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EFFECTS OF CHANGES IN THYLAKOID MEMBRANES — A MEASURE FOR EVALUATION OF RESISTANCE AND ADAPTABILITY OF MAIZE INBRED LINES TO HIGH TEMPERATURE

ABSTRACT: The chlorophyll delayed fluorescence (DF) method, as a non-invasive bioluminescence method, is recommended for application in maize breeding and seed production in order to evaluate maize inbred lines for resistance and adaptability to increased and high temperatures.

The following thermal properties of the photosynthetic apparatus of the studied maize inbred lines have been determined: the temperature dependence within a range of 23—60°C; critical temperatures at which phase transitions occur in the thylakoid membrane were established and by means of it significant functional changes in the photosynthetic apparatus of the investigated maize inbred lines were detected. Activation energies (E_a, kJ mol^{-1}) alongside the straight lines prior and after critical temperatures were calculated. E_a is a measure of occurrence of chlorophyll DF recombination processes and, simultaneously, a measure of total changes in the structure and functioning of thylakoid membranes.

Thylakoid membranes in maize inbred lines underwent significant conformational changes in the part following the maximum intensity, i.e. in the part of its sharp linear decline. Such a state fits more a non-living than a living organism in relation to its both, structure and functions.

The presented parameters of total thermal processes of chlorophyll DF such as temperature dependence, critical temperatures and activation energy can be an important factor for a more exact characterization of maize inbred lines in relation to their resistance and adaptation to increased and high temperatures, contributing to a more rapid and rational development of the selection process.

KEY WORDS: maize inbred lines, thylakoid membrane, photosynthetic process, delayed fluorescence, thermal process, critical temperature, activation energy

INTRODUCTION

Although photosynthetic processes are ubiquitous, highly intensive, very complex and broadly observed, they still do not have sufficient application in plant breeding. This situation is probably a consequence of the existence of several functional interrelationships that unite structural and dynamic changes within chloroplasts and their thylakoid membranes on the one hand, and effects of numerous environmental factors on the other.

Chlorophyll DF can be phenomenologically described as the occurrence of luminescence (bioluminescence) within the red range of the visible spectrum produced by plant systems, bacteria, algae and higher plants, immediately after their intermittent illumination (Radenović, 1992, 1994, 1997). Chlorophyll DF was discovered by Stehler and Arnold (1951) in their attempt to reveal a nature of induction illumination in a form of bioluminescence. Numerous studies, especially those conducted over last 20 years (Jursinic 1986, Marković et al., 1993, 1996, Veselovski and Veselova 1990), revealed a direct connection between chlorophyll DF and photosynthetic processes, in which DF was considered an unavoidable indicator — a susceptible „probe" for experimental photosynthetic studies (Radenović et al, 1994a, 1994b, Radenović and Jeremić, 1996, Marković et al., 1987, 1993, 1996, 1999). Chlorophyll DF has become, and it will remain in immediate future, a modern methodological approach in the study of certain photo-processes in the light phase of photosynthesis. Consequently, thermal processes of chlorophyll DF and the application of activation energy in these processes are topical issues (Radenović, 1997, Radenović and Jeremić, 1996).

During last 20 years, attempts were made at the Maize Research Institute in Zemun Polje to employ complex photosynthetic processes in characterizing maize inbred lines. Such an approach can improve the cost efficiency of the breeding process. Therefore, a new, non-invasive method for chlorophyll DF measurements was developed. The measurements were performed on intact leaves of maize inbred lines (Radenović, 1997, Vučinić et al., 1982, Marković et al., 1987, 1993, 1996) and introduced into maize breeding.

The objective of the present study was to determine effects of increased and high temperatures on changes of thermal processes of chlorophyll DF in thylakoid membranes of observed maize inbred lines. The obtained changes in chlorophyll DF intensities, activation energies, as well as the different values of critical temperatures at which evident conformational changes in thylakoid membranes occurred can be good parameters for the evaluation of resistance and adaptation of the observed maize inbred lines to increased and high temperatures.

MATERIAL AND METHODS

Maize inbred lines from the collection of the Maize Research Institute, Zemun Polje, were used as objects in this study. Two inbreds (ZPPL 14 and ZPPL52) were developed at the Institute, while the inbred H108 is of the USA origin.

The principal traits of these inbreds are:

1) The inbred ZPPL 14, derived from crosses of the inbred A82 with a multirow dent population originating from Serbia, belongs to the FAO maturity group 450. Its dent-type kernel is yellow, the cob is red.

2) The inbred H108 of the FAO maturity group 550 is derived from the cross (Mo17 x H98) x Mo17, and it belongs to USA public inbreds. Its dent-type kernel is yellow, the cob is red.

3) The inbred ZPPL 52, derived from self-pollinated maize varieties originating from Istria, belongs to the FAO maturity group 600. Its flint — semi-flint kernel is red-yellow in color, the cob is white. This inbred is a component of commercial ZP maize hybrids.

Figure 1 shows a schematic presentation of the measuring equipment for chlorophyll DF. This scheme of the bioluminescence method has been developed and mainly used at the Biophysical Laboratory of the Maize Research Institute, Zemun Polje. Measurements of changes in chlorophyll DF intensities were performed after a method that was both, in principle and detail, described in previous papers (R a d e n o v i ć, 1979, 1992, 1994, 1997; M a r k o v i ć et al., 1996).

The plant materials were grown at the experiment field of the Institute. During July and August, maize plants were taken to the laboratory during morning hours (between 7 am and 8 am). Plants, when sampled in the field, were transversely cut at the ground internode. In the laboratory, plants were internode lengthwise placed in water. Two hours prior to the bioluminescence experiment, plants were kept under the ball glass. A segment of intact leaf was taken from such plants and placed into the chamber of the phosphoroscope and kept (in dark) for at least 15 minutes, and then measured for chlorophyll DF.

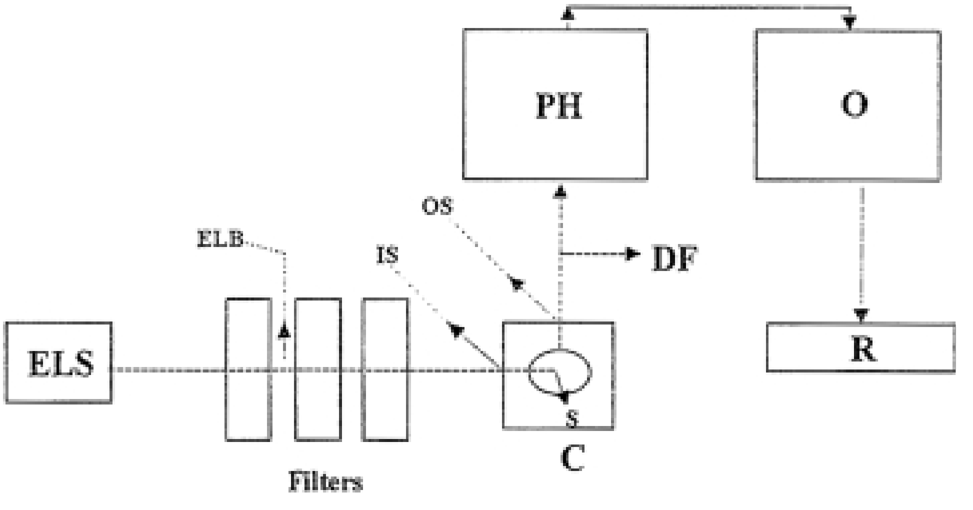

Fig. 1 — Potential scheme of the method and measuring equipment for chlorophyll delayed fluorescence: C — dark chamber with a sample stand; s — sample (leaf segment); Filters; ELS — excitation light source; PH — photo-multiplier; O — oscilloscope; R — printer; ELB — excitation light beam; DF — luminescent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

RESULTS

The obtained experimental results on chlorophyll DF intensity changes in the function of initiation time served for further processing and as such are presented in this paper for each inbred separately.

The inbred ZPPL 14

Figure 2 shows the temperature dependence of this inbred within the temperature range of 23—58°C. The intensity of chlorophyll DF commences to increase at 27°C. The intensity increases at the temperature of approximately 30°C, and then at 34 and 47°C. The rounded maximum occurs at 48—49°C. Further temperature increase resulted in a monotonous, steep and linear descend of chlorophyll DF intensity (Figure 2).

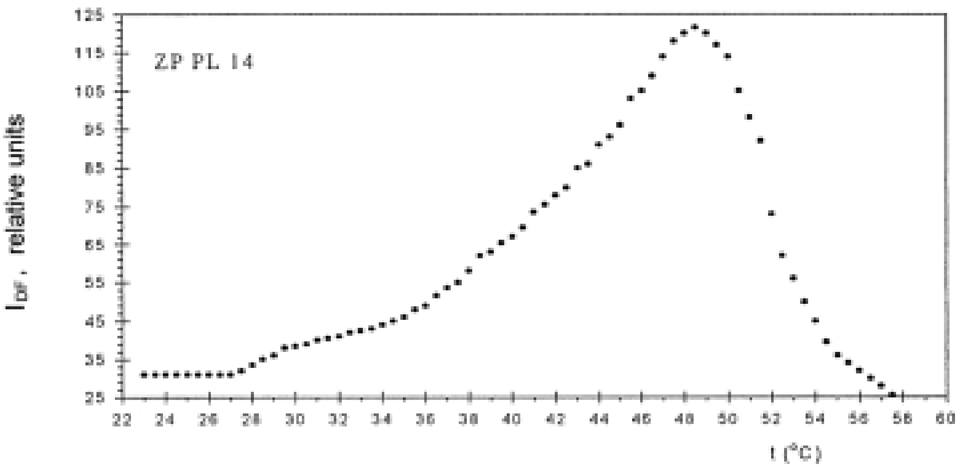

Fig. 2 — Changes of chlorophyll-delayed fluorescence intensities (I_{DF}) of thermal processes in dependence on temperature impacts in thylakoid membranes of the intact leaf of the maize inbred line ZPPL 14

Critical temperatures at which conformational changes occurred in thylakoid membranes of inbred line ZPPL 14 were determined by the Arrhenius plot of linearization of the chlorophyll DF temperature dependence (Figure 3, Table 1). The values of critical temperatures in °C, their number and mutual distances characterize the maize inbred line in relation to its resistance to increased and high temperatures. This inbred was characterized by four temperatures (27.0, 29.5, 35.0 and 47.5°C) in the ascending part of the chlorophyll DF intensity, and two temperatures (49.5 and 53.0°C) in the descending part of the temperature dependence (Figure 3, Table 1).

Activation energies (E_a) pertaining to the ascending temperature dependence had a negative sign (—62.9, —26.1 and —61.5 kJ mol^{-1}). E_a pertaining to the descending temperature dependence showed positive values (24.2, 227.2 and 124.2 kJ mol^{-1}) (Table 1).

Tab. 1 — Changes of activation energy (Ea) and critical temperatures during thermal processes in thylakoid membranes of the intact leaf of the maize inbred line ZPPL 14

Ea, kJ/mol	t°C
—	27.0
–62.9	29.5
–26.1	35.0
–61.5	47.5
24.2	49.5
227.2	53.0
124.2	—

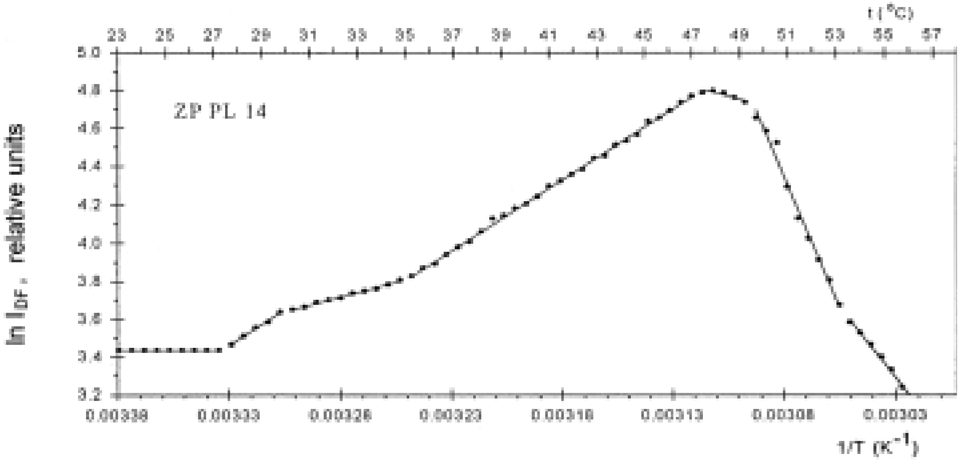

Fig. 3 — Arrhenius plot for evaluation of critical temperatures that cause conformational changes in thylakoid membranes of the intact leaf of the maize inbred line ZPPL 14

The inbred H 108

The temperature dependence of this inbred occurred initially at 32.5°C, and then a gradual increase of the chlorophyll DF intensity followed to the temperature of 49.5°C (Figure 4). The maximum intensity, not in the form of a sharp peak, occurred at approximately 50—51°C. Further temperature increase led to a steep ascent of chlorophyll DF intensity (Figure 4).

Four critical temperatures (33.0, 39.5, 44.0 and 49.5°C) were obtained by the Arrhenius plot of linearization in the ascending part (Figure 5) and only one value (51.0°C) in the descending part of the temperature dependence. Activation energies pertaining to the ascending trend had negative values (—46.3, —84.3 and —98.9 kJ mol^{-1}) (Table 2), while E_a accompanying the descending temperature dependence had positive values (7.0 and 425.8 kJ mol^{-1}) (Table 2).

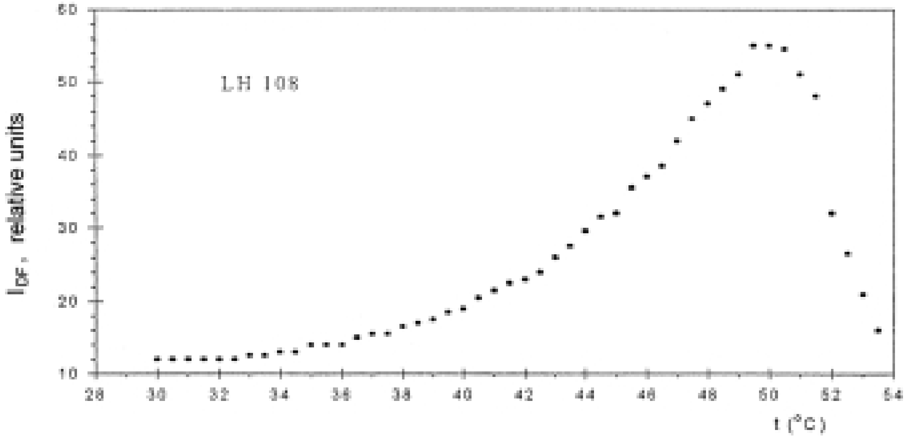

Fig. 4 — Changes of chlorophyll delayed fluorescence intensities (I_{DF}) of thermal processes in dependence on temperature impacts in thylakoid membranes of the intact leaf of the maize inbred line H-108

Tab. 2 — Changes of activation energy (Ea) and critical temperatures during thermal processes in thylakoid membranes of the intact leaf of the maize inbred line H 108

Ea, kJ/mol	t°C
—	33.0
–46.3	39.5
–84.3	44.0
–98.9	49.5
7.9	51.0
425.8	—

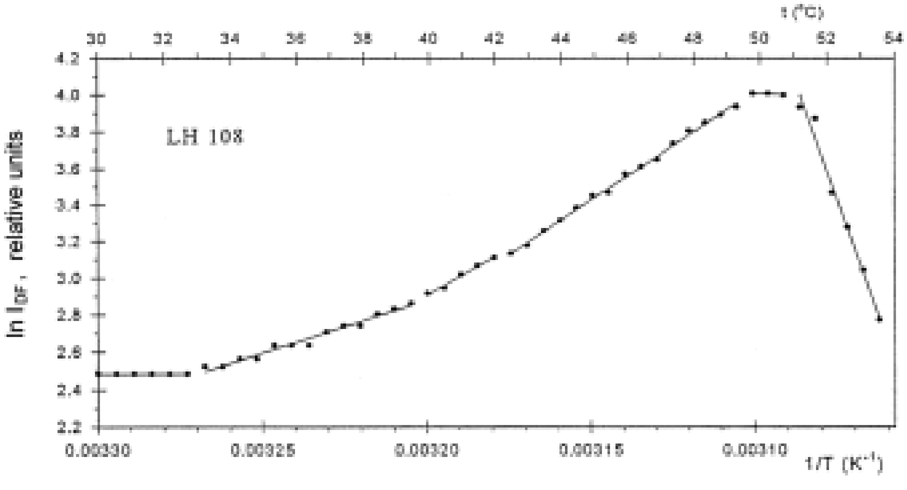

Fig. 5 — Arrhenius plot for evaluation of critical temperatures that cause conformational changes in thylakoid membranes of the intact leaf of the maize inbred line H-108

The inbred ZPPL 52

This maize inbred line underwent the same order of study — temperature dependence (Figure 6), Arrhenius plot (Figure 7), and the same processing of results on activation energies and critical temperatures (Table 3).

Tab. 3 — Changes of activation energy (Ea) and critical temperatures during thermal processes in thylakoid membranes of the intact leaf of the maize inbred line ZPPL 52

Ea kJ/mol	t°C
—	34.0
–74.41	53.0
–41.05	56.5
104.58	59.9
241.44	—

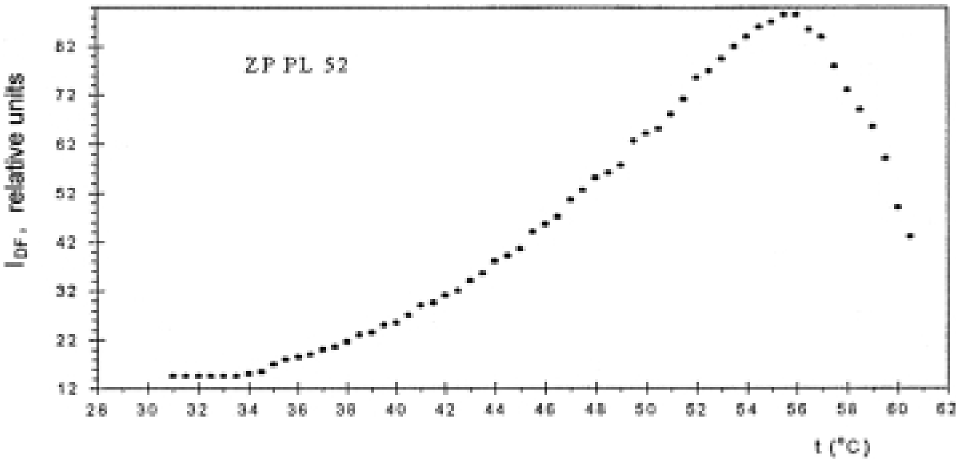

Fig. 6 — Chanres of chlorophyll delayed fluorescence intensities (I_{DF}) of thermal processes in dependence on temperature impacts in thylakoid membranes of the intact leaf of the maize inbred line ZPPL 52

DISCUSSION

The temperature dependence of the chlorophyll DF was only partly characterized by four typical points in the investigated maize inbred lines (Figures 2, 4 and 5). The first point pertains to the lowest temperature at which the first change of the chlorophyll DF intensity was observed. The second point was related to the linear monotony and dynamics of the ascending part of the chlorophyll DF intensity. The third point expressed smaller or greater roundness of the chlorophyll DF peak intensity. Finally, the fourth point pertains to the linear monotony and dynamics of the descending part of the chlorophyll DF intensity. The stated points, although characteristic, only hinted at possible confor-

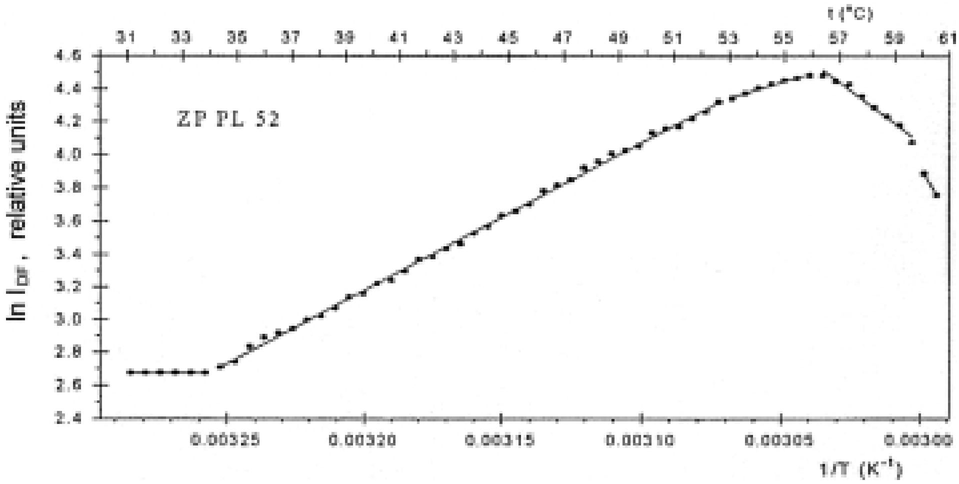

Fig. 7 — Arrhenius plot for evaluation of critical temperatures that cause conformational changes in thylakoid membranes of the intact leaf of the maize inbred line ZPPL 52

mational changes in the thylakoid membrane of the observed maize inbred lines and there are scarce literature data on the subject (V u č i n i ć et al., 1982, R a d e n o v i ć , 1994, M a r k o v i ć et al., 1987).

All critical temperatures at which even slightest conformational changes occurred in thylakoid membranes of the studied maize inbred lines were determined by the Arrhenius plot of linearization for chlorophyll DF temperature dependence. The value of critical temperatures (expressed in °C), their number and distances characterized the maize inbred lines in relation to their resistance and adaptation to increased and high temperatures. The Arrhenius plot is based on the existence of straight lines. Each of Arrhenius straight lines represents activation energy (E_a). The intersecting points of the straight lines are determined by critical temperature. There is one value of activation energy that precedes each critical temperature, and 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). Chlorophyll DF thermal processes in the observed maize inbred lines had activation energies with a negative sign in the ascending part of the chlorophyll DF intensity, while their values were positive in the descending part of the chlorophyll DF intensity. Such negative activation energies in thermal processes of thylakoid membranes in maize inbred lines were observed for the first time in the present study. This phenomenon is explained by the fact that the temperature increase results in smaller or greater molecular conformational changes in the thylakoid membrane, due to which molecules become more reactive and by means of that they acquire a new energy that is used in the recombination process of the chlorophyll DF occurrence.

E_a was positive in the descending part of the thermal curve, as it commonly is the case in all chemical reactions. Namely, thylakoid membranes underwent significant conformational changes, especially in the part of the thermal curve following the maximum intensity and its sharp linear decline. Such a state fits more a non-living than a living organism in relation to its structure and functions. The presence of positive E_a in the photosynthetic process originates from solar energy.

With much regret we have to state that there are no references regarding chlorophyll DF in intact leaves of higher plants that would encompass the results with parameters necessary to characterize maize inbred lines in relation to their resistance and adaptation to increased and high temperatures. The existing literature refers more to the mechanisms and processes of occurrence and development of chlorophyll DF that are of interests for biophysical, biochemical and physiological studies of the photosynthetic process (Jursinic 1986; Kerečki et al., 1986; Marković et al., 1987, 1993, 1999; Radenović 1992, 1994, 1997).

An effort was made in this study to apply the knowledge gained and the long experience for determining parameters that would make the processes of maize breeding more cost efficient. We have completed the bioluminescence method of chlorophyll DF which, being non-invasive, is not only an original but also a prospective method within the field of plant breeding.

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ЕФЕКТИ ПРОМЕНА У ТИЛАКОИДНИМ МЕМБРАНАМА — МЕРА ЗА ОЦЕНУ ОТПОРНОСТИ И АДАПТИВНОСТИ ИНБРЕД ЛИНИЈА КУКУРУЗА ПРЕМА ВИСОКИМ ТЕМПЕРАТУРАМА

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Резиме

Метод о закаснелој флуоресценцији хлорофила, као неинвазивни биолуминисцентни метод, кандидује се за примену у оплемењивању и семенарству ради утврђивања оцене инбред линија кукуруза на отпорност и адаптивност према вишим и високим температурама.

Утврђене су термалне карактеристике фотосинтетичног апарата проучаваних самооплодних линија кукуруза и то:

— температурна зависност у опсегу 23°C—60°C;

— вредности за критичне температуре на којима долази до мањих и већих структурних промена у тилакоидним мембранама,

— вредности за енергије активације (Ea, кJ/mol) дуж правих линија пре и после појаве критичне температуре у термалном процесу.

Утврђена је различита монотоност у растућем и опадајућем делу интензитета термалне криве, што указује на неједнаку отпорност и адаптираност испитиваних самооплодних линија кукуруза према вишим и високим температурама.

Проучаване самооплодне линије кукуруза у односу на њихову отпорност према вишим и високим температурама рангирају се на следећи начин:

— показано је да је самооплодна линија кукуруза ЗППЛ 52 врло отпорна према високим температурама,

— самооплодне линије кукуруза ZZPL 14 и H 108 имају задовољавајућу отпорност према високим температурама.