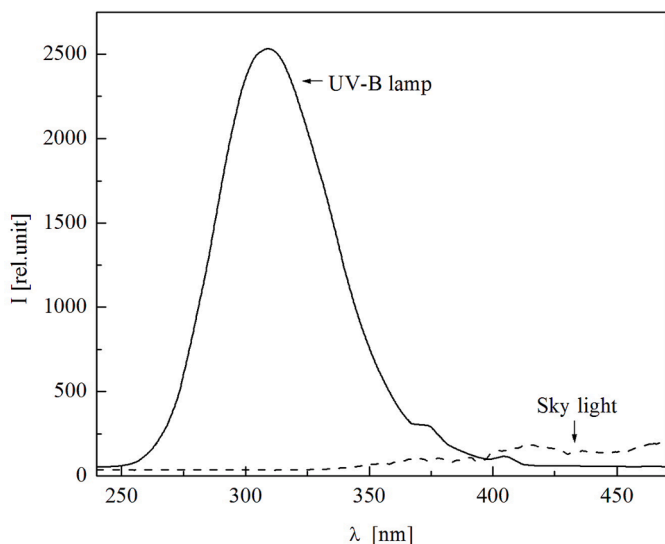


Fig. 1. UV-B spectra of the lamp used for laboratory irradiation of the plants (—) and clear-sky solar spectra (---).

an average value of the performed measurements.

Theoretical framework

It is well known that the photosynthesis status of stressed and normal plants can be best obtained from the fluorescence spectrum. Or in other words, literature shows that monitoring the optical activity of chlorophyll molecules is a useful method for monitoring plant health [8;13;14].

Amount of energy which may potentially be stored through primary products of photosynthesis for a given time interval is called photosynthesis energy of radiation, W_{ph} [15]:

$$W_{ph} = \alpha_{\max} \int_{t_1}^{t_2} I_{NPAR} dt. \quad (1)$$

Here t_1 and t_2 are the beginning and the end, respectively, of the radiation energy input, α_{\max} is the efficiency coefficient of energy use for photosynthesis at a wavelength of 680 nm. I_{NPAR} is the irradiance which can be expressed as

$$I_{NPAR} = \int_{\lambda_1}^{\lambda_2} K(\lambda)_{ph} I(\lambda) d\lambda, \quad (2)$$

where $I(\lambda)$ is the radiant flux incident on the receiving surface, $\lambda_1 = 410$ nm, and $\lambda_2 = 800$ nm. Coefficient $K(\lambda)_{ph}$ has been determined from the action spectrum of photosynthesis and is calculated as the ratio of the photosynthesis rate at the wavelength λ and the maximal photosynthesis rate at the wavelength 680 nm. Finally, by combining Eq. (1) and Eq. (2), and taking into account $\Delta t = t_2 - t_1 = 1$ h, which is regularly used in the literature [15,16], we can express the photosynthesis energy of radiation in the following way:

$$W_{ph} = \alpha_{\max} \int_0^1 [K(\lambda)_{ph} I(\lambda) d\lambda] dt. \quad (3)$$

Let us assign W_{ph}^C and W_{ph}^S as amounts of energy potentially stored through primary products of photosynthesis for plants in control (index C) and experimental groups (index S). Then, the rate of change of photosynthetic apparatus due to stress, in our case due to radiation, can be determined. We can assume that the energy stored through primary photosynthesis products is maximized in a plant residing in optimal condition for photosynthesis; the deviation from this value determines the rate of change of photosynthetic apparatus. Since this deviation is more or less present in all plants, it is much more convenient to observe their relative change usually referred to as a plant's loss of ability to store absorbed energy. Therefore, in agreement with the literature data, relative change of a plant's loss of ability to store absorbed energy $\Delta\varepsilon$ [%] can be expressed with [17]:

$$\Delta\varepsilon [\%] = 100 \cdot \left(1 - \frac{W_{ph}^S}{W_{ph}^C} \right) \quad (4)$$

Using chlorophyll fluorescence spectra and Eq. (3) and (4) it is possible to determine the rate of efficiency change in photosynthesis apparatus under stressed conditions. This method was successfully tested during researching the effect of γ -nuclear radiation on bean and pumpkin [17] and the influence of mineral nutrition on maize [15].

A known fact in literature is that the ratio of the two chlorophyll fluorescence peaks (F_{730} / F_{690}) in the leaves correlates well with the amount of chlorophyll content in the plant leaves [13,16,18,19,20]. Therefore chlorophyll content was determined using: a) maize fluorescence spectra, and b) relation between chlorophyll content and the fluorescence intensity ratio FIR defined as the ratio of the fluorescence

intensity measured at 730nm (F_{730}) and 690nm (F_{690}) $FIR = FIR_{730}/FIR_{690}$. In order to eliminate errors that may arise due to different chlorophyll concentration in a different kind of plant species, we have decided to use correlation between relative change of chlorophyll fluorescence ratio ΔFIR and relative change of chlorophyll content ΔChl (a,b). Therefore in order to eliminate the differences in the individual chlorophyll content in the different corn hybrids relative change of the total chlorophyll content $\Delta chl(a,b)$ we have used the equation obtained from literature data [8]:

$$\Delta Chl(a, b) [\%] = 0.1683 \times \Delta FIR^{1.386} \quad (5)$$

where: $\Delta FIR = 100 (FIR_{UV})/(FIR_{Cont})^{-1} - 100$, FIR_{Cont} and FIR_{UV} are the fluorescence intensity ratio for control and stressed plants. The described method was successfully used on pumpkins exposed to the permanent magnetic field [8].

Results

Figure 2 presents fluorescence spectra of corn plants from control group (not treated with UV-B radiation) and experimental group (treated with UV-B radiation).

Figure 3 shows the dynamics of change of $\Delta \epsilon$ during exposure to the UV-B radiation for five corn hybrids. There is no significant decrease of $\Delta \epsilon$ with two hybrids (ZP-677 and ZP-704) during the complete investigated period. Starting from the first day, $\Delta \epsilon$ remained almost unchanged until the fifth day when the slow decrease of $\Delta \epsilon$ was noted at both hybrids and lasted until the end of treatment. After 19 days, the value of $\Delta \epsilon$ was close to 5 % in comparison to the control specimens (plants not treated with UV-B radiation).

For the corn hybrid ZP-434, $\Delta \epsilon$ remained constant until the fifth day, and then started to decrease rapidly as the exposure to UV-B persisted. At the end of the period of investigation, $\Delta \epsilon$ decreased below 50 % comparing to the plants not treated with UV-B radiation. For the ZP-42A, $\Delta \epsilon$ was decreasing from the beginning of treatment during the complete period of investigation. On the last day of treatment, $\Delta \epsilon$ was reduced to 30 % in comparison to the control plants. For the hybrid ZP-735, $\Delta \epsilon$ was slowly decreasing till the fifth day (5 %) and then varied a bit until the 16th day when started to decrease rapidly. On the last day, $\Delta \epsilon$ was reduced to 30 % in comparison to the control specimen.

Figure 4 shows a relative change of the total concentration of chlorophyll ΔChl (a,b) during the exposure of five corn hybrids to UV-B

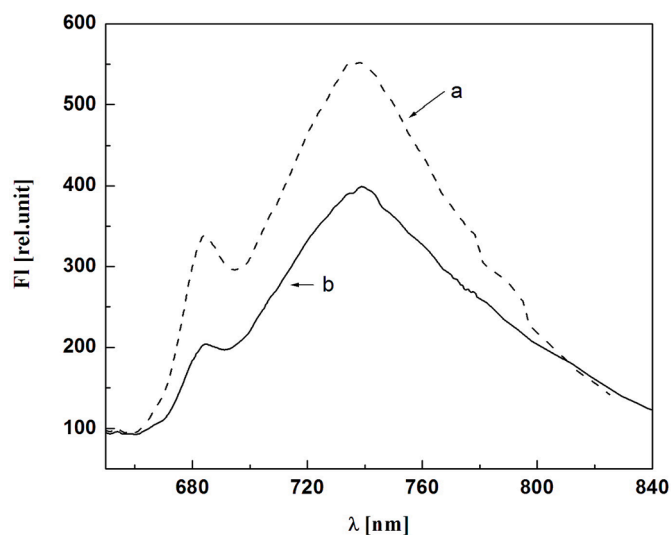


Fig. 2. Typical fluorescence spectra for corn plant (ZP-434 hybrid). (a) no treating with UV-B radiation ($t = 19$ days); (b) treating with UV-B radiation ($t = 19$ days).

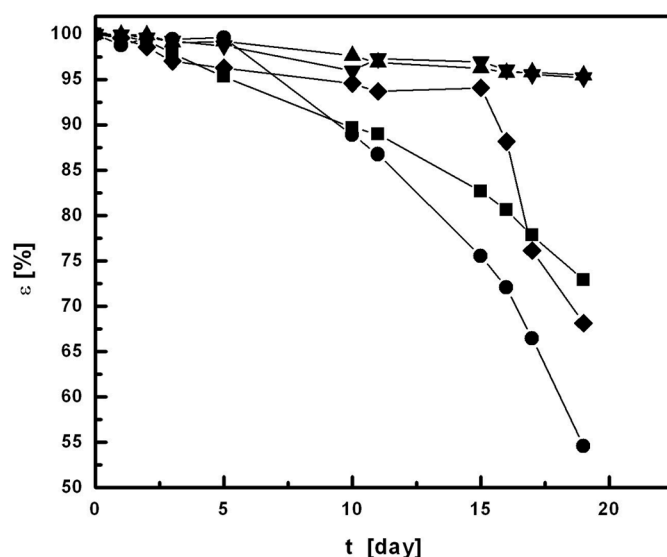


Fig. 3. Relative changing in $\Delta \epsilon$ during exposing to the UV-B radiation for five corn hybrids: (▲) – ZP-677, (▼) – ZP-704, (■) – ZP-42A, (◆) – ZP-735, (●) – ZP-434.

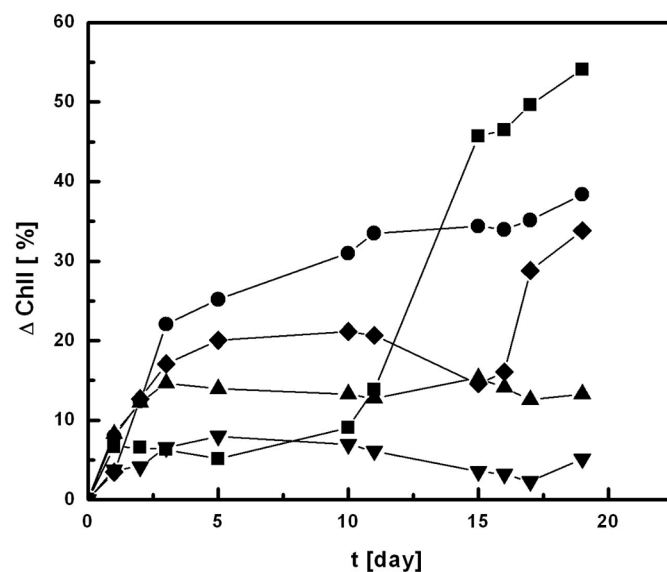


Fig. 4. Relative change of the chlorophyll(a,b) content during exposing to the UV-B radiation for five corn hybrids: (▲) – ZP677, (▼) – ZP704, (■) – ZP42A, (◆) – ZP735, (●) – ZP 434.

radiation. It is easy to notice that ΔChl (a,b) changed differently at all five hybrids during the period of investigation. The smallest changes were noted at the line ZP-704. The change of the chlorophyll concentration ΔChl (a,b) was increasing up to the fifth day when it was higher for 8 % in comparison to the control specimens; then it was slowly dropping until the 17th day to about 2 %. On the last day of investigation, a small increase of ΔChl (a,b) was noted, for 5 % in comparison to the control specimen. ZP-677 reacted similarly, except the change level which was a bit higher.

Changes of ΔChl (a,b) increased until the third day when they were higher by 15 % in comparison to the control specimen; after that period, up to the 19th day there were no significant changes and ΔChl (a,b)

varied between 12 % and 15 %. ZP-434 reacted in a completely different way. Starting from the first day, the decrease of ΔChl (a,b) was constantly increasing up to the 19th day when it was the highest amounting to 38 % in comparison to the control specimen.

For ZP-735, in the first days up to the 10th day, UV-B radiation-induced constant decrease of the change of ΔChl (a,b), when it amounted to 22 %, and then slowly decreased until the 15th day. After that, UV-B radiation-induced rapid increase of the change of ΔChl (a,b) until the end of the investigated period, when the change of ΔChl (a,b) was the biggest 55 %. This means that in chlorophyll concentration Chl (a,b) is more sensitive than in other mentioned hybrid. For ZP-42A on the first day of treatment with UV-B beams, the change of ΔChl (a,b) was noted amounting to 7 %, afterward remaining unchanged until the 10th day. After the 10th day, there was a rapid increase in the change of ΔChl (a,b) on daily basis, until the 19th day, when the change was the largest in comparison to the other hybrids, amounting to 57 %.

Discussion

The obtained results show the difference in plant leaves fluorescence spectra for genetically different plants species, which is not unusual. Namely, in literature data one can find a lot of examples proving that genetic difference in material has a significant effect on fluorescence spectra characteristics. Examples include plant species such as tomato [22], cucumber [23], mung (*Vigna radiata*) [24]. Also, the difference in plant leaves fluorescence spectra for genetically different maize species was observed [25].

Since the beginning of UV-B exposure, the decrease of $\Delta\epsilon$, i.e. photosynthesis was noted during the complete period of investigation for two hybrids (ZP434 and ZP735). At ZP-434 there was a rapid decrease of photosynthesis – on the 19th day the activity dropped to 55 %, and at line 735 to 75 %. Rapid changes of $\Delta\epsilon$ could be expected because the plants were exposed to UV-B radiation of high intensity (0.75 mW/cm²), twice higher than the radiation which induced a decrease of photosynthesis at trees for 70 % [21], four times higher than the radiation which induced total reduction of plant mass [26] and 25 times higher than the radiation which inhibited electron transport in Photosystem II for 95 % in spinach leaves [27]. Here, the exposure of two corn hybrids to UV radiation resulted in significant changes in the amount of energy that could have been stored through primary photosynthesis products, which is a clear indicator that changes in photosynthesis occurred. This is in accordance with the studies reporting that: a) UV-B radiation induces a significant change in photosynthesis, b) UV-B photons can cause cellular damage in biomolecules but without significant damage of photosynthesis or pigment levels; this means that the treatment is not lethal and that corn leaf physiology readily recovers [28] c) that UV-B radiation inhibits photosynthesis at wheat [29], soybean [30] and rice [31]. According to previously said, it may be supposed that there are more reasons for such inhibition of photosynthesis and with the change of $\Delta\epsilon$ due to UV-B radiation. First, permanent damage of the ribosome probably occurred due to the long exposure to UV radiation, as experimentally shown at *Zea mays* after 16-hour-long leaf exposure [28]. Second, UV-B radiation may induce the uncoupling of chlorophyll molecules in the light-harvesting system leading to inhibition of energy transfer [1], which affected directly to decrease of $\Delta\epsilon$, i.e. of photosynthesis [32,33,34][35]. Third, UV-B radiation may induce a shift in the ontogenetic sequence of photosynthetic capacity [4]. Fourth, damage to photosynthetic electron transport probably occurred due to UV-A radiation targeted at the Photosystem II complex [3,36]. Finally, the obtained decrease in $\Delta\epsilon$ can be connected with UV-B induced inhibition of photosynthesis and can be attributed to the reduction in the activity of Photosystem II [34]. On the other hand, photosynthetic activity linearly decreases in the corn due to UV-A radiation in 10 days [36].

At Z-42A, $\Delta\epsilon$ changed only a little until the 16th day when it began to decrease rapidly. The explanation of such behavior is highly complex.

One of the reasons is that the mechanisms for photo repair of daily UV-induced damage [26] probably ceased to function after the 16th day. Also, it was shown that this hybrid was partly sensitive to the duration of UV treatment and that a period longer than 16 days was lethal, after which the plant lost the ability to recover. This fact is not in contradiction to the information found in literature stating that for some corn hybrids, neither photosynthesis nor pigment levels were affected significantly by UV-B radiation during short-term exposure (6-16 hours) demonstrating that the treatments applied was not lethal and that corn leaf physiology readily recovers [28]. The concentration of flavonoids most likely was insufficient to protect the plant from longer exposure to UV radiation.

At the remaining two hybrids (ZP-704 and ZP-677) there was no significant decrease of $\Delta\epsilon$, indicating that both were resistant to UV-B radiation. Non-sensitivity to UV-B radiation has been shown on other plants as well. For example, a cucumber endured a 33-day exposure to UV-B radiation with no change in photosynthetic efficiency [37]. In the case of corn plants, they can prevent the dangerous effects of UV-B radiation by synthesizing flavonoids, a class of UV absorbing compounds located mainly in the epidermis and acting as an internal filter [38]. It was reported that the concentration of flavonoids accumulates in corn leaves when they are exposed to the increased UV-B radiation the flavonoid accumulation in maize leaves increased [39]. Also, it was shown that the flavonoids protect DNA in corn plants from the potential damage caused by UV radiation in comparison to the DNA in plants that are genetically deficient in these compounds [40]. On the other hand, experimental results indicate that physiological levels of UV-B radiation have a positive effect on the induction of the synthesis of a photosynthetic enzyme involved in corn photosynthesis [41]. UV-B radiation is highly damaging for Photosystem II [3] and in accordance with that, the first reason for the unchanging rate of the efficiency change in photosynthetic apparatus ϵ induced by stress at the said two hybrids might be connected to the higher concentration of flavonoids in comparison to the other three which, possibly, were genetically deficient in those compounds. Flavonoids are known to act as an effective internal screen in epidermal cells protecting a plant from UV-B radiation. It was shown that in eight soybean hybrids the degree of damage by UV-B radiation should be strongly dependent on the efficiency of constitutive and UV-induced mechanisms of protection [42]. The unchanging of ϵ during the whole period of treatment with UV-B radiation at these two species might be connected to the experimentally verified fact that corn has efficient mechanisms for photo repair of daily UV induced damage [40]. Finally, it might be said that photo repair, in combination with the presence of flavonoids, makes these two hybrids (ZP-704 and ZP-677) show the great resistance to UV radiation as well as the ability of adjustment for survival in conditions of the increased UV radiation. Likewise, some results indicate that physiological levels of UV-B radiation have a positive effect on the induction of the synthesis of a photosynthetic enzyme which is involved in corn photosynthesis [41].

Figure 4 presents the results of the relative change of the total concentration of chlorophyll ΔChl (a,b) in the leaves of five hybrids of corn ZP-42A, ZP-434, ZP-677, ZP-704, ZP-735 occurring due to intensive UV-B radiation during 19 days. Literature data indicates that there are contradictory results regarding the effect of UV-B radiation on the concentration of two pigments very important for photosynthesis (chlorophyll a and chlorophyll b). Some studies show that UV-B radiation did not have a significant effect on chlorophyll concentration at rice and pea plants [43]. After 33 days, UV-B radiation did not cause significant changes in the total concentration of chlorophyll which remained almost unchanged [37]. Total concentration of chlorophyll at white clover remained unchanged even after four weeks of exposure to UV-B radiation – 3 % [45]. At five tree species, the total concentration of chlorophyll changed only a bit and varied between 2-14 % after five years of exposure to UV-B radiation [21]. Other studies show that UV-B radiation induces a decrease of concentration of chlorophyll in terrestrial plants [4]. Chlorophyll concentrations (leaf area basis) in

UV-B-irradiated spinach leaves were significantly lower than in control plants after 4, 8 and 12 days of exposure [46]. Even small doses of UV-B radiation induce a decrease in chlorophyll concentration in duckweed [47]. On the contrary, there are experiments which proved that concentrations of chlorophyll a and chlorophyll b significantly increased in leaves due to UV-B radiation [48]. Also, in soybean leaves exposed to UV-B radiation increase of concentration of photosynthetic pigments was induced [2]. Having in mind all previously said, it is certain that variations in the relative composition of photosynthetic pigments may be an indicator of perturbations in the photosynthetic apparatus [38].

The situation with corn is similar. Results describing the effect of UV-B radiation on the total concentration of chlorophyll in corn leaves are contradictory as well. Namely, some experiments show that in the first three days of UV-B radiation treatment in corn leaves there is a significant decrease of the total concentration of carotenoids (6-15 %) and the biggest change in chlorophyll concentration (4-16 %) [38]. Also, data are showing that the increase of UV-B radiation significantly reduces chlorophyll concentration in corn leaves [39]. On the contrary, some experiments show that UV-B radiation induces increase of chlorophyll concentration [38,39]. As a reaction to UV-B radiation and as a tendency to adapt to unfavorable conditions, ZP-677 and ZP-704 probably intensified mechanisms for flavonoid synthesis, as shown in some experiments with corn [36]. Literature shows that corn plants that contain flavonoids (primarily anthocyanins) are protected from the induction of damage [36]. Such an increase of flavonoids protected the photosynthetic apparatus of these two hybrids, which affected the constancy of $\Delta\epsilon$ (Figure 3). ZP-42A and ZP-434 did not show any ability to adjust to the conditions they were grown in, as shown in the constant big change of total chlorophyll concentration (Figure 4) undoubtedly leading to rapid perturbations within the photosynthetic apparatus [38]. The direct consequence of induced perturbations within the photosynthetic apparatus revealed itself as a significant decrease of $\Delta\epsilon$ (Figure 3). In accordance with the literature data, such rapid changes of chlorophyll concentration may be explained by the hypothesis stating that these two hybrids are genetically deficient in these compounds [36]. The ability of line ZP735 to adapt in the beginning period of treatment (the first 15 days) was prominent, but after that, it disappeared and was followed by the increase of the change of total chlorophyll concentration ΔChl (Figure 4) and by the simultaneous decrease of $\Delta\epsilon$. In agreement with the mentioned above discussions and literature data [49], we assumed that change in $\Delta\epsilon$ (photosynthesis) can be used as a useful tool for determination of plant tolerance on UV-B radiation.

Conclusions

Having in mind previously given results, we may conclude that: a) present studies have demonstrated that significant variation exists in the plant's response to UV-B radiation between different maize lines, b) variations in the relative concentration of photosynthetic pigments (chlorophyll) may be an indicator of perturbations in the photosynthetic apparatus, c) regardless of what may be the reason of the plant's inability to deposit a part of the absorbed energy during the process of photosynthesis $\Delta\epsilon$ or total chlorophyll concentration, it has been shown that two out of five investigated maize hybrids show great resistance to UV-B radiation, and at last d) $\Delta\epsilon$ can either decrease or increase depending on UV characteristics and the plant species and e) $\Delta\epsilon$ parameter can be used as a measure of plant resistance to harmful effect of UV-B radiation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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