

INFLUENȚA CONDIȚIILOR ANTROPICE ALE ORAȘELOR PITEȘTI, MIOVENI ȘI MĂRĂCINENI ASUPRA EFICIENȚEI FOTOSISTEMULUI II AL BURUIENILOR DOMINANTE, FOLOSIND FLUORESCENȚA CLOROFILEI INFLUENCE OF ANTHROPIC CONDITIONS ON PITEȘTI, MIOVENI AND MARACINENI DOMINANT WEEDS PHOTOSYSTEM II EFFICIENCY USING CHLOROPHYLL FLUORESCENCE

Marinela Roxana Roșescu¹, Emil Chițu²

¹National High School "Alexandru Odobescu" Pitesti, Romania

²Research Institute for Fruit Growing Pitesti, Romania

Abstract

The plant's life is influenced in various ways by the human activity. The study of the physiological processes of weeds offers information about their ability to adapt to the conditions of the anthropic environment. The experiments were realized during the month of July 2009 on six plant species dominant in the Pitesti, Mioveni and Maracineni area: *Cichorium intybus* L., *Conyza canadensis* (L.) Cronq., *Erigeron annuus* L. (Pers.), *Lactuca serriola* Torn., *Polygonum aviculare* L. și *Echinochloa crus-galli* (L.) Beauv. Measurements of the photosynthesis and transpiration processes, on one hand and of the chlorophyll fluorescence, the OJIP and the NPQ tests, in order to determine the efficiency of photo-system II (PS II) on the other hand, were taken. The efficiency of PS II was correlated to the main environmental factors which influence the photosynthesis process and with the region's natural layers in which the experiments were conducted.

Cuvinte cheie: fotosinteză, fluorescența clorofilei, testul OJIP, testul NPQ, stresul ambiental

Keywords: photosynthesis, chlorophyll fluorescence, OJIP test, NPQ test, ambient stress

1. Introduction

The synanthropic plants (like weeds) are plants related to the human activities. The study of the physiological processes of weeds offers information about their ability to adapt to the conditions of the anthropic environment. In the anthropic environment plants are highly stressed (high pollution levels, pH soil modifications, extreme low and high fertilizations as well as other anthropic pressures – irrigation, mowing etc.). Therefore, the plants are forced to find surviving strategies to which can be revealed through the recording of physiological processes (photosynthesis, respiration, transpiration). Numerous studies were conducted related to the effect of the environment on the physiological processes of the plants with economic importance (fruit and ornamental trees, various crops, etc.) Chitu et al., 2009, have studied the sensibility of the chlorophyll fluorescence at various environmental nutritional, climacteric and stress factors for various apple cultivars. Water and nutrients are often the most yield limiting factors for the growing plant. A number of studies have shown that chlorophyll fluorescence parameters provide good indicators of nutrient deficiency (Subhash and Mohanan, 1997; Freedman et al., 2002, quoted by Chitu et al., 2009). Long-term stress events and mineral deficiencies may eventually reduce the chlorophyll and carotenoid content of leaves, which could be monitored by fluorescence measurements (Apostol et al., 2003; Gitelson et al., 1996, quoted by et al., 2009). Cosmulescu et al., 2008, were preoccupied by the study of physiological "answers" of the black currant cultivars to the environmental impact. It was revealed that the photosynthesis' rate is influenced by the leaf temperature and by the active photosynthetic radiation. The same authors have found that, on the case of black currant, the stomatal conductance decreases as the leaf's temperatures rises and this is explained by the existence of adaptive mechanisms through which, at high temperatures and water deficits, the plant is reducing its water losses by closing and opening its stomata. Siddique et al., 1999, have studied the drought effects as stress factor over the photosynthesis rate and the wheat gas exchange. Nabil, 2000, had studied the influence of leaf's age and position over the stomatal conductance for apple, and Gutierrez-Rodriguez, 2005, has determined the photosynthesis rate and stomatal conductance for two species of *Phaseolus*, Bunce, 2000, has studied the stomatal conductance response to light, humidity and temperature for the fall wheat and barley. Oneata, 2007, had studied the influence of CO₂ variation in atmosphere and that of photosynthetic active radiation (PAR) over the physiological processes of the magnolia species, underlining that the CO₂ concentration and PAR play a significant role influencing the researched processes.

2. Materials and methods

The experiments were realized in July 2009 on six weeds: *Cichorium intybus* L., *Conyza canadensis* (L.) Cronq., *Erigeron annuus* L. (Pers.), *Lactuca serriola* Torn., *Polygonum aviculare* L. and *Echinochloa crus-galli* (L.) Beauv., dominant species on unmanaged lands in the cities of Pitesti, Mioveni and Maracineni. The areas over which the research was conducted registered for the month of July 29°C ground temperature (vs. the normal average 24.2°C), 29.1°C, air temperature (vs. the normal average 27,4°C), 32.7% air humidity (vs. the normal average 47.2%) and 8.4 hours/day sunshine (260.4 vs. normal average 303.5 hours/month). For the six species the following were measured: photosynthesis and transpiration intensity and the chlorophyll fluorescence; the Chlorophyll Fluorescence Induction Kinetics (OJIP) and Non-photochemical Quenching (NPQ) tests were also conducted. The photosynthesis and transpiration rates were measured using a portable LCpro+ system which also measured other parameters such as the stomatal conductance, the leaf's temperature, the photosynthetic active incidental radiation on the leaf's surface. The fluorescence measurements were done using an OS-30 (Opti-Sciences) fluorometer with the following settings: two seconds for the action time of the light spot and 2000 micromole/ m²/s for the source intensity. The subjected leafs of the experiment were dark adapted for a minimum of 15 minutes in order to determine the Fv/Fm ratio. The two tests were conducted using the the FluorPen FP 100. The OJIP test permitted the computing of the following 25 indicators:

F₀ (F_o): = F50μs; fluorescence intensity at 50 μs;

F_k: = fluorescence intensity at k-step (at 300 μs);

F_j: = fluorescence intensity at j-step (at 2 ms);

F_i: = fluorescence intensity at i-step (at 60 ms);

F_M (F_m): = maximal fluorescence intensity;

F_v: = F_M - F₀ (maximal variable fluorescence);

V_j = (F_j - F₀) / (F_M - F₀) - relative variable fluorescence at the j-step. For unconnected PSII units, equals the fraction of closed RCs at 2 ms expressed as a proportion of the total number of RCs that can be closed;

V_i = (F_i - F₀) / (F_M - F₀) - relative variable fluorescence at the i-step;

F_v / F₀ = Performance due to trapping probability Φ_{P₀} (P_{TR}) [Φ_{P₀} / (1 - Φ_{P₀})] or the contribution to the PI of the light reactions for primary photochemistry. The contribution of the light reactions to primary photochemistry is estimated according to the JIP test as [Φ_{P₀} / (1 - Φ_{P₀})] = F_v / F₀;

F_v / F_M = related to the quantum efficiency of PS II (dark); quantum efficiency or potential quantum yield of PS II in a dark adapted leaf. F_v/F_M gives the potential quantum yield or potential quantum efficiency of the leaf and is thus an indicator of plant health. A healthy terrestrial plant will almost always have a dark adapted F_v/F_M value close to 0.8. A decrease from this indicates a stress (either short-term or long-term) and the presence of a quenching mechanism;

M₀ or (dV / dt)₀ = TR₀ / RC - ET₀ / RC = 4 (F₃₀₀ - F₀) / (F_M - F₀) - Approximated initial slope (in ms⁻¹) of the fluorescence transient V = f(t); Net rate of PSII closure: (dV/dt)₀ or **M₀** = 4 (F_{300μs} - F₀) / (F_M - F₀). An approximation of the slope at the origin of the fluorescence rise (dF/dt)₀ which is a measure of the rate of the primary photochemistry. It is a net rate because the reduced Q_A can be reoxidized via electron transport beyond Q_A;

Area = area between fluorescence curve and F_M (background subtracted). The area above the fluorescence curve between F₀ and F_M is proportional to the pool size of the electron acceptors Q_A on the reducing side of Photosystem II. The Area measurement is a very useful parameter as it highlights any change in the shape of the induction kinetic between F₀ and F_M which would not be evident from the other parameters e.g. F₀, F_M, F_v/F_M which only express changes of amplitude of the extreme F₀ and F_M;

Fix Area = total area above the OJIP fluorescence transient - between F_{40μ} and F_{1s} (background subtracted);

S_M = area / F_M - F₀ (multiple turn-over);

S_s = the smallest S_m turn-over (single turn-over);

N = S_M · M₀ · (1 / V_j) - turn-over number Q_A;

Phi_P₀ (Φ_{P₀}) = 1 - (F₀ / F_M) (or F_v / F_M) - maximum quantum yield of primary photochemistry at t = 0; Trapping probability or maximum quantum yield of primary photochemistry: Φ_{P₀} or TR₀/ABS; the probability that an absorbed photon will be trapped by the PSII RC, with the resultant reduction of Q_A, relates to the whole measured sample that may be heterogeneous in terms of Q_A reducing and non-reducing RCs;

Psi_{l0} (Ψ₀ or ET₀/TR₀) = 1 - V_j - probability (at time 0) that a trapped excitation moves an electron into the electron transport chain beyond Q_A; Electron transport probability or the probability that an electron residing on Q_A will enter the electron transport chain;

Phi_E₀ (Φ_{E₀}) = (1 - F₀ / F_M) · Psi_{l0} - Quantum yield for electron transport at t = 0;

Phi_D₀ (Φ_{D₀}) = 1 - Phi_P₀ - (F₀ / F_M) - Quantum yield at t = 0 for energy dissipation;

Phi_P_{av} (Φ_{P_{av}}) = Phi_P₀ - (S_M / t_{FM}); t_{FM}) = Time to reach F_M (in ms);

Pi_Abs = Performance index (PI) on absorption basis. $(\gamma_{RC}/(1 - \gamma_{RC}))((\Phi_{P_0}/(1 - \Phi_{P_0}))(\Psi_0/(1 - \Psi_0))) = (RC/ABS)(P_{TR})(P_{ET})$ a multi-parametric expression of these three independent steps contributing to photosynthesis. The Performance Index is essentially an indicator of sample vitality. It is an overall expression indicating a kind of internal force of the sample to resist constraints from outside. It is a Force in the same way that redox potential in a mixture of redox couples is a force. PI (on an absorption basis or on a chlorophyll basis) has three components: 1) The first component shows the force due to the concentration of active reaction centers. **RC/ABS** is a parameter of the JIP test and it is related to the force generated by the RC concentration per antenna chlorophyll. 2) The second component **-Phi_P₀** (Φ_{P_0}), is the force of the light reactions, which is related to the quantum yield of primary photochemistry. 3) The third component **-Psi₀** (Ψ_0 or ET_0/TR_0) is the force related to the dark reactions (after QA⁻). These are normal redox reactions in the dark;

Specific fluxes or activities expressed per reaction center (RC)

$$ABS / RC = M_0 (1 / V_J) \cdot (1 / \Phi_{P_0})$$

$$TR_0 / RC = M_0 \cdot (1 / V_J) - \text{trapped energy flux per reaction centre (RC) at } t=0;$$

$$ET_0 / RC = M_0 \cdot (1 / V_J) \cdot \Phi_{P_0} - \text{electron transport flux per RC at } t=0;$$

$$DI_0 / RC = (ABS / RC) - (TR_0 / RC) - \text{dissipated energy flux per RC.}$$

All measurements and tests were conducted in the field; the temperature and humidity were recorded using a digital datalogger (Mannix DL8829). Correlations measure how variables or rank orders are related. Pearson's correlation coefficient is a measure of linear association. Two variables can be perfectly related but if the relationship is not linear Pearson's correlation coefficient is not an appropriate statistic for measuring their association. The statistic method used to process the experimental data was the analysis of variance for polifactorial experiments and the Duncan test ($\alpha=0.05$ confidence level) was used to determine the statistic significance.

3. Results and discussions

The chlorophyll fluorescence is an indicator of the energy conversion in the photosynthesis process, offering efficiency information for the photosystem II. Functioning of photosystem II (PSII) is the most sensitive indicator of environmental stress in plants. Changes in PSII activity can be assayed rapidly and non-destructively by measurement of chlorophyll fluorescence. Factors such as light levels, light quality, water availability, nutrient availability, heat, cold, herbicides, pesticides, pollution, heavy metals, disease, and genetic make up can all have an impact on CO₂ assimilation, plant health and condition. They also are reflected in the fluorescence signal in PSII. Therefore, by using a chlorophyll fluorometer one can quantify the impact of these factors on plants to understand better plant functions. F_v/F_m – dark adapted test - a measurement ratio that represents the maximum potential quantum efficiency of Photosystem II (PSII) is a very robust test that have been shown to correlate well with carbon fixation under most conditions. The 155 values of the sample represent the total number of experiments of the following indicators: Fo, Fj, Fi, Fm, Fv, Fm/Fo, Fv/Fo and Fv/Fm (of the 25 researched indicators). The fluorescence indicators statistic is presented in table 1. The sample average for Fv/Fm was 0.741, and it was close to the maximum value of Fv/Fm (0.80-0.83), which indicates a normal working reaction center for PS II. The average is representative for the sample only if the normality is accepted. The normality was tested using the Shapiro-Wilk test and the W indicator permitted the acceptance.

The histogram associated reveals the frequency distribution of the Fv/Fm values by class (figure 1):

Table 2 has the data following the computing of the simple correlation coefficients of the chlorophyll fluorescence indicators. We observe the existence of very significant positive and negative correlations among the analyzed indicators. In all cases the significance level is $p < 0.01$.

By analyzing figure 2, which introduces The PS II efficiency variance by the environment in which the experiment was conducted, on constant species levels we observe the existence of four homogenous classes for the values F_v/F_m , the PS II efficiency recording the highest values in Maracineni, soil conditions, (0,797). Generally, the average tendency was kept for *Cichorium intybus* and for *Lactuca serriola* at which the asphalt's positive effect was accentuated, regardless of the location, over the F_v/F_m . For *Erigeron annuus* the differences between species, with respect to the anthropic's environment influence of the PS II efficiency, (F_v/F_m), have vanished. In the case of *Conyza canadensis*, the Pitesti conditions have a favorable influence on the chlorophyll fluorescence (F_v/F_m) in contrast to the average effect. In the same environment, *Echinochloa crus-galli* is positively influenced photosystem II maximum efficiency while, the same species, the unfavorable influence of the Mioveni environment, soil conditions, is accentuated. *Polygonum aviculare* is prospering when vegetating in asphalt cracks ($F_v/F_m=0.766$).

Figure 3 presents the maximum efficiency variance of PS II adapted for darkness by species, on constant levels in the environment where experiments were conducted and it shows that, on the experiment average, two homogenous classes had formed for the computed F_v/F_m values. The first class of values has five species while the second contains *Echinochloa crus-galli* with the lowest PS II efficiency (0.687). *Echinochloa crus-galli* is doing well in Pitesti, asphalt conditions, in contrast with the average findings were values were unfavorable for *Erigeron annuus* and *Lactuca serriola* (which, on

average, had higher Fv/Fm values). Also in Pitesti but in soil conditions, *Conyza canadensis* had the highest Fv/Fm value, significantly different than that of other species which had a uniform behavior. In Mioveni, asphalt conditions, the differences among species had accentuated, *Conyza canadensis* registering a decrease in its PS II efficiency over the average tendency. The soil conditions in Mioveni, had kept the average tendency and the lowest PS II efficiency was recorded for *Echinochloa crus-galli*. Also, the species effect over Fv/Fm had amplified so *Erigeron annuus* și *Polygonum aviculare* had been less efficient while *Echinochloa crus-galli* more efficient, with respect to the activity of the photosystem II (adapted to darkness – Fv/Fm), over the average tendency.

The 155 determining experiments of the 25 OJIP parameters, executed on leaves adapted to darkness were correlated with PAR, with leaf temperature, transpiration rate, stomatal conductance and with the photosynthesis rate. Tabel 3, which has the values of simple correlation coefficients, shows that of a number of 25 analyzed parameters, 19 had correlated synthetic significantly, positive or negative, with the following parameters: PAR, leaf temperature and the photosynthesis rate. Fm/Fo and Fv/Fm had correlated significantly negative with PAR ($r = -0.383^*$ in both situations), Ss had correlated distinct significantly, positive, with the photosynthesis rate ($r = 0.400^{**}$), while Tro/RC had correlated significantly negative cu the photosynthesis rate ($r = -0.367^*$). A number of 18 OJIP parameters had correlated with the leaf temperature. The stomatal transpiration rate had not correlated with any OJIP parameter.

4. Conclusions

1. Analyzing the photosynthesis and chlorophyll fluorescence indicators of leafs adapted to dark conditions the following were observed:
2. Fv/Fm was correlated most intense negative with Fo ($r = -0.471^{**}$) and most intensely positive cu Fv ($r = 0.700^{**}$). The average value for the entire experiment was 0.741, close to the optimum value (0.830) that indicates a low level of stress (either short-term or long-term) and the absence of a strong quenching mechanism.
3. Fm/Fo and Fv/Fm were significantly correlated negative with PAR ($r = -0.383^*$, in both situations) the photosynthesis rate correlated distinct significantly, positive cu Ss ($r = 0.400^{**}$) and negative with Tro/RC ($r = -0.367^*$); the leaf temperature correlated significantly, positive and negative, with 18 OJIP parameters.

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Table 1. Chlorophyll fluorescence statistic indicators determined on leaves adapted to darkness

	Fo	Fj	Fi	Fm	Fv	Fm/Fo	Fv/Fo	Fv/Fm
N Valid	155	155	155	155	155	155	155	155
Mean	430.2	1083.5	1337.3	1716.4	1286.1	4.0742	3.0742	0.741
Std. Error of Mean	7.254	25.125	27.812	29.517	26.960	0.0665	0.0665	0.005
Median	427.00	1030.0	1280.00	1723.33	1264.0	4.0990	3.0990	0.7560
Mode	355(b)	822(b)	1030(b)	1357(b)	1115(b)	3,992	2,992	0,749
Std. Deviation	90.317	312.80	346.255	367.485	335.65	0.8287	0.8287	0.0668
Variance	8157.1	97846.1	119892.2	135045.3	112660.	0.687	0.687	0.004
Skewness	0,501	0,772	0,379	0,123	-0,039	-0,273	-0,273	-2.032
Std. Error of Skewness	0.195	0.195	0.195	0.195	0.195	0.195	0.195	0.195
Kurtosis	0.343	0.279	0.233	-0.226	-0.067	0.421	0.421	5.350
Std. Error of Kurtosis	0.387	0.387	0.387	0.387	0.387	0.387	0.387	0.387
Range	479	1552	2009	1926	1770	4.549	4.549	0.403
Minimum	227	564	546	839	368	1.781	0.781	0.439
Maximum	706	2116	2555	2765	2138	6.330	5.330	0.842
Sum	66,690	16,7951	20,7285	266,049	199,359	631.51	476.51	114.99

a Calculated from grouped data; b Multiple modes exist. The smallest value is shown

Table 2. Correlation matrix (Pearson coefficient) between selected fluorescence indicators determined on leaves adapted to darkness

	Fo	Fj	Fi	Fm	Fv	Fm/Fo	Fv/Fo	Fv/Fm
N	155	155	155	155	155	155	155	155
Fo	1	0.884**	0.568**	0.460**	0.235**	-0.491**	-0.491**	-0.471**
Fj	0.884**	1	0.746**	0.647**	0.470**	-0.214**	-0.214**	-0.147
Fi	0,568**	0,746**	1	0,950**	0,888**	0,359**	0,359**	0,388**
Fm	0.460**	0.647**	0.950**	1	0.971**	0.528**	0.528**	0.523**
Fv	0.235**	0.470**	0.888**	0.971**	1	0.711**	0.711**	0.700**
Fm/Fo	-0.491**	-0.214**	0.359**	0.528**	0.711**	1	1.000**	0.938**
Fv/Fo	-0.491**	-0.214**	0.359**	0.528**	0.711**	1.000**	1	0.938**
Fv/Fm	-0.471**	-0.147	0.388**	0.523**	0.700**	0.938**	0.938**	1

** Correlation is significant at the 0.01 level (2-tailed)

Table 3. Correlation matrix (Pearson coefficient) between selected OJIP and photosynthesis parameters

Parametri OJIP	PAR on leaf surface	Leaf temperature	Photosynthetic rate
Fo		0.561**	
Fj		0.471**	
Vj		0.577**	
Fm/Fo	-0.383*	-0.629**	
Fv/Fm	-0.383*	-0.629**	
Mo		-0.509**	
Ss		0.545**	0.400**
N		0.426*	
Phi_Po		-0.509**	
Psi_o		-0.577**	
Phi_Eo		-0.614**	
Phi_Do		0.509**	
Phi_Pav		0.394*	
Pi_Abs		-0.693**	
ABS/RC		0.404*	
Tro/RC		0.388*	-0.367*
Eto/RC		-0.488**	
Dlo/RC		0.394*	

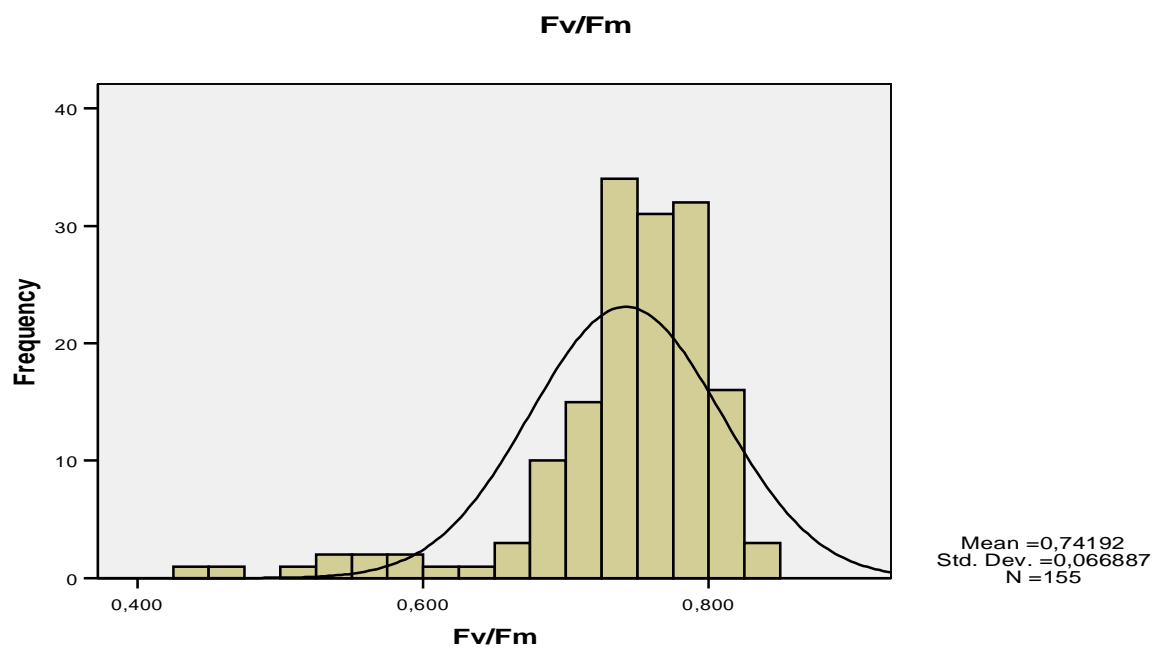


Figure 1. Frequency distribution of the Fv/Fm values by class

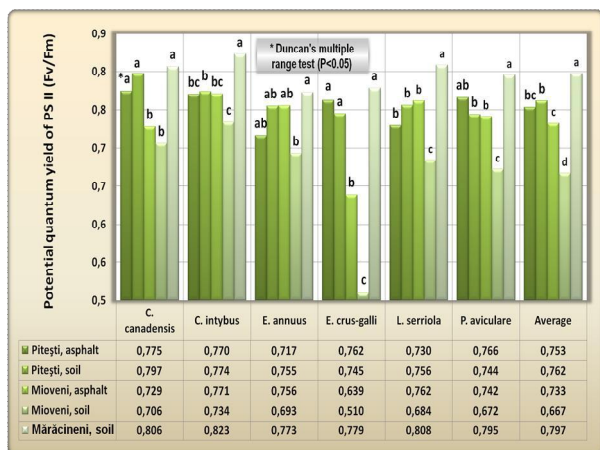


Figure 2. The PS II efficiency variance by the environment in which the experiment was conducted, on constant species levels

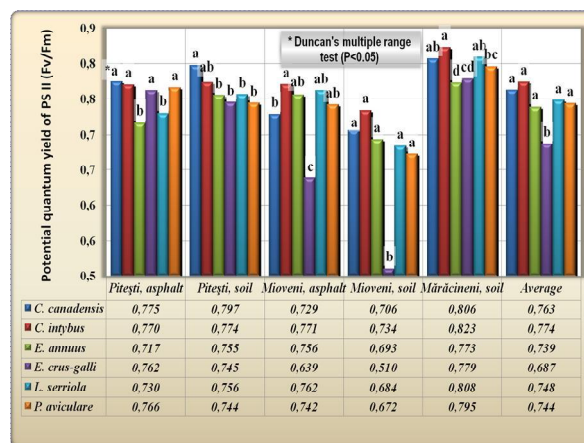


Figure 3. The maximum efficiency variance of PS II adapted for darkness by species, on constant execution environment levels