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Chlorophyll fluorescence analysis of photosynthetic performance in seven maize inbred lines under water-limited conditions

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Background and Purpose: *Photosynthetic efficiency in crops can be associated with stress resistance and yield increase. In maize, photosynthetic efficiency is important in inbred lines during breeding process and in seed production, as well as in hybrids. Objective of this study is to determine differences in photosynthetic efficiency under the water-limited conditions between seven flint and dent maize inbred lines belonging to various heterotic groups. Moreover, this investigation will serve as a preliminary study for the QTL analysis of chlorophyll fluorescence parameters in order to understand the genetic and physiological background to drought stress tolerance in maize.*

Materials and Method: *Photosynthetic efficiency was measured in maize field nursery in Osijek during silking by Hansatech Handy-PEA analyzer in the elite inbreds Os1767/99, Os1252/99, Os163_9, B73, Mo17, Os6_2 i B84. The data obtained were used to calculate two biophysical parameters that describe the photochemistry of PSII: maximum quantum yield of photosystem II (Fv/Fm) and performance index on absorption basis (PI_{ABS}). Obtained data were subjected to statistical analysis applying least significant difference (LSD) and cluster analysis.*

Results and Conclusions: *Differences in photosynthetic efficiency appeared to be higher within dent inbred lines than between dents and flints. Although investigated parameters of chlorophyll fluorescence (Fv/Fm and PI_{ABS}) revealed similar clustering of inbred lines, there was slight difference concerning the grouping of the line Os6_2. Therefore we recommend the combined use of these two main parameters of chlorophyll fluorescence when the investigation includes photosynthetic performance in stress challenged plants, such as water-limited conditions.*

INTRODUCTION

Inbred lines as parental components for hybridization are essential for producing commercial hybrid seed (*1*) and they are the most valuable source in maize biology, genetics and breeding. They have been crucial for various genetic studies including the development of linkage maps (*2*), genetic diversity (*3*), quantitative trait loci mapping(*4*), molecular evolution (*5*) and developmental genetics (*6,7*).

Analysis of chlorophyll *a* fluorescence is appreciated as widely used method in research of photosynthetic efficiency in higher plants (*8*).

One of the most often employed parameters is maximum quantum yield of PSII (Fv/Fm) or so called Genty's parameter (*9*), which gives the information about the proportion of the light absorbed by chlorophyll in PSII that is used in photochemical processes. Further advances in chlorophyll fluorescence techniques as well as development of new apparatuses for its measurement (*10*) allowed the introduction a plenty of new parameters that describe PSII photochemistry in detail. One of the most powerful and most comprehensive parameter is performance index (PI_{ABS}). This is a multiparametric expression that takes into account all of the main photochemical processes, such as absorption and trapping of excitation energy, electron transport further than primary plastoquinone (Q_A) and dissipation of excess excitation energy. Unlike Fv/Fm which utilizes only extreme values of chlorophyll fluorescence, namely minimal (F_0) and maximal (Fm) fluorescence, very fast and accurate measurement of fluorescence transient between these two extremes is necessary for PI_{ABS} calculation. Therefore, PI_{ABS} appeared to be very suitable and sensitive parameter to investigate plant overall photosynthetic performance under different abiotic and biotic stresses (*11,12,13,14, 15,16,17*) as well as during development of photosynthetically active plant organs (*18,19,20,21*).

This experiment was done in order to estimate variation among diverse inbred lines of maize (*Zea mays* L.) for chlorophyll fluorescence parameters (Fv/Fm and PI_{ABS}) during flowering phenophase. It is known that a common characteristic of maize response to stresses near flowering is a reduction of reproductive fertility (*22*) affecting grain formations. In the present study we analyzed the photosynthetic performance capability in seven maize inbred lines under the water-limited conditions (Fig. 1). The main goal of the study was to compare grouping of selected inbred lines in respect to two investigated chlorophyll fluorescence parameters (Fv/Fm and PI_{ABS}). We also hypothesized that differences in photosynthetic performance under the water-limited conditions between flint and dent inbred lines would appear since there are considerable genetic and physiological differences between them. Moreover, this investigation will serve as a preliminary study for the QTL analysis of chlorophyll fluorescence parameters in order to understand the genetic and physiological background to drought stress tolerance in maize.

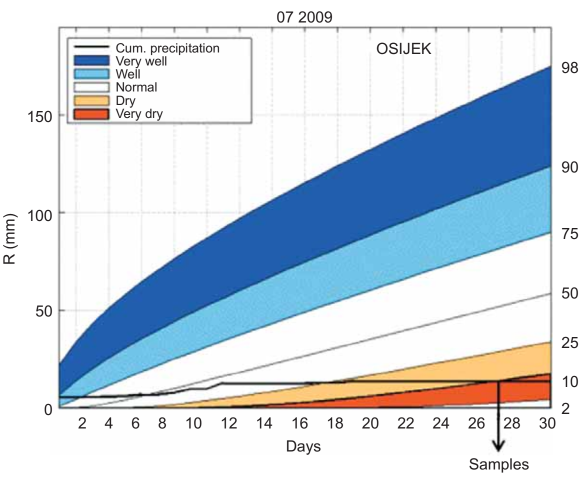

Figure 1. *Cumulative precipitation (mm) in July 2009 with theoretical percentiles (2, 10, 25, 50, 75, 90 and 98) curves for the period 1961–2000 at the location Osijek, Croatia (Source: Meteorological and Hydrological Service, Republic of Croatia). Arrow indicates the dates when samples for chlorophyll fluorescence measurement were taken under water-limited conditions.*

MATERIALS AND METHODS

In 2009, total of seven maize inbred lines of different origin were grown in the field at the experimental station of the Agricultural Institute Osijek in Osijek, Croatia (45°32' N, 18°43' E) in a completely randomized design. Two inbred lines were of flint type (Os1767, Os1252), and other five belong to dent gene pool, either of Lancaster (Os163_9, Mo17, Os6_2) or BSSS origin (B73, B84). Liu *et al.* (2003) presented in detail genetic and genomic relations among the flint pool, Lancaster and BSSS inbred lines of maize, as well as relations between Mo17, B73 and B84. The inbred lines designated with »Os« were developed at the Agricultural Institute Osijek, Croatia. All inbred lines in the experiment are fully adapted to climate conditions of southeast Europe (Croatia). The field trial consisted of four-row plots (20 plants per row resulting 80 plant in each plot) and two replications. According to official data of Meteorological and Hydrological Service, Republic of Croatia, in Osijek during July 2009 water deficit occurred (Fig. 1). There were very dry weather conditions in the field confirmed by cumulative precipitation curve being on the 10 theoretical percentiles level when samples have been taken on June 28.

Sample of nine ear-leaves per inbred line was taken for the chlorophyll fluorescence analysis. Ear-leaves at the beginning of flowering were measured since it had been demonstrated that it could represent chlorophyll related traits in a maize canopy (*23*). In each experimental unit, the first two plants were considered border plants and were not used for measurement. Samples for chlorophyll fluorescence measurement were taken on second half of July (Fig. 1) during maize tasseling, i.e. during VT phenological stage, when maize plant is particularly sensitive to stress. The chlorophyll *a* fluorescence transient was measured by a Plant Efficiency Analyser (PEA, Hansatech, UK) on attached leaves at ambient temperature. After dark adaptation (30 min) the chlorophyll fluorescence transient was induced by applying a pulse of saturating red light (peak at 650 nm, 3000 μmol m^{-2} s^{-1}). The application of the saturating light pulse induces chlorophyll *a* fluorescence increases from minimal fluorescence (F_0), when all reaction centers are open, to maximal fluorescence (Fm), when all reaction centers are closed. During the first 2 ms, changes were recorded every 10 μs and every 1 ms thereafter. The data obtained were used to calculate two biophysical parameters that describe the photochemistry of PSII (*24*): maximum quantum yield of photosystem II (Fv/Fm) and performance index on absorption basis (PI_{ABS}). Obtained data were subjected to statistical analysis applying least significant difference (LSD) and cluster analysis, using the program Statistica 6.0.

RESULTS AND DISCUSSION

Generally, there were significant differences among seven inbred lines for both investigated chlorophyll fluorescence parameters (Figs. 2, 4). Also, there were neither consistent differences according to the gene pools (flint, dent), nor within the dent gene pool (Lancaster, BSSS). Values of maximum quantum yield of photosystem II (Fv/Fm) were ranging from 0.742 in Mo17 to 0.798 in lines B84 and Os 1252/99, respectively (Fig. 2). Cluster analysis (Fig. 3) of Fv/Fm values revealed two groups of investigated inbred lines, where Mo17 and Os 163_9 had lower Fv/Fm values while other lines with higher Fv/Fm values were grouped together. Also, it should be emphasized that Mo17 inbred line was the only one with Fv/Fm values lower than 0.750, which is considered to be a boundary value for fully functional PSII (*25*). Values of performance index (PI_{ABS}) are shown in Fig. 4 and were ranging from 0.800 in Mo17 to 2.004 in line Os 1252/99. Although the PI_{ABS} cluster analysis (Fig. 4) revealed two groups, as well as for Fv/Fm values, the variations within and between investigated inbred lines lead to slightly different positioning of Os6_2 line. Here Os6_2 line was grouped together with Mo17 and Os 163_9 lines.

Based on the presented results it can be concluded that differences in photosynthetic efficiency is higher within dent inbred lines than between dents and flints. Although investigated parameters of chlorophyll fluorescence (Fv/Fm and PI_{ABS}) revealed similar clustering of inbred lines, there was slight difference concerning the grouping of the line Os6_2. Therefore we recommend the combined use of these two main parameters of chlorophyll fluorescence when the investigation includes photosynthetic performance in stress challenged plants, such as water-limited conditions. Recent investigations on the use of chlorophyll fluorescence parameters for selection of drought-tolerant maize cultivars gave conflicting conclusions. Some reports (*26, 27*) supported our findings by recognition of chlorophyll fluorescence measurements as valuable tool for screening drought-tolerant maize culti-

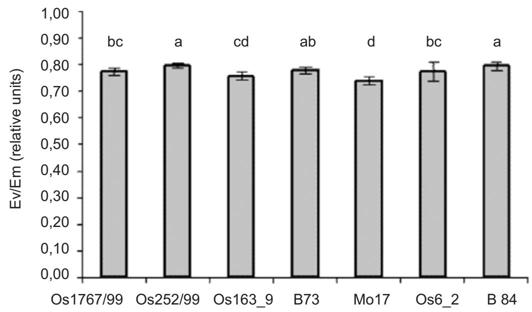

Figure 2. *Mean values of maximum quantum yield of photosystem II (Fv/Fm) in seven maize inbred lines under water-limited conditions in the field. Bars represents standard deviations and significant differences between investigates inbred lines were designated by different letters (a, b, c and d) placed on the top of the column.*

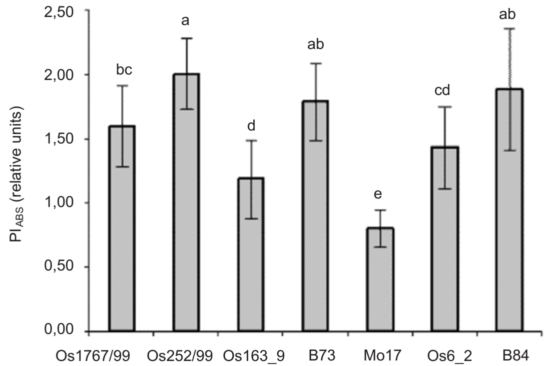

Figure 4. *Mean values of performance index (PI_{ABS}) in seven maize inbred lines under water-limited conditions in the field. Bars represents standard deviations and significant differences between investigates inbred lines were designated by different letters (a,b,c and d) placed on the top of the column.*

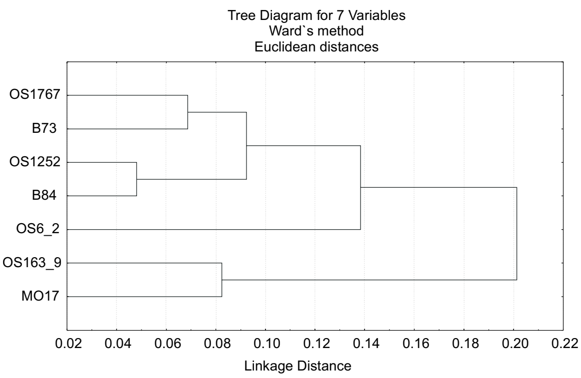

Figure 3. *Tree diagram of clustering showing the natural grouping of seven maize inbred lines according to the values of maximum quantum yield of photosystem II (Fv/Fm) under water-limited conditions in the field.*

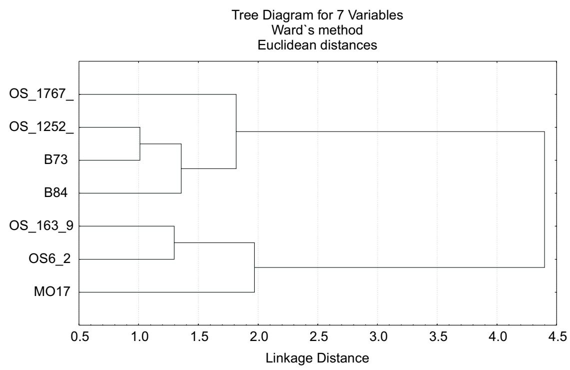

Figure 5. *Tree diagram of clustering showing the natural grouping of seven maize inbred lines according to the values of performance index (PI_{ABS}) under water-limited conditions in the field.*

vars, while Ashraf *et al.* (*28*) found no relationship between drought-tolerance of investigated maize cultivars with Fv/Fm parameter.

Further, the screening experiment revealed that inbred lines B73 and Mo17 differed remarkably for both investigated chlorophyll fluorescence based parameters, providing thereby contrasting parents for the mapping population. It was crucial finding, since their biparental population IBM is unique among publicly available maize mapping populations for two reasons: four generations of intermating at the F_2 stage have increased the observed numbers of recombination expanding the genetic map approximately fourfold compared to non-intermated, conventional RIL populations (*29*), and the IBM population consisting of a relatively large number of RILs (302) has been densely genotyped with >2000 molecular markers (*30*). It can be served as an excellent resource for further physiological, genetic, genomic or postgenomic studies of photosynthesis.

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