



Research article

Detecting the phase transition in thylakoid membranes of maize inbred lines by means of delayed fluorescence



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ABSTRACT

In this paper the changes on growth, photosynthesis and water relations were tested by non-invasive fluorescence method. The applications of this method allow to determine some functional properties of prestigious maize inbred lines with erect top leaves. So the temperature dependency of delayed fluorescence intensity maximum of ZPPL 16 is observed at higher temperatures than for ZPPZ 62. This fact correlates with low values of phase transition of activation energy E_a in thylakoid membrane and accompanied by a decrease of the angle and area of the leaf, as well as with the content and the rate of water release from the seed. It seems reasonable to assume that, DF can be applied in breeding and maize hybrid seed production for the estimation of prestigious maize inbred lines and their resistance adaptability to increased and high temperatures, as well as, to drought.

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1. Introduction

The studies on maize photosynthesis carried out in the previous period did not have a more important application in breeding and the maize hybrid seed production. It was almost impossible to found correlation between some photosynthetic parameters, breeding and production of both, maize hybrid seed and commercial maize. During the last years, new and important studies within the field of bioluminescence and fluorescence phenomena and processes within the plant systems have been carried out (Barber and Neumann, 1974; Govindjee et al., 1990; Krause and Weis, 1991; Veselovski and Veselova, 1990). Delayed chlorophyll fluorescence (DF) is the light emitted from green plants, algae and photosynthesizing bacteria in the red-infra-red region of the spectrum for a short time after they have been exposed to light, but after the prompt fluorescence has decayed. The source of this emission is either chlorophyll a, or bacteriochlorophyll molecule, depending upon the organism.

One of the major advantages of DF is the possibility to measure it in native samples. The high sensitivity of photosynthesis to environmental factors, and the sensitivity of DF to changes in the different photosynthetic processes have made DF a useful tool for testing the plant reactions under stress conditions (Bilger and Schreiber, 1990). The direct dependence of the delayed chlorophyll fluorescence intensity on changes of photosynthetic processes in thylakoid membranes of maize intact leaves was determined (Radenović et al., 1994). The DF decay up to ms time domain is a result of the equilibrium reactions in PS II. The DF induction curve reflects processes that occur in photosynthetic samples when they are exposed to light after a period of dark adaptation. A stationary level of DF is reached after the induction period, which lasts about 2–3 min at room temperature and saturating light intensity, (Veselovski and Veselova, 1990; Radenović et al., 1994). Conditions that provided monitoring of complex photosynthetic processes in the maize intact leaf over a photosynthetic and fluorescence response in the form of DF were developed a group of researchers from the Maize Research Institute, Zemun Polje [see 7–9].

This study is an attempt to answer the following questions: (1) are there reliable and dominant parameters of maize inbred lines with erect top leaves by which planned and satisfactory progress in maize breeding and the high-quality hybrid seed and commercial

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maize production can be detected using DF (2) which maize inbred lines morphological properties could be compared with changes in DF intensity. The purpose of this study was to prove that inbred lines with erect top leaves and high-yielding maize hybrids derived from them could be tested by the DF. This can make a contribution to the effective selection, increased production and use of varied commercial maize.

2. Materials and methods

In this study three elite maize (*Zea mays* L.) inbred lines with erect top leaves, ZPPL 16, ZPPL 218 and ZPPL 62, from the collection of the Maize Research Institute, Zemun Polje were investigated. The inbred line ZPPL 16 was derived from the BSSS population and belongs to the FAO 700 maturity group. The inbred line ZPPL 218 belongs to the Lancaster heterotic group and the FAO 650 maturity group. The inbred line ZPPL 62 represents the BSSS heterotic group and belongs to the FAO 350 maturity group.

Systematically studies of the inbred lines and selected maize hybrids with erect top leaves have been made in series of experiments in which standard and other corresponding procedures were applied. An especially protractor was used to measure the angle between the position of the above-ear leaf and the position of the plant stalk. The leaf area was measured using the portable area meter (Model LI-3000 A, LI-COR, Lincoln, NE). Measurements of the angle between the above-ear leaf and the stalk and the leaf areas were carried out on 218 plants for each inbred line (Radenović et al., 2004).

The temperature dependence of the delayed chlorophyll fluorescence of leaf (the thermal-dependent DF) was used for identification of the optimal temperatures and the activation energy. The maize inbreds grown in the experimental field of the Maize Research Institute, Zemun Polje, were brought to the laboratory during morning hours. Plants sampled in the field were transversally cut in the ground internodes. In the laboratory, plants were internode lengthwise placed in distilled water. Before DF measurements, the plants were kept under the black ball glass (2 h). A segment of intact leaves was placed into the phosphoroscope chamber and dark adapted for at least 15 min (Radenović et al., 1994; Rubin et al., 1988). In experiments 268 plants of each inbred line was studied. By means of non-invasive DF method was used (Radenović et al., 1994; Rubin et al., 1988; Marković et al., 1999; Radenović et al., 2004). The water state and the dynamics of its grain dry-down rate in the maturation period was observed by the application of the thermal method of oven-drying at 105 °C to the constant weight. The measurements of the grain water status changes were done seven days later and lasted for 35 days. The dynamics of grain dry down at the grain maturation was observed in the course of five years, because of a great instability of this trait in the majority of maize inbred lines.

3. Results and discussion

Intensive studies and the development of maize inbred lines with erect top leaves were carried out at the Maize Research Institute, Zemun Polje. The ear leaves have been particularly

observed, but also other top leaves up to the tassel. The most efficient and the longest photosynthetic processes necessary for the maize plant are achieved by these leaves. According to the stated, a new hypothesis that top leaves (above-ear leaves) achieve the efficient photosynthesis has been proposed. The overall studies on maize inbred lines with erect top leaves encompassed several series of experiments.

The first series of experiments included measurements of the angle and the leaf area and grain dry down rates of prestigious maize inbred lines (Table 1; Fig. 1). Correlation between the plant morphological parameters (angle and leaf area) and maize varieties was found. Obviously, an optimal physiology and morphology is improved due to the genetic status of the plant. These results are very important not only for seed breeding and production, but for the comparison of fluorescence parameters and leaf morphology of plants.

At next series of experiments the studies of the specific grain water status and grain dry down rates in the maturation period was investigated (Fig. 1). The seed water redistribution rate may be associated with genetic status of plants and dry down rates at grain maturation. Earlier these parameters were not investigated in case of the development of maize inbreds and hybrids with erect top leaves, but also in the organisation of the hybrid maize seed production. The grain dry down rate in the maturation period is a very complex process and depends on the following several parameters: a) the osmotic pressure in the grain in the maturation period; the osmotic pressure is prone to the external atmospheric pressure, as well as, frequency and intensity of air currents and significant changes in relative air humidity; 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 and protein bodies, including their binding affinity to water; d) morphological properties of the ear; e) morphological properties of the grain; f) other physical and chemical parameters of a chemical structure of the grain, which interact with water.

For plant diagnostics development at the second series of experiments fluorescence studies on functional changes in the intact above-ear leaf of prestigious maize inbred lines was investigated. The DF temperature dependence of maize inbred lines are presented in Figs. 2 and 3.

In the course of the study it was found that the temperature dependence of the DF intensity is not monotonic and has a characteristic maximum (critical temperature) for each hybrid: the optimum for ZPPL 62 was found at 47 °C, which is significantly lower than corresponding maxima of ZPPL 16 at 52 °C (Fig. 2C and A). It is possible that temperature dependence of the DF chlorophyll intensity changes of the above-ear leaf of the maize inbreds is optional diagnostic method of plant genetic status (Radenović et al., 1985; Radosavljevic et al., 2000). Temperature dependences of DF changes were analysed by the Arrhenius plot (Fig. 3) which displays the logarithm of kinetic constants ($\ln(I)$, ordinate axis) plotted against inverse temperature ($1/T$, abscissa). The Arrhenius criterion allows to determine activation energy (E_a) of a single rate-limited

Table 1
The DF parameters and the leaf morphology and seed water redistribution.

Inbred line	t , °C	E_a , kJ mol ⁻¹	E_a , kJ mol ⁻¹	Angle of the above-ear leaf (°)	Area of the above-ear leaf ($\times 10^3$ cm ²)	Grain water content (%)	Dynamics of grain dry down (rel.un)
ZPPL 16	53	51.9	–150	18.3	3.63	32	0.47
ZPPL 218	48–49	33.2	–159	22.1	3.91	29	0.45
ZPPL 62	46	99.2	–262	20.3	3.3	27	0.40

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