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PHOTOSYNTHETIC PROPERTIES OF ELITE ERECT LEAF MAIZE INBRED LINES AND THEIR CONTRIBUTION TO SEED PRODUCTION IMPROVEMENT*

ABSTRACT: A hypothesis that elite erect leaf maize inbred lines are characterized by properties of an efficient photo-model and that as such are very desirable in increasing the number of plants per unit area (plant density) in the process of seed production has been confirmed in the present study.

The properties of the observed elite erect leaf maize inbred lines were based on the effects and characteristics of thermal processes of delayed chlorophyll fluorescence occurring in their thylakoid membranes. The temperature dependence of the delayed chlorophyll fluorescence intensity, the Arrhenius plot for the determination of phase transitions (critical temperatures) and activation energy are the principal parameters of the thermal processes.

Based on the obtained results on photosynthetic properties it was also possible to estimate the tolerance and adaptation of elite erect leaf maize inbred lines to high temperatures and drought.

KEY WORDS: maize inbred lines, erect leaves, maize seed, photosynthetic model, thermal and photosynthetic processes, thylakoid membrane, delayed chlorophyll fluorescence

INTRODUCTION

This study considers two evident facts from the most recent history of maize breeding and seed production in our country. The first one is with a plus sign, based on the results of maize breeding and seed production that have been intensively developing during the last 50 years. As a result of these activities, approximately a thousand grain and silage maize hybrids were developed; at the same time, conditions necessary for a highly developed seed production had been provided: drying and processing plants, choice of land, irri-

* This paper is contributed to the memory and long reminiscence of a countenance and deeds of Dr. Jovan Smiljaković's.

gation systems, sufficient quantity of basic seed and qualified staff (Duvick, 1984; Trifunović, 1986; Ivanović et al., 1995; Radenović and Somborac, 2000). Regardless of such a colossal success in maize breeding and seed production, eagerness and enthusiasm of the total research body did not slow down. The search for new methods and exact approaches in the completion and enrichment of the research within maize breeding and seed production was continued. The other fact, with a minus sign, is related to the interrelation between photosynthesis and maize seed production. Although photosynthetic processes are highly productive in their intensity, very complex in their nature, and vastly studied in their scientific actuality, their application in maize seed production is still insignificant. Such a state of affairs is probably a consequence of the existence of several functional interrelations that unify conformational and dynamic changes within chloroplasts and their thylakoid membranes on the one hand, and the effects of environmental stress factors on them on the other.

Delayed chlorophyll fluorescence (DF) can be phenomenologically described as an occurrence of luminescence (bioluminescence) within the red range of the visible spectrum produced by plant systems, bacteria, algae and higher plants (maize), immediately after their intermittent illumination (Radenović, 1992, 1994, 1997). DF was discovered by Strehler and Arnold (1951). Important studies, especially those conducted over last 20 years (Jursinic, 1986; Veselovski and Veselova, 1990; Marković et al., 1993, 1996) revealed a direct connection between DF and the photosynthetic processes, in which DF was considered an unavoidable indicator — a sensitive „probe" for experimental photosynthetic and bioluminescence studies (Radenović et al., 1994a, 1994b; Radenović and Jeremić, 1996; Marković et al., 1987, 1999).

The objective of the present study was to determine general and photosynthetic properties of elite erect leaf maize inbred lines, which serve as an efficient photo-model, using a non-invasive photosynthetic and bioluminescence method in maize breeding and seed production (Radenović et al., 2000, 2001a, 2001b, 2002a, 2002b).

MATERIAL AND METHODS

A brief survey of the studied elite erect leaf maize inbred lines is given in Table 1. The material selected for this study was grown at the experiment field of the Maize Research Institute, Zemun Polje.

During July and August, maize plants were brought from the field to the laboratory during morning hours (between 7 a. m. and 8 a. m.). During sampling in the field, plants were transversely cut at the ground internode. In the laboratory, plants were internode-lengthwise placed in water. Two hours prior to the bioluminescence experiment, the plants were kept under the black ball glass. A segment of ear intact leaves (or leaves just above the ear) was taken from such plants and placed into a chamber of the phosphoroscope (Figure 1) and kept in the dark for at least 15 minutes, and then DF was measured. The non-invasive photosynthetic bioluminescence method used to measure DF is presented in Figure 1. This block scheme of the bioluminescence method was developed at the Maize Research Institute, Zemun Polje. Measurements of changes in DF intensities were performed after a method that had been described, both in principle and detail, in previous papers (Radenović, 1992, 1994, 1997; Radenović et al., 2001a, 2001b, 2002a, 2002b; Marković et al., 1996).

Table 1. Description of elite erect leaf maize inbred lines

Ordinal number	Designation of inbred lines	FAO maturity group	Origin	The axil between ear leaf and stalk	Ear leaf area (cm^2)
1	ZP PL 16	700	BS13(S2)CO	31.60°	5260.30
2	ZP PL 111	600	Iowa SSS	32.10°	4986.80
3	ZPL 773	650	B73 rec	31.98°	4904.10
4	ZP PL 121	600	Mo17 x 25-10-1	32.50°	5320.20

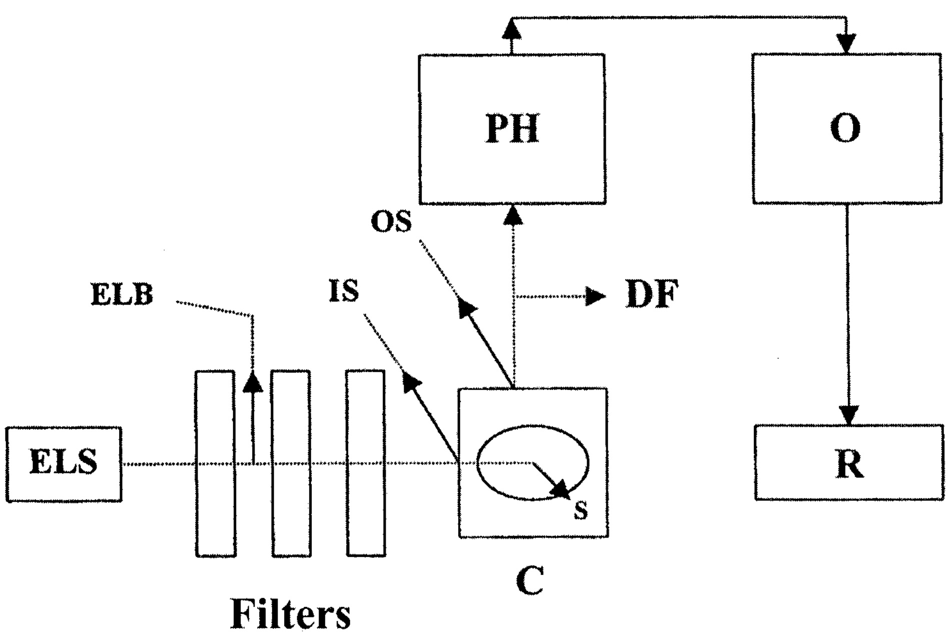


Figure 1. Experimental setup of the method and measuring equipment for delayed chlorophyll fluorescence: C — dark chamber with a sample stand; s — sample (leaf segment), filters, ELS — excitation light source, PH — photo-multiplier; O — oscilloscope, R — printer, ELB — excitation light beam, DF — luminescent light, IS — input chamber slot, OS — output chamber slot

RESULTS

The results of photosynthetic properties of the elite erect leaf maize inbred lines, as optimal photo-models, are presented through five sections.

1. *The measure of the axil and leaf area of the elite erect leaf maize inbred lines*

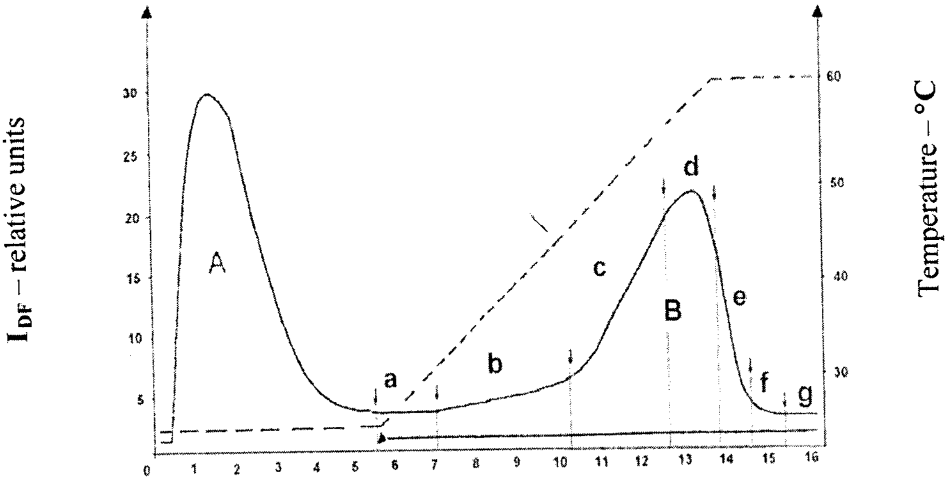
A specially designed protractor was used to measure the axil of each leaf of the elite erect leaf maize inbred plants. The results of the measurements of the axils (ranging from 31.60° to 32.50°) between the ear leaves and the stalks are presented in Table 1. Average leaf areas of the elite erect leaf maize inbreds are also presented in Table 1. It is clear that the leaf area has no properties that would particularly distinguish the studied elite erect leaf maize inbred lines.

2. *Parameters of thermal processes of the delayed chlorophyll fluorescence in the studied elite erect leaf maize inbred lines*

A detailed study on thermal processes of chlorophyll DF was carried out for the elite erect leaf maize inbred lines. These processes were characterized by time parameters in regard to the duration of conventionally sampled segments: **a**, **b**, **c**, **d**, **e**, **f** and **g** on the thermal curve of chlorophyll DF (Figure 2). The results on time parameters of the chlorophyll DF thermal curve for the stated segments are presented in Table 2.

Table 2. Duration (in seconds) of segments on the thermal curve of delayed chlorophyll fluorescence for elite erect leaf maize inbred lines

Segment of thermal curve	Segment designation	MAIZE INBRED LINE				Temperature range for establishing thermal curve (°C)
		ZPPL 16	ZPPL 111	ZPL 773	ZPPL 121	
Stationary level of DF intensity	a	>130	>130	>130	>130	15—45
Initial increase of DF intensity	b	460	488	496	448	15—45
Linear increase of DF intensity	c	150	162	166	138	15—45
Maximum level of DF intensity	d	58	66	52	56	15—45
Linear decrease of DF intensity	e	132	120	126	138	15—45
Decelerated decrease of DF intensity	f	119	102	96	88	15—45
Minimum level of DF intensity	g	26	22	18	24	15—45



Time elapsed (minutes)

Figure 2. Schematic presentation of typical changes of chlorophyll DF intensities in the intact leaves of the observed elite erect leaf maize inbred lines (solid line) and changes of temperatures (dashed line): curve **A** indicates induction processes of chlorophyll DF, while curve **B** encompasses thermal processes of chlorophyll DF. Segments on the thermal curve (**a, b, c, d, e, f, g**) are interception points in which conformational and functional changes in the thylakoid membrane occur

3. *Temperature dependence of the delayed chlorophyll fluorescence intensity for the thylakoid membrane of the elite erect leaf maize inbred lines*

Figure 3a, b, c, d presents the changes in the intensity of the stationary DF level in the function of temperatures ranging from 15°C to 45°C in the thylakoid membrane of the following elite erect leaf maize inbred lines: ZPPL 16, ZPPL 111, ZPL 7739 and ZPPL 121.

4. *The Arrhenius plot for the determination of conformational changes in the thylakoid membrane of the elite erect leaf maize inbred lines*

Figure 4a, b, c, d presents results of conformational changes that occurred in the thylakoid membrane of the elite erect leaf maize inbred lines. These changes were caused by the temperature impact on the intact leaf segment of the following erect leaf maize inbred lines: ZPPL 16, ZPPL 111, ZPL 7739 and ZPPL 121.

Figure 3a

Figure 3b

Figure 3c

Figure 3d

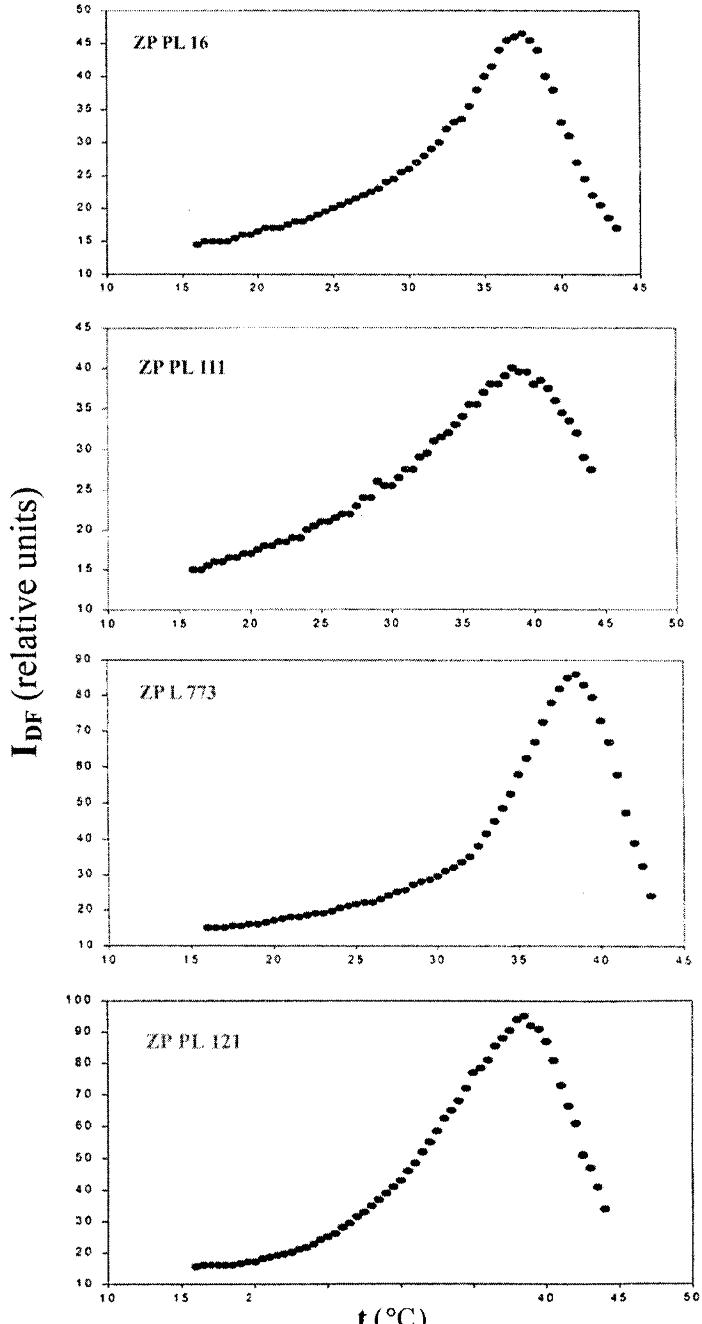


Figure 3 a, b, c, d. Changes of the intensity of the stationary chlorophyll DF level in dependence of the temperature in the thylakoid membrane of intact leaves of the elite erect leaf maize inbred lines

Figure 4a

Figure 4b

Figure 4c

Figure 4d

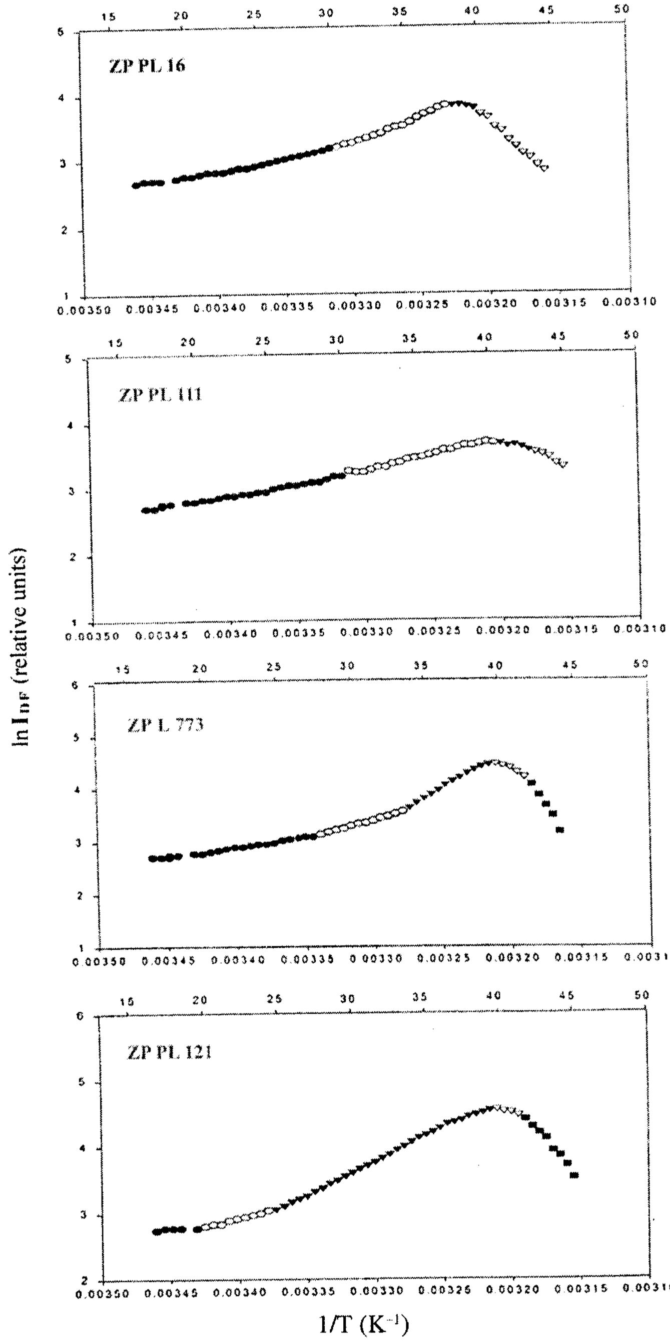


Figure 4 a, b, c, d. Arrhenius plot for a change of the logarithm of intensity of chlorophyll DF in dependence of the reciprocal values of temperatures on the thylakoid membrane of the elite erect leaf maize inbred lines

5. *Activation energies and critical temperatures in the thylakoid membrane of the elite erect leaf maize inbred lines*

Activation energies and temperatures of phase transitions (critical temperatures) in the thylakoid membrane of the elite erect leaf maize inbred lines ZPPL 16, ZPPL 111, ZPL 7739 and ZPPL 121 are presented in Table 3.

Table 3. Activation energies and temperatures of phase transitions in the thylakoid membrane of the studied elite erect leaf maize inbred lines

ZPPL 16		ZPPL 111		ZPL 773		ZPPL 121	
Ea ($kJ\ mol^{-1}$)	$t_{p.t.}$ (°C)	Ea ($kJ\ mol^{-1}$)	$t_{p.t.}$ (°C)	Ea ($kJ\ mol^{-1}$)	$t_{p.t.}$ (°C)	Ea ($kJ\ mol^{-1}$)	$t_{p.t.}$ (°C)
—	18.0	—	18.5	—	18.6	—	18.6
28.1	29.0	26.7	28.9	29.7	26.8	6.5	24.0
65.3	36.5	29.0	39.8	56.3	33.6	42.6	38.0

DISCUSSION

As already mentioned, the second half of the 20th century will be remembered, recognized and hardly excelled for the great success achieved in maize breeding and seed production. This immense activity was based on a very broad and complex program of maize breeding and seed production. Its goal was clear and concrete — to provide the highest possible grain yield in the newly developed maize hybrids and to provide a sufficient amount of quality seed for the domestic and foreign markets. The number of plants per area unit kept growing ever since 1980. This trend in maize breeding was referred to as „plant density" program and it most directly affected further yield increase of seed and commercial maize. In addition, a subprogram on maize breeding and seed production of erect leaf maize inbred lines was established. In pursuance of our hypothesis it was considered that these inbreds were closest to the projected photosynthetic model. These two subprograms of maize breeding and seed production were not only complementary, but also they were considerably expanded. Their implementation led to new results in both, maize breeding and seed production. New hybrids with high grain and silage yields were developed (Ivanović and Stojnić, 1992; Ivanović et al., 1995; Kojić, 1993; Trifunović, 1986; Duvick, 1984). Furthermore, seed production volume and quality were improved, reaching the amount of 70,000 tons (Selaković, 1999). In view of the above, it was quite expected for the breeding and seed production program of erect leaf maize inbred lines to be further expanded. A large number of erect leaf maize inbred lines, including the inbreds ZPPL 16, ZPPL 111, ZPL 773 and ZPPL 121 that were the objects of the present study, was in fact developed in an attempt to achieve the envisaged objective and to confirm the proposed hypothesis on the photosynthetic model.

It was necessary to characterize in detail the photosynthetic properties of the elite erect leaf maize inbred lines (Table 1). For that purpose, a non-invasive photosynthetic-bioluminescence method was applied (R a d e n o v i ć et al., 2001a, 2001b, 2002a, 2002b). The application of this method provided the characterization of the studied elite erect leaf maize inbred lines in relation to their resistance to both drought and high temperatures.

Actually, the temperature dependence of DF in all of the studied elite erect leaf maize inbred lines is, to a certain extent, characterized only by typical points on the thermal curve (Figure 2). The first typical point occurred at the contact point of segment **a** and segment **b** and it was related to the lowest critical temperature at which the initial change in DF intensity was observed. The second typical point occurred at the contact point of segment **b** and segment **c** and it was related to linear monotony and the angle of the rising part of the DF intensity. Throughout the duration of this typical point, evident changes in the structure of the thylakoid membrane occurred. The third typical contact point reflected a smaller or greater rotundity of DF intensity peaks. The „breaking" conformational changes occurred at two interception points of segments **c** and **d** and segments **d** and **e**. The fourth typical contact point was related to the linear monotony and the inclination angle of the declining part of the DF intensity. This segment bore the last conformational changes that had occurred in the thylakoid membrane. These changes can hardly be described as characteristic for the of functioning of a living leaf segment. The typical points designated **f** and **g** almost had no physiological role at all.

The observed typical points (Figure 2) can be considered as points that characterize elite erect leaf maize inbred lines, especially as these points are also points of possible conformational and functional changes in the thylakoid membranes. This statement is in accordance with literature data (V u č i n i ć et al., 1982; R a d e n o v i ć, 1992, 1994; R a d e n o v i ć et al., 2001a, 2001b, 2002a, 2002b; M a r k o v i ć et al., 1987).

All critical temperatures at which even the slightest conformational changes occurred in the thylakoid membranes of the studied elite erect leaf maize inbred lines were determined by the Arrhenius criterion and the linearization of the DF temperature dependence. The value of critical temperatures (°C), their frequency and intermediate distance on the thermal curve, characterized the elite erect leaf maize inbred lines in respect to their resistance and adaptation to high temperatures. The Arrhenius criterion is based on the existence of straight lines. Each Arrhenius straight line represents its activation energy (Ea). The interception point of two straight lines is determined by a critical temperature. Each critical temperature is preceded by a certain value of activation energy and then it is followed by another value of activation energy (R a d e n o v i ć 1985, 1997; M a r k o v i ć et al., 1993, 1996; R a d e n o v i ć et al., 2001a, 2001b, 2002a, 2002b). The values of activation energies in the rising and declining part of the thermal curve are explained by smaller or greater conformational changes that occur in the molecules of the thylakoid membranes with the temperature increase. Due to such changes these molecules become more reactive and thereby gain an additional energy that is used in the recombining process of DF occurrence (Table 3). This study was an attempt to show that there are elite erect leaf maize inbred lines having properties of an efficient photosynthetic model and that as such, they are very desirable in the process of contemporary selection and seed production.

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ФОТОСИНТЕТИЧНЕ КАРАКТЕРИСТИКЕ ЕЛИТНИХ САМООПЛОДНИХ ЛИНИЈА КУКУРУЗА СА УСПРАВНИМ ПОЛОЖАЈЕМ ЛИСТОВА И ЊИХОВ ДОПРИНОС У УНАПРЕЂЕЊУ ПРОИЗВОДЊЕ СЕМЕНА

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Резиме

У овом раду потврђује се наша хипотеза да елитне самооплодне линије кукуруза са усправним положајем листова имају својство ефикасног фотосинтетичног модела и да се, као такве, у семенарству успешно користе при повећавању броја биљака на јединици површине (густина биљака). Ова хипотеза доказана је егзактном применом неинвазивног фотосинтетично-биолуминисцентног метода са закаснелом флуоресценцијом хлорофила, погодног за оцену ефикасности фотосинтетичног модела.

Добијене фотосинтетичне карактеристике елитних самооплодних линија кукуруза: ZPPL 16, ZPPL 111, ZPL 773 и ZPPPL 121, са усправним положајем листова, засноване су на ефектима и природи промена закаснеле флуоресценције хлорофила које се одигравају у њиховим тилакоидним мембранама. Главни показатељи су температурна зависност интензитета закаснеле флуоресценције хлорофила, Аренијусов критеријум за утврђивање фазних прелаза (критичне температуре) у тилакоидним мембранама и енергије активације.

Утврђене фотосинтетичне карактеристике још омогућавају да се проучаване елитне самооплодне линије кукуруза са усправним положајем листова оцене и на њихову толерантност и адаптацију према деловању високих температура као и суше.