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Original scientific paper

ACTUAL PRESTIGIOUS PROPERTIES OF MAIZE INBRED LINES – A GOOD INITIAL BASIS FOR THE EFFICIENT DEVELOPMENT OF NEW AND YIELDING MAIZE HYBRIDS*

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This study conforms our hypothesis that there are elite maize inbred lines, which can be considered actual and prestigious as they have not only a property of the water status and a greater grain dry down rate during the maturation period, but also a property of the efficient photosynthetic-fluorescence model that is successfully used in the contemporary processes of breeding, and thereby in the development of new and yielding maize hybrids. Presented results obtained on the dynamics of grain dry down during the maturation period and on photosynthetic-fluorescence parameters (temperature dependence of the chlorophyll delayed fluorescence intensity, the Arrhenius plot for the determination of critical

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*This study is dedicated to the memory of Mr. Miladin Vuković and his exemplary devotion to maize breeding and selection, which aligned him with the leaders within this field in Serbia.

temperatures, i.e. phase transition temperatures and the activation energy) show that properties of the observed inbreds are based on effects and nature of conformational and functional changes occurring in their thylakoid membranes and other chemical structures of grain tissues. Summarised results of studies on actual and prestigious properties of maize inbreds will contribute to more exact, rational and expeditious proceedings of contemporary processes of breeding.

Key words: adaptability, chlorophyll delayed fluorescence, drought, grain dry down, high temperature, inbred line, intact leaf, thylakoid membrane, transport processes..

INTRODUCTION

In recent times, binding complex processes of fundamental sciences with multidisciplinary ones has become the necessity. This study encompasses such attempts that have been made between breeding, photosynthesis, biophysical chemistry and fluorescence in maize inbreds and hybrids. The present study analyses the development of dominant processes of the stated scientific disciplines and discovers the functional sites of their efficient interdependence.

Maize breeding has been intensively developed during the last 60 years. As a result of such an activity, over 1,100 maize hybrids for grain and silage have been developed. Contemporary technical and technological prerequisites for conducting a modern process of breeding were provided (DUVICK, 1984; TRIFUNOVIĆ, 1986; IVANOVIĆ *et al.,* 1995; RADENOVIĆ and SOMBORAC, 2000). Regardless of such a colossal success in maize breeding, eagerness and enthusiasm of the overall research have not been slowing down. The search for new methods and exact approaches in the completion and enrichment of the research within maize breeding and the seed production has been continued.

Our methods of photosynthetic fluorescence studies are a good example of such successful attempts.

The development of studies on maize photosynthesis was quite different. Namely, although photosynthetic processes are very spread, highly productive in their intensity, very complex in their nature, and vastly studied in their scientific actuality, their application in maize breeding is still insignificant. It is almost impossible to make a distinct, direct interrelationship between photosynthesis and breeding. Such a state 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 effects of numerous environmental stress factors on them, on the other hand (RADENOVIĆ *et al.*, 2003a, 2007b, 2007c).

Biophysical chemistry methods contributed, to a great extent, to connecting studies on photosynthetic and transport processes in the thylakoid membrane and in different chemical grain structures to the processes of fluorescence spectroscopy, chemical kinetics and the dynamics of grain dry down during its maturation period (RADENOVIĆ, 1994, 1998; RADENOVIĆ *et al.,* 2007a, 2007b; RUBIN *et al*., 1988).

The delayed chlorophyll fluorescence (DF) phenomenon can be 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 upon their intermittent illumination (excitation) (in case of maize upon illumination of the intact leaf) (RADENOVIĆ, 1992, 1994, 1997; MARKOVIĆ *et al.,* 1987, 1993, 1996, 1999). DF was discovered by STREHLER and ARNOLD (1951) in their attempt to reveal a nature of induction illumination in a form of bioluminescence. Numerous studies, especially those of the last 25 years (JURSINIC *et al*., 1982; JURSINIC, 1986; MARKOVIĆ *et al.,* 1996, 1999; VESELOVSKI and VESELOVA, 1990; RADENOVIĆ, 1992, 1994, 1997; RADENOVIĆ *et al*., 1994a, 1994b, 2000, 2001a, 2001b, 2002a, 2002b, 2003a, 2003b, 2004) revealed the direct connection between DF and photosynthetic processes, in which DF was considered as an unavoidable indicator - a susceptible "probe" for experimental photosynthetic studies in the intact leaf of maize inbreds RADENOVIĆ, 1992, 1994, 1997; RADENOVIĆ *et al*., 1994a, 1994b, 2000; RADENOVIĆ and JEREMIĆ, 1996; MARKOVIĆ *et al*., 1987, 1993, 1996, 1999; KALAUZI *et al.,* 2006).

Today, as well as, in the near future, DF shall be an efficient tool, i.e. a modern methodological approach in studies of certain, often very complex photoprocesses in the light phase of photosynthesis. In relation to this approach, thermal processes of chlorophyll DF, are considered the actual scientific issues. Within the scope of the stated, issues on the activation energy and critical temperatures within activities of the total induction processes of chlorophyll DF, arise. (RADENOVIĆ *et al*., 2000, 2003a, 2004).

In relation to the stated above, studies on photosynthetic fluorescence of thermal processes of chlorophyll DF in the intact leaf of observed maize inbreds lines, i.e. the determination of critical temperatures at which conformational and functional changes occur in the thylakoid membrane and revealing the activation energy in them, are considered the actual scientific issues. Moreover, the objective of this study was to determine the dynamics of the grain dry down in the maturation period of maize inbreds.

MATERIALS AND METHODS

The studies were carried out with two maize inbred lines, ZPPL 233 and ZPPL 62, belonging to the collection of the Maize Research Institute, Zemun Polje. The principal traits of the stated inbreds are as follows: the inbred line ZPPL 233 is a result of the ZPL 375 x ZPPL 25-10-1 cross, while it has not to be forgotten that the inbred line ZPL 375 was derived from the following cross: H108 x Mo17. Hence, the observed inbred ZPPL 233 belongs to the FAO 500 maturity group. The kernel is classified as the semi-flint/semi-dent type and is yellow, while the cob is pink. This inbred, as a female component, is included into the hybrid ZP 578. On the other hand, the inbred line ZPPL 62 is of the FAO 350 maturity group and represents a BSSS heterotic group. The kernel of this inbred belongs to semi-dent/dent type and the cob is red. This inbred, as one of components, is included into hybrids ZP 260, ZP 341, ZP 360 and ZP 434. Traits of greater grain dry down rates at the maturation and satisfactory tolerance to high and very high temperatures and drought are prestigious properties of these inbreds. The overall studies of the stated maize inbred lines encompassed the following two series of experiments: 1) photosynthetic fluorescence measurements done on the intact leaf and 2) observations of the water status and the dynamics of greater grain dry down rates at the maturation carried out by the method of oven-drying at 105^{o}C to the constant weight. Photosynthetic fluorescence measurements were performed for three years during July, August and September. The grain dry down rates at the maturity were estimated on the basis of an average sample drawn from five ears. In order to observe the water status, the plants were picked up at the black layer maturity, i.e. at the physiological maturity. Measurements of the grain water status were done seven days later and lasted for about 35-42 days. The dynamics of grain dry down at the maturation was observed not less than five years, because of a great instability of this property in the majority of maize inbred lines.

The test plants were grown in the experimental field of the Maize Research Institute and were brought to the laboratory during morning hours (between 7 a.m. and 8 a.m.). Plants sampled in the field were transversally cut in the ground internodes. In the laboratory, plants were internode lengthwise placed in water. Two hours prior to the fluorescence experiment, the plants were kept under the black ball glass. A segment of ear intact leaves was taken from such plants and placed into a chamber of the phosphoroscope (Fig. 1). The intact leaf segments were kept in the chamber (in the dark) for at least 15 minutes. These tests were performed with 180 plants of each inbred line.

The improved non-invasive photosynthetic fluorescence method used to measure chlorophyll DF is schematically presented in Fig. 1. This block scheme of the photosynthetic fluorescence method was developed at the Maize Research Institute, Zemun Polje. Measurements of changes in the intensity of chlorophyll DF were performed after a method that had been both, in principle and details, described in previous papers (RADENOVIĆ 1992, 1994, 1997; MARKOVIĆ *et al.* 1996; RADENOVIĆ *et al.* 2001a, 2002a).

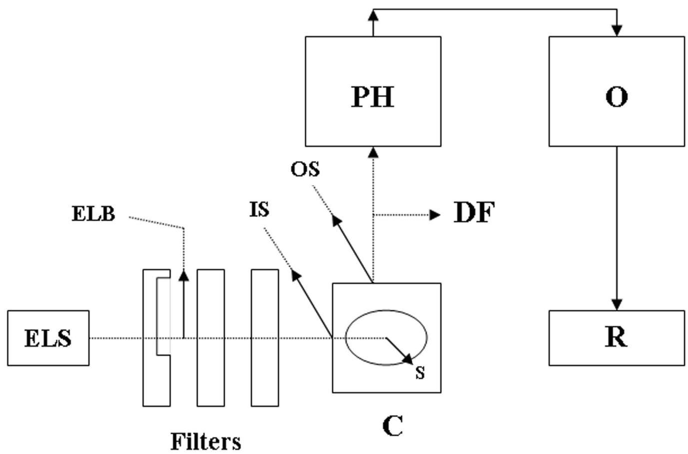

Fig. 1. Experimental setup of the photosynthetic fluorescence method and measuring equipment for delayed chlorophyll fluorescence: **ELS** - excitation light source, **ELB** - excitation light beam, **IS** - input dark chamber slot, **C** - dark chamber with a sample stand; **s** - sample (intact leaf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

RESULTS

1. The temperature dependence and temporal parameters of thermal processes of the delayed chlorophyll fluorescence of the observed maize inbred lines

The impacts of temperature, ranging from 15 to 60^{o}C, on the dynamics of the changes in stationary DF level were studied. The thermal curve is a curve that shows the dynamics of changes in the stationary DF level intensity in dependence on a temperature. The trend of its establishing is presented in the Fig. 2 and relates to both tested maize inbreds. The dynamics of the changes in chlorophyll DF depending on a temperature is presented in Tab. 1. Namely, the temperature dependence of observed maize inbreds is expressed over the temporal parameters of thermal processes of chlorophyll DF. The observation of the thermal curve course and the analysis of the duration of certain segments point out to the existence of several critical temperatures (phase transition temperatures) at which smaller or greater conformational and functional changes occur in the thylakoid membrane. The temperature dependence of the chlorophyll DF intensity is an important photosynthetic fluorescence parameter. There are two obvious trends in the dynamics of chlorophyll DF intensity changes - ascending with segments b, c, and d, and descending with segments d, e and f. Results obtained on the segment duration are presented in Tab. 1.

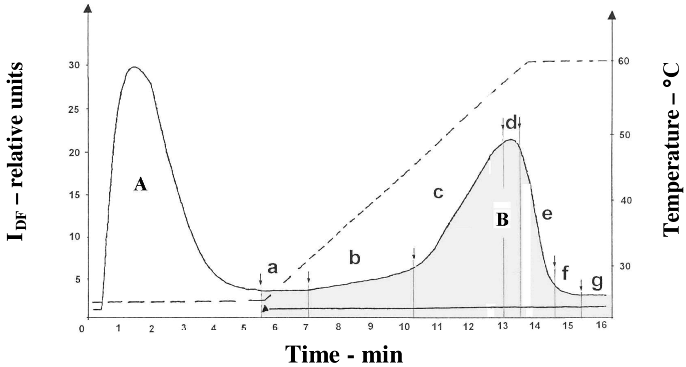

Fig. 2. Schematic presentation of typical changes of chlorophyll DF intensities (**I_{df}**) on the intact leaf of the observed maize inbred lines (solid lines **A** and **B**) and changes of temperatures (dashed line): curve **A** indicates induction processes of chlorophyll DF, while curve **B** encompasses photosynthetic thermal processes of chlorophyll DF. Typical temporal segments (**a**, **b**, **c**, **d**, **e**, **f** and **g**) on the thermal curve **B** correspond to dynamics of **I_{df}** changes at the time of a chlorophyll DF formation. Conformational and functional changes in the thylakoid membrane occur at interception points of typical temporal segments.

Table 1. Duration (in seconds) of certain segments on the thermal curve of delayed chlorophyll fluorescence

Segments of thermal curve	Segment designation	Tested maize inbreds: ZPPL 233	Tested maize inbreds: ZPPL 62	Temperature range for establishing thermal curve, °C
Stationary level of DF intensity	a	>30	>30	15 – 60
Initial increase of DF intensity	b	64±6	72±5	
Linear increase of DF intensity	c	30±4	36±3	
Maximum level of DF intensity	d	17±3	12±2	
Linear decrease of DF intensity	e	21±4	19±3	
Decelerated decrease of DF intensity	f	29±5	41±4	
Minimum level of DF intensity	g	30±2	45±4	

2. The Arrhenius plot for the determination of critical temperatures and conformational changes in the thylakoid membrane of the tested inbreds

All critical temperatures (phase transition temperatures) at which even the smallest conformational and functional changes occurred in thylakoid membranes of the studied maize inbreds were determined by the application of the Arrhenius plot on the linearisation of the chlorophyll DF temperature dependence and are presented in Fig. 3 and Fig. 4.

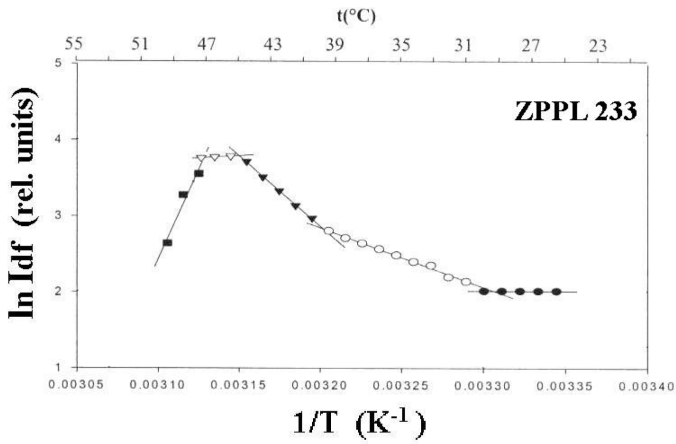

Fig. 3. Arrhenius plot for the determination of critical temperatures (intersection of two straight lines) that cause conformational and functional changes in the thylakoid membrane of the intact leaf of the studied maize inbred ZPPL 233.

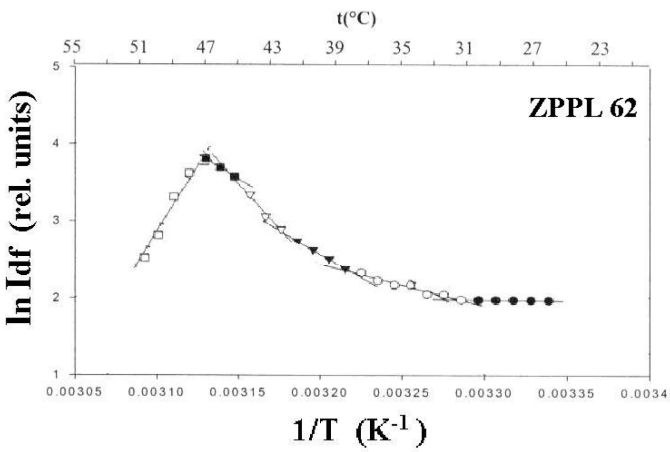

Fig. 4. Arrhenius plot for the determination of critical temperatures (intersection of two straight lines) that cause conformational and functional changes in the thylakoid membrane of the intact leaf of the studied maize inbred ZPPL 62.

3. Activation energies and critical temperatures in the thylakoid membrane of studied inbreds

Studies on the thermal curve of chlorophyll DF encompassed not only the temperature dependence with temporal parameters and Arrhenius plot, but also the estimation of values of activation energies and critical temperatures (phase transition temperatures) in the thylakoid membrane of the studied inbreds. Obtained results on activation energies and critical temperatures are shown in Tab. 2.

Table 2. Changes in activation energies (Ea) and critical temperatures in the course of thermal processes in the thylakoid membrane of the intact leaf of studied inbreds ZPPL 233 and ZPPL 62

ZPPL 233		ZPPL 62	
Ea ($kJ\ mol^{-1}$)	t, °C	Ea ($kJ\ mol^{-1}$)	t, °C
-	25,0	-	25,0
32.0	30.0	45.0	32.0
100.3	38.0	91.7	38.0
176.7	42.0	220.0	41.0
259.9	47.0	149.7	46.9
-	50.0	-	49.0

4. Dynamics of changes in the grain water status during the maturation of the studied inbreds

Dynamics of changes in the grain water status and dry down during the maturation of the studied inbreds are a prestigious property of these inbreds to which great attention is paid in the process of contemporary selection. Obtained results are presented in Tab. 3.

Table 3. Dynamics of dry down during the maturation of the studied inbreds

Inbred lines	Water content (%) in initial and subsequent measurements						Daily dry down
	I initial measurem.	II measurement after 7 days	III measurement after 14 days	IV measurement after 21 days	V measurement after 28 days	VI measurement after 35 days	
ZPPL 233	27.44±3.80	24.36±3.51	21.28±3.33	18.20±3.07	15.12±2.81	12.04±2.24	0.44±0.09
ZPPL 62	28.09±3.28	25.29±3.09	22.49±2.88	19.69±2.56	16.89±2.04	14.09±1.94	0.40±0.07

DISCUSSION

This study was an attempt to answer the following questions by setting up two series of contemporary and authentic tests: 1) are there reliable and prestigious properties of elite maize inbreds by which planned progress in maize selection can be achieved? and 2) what kind of maize inbreds should they be?

The first series of experiments included photosynthetic fluorescence studies on conformational and functional changes in the thylakoid membranes of the intact leaf of studied maize inbreds. The temperature dependence of thermal processes of chlorophyll DF for the studied maize inbreds is presented in a form of establishing (Fig. 2) and final magnitudes for segments of the thermal curve (Tab. 1). In such a way, overall thermal properties of the photosynthetic apparatus were determined (RADENOVIĆ *et al.*, 2000, 2001a, 2007b, 2007c). The results and the discussion of presented parameters of the total thermal processes of chlorophyll DF, such as, the temperature dependence of the chlorophyll DF intensity, critical temperatures and the activation energy can contribute to more exact characterisation of studied maize inbreds in relation to their adaptability, tolerance and resistance to stress effects of temperatures and drought (RADENOVIĆ et al., 2000, 2003a, 2007b, 2007c). Presented photosynthetic fluorescence properties of studied inbreds can contribute to more exact, rational and expeditious proceedings of selection processes.

The second series of experiments encompassed the thermal studies of the specific grain water status and grain dry down rates at the maturation. Transport processes and dry down rates at the grain maturation are a very important and prestigious property to which a great economic and scientific importance is ascribed in the process of studying and the development of maize inbreds and hybrids (RADENOVIĆ, 1998). The grain dry down rate at the maturation period is a very complex process and depends on the several following parameters: a) the osmotic pressure of the grain in the maturation period; the osmotic pressure is prone to the external atmospheric pressure, which is inclined to great changes (frequent air currents, changes in relative humidity and the like) that contribute to its uneven alterations; furthermore, the osmotic pressure in the grain depends on the structural properties of chemical compounds and the nature of their chemical bonds with water; b) the pericarp structure and thickness and its water permeability, that is water transport capacity through such a structure; c) the content and structure of starch grains, including their binding affinity to water; d) morphological properties of the ear; e) morphological properties of the grain; and f) other physical and chemical parameters of a chemical structure of the grain, which establish the interaction with water.

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AKTUELNA PRESTIŽNA SVOJSTVA SAMOOPLODNIH LINIJA KUKURUZA – DOBRA POLAZNA OSNOVA ZA EFIKASNO KREIRANJE NOVIH I RODNIH HIBRIDA KUKURUZA

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Idejom za ovaj rad potvrđuje se naša hipoteza da postoje elitne linije kukuruza koje se smatraju aktuelnim i prestižnim i koje poseduju, kako svojstvo stanja vode i njenog bržeg otpuštanja iz zrna u periodu sazrevanja, tako i svojstvo efikasnog fotosintetično-fluorescentnog modela, koji se uspešno koristi u savremenim procesima oplemenjivanja, a time i za stvaranje novih i rodnijih hibrida kukuruza. Izloženi rezultati o dinamici otpuštanja vode iz zrna u periodu sazrevanja i o fotosintetično-fluorescentnim pokazateljima: teperaturnoj zavisnosti intenziteta zakasnele fluorescencije hlorofila, Arrhenijus-ovim kriterijumom za određivanje kritičnih temperatura i energija aktivacije, pokazuju da su svojstva proučavanih linija zasnovana na efektima i prirodi strukturnih i funkcionalnih promena koje se odigravaju u njihovim tilakoidnim membranama i drugim hemijskim strukturama tkiva zrna. Sumarni rezultati proučavanja aktuelnih i prestižnih svojstava linija kukuruza doprineće egzaktnijem, racionalnijem i bržem odvijanju savremenih procesa oplemenjivanja.

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