Photosynthetic properties of maize hybrids under different environmental conditions probed by the chlorophyll a fluorescence

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Abstract

Temperatures and light intensities that deviate from optimum often affect maize (*Zea mays* L.) production in temperate regions. Objectives of the current study were (i) to investigate the effects of extreme temperatures and low light conditions on photosynthesis in maize hybrids, (ii) to test whether maize hybrids show distinctive reactions when subjected to different stresses, and (iii) to investigate informativeness of different fluorescence parameters in different stress conditions. Plants of five maize hybrids were subjected to: cold (Co), low light (LL), heat (H), and severe heat (SH) treatments and field (F) conditions along with control (C), and chlorophyll *a* fluorescence (ChlF) was measured. ChlF transients were shown to respond to different treatments by changing the appearance of OJIP band, mostly at steps J and I, while the appearance of the K step was confirmed as a good indicator of temperature stress. PC analysis revealed grouping of parameters with PCs according to their informativeness. Parameter ψEo, was shown to be in most defined by the F treatment, probably due to higher light intensity compared to C. Variables regarding electron transport and dissipation showed specific reactions to H and SH treatments. Co and LL conditions caused specific changes in in parameters V_J and V_I. Further research on usability of ChlF in maize breeding is needed, as it represents the data-rich, easy to measure method, providing the biological framework for explanation of reactions to different stresses.

Introduction

Agricultural production worldwide is affected by different abiotic stress conditions which cause extensive economic losses (Boyer 1982). Abiotic stress conditions such as heat, drought, salinity and low temperatures have been subjects of intense research (Bray et al. 2000, Cushman and Bohnert, 2000). Plants that are grown in temperate climate regions are constantly exposed to temperatures that deviate positively or negatively from optimum which in most plants results in physiological and biochemical damage (Lyons 1973, Grace et al. 1998). This damage is usually reflected in metabolic processes such as generation of ROS (reactive oxygen species), changes in photosynthetic activity, downregulation of Rubisco activity and plant hormone synthesis (Anderson et al. 1994, Salvucci et al.2004, Strauss et al.2006, Brestic et al. 2013). Generally, heat stress in plants causes unique physiological conditions: increase in respiration, stomatal conductance and leaf temperature, reduced activity of antioxidant enzymes, increase in ROS production (Rizhsky et al. 2002, 2004; Gong et al. 1997). Cold stress inhibits metabolic reactions and induces osmotic, oxidative and other stresses along with changes in photosynthetic activity (Chinnusamy et al. 2007, Strauss et al. 2006). Majority of abiotic stress experiments are conducted in controlled conditions and therefore do not reflect actual conditions that plants are subjected to in the field that are usually a specific combination of different stresses.

Maize (*Zea mays* L.) planting in South East Europe mainly occurs at lower temperatures than within the U.S. Corn Belt, median temperature being 12.1°C, compared to 15.4°C in U.S. (Sacks et al. 2010). Early planting dates mainly cause reduction of growth in early developmental stages because of low temperatures and lower intensity of solar radiation captured by the young photosynthetic apparatus (Otegui et al. 1995). Besides the abiotic factors affecting light intensity of light available in field conditions like clouding or day periodicity, strong light intensity gradients are also created by leaves of plants within dense populations (Hirth et al. 2013). Efficiency of plants to utilize captured radiation is negatively affected by low temperatures (Andrade et al. 1993). In short-season environments on the North of the US Corn belt, radiation-use efficiency (RUE) presents a major constraint for higher yields in maize (Westgate et al. 1997) since there is a high incidence of occurrence of low light conditions. Low light conditions may also occur in field when the planting density is high or there is a high level of leaf interception. Heat stress in vegetative growth stages can delay silking, increase male and female sterility and alter plant height and leaf area index (Cichino et al. 2010). Late planting of maize is mostly an option used by farmers deciding to replant or to plant maize as a cover crop after other cereals like wheat or barley. Later planting dates negatively affect the grain yield while grain harvest moisture stays high, thus increasing the costs of drying (Lauer et al. 1999), though vegetative growth as a result of higher RUE is positively affected by higher, non-stressful temperatures (Andrade et al. 1993).

Stem reserve mobilization and the effectiveness of photosynthesis have a crucial role on the formation of generative organs in cereals, and therefore yield (Blum et al. 1994). Since photosynthesis is a vital part of plant metabolism, and is sensitive to different kinds of abiotic and biotic stresses it can be used for screening of plant material for tolerance or sensitivity. Effectiveness of photosynthesis can be described through measurements of ChlF which is an *in vivo*, nonintrusive, convenient and extensively used method in abiotic and biotic stress research (Galic et al. 2019a,b; Pérez-Bueno et al. 2019; Kalaji et al. 2007, 2014, 2017; Strasser et al. 2000; Bilger et al. 1995). Illuminating a dark-adapted leaf results in changes of ChlF intensity in a characteristic way, which when plotted on a logarithmic scale, exhibits characteristic J and I steps (Strasser and Govindjee 1992) between initial O (F_0) and maximum P (F_m) level. Strasser and Strasser (1995) have developed the JIP-test which outputs original OJIP transient measurements into multiple biophysical and phenomenological parameters quantifying the cascade of chloroplast redox reactions at microsecond or millisecond scales (Kalaji et al. 2016). It has been previously shown by Krüger et al. (1997) that the shape of the OJIP fluorescence transient is sensitive to stress in many environmental conditions.

Five maize hybrids used in the current study represent the maturity groups from FAO 300 to FAO 600. The objectives of the current study were: (i) to investigate the individual effects of extreme temperatures and low light conditions on photosynthetic apparatus in young plants of five maize hybrids by the means of ChlF, (ii) to test whether maize hybrids from different maturity groups show distinctive reactions when subjected to different stresses, and (iii) to investigate informativeness of different measured and phenomenological parameters of ChlF in physiological assessment of different stress conditions.

Materials and Methods

Plant material and growth conditions

Five maize (*Zea mays* L.) hybrids from different maturity groups were used to conduct the current study. Hybrid 378 (FAO 350) represented early maturity group, Drava 404 (FAO 420) and 444 (FAO 450) represented the most widely used in Croatia, middle maturity group. Hybrid 505 (FAO 510) represented middle to late maturity group and Veli (FAO 590) represented the late maturity group. Maturity groups of hybrids used in the current study are the ones most widely represented for grain production on farmer's fields in South-eastern Europe.

Seeds of five genotypes were planted in trays (21×35×7 cm) filled with organic soil and placed in a growth chamber (25°C, 16/8 day/night, 200 μmol m^{-2} s^{-1}). Soil properties were: nitrogen (NH_3 + NO_3^-) 70 mg L^{-1}, phosphorous (P2O5) 80 mg l-1, potassium (K2O) 90 mg l^{-1}, organic matter 70% (DW), and pH = 5.7 ($CaCl_3$). Contents of heavy and other potentially toxic metals (Cd, Cr, Cs, Hg, Ni, Pb, Zn) and other toxic substances (polycyclic aromatic hydrocarbons, polychlorinated byphenyls) were below permitted amounts for EU. Experiment was replicated three times. The 15 plantlets in a single tray for each genotype and each of the treatments was considered a replicate. Trays were watered with 200 mL of tap water using a spray

Table 1- Names, abbreviations and conditions of the treatments

Treatment	Abbreviation	Light intensity (μmol m^{-2} s^{-1})	Temperature (°C)	Duration
Control	C	200	25	Continuous
Low light	LL	50	25	12 h
Cold	Co	200	5	12 h
Heat	H	200	30	12 h
Severe heat	SH	200	38	12 h
Field	F	variable	variable	Continuous

Table 2 - Definition of terms and formulae of JIP-test parameters and expressions (Source: Strasser *et al.* 2004)

Parameter	Description
	Data extracted from the recorded fluorescence transient
F_J	Fluorescence Intensity At 30 ms (J Step)
F_K	Fluorescence Intensity At 300 ms (K Step)
V_J	Relative Variable Fluorescence At J Step; $V_J=(F_J–F_0)/(F_m–F_0)$
V_I	Relative Variable Fluorescence At I Step; $V_I=(F_I–F_0)/(F_m–F_0)$
M_O	Initial Slope Of Relative Variable Fluorescence; $M_0=4(F300\mu s–F_0)/(F_m–F_0)$
N	Turnover number, number of times Q_A has been reduced in the time span from 0 to t_{max}; $N = Sm\cdot [(dV/dt)0]/V_J$
S_M	Normalized area; $Sm = Area/(F_M – F_0)$
	Flux Ratio Or Yield
ΨEo	Efficiency/probability for electron transport (ET), i.e. efficiency/probability that an electron moves further than Q_A–; $\Psi E0 = ET_0/TR_0 = (1-V_J)$
φPo	Maximum quantum yield for primary photochemistry; $\varphi P0 = TR_0/ABS = [1-(F_0/F_m)]$
	Specific Fluxes Per Active Reaction Center
ETo/RC	Electron Transport Per Active Reaction Centre: $ET_0/RC=M_0\cdot(1/V_J)\cdot(1–V_J)$
DIo/RC	Dissipation Per Active Reaction Centre; $DI_0/RC=(ABS/RC)–(TR_0/RC)$
REo/RC	$RE_0/RC = M_0(1/V_J)\Psi E0\ \delta R0$
	Performance Index
PI_{ABS}	Performance Index On Absorption Basis; $PI=(RC/ABS)\cdot(TR_0/DI_0)\cdot[ET_0/(TR_0-ET_0)]$
RC/ABS	Density Of Reaction Centres On Chlorophyll a Basis; $RC/ABS=(RC/TR_0)\cdot(TR_0/ABS)=[(F_J–F_0)/4(F300\mu s–F_0)]\cdot(Fv/Fm)$

bottle every two days. Plantlets were grown for 10 days when ChlF measurements were performed using the fluorimeter Handy PEA (Hansatech, King´s Lynn UK) in 10 equally developed young plants.

In LL, Co, H and SH treatments, plants were grown in controlled conditions similar to C and transferred to treatment conditions at the beginning of daytime cycle (6:00 AM). In F treatment, plants were grown in trays left in field after planting and measurements of chlorophyll fluorescence were performed when the plants were at similar growth stage as plants in C. For field treatment (F) soil was the same as in C but trays were collocated in the field and ChlF was measured when the plants were at same growth stage as plants in C. Lowest temperature observed in the field was 4°C while the highest was 27°C, with median being 15°C. Precipitation during the time of the experiments was 65 mm of rain and cumulative insolation duration was 180 hours. Light intensity exceeded control light intensity reaching more than 900 µmol m^{-2} s^{-1}. Planting date was May 5th as advised by Lauer et al. (1999) on the middle date between optimal (April 30th) and maturity switching (May 14th) dates

ChlF measurements

ChlF was measured on the middle section of the first fully developed leaf. 10 measurements per replicate (tray) were conducted giving 30 measurements for each genotype per treatment. For F treatment measurements were done in the morning between 8 and 9 a.m. For Co, LL, H and SH treatments ChlF was measured after 12 hours of acclimatization. After 30 minutes of dark adaptation chlorophyll fluorescence transient was induced by applying a pulse of saturating red light (peak at 650 nm, 3,200 mmol m^{-2} s^{-1}) on the leaf surface that is exposed by the leaf clip (4 mm in diameter). Saturating pulse of light induces ChlF increase from minimal fluorescence (F_o), when all reaction centers of photosystem II are open, to maximal fluorescence (F_m), when all reaction centers are closed. During the one second measurement 120 data points are collected. ChlF data was processed with PEA Plus software (V1.10) provided with the fluorimeter. Genotypic means of normalized ChlF transients were plotted on a logarithmic time scale. Such transients show characteristic polyphasic rise in ChlF, clearly exhibiting two intermediate steps between minimum and maximum fluorescence intensities (O and P steps respectively) known as J and I (Strasser and Govindjee, 1992). The JIP-test (Strasser et al. 1995, 2000, 2004, 2010) was used to interpret ChlF transients. JIP-test is a representation of the original data to biophysical parameters that quantify the energy fluxes through photosystem II (PSII). The formulas in Table 2 illustrate how each of the mentioned biophysical parameters can be calculated from the original fluorescence measurements. JIP-test data was used to perform principal component analysis.

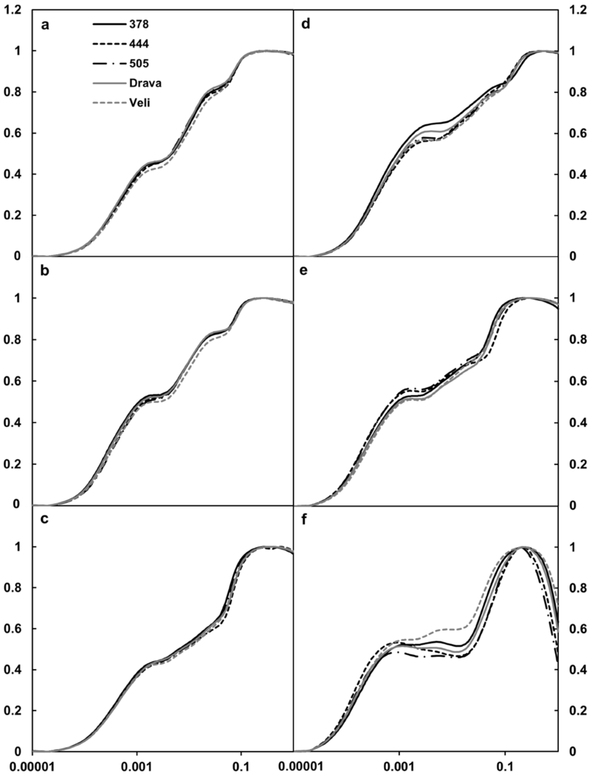

Fig. 1 - Normalized ChlF transients of five maize hybrids (378, 444, 505, Drava and Veli) under different environmental conditions: control (a), low light (b), field (c), cold (d), heat (e), and severe heat (f). ChlF was measured on the middle section of the first fully developed leaf. Each transient represents average (n = 30)

Statistical analysis

Plotting logarithm of $(PI_{ABS})rel$ against $(ET_0/ABS)_{rel}$ reveals what is considered a typical property of plants to transform absorbed light energy into chemical energy that is diverted to further metabolic reactions. The linear least squares model was fitted to the log function of relative performance index on absorption basis (log $(PI_{ABS})_{rel}$) with the probability that an absorbed photon moves further than QA $^-$ $(ET_0/ABS)_{rel}$. Values of treatments were normalized relatively to control. Statistical analyses were performed in R (R core team, 2019). PC analysis was performed with log-transformed, normalized and centred data using R´s *prcomp* command and applied to fourteen selected variables of the JIP test (Table 2). Dunn’s test for multiple comparisons was used to test for significant effects of environments on ChlF parameters. Differences between genotypes were tested using Kruskal-Wallis one-way ANOVA.

Results and discussion

Statistical analysis of selected ChlF parameters revealed significant differences between control and all other treatments (Environments) (P < 0.001) for all selected parameters (data not shown). Dunn´s test revealed that only parameters where there was no significant difference from the control treatment were: V_J, M_O, ψ_{Eo}, and PI_{ABS} for field treatment (F), S_M for heat treatment (H), and RE_o/RC for low light treatment (LL). Kruskal-Wallis test has revealed significant differences between genotypes for parameters: K, J, S_M, N, and φ_{Po}. Means and standard errors of selected biophysical parameters are shown in Supplementary table.

ChlF transients are distinctly affected by different environmental conditions

In all five maize hybrids transients of untreated plants (C) were similar. There were slight differences mostly regarding J and I steps (Fig. 1a). In LL treatment responses of different genotypes were also similar, hybrid 505 deviated the least from the control but minor differences from other genotypes were observed. In general, low light treatment caused slight increases in K and J steps (Fig. 1b). Appearance of the K step at 300 µs in the LL treatment (Fig. 1b) suggests an imbalance in the electron donor and acceptor sides of PSII and could be related to deactivation of oxygen evolving center (OEC) (Strasser et al. 1995, Jiang et al. 2006). Bertamini and Nedunchezhian (2003) found that the activity of OEC is often inhibited under stress which leads to the blockage of electron transfer from the electron donor side to the electron acceptor side. This imbalance is confirmed by the increase in J step which reflects the start of Q_A re-oxidation by Q_B (Strasser et al. 1995); hence increase in J step would suggest impairment of Q_A re-oxidation and a consequent build-up of reduced Q_A. Increases in K and J steps under low light were observed previously by Zhang et al. (2016) on two *Physocarpus* cultivars. Low light intensities cause changes in antennae structures and light-harvesting complexes (LHC) which were observed in maize by Hirth et al. (2013). Such changes can be linked to increase in F_o that occurred under low light conditions which can indicate the dissociation of LHCs from the PSII core complex and the accumulation of inactive, non Q_A reducing RCs. A decrease in J to I step compared to control was observed in F. In general, transients were similar between genotypes and their shape was similar to the ones from control (Fig. 1c) the only difference being the reduction in J–I rise. Contrary to the “photochemical phase” (OJ rise) of the fluorescence transients there is a controversy in the literature about the molecular mechanisms behind the kinetics at the “thermal phase” (JIP rise), for review see Stirbet and Govindjee (2012). The plastoquinone pool, plastocyanin and PSI are involved in the J–I–P part of the induction curve but the interpretations of

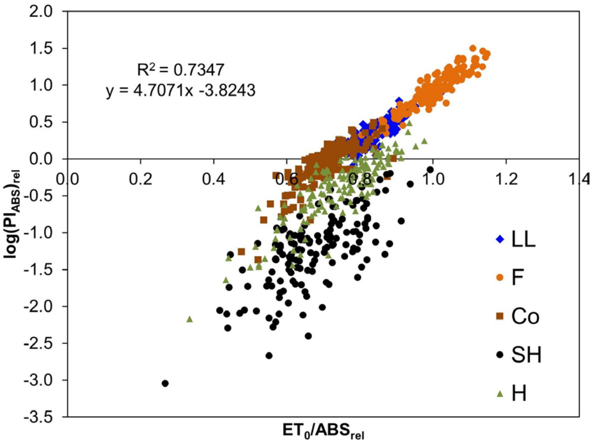

Fig. 2 - Linear regression model between the log function of relative performance index on absorption basis (log(PIABS)rel) relative yield of electron transport (ET0/ABS) of five maize plants subjected to different environmental conditions: low light (LL), field (F), cold (Co), heat (H), and severe heat (SH). For definitions and formulas, see Table 2.

these steps are still tested. According to Lazár (2006), relative height of the I-step (plateau) is a measure of the relative amount of the Q_B–nonreducing PSIIs. Also, the inactivation of the ferredoxin-NADP$^+$–oxidoreductase (FNR) has been suggested as a factor that could contribute to the appearance of the-I step (Schansker et al. 2003). Co treatment induced changes in fluorescence transients visible as an increase in K and J steps and decrease in I step. The I to P rise was very small due to increases in J step which caused the transient to be visibly different from the usual OJIP transient shape. Genotypes 378 and Drava showed the largest deviation from their controls (Fig. 1d). All maize genotypes used in this study have shown similar reactions in relative variable fluorescence transients as moderately chilling sensitive soybean (*Glycine max* L.) cultivar used in study by Krüger et al. (2014) after three nights of dark-chilling and sensitive cultivar PAN809 after seven nights of dark-chilling used in study by Strauss et al. (2006). Reason for such similarity in appearance of transients might be sensitivity of maize to chilling stress compared to C3 type plants including soybean. Contrarily to the study of Zushi et al. (2012) in tomato, the slight K step was observable in our study. The appearance of K step in Co treatment (Fig. 1d) which is usually hidden in the OJ rise, is considered an indicator of OEC damage and can be attributed to inhibition of electron transfer to secondary electron donor of PSII (YZ) (Nash et al. 1985; Strasser et al. 2004; Kalaji et al. 2016). Increase in K and J steps have been reported in tobacco leaves under low temperature stress (Wang et al. 2008; Strauss et al. 2003). It has been hypothesized that the decrease in the electron transport in cold stress could be due to Rieske center of Cyt b_6/f involvement in photoinhibition process (Wang et al. 2008). Also, cold stress strongly negatively affects chloroplast development (Marocco et al. 2001). H treatment caused changes in fluorescence transients that were visible mostly as an increase in J and K steps and decrease in I step (Fig. 1e). Changes were similar to Co treatment except in H increases in K step were larger than the increases in J step. Genotypes 444 and 505 deviated the most from the C in the K and J steps. Moderately elevated temperature in H treatment induced in changes in the ChlF transients shape most notably as the increase in K step which indicates OEC impairment resulting in inhibition of electron transport chain (Oukarroum et al. 2016). Related to the increased K step is the increase in rate of primary photochemistry visible as increased J step which reflects the start of Q_A re-oxidation by Q_B (Strasser et al. 1995); hence the impairment of Q_A re-oxidation that consequently leads to build-up of reduced Q_A. SH treatment caused discernible differences in fluorescence transients at K, J and I steps. Shapes of the transients were visually very different from the usual OJIP transient shape, due to large increase in K step followed by the dip at J step and almost flat J-I transition with a large jump in fluorescence intensity from I to P (Fig. 1f). The genotype Veli had the most similar transient to control mostly due to smallest decrease in I step while the largest difference from the control value was in the genotype 505. Loss of oxygen evolving activity due to destruction of manganese cluster are usually observed under heat stress (Strasser et al. 2004) and the PSII units are still able to perform limited electron transfer (K step) (Kalaji et al. 2011). The K step is probably a result of imbalance of donation rate of PSII and reoxidation

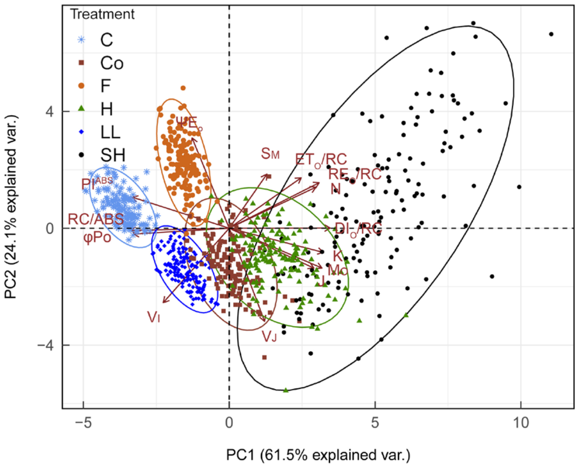

Fig. 3 - Principal component analysis of variability of selected JIP-test parameters in leaves of five maize hybrids (378, 444, 505, Drava and Veli) subjected to different environmental conditions: control (C), low light (LL), field (F), cold (Co), heat (H), and severe heat (SH).

Table 3 - Component loadings for the first three principal components (PC1, PC2 and PC3) shown as correlation matrix complemented with their respective communalities (correlations of parameters with PCs)

Parameter	Loadings			Communalities		
	PC1	PC2	PC3	PC1	PC2	PC3
K	0.91	-0.23	0.03	0.91	-0.23	0.03
J	0.82	-0.39	0.00	0.82	-0.39	0.00
V_J	0.34	-0.90	0.25	0.34	-0.90	0.25
V_I	-0.64	-0.72	-0.18	-0.64	-0.72	-0.18
M_O	0.90	-0.38	-0.18	0.90	-0.38	-0.18
S_M	0.36	0.52	0.76	0.36	0.52	0.76
N	0.84	0.40	0.36	0.84	0.40	0.36
DIo/RC	0.98	0.00	-0.14	0.98	0.00	-0.14
ETo/RC	0.70	0.49	-0.51	0.70	0.49	-0.51
REo/RC	0.87	0.44	0.02	0.87	0.44	0.02
φPo	-0.93	-0.06	0.01	-0.93	-0.06	0.01
ΨEo	-0.36	0.87	-0.30	-0.36	0.87	-0.30
RC/ABS	-0.96	-0.02	0.27	-0.96	-0.02	0.27
PI_{ABS}	-0.94	0.30	0.01	-0.94	0.30	0.01

of QA^-. Concomitant increase in the rate of primary photochemistry (M_O) was also observed (Supplementary table). Similar like in H treatment, blockage of electron flow from the electron donor side of PSII could be the cause of increase in J step; the impairment of Q_A re-oxidation consequently leads to build-up of reduced Q_A. Lack of J-I rise and the re-reduction of $P700^+$ and PC^+ suggests that there is an electron donor pool (such as ascorbate) supporting electron transport through PSII in the absence of functioning OEC or there is an increased cyclic electron transport around PSI (Toth et al. 2007, Joliot and Joliot 2002). Another interpretation of the impaired J-I rise or "dip" after the K step is the impairment of other components of photosynthetic apparatus other than OEC (Chen et al. 2016). The change in variable fluorescence at K step can be used as a convenient measure of heat tolerance (Oukarroum et al. 2016). Hybrid Veli thus showed relatively highest SH tolerance and unlike the other hybrids, at least some J-I rise. The presence of I-P rise suggests that the FNR is transiently blocked, as it is a condition for forming the I-P rise, and that P700+ and PC+ are being re-reduced. The distinct reaction of hybrid Veli could be explained by greater heat tolerance of Iowa Stiff Stalk Synthetic germplasm as shown by Šimić et al. (2014) which makes the maternal line of this hybrid. The relevance of ChlF transient analysis was recently confirmed through feasibility of their use in modelling of maize growth and biomass accumulation (Galic et al. 2019a). Further research of ChlF transients in context of modelling plant responses is needed, as they are easily measured and show distinct reactions to different environmental factors that deviate from optimum.

Cold and heat stress deteriorate efficiency of light conversion

Plotting log $(PI_{ABS})_{rel}$ against $(ET_o/_{ABS})_{rel}$ reveals what is considered a typical property of plants to transform absorbed light energy into chemical energy that is diverted to further metabolic reactions (Hermans et al. 2003). The log function of relative performance index on absorption basis (log $(PI_{ABS})_{rel}$) was linearly correlated with the probability that an absorbed photon moves further than Q_A^- $(ET_O/ABS)_{rel}$ (R = 0.86). F treatment had the highest values and was in the positive quadrant meaning that the efficiency of transforming light energy into chemical energy was the highest in this treatment (Fig. 2). LL treatment was also in positive quadrant while Co treatment was in part positive showing signs of reduced ability to transform light energy to chemical energy. H and SH treatments were in the negative quadrant showing signs of stress and reduced ability of energy transformation. Such relationships have been previously used to assess plant species in stressful conditions (Hermans et al. 2003, Christen et al. 2007). Plot of logarithm of PI_{ABS} and $ETo/_{ABS}$ shows there is tight correlation between these two parameters (R = 0.86). The reduction in conversion of light into chemical energy was not impaired in F and LL treatments. Lower efficiencies of conversion were observed in other treatments in decreasing order: Co, H and SH, respectively, with more stressful environments having larger variance and fitting the linear model worse.

Different parameters enable classification of stress effects

The first three principal components accounted for 95.9% of total variance. Selected 14 parameters had different sensitivity to environmental effects and hence the different contribution in formation of principal components. Standardized loadings based upon correlation matrix are shown in Table 3. The plot of component loadings showed that all five treatments were visible as separate clusters and placed in different regions of the Cartesian coordinate system (Fig. 3). SH treatment was dispersed the most which indicated the severity of heat stress to maize plants visible through changes in all examined parameters.

The strongest correlations of PC1 were with DIo/RC (0.98) and RC/ABS (–0.96), hence PC1 can be considered as a measure of functioning reaction centres with extrema on two sides being C and SH. The strongest correlation of PC2 was observed with variable fluorescence at J step (V_J, -0.90) and ψ_{Eo}(0.87), and PC3 with normalized area above the OJIP transient curve (0.76). On the opposing ends of the second principal component are F and both Co and LL treatments where LL and Co are mostly characterized by V_J and V_I parameters and F treatment by ψ_{Eo} and ET_o/RC parameters which explain electron transport flux. Similar grouping of parameters was shown in the recent study in classification of environmental effects on several plant species (Bussotti et al. 2020). It appears that PIABS offers only a single level of classification to well and poor performance at this growth stage, while the rest of the parameters should be used to test for specific effects of each environmental condition. For example, opposing sides of the first principal component show two environmental extrema that maize plants have been subjected to: (i) Co – characterized mostly by V_J and V_I parameters and on the other side SH – characterized mostly by K, M_0, RE_o/RC and DI_0/RC. This was also confirmed by Stirbet et al. (2018) who suggested designing new indices using ChlF parameters with known specific reactions to certain conditions. However, Galic et al (2019b) showed that PIABS can be efficiently used in selection for grain yield in heat-stressed environments.

Conclusions

Crop phenotyping represents new frontier of plant sciences and high throughput methods sensitive to various stresses are needed. Coping with climate change from crop breeding perspective demands phenotypes that are heritable, easily measured and show specific responses to different stressful conditions often found in growing environments. ChlF was shown to respond to different treatments by changing the appearance of OJIP band, mostly at J and I steps, while appearance of K step was confirmed as a convenient indicator of temperature stress. Relative to control, efficiency of utilization of photochemical energy was the lowest in severe heat treatment, while the highest values obtained in field were probably caused by higher light intensity compared to control. In PCA it was shown that first two PCs provide information about different photochemical processes facilitating classification of different stresses according to their effects on ChlF. Further research of usability of ChlF in maize breeding is needed, as it represents the data-rich, easy to measure method, providing the biological framework for explanation of reactions to different stresses

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