

Correlations between delayed fluorescence of chlorophyll, metabolism and yield of plants.

I. Influence of fertilizers on correlations

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ABSTRACT

The increase of the potash fertilizer dose induced a raise in efficiency influence of the nitrogen fertilizer, optimisation of phosphorous fertilizer effect, enhancement of leaf protein production, expansion of assimilating surface and yield growth. In the period of yield formation, the parameters of delayed fluorescence of chlorophyll (DF) of leaf wholly corresponded with key factors that had a dramatic influence on the effectiveness of yield formation. The maximum level of DF amplitude mostly depended on the activity of nitrogen metabolism and presumably on active PSII concentration changes per square unit. Half-decay time of this amplitude was predominantly identified by the level of carbohydrate metabolism in the overall plant system, including the quantity of its products and, therefore, mostly by correspondence with yield. This is a biological base triggering the use of DF parameters for system analyses of plant production process.

Keywords: Delayed Fluorescence of Chlorophyll; Metabolism; Nitrogen; Phosphorus and Potash Fertilizers; Moisture; Poisonous; Yield

1. INTRODUCTION

Plant growth is affected by numerous environmental factors. Therefore, it is difficult to predict the amount of yield, without system analyses of interaction among all key components of crop yield formation. Finding general relationship in the complex system of the plant also required selection of appropriate key objects. Our choice was PSII, which is the main producer of the DF (the level of PSI DF is several times lower [1]). DF transients have been shown to be driven by many mechanisms,

including electrical and pH gradients [2], acceptor availability, donor availability [3], and redox state of the oxygen-evolving system [4,5]. The DF induction curve, registered in a several-minute period, encompasses two major phases—a fast one taking place in the first second of induction, and a slow one, that can last for minutes. Each phase is shaped by several processes running at the same time [4,5]. When applying the phosphoroscope-based method of registration, the fast phase I observed as maximum intensity of leaf DF curve. Many authors attribute this initial phase to the light induced changes of the electrical potential and proton gradient across the thylakoid membrane, depending from the state of the PSII reaction center, which result manifests in the decrease the amount of activation energy necessary for the back reaction between QA^- and Z^+ [2,4,6] as well as may depend from quantity of oxidized QA (**Figure 1**).

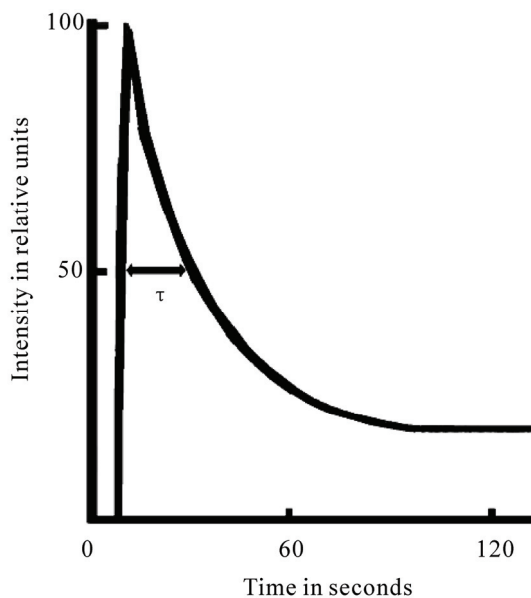


Figure 1. Induction curve of delayed fluorescence of chlorophyll.

Previously, it assumed that kinetics of fast phase further decay depend from the photosynthetic dark processes intensity [4-6]. This decay of induction curve induced the membrane energisation decreasing as result of cation efflux from thylakoids. These are a biological base triggering the use of these DF parameters in physiological research. The interest of using the parameters of DF for the diagnosis of plants state identified as the possibility of their fast measurements on intact objects by the use of comparatively simple and accessible equipment. Therefore, our goal was research the correspondence of DF parameters, metabolism and yield changes of plants in order of the use of these parameters for the system analyses of production process of field plants.

2. MATERIALS AND METHODS

2.1. Plant Material and Experimental Design

The tomato (*Lycopersicon esculentum*) variety Nver seedling and the potato (*Solanum tuberosum*) variety Pirmunes were planted on May 30 and March 30, respectively, in hot and draughty conditions of loamy clay soils of Ararat Valley, Armenia. The sprouts of pea (*Pisum sativum*) variety Pobeditel were transferred into solutions of distilled water after seeds germination (24 h up to 2-3 cm rootlets) and grown at temperature of 25°C, photosynthesis photon flux density of 605 $\mu\text{mol m}^{-2}/\text{s}$ and a photoperiod of 16/8 h. The experiment with pea performed in six replications.

The tomato sprouts planted in three replications on an each field plot of 91 m^2 . A part of nitrogen at dose of N_{30} (in kg/ha) and a full dose of phosphoric and potash fertilizers were added to the soil during the pre-sowing cultivation. The other parts of nitrogen fertilizer added twice on May 17 and June 13. For the $\text{N}_0\text{P}_0\text{K}_0$ variant, the fertilizers not added to the soil.

The potato planted in four replications on each field plot of 50 m^2 . During experiment of potato, the average air temperature was at 26°C, and the maximum air temperature was at 33.6°C. The potato plants were watered only on June 25 (on June 19 the soil humidity was 16.9%, June 24—14.0%, June 26—21.4%, July 8—14.3% and 15—12.2%).

2.2. Measurement and Sample Protocol

The yield of plants obtained per plant and for each plot. The analysis of chemical compounds quantity was performed by the use of standard methods in air-dry samples by methods of Ginsburg for leaves and Maslova (K_2O) and Arrhenius (P_2O_5) for soil [7]. The humidity of soil (in the ground layer 0-30 cm) and leaves measured through samples drying at 105°C and 80°C, respectively, in oven through 3-4 repetitions. The square of assimilat-

ing surface of plants measured by using weighing method and device AAT-5 (Hayasaki Denco Co., Japan) in four repetitions.

The parameters of the leaves DF induction curve were measured from the center of lobes by using a single-disc phosphoroskope (time between excitation and recording of DF was 7 ms) at temperature of 22°C. Exciting light was obtained from a 200 W glow lamp (KGM-200, provided maximum intensity of modulated visual light at the sample surface of 2000 $\mu\text{mol photons m}^{-2}/\text{s}$) and photodiode. Each detached-from-leaf lobe incubated about 1.5 hour in the dark between paper sheets at temperature of 4°C.

The leaf DF parameters and concentration of chemical compounds of tomato leaves measured since fructification phase, per 10 dates. The DF measurements were made with fields leaves detached from 50 tomato plants of each variant for each data of test. The calculation of yield (plot-based weighing method) and concentration of chemical compounds of fruits was carried out on 3 different dates in harvesting stage (July 25, August 6 and 18). The leaf DF parameters and concentration of chemical compounds of potato leaves were measured since bud phase, per 5 dates, concentration of chemical compounds of soil at the end of vegetation.

2.3. Statistical Analyses

The replications dates were processed from a purely mathematical standpoint by the use of the Student's and Fisher's exact test, and the standard methods of correlation analyses with 5% significance level.

3. RESULTS AND DISCUSSION

The research of the effects of mineral nutrition on DF parameters was carried out on pea germ fully provided with necessary nutrient components, contained in seed at the early stage of form-building processes. The data shows that in the growing period of germs I increased dramatically by the raise in mineral salts amounts in solution at the initial stage and then it generally fell, compared to salt-free grown plants (Tables 1-3). I reduction correlated with suppression level of germs' vital activity. It is well known that the increase of mineral salt concentration in the nutrient medium stimulates their quantity increase in plants [8]. In the case of an unbalanced heightened amount of separate ions in the nutrient medium, a poisonous effect on plants or unfavourable effect on their yield was observed [8-10]. In the event of addition of mineral salts to nutrition solution, the highest level of poisonous effect was detected for phosphorus anions (Tables 1-3). In complex with NO_3^- versus Cl^- anion cation K^+ reduced I more rapidly. This was probably based on the obtained capability of plants to

Table 1. Effect of salt concentration in solution on I of 6-day-old pea and the correlation coefficient between I and salt amount*.

Quantity in mM	I in rel. units					Salt	Correlation coefficient in rel. units
	NaCl	KCl	KNO ₃	KH ₂ PO ₄	Ca(NO ₃) ₂		
0	100.00	100.00	100.00	100.00	100.00	NaCl	0.467
2	93.55	119.82	105.76	97.24	101.92	KCl	0.295
4	101.61	114.00	94.93	89.40	104.26	KNO ₃	-0.775
20	101.84	114.29	90.78	70.28	113.22	KH ₂ PO ₄	-0.985
LSD ₀₅	24.17	3.96	24.67	27.67	14.31	Ca(NO ₃) ₂	0.992

*At the significance level of 5%, the critical value of correlation coefficient is 0.950.

Table 2. Temporal variation of pea I, planted in salt solution.

Quantity in mM	I in rel. unit					
	Ca(NO ₃) ₂			CaCl ₂		
	6 day	13 day	20 day	6 day	13 day	20 day
0	100.00	102.56	84.01	100.00	103.70	95.91
2	101.02	106.6	88.05	103.70	121.60	98.77
4	104.26	110.02	105.32	103.77	125.52	94.44
8	118.33	110.44	91.47			
20				105.77	100.00	84.57
LSD ₀₅	11.10	14.07	30.39	14.72	20.53	20.88

Table 3. Correlative correspondence between pea DF I and mineral salts concentration*.

Quantity in mM	Quantity of repetitions	Correlation Coefficient in rel. unit					
		Ca(NO ₃) ₂			CaCl ₂		
		6 day	13 day	20 day	6 day	13 day	20 day
0 → 4	3	0.999	0.999	0.941	0.779	0.938	-0.569
0 → 8	4	0.959	0.794	0.402			
0 → 20	4				0.817	-0.530	-0.973

*At the significance level of 5%, the critical value of correlation coefficient is 0.997 for three, and 0.950 for four repetitions.

reduce the effects of highly concentrated Cl⁻ anion [8]. The NO₃⁻ anion far more increased I in case of the complexity with Ca²⁺ compared with K⁺ (**Table 3**). This is probably due to the explicit favorable effect of cation Ca²⁺ on the increase of nitrate reductase activity in leaves [11]. At the same time, in the event of cation Ca²⁺ with anion NO₃⁻ induced the increase of I proves to be more prolonged than in case with anion Cl⁻ (**Tables 2 and 3**). As known, the influence of cations and anions may cause changes in the ECP (thus affecting on only the DF parameters) and quantity of active PSII [3-5], but could reflect on the interaction between weakly associate polypeptides and membrane [12], and on the packaging

of tilakoids in grana [13]. Our results confirmed that in the earlier stage of pea seedling development the mineral salts induced influence on I value mostly depended on the affects of salt concentration in solution on metabolism of plants, compared to the changes of ECP of membranes [14].

A significant reduction in N, N_p and P₁ tomato leaves of all variants was observed in field conditions from fructification stage up to harvesting (**Figure 2, Table 4**). During the experiment, it was obtained that correspondence between N and P₁ changes was significant in all variants (**Table 5**). At the same time, in all variants a reduction in N_p reliably correlated (versus the variant

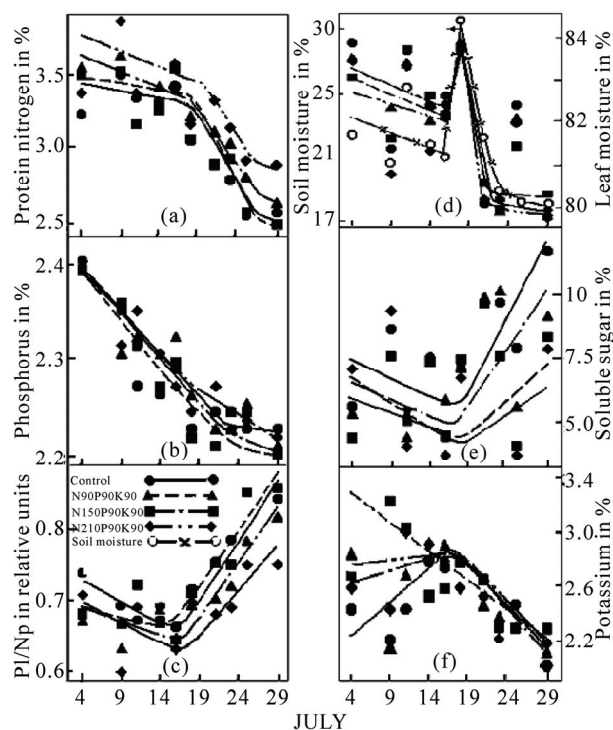


Figure 2. Temporary variation of protein nitrogen (a) and phosphorus (b) concentrations, their ratio (c), leaves and soil moistures (d), soluble sugar leaves (e) and potassium (f) amounts.

Table 4. Fertilizer dose affect on temporary variation of leaf chemical compounds quantities*.

Coefficient Description	Correlation Coefficient in rel. unit			
	$N_0P_0K_0$	$N_{90}P_{90}K_{90}$	$N_{150}P_{90}K_{90}$	$N_{210}P_{90}K_{90}$
$r_{N T}$	-0.880	-0.826	-0.889	-0.863
$r_{N_p T}$	-0.854	-0.866	-0.885	-0.747
$r_{P_1 T}$	-0.860	-0.920	-0.904	-0.930
$r(P_1/N_p) T$	0.728	0.768	0.806	0.531
$r_{S_1 T}$	0.765	0.561	0.368	-0.014
$r(S_1/N_p) T$	0.797	0.742	0.605	0.195
$r_{K_1 T}$	-0.227	-0.512	-0.732	-0.606
$r_{K_1/N_p} T$	0.556	0.289	0.166	-0.094
$r_{M_1 T}$	-0.640	-0.711	-0.668	-0.570
$b_{N/T}$	0.774	0.683	0.796	0.745
$b_{N_p/T}$	-0.038	-0.036	-0.043	-0.028
$b_{K_1/T}$	-6.46	-13.73	-17.67	-17.16
$b(P_1/N_p) / T$	0.007	0.006	0.009	0.003
$b_{M_1/T}$	-0.139	-0.150	-0.128	-0.123

*The concentration of chemical compounds of leaves were measured at the beginning of fructification, per 10 dates. At the significance level of 5%, the critical value of correlation coefficient is 0.632.

Table 5. Fertilizer dose affect on correlative correspondence between concentrations of chemical compounds*.

Coefficient Description	Correlation Coefficient in rel. unit			
	$N_0P_0K_0$	$N_{90}P_{90}K_{90}$	$N_{150}P_{90}K_{90}$	$N_{210}P_{90}K_{90}$
$r_{N_p N}$	0.885	0.853	0.845	0.439
$r_{N_p M_1}$	0.497	0.545	0.521	0.153
$r_{N M_1}$	0.553	0.655	0.614	0.740
$r_{N P_1}$	0.707	0.691	0.892	0.731
$r_{N_p P_1}$	0.633	0.783	0.798	0.560
$r_{N_p K_1}$	0.357	0.527	0.650	0.442
$r_{N_p (P_1/N_p)}$	-0.950	-0.970	-0.968	-0.946
$r_{M_1 K_1}$	0.613	0.656	0.768	0.798

*Experimental design of data measurements was as in Table 4. At the significance level of 5%, the critical value of correlation coefficient is 0.632.

$N_{210}P_{90}K_{90}$) with N and P_1 decrease ($d_{P_1 N_p} = 61-64\%$, **Figures 2(a) and 2(b)**, **Table 5**). It was previously shown that ATF, synthesised in LR, is used in phosphorylation reactions of monosaccharides at first priority [15]. Besides, a reduced mineral part of P_1 was observed in leaves, resulting from this reaction. In this case, the efficiency of phosphorylation fell significantly, inducing ADF/ATF ratio increase. As a result, it led to the inhibition of protein synthesis [16,17]. During the experiment, in case of N_p and P_1 reduction a significant P_1/N_p ratio increase was obtained in all variants, except for variants $N_{210}P_{90}K_{90}$ (**Figure 2(c)**, **Table 4**). Simultaneously, during the experiment, P_1/N_p ratio growth was reliable correspondence with N_p reduction in all variants. Thus, the N_p decrease could be induced only by the deficiency in P_1 mineral part.

The N and N_p reduction degree was higher particularly in the unfertilized $N_0P_0K_0$ variant and high-yield variants $N_{150}P_{90}K_{90}$, compared with other variants of the second group (**Tables 4 and 7**). Only first group variants showed a deficiency in reliable correspondence between the changes N and M_1 (**Table 5**). The existence of correspondence between the nitrogen amount in leaves and fruits and the lack of correspondence between the applied dose of nitrogen fertilizer with concentrations N, N_p and N_f were also obtained (**Tables 6 and 7**). This allows to assume that the quantity of added nitrogen fertilizer and the amount of leaf nonprotein nitrogen have not generally determined N_p differences between variants. The degree of N_p regression decrease was lowerest for $N_{210}P_{90}K_{90}$ variant (**Table 4**) (which has integrated with a high concentration of N and N_f and absence of reliable changes of M_1 and ratio P_1/N_p , as well as correspondence between N and N_p changes, compared with other variants (**Tables 4-7**)) that could, presumably, be caused by

Table 6. Effect of fertilizer dose on average concentration and quantities of leaves chemical components*.

Doze	Concentration in %							Ratio of Amounts in rel. unit			Total Quantity in rel. unit	
	N	N _p	S _i	St	P _i	K _i	M _i	S _i /N _p	P _i /N _p	K _i /N _p	N _{p-total}	St _{total}
N ₀ P ₀ K ₀	3.65	3.07	7.82	5.73	2.254	2.47	81.75	2.63	0.741	0.812	43.4405	110.65
N ₉₀ P ₉₀ K ₉₀	3.89	3.22	7.28	4.95	2.264	2.52	81.66	2.31	0.709	0.785	51.3912	116.19
N ₁₅₀ P ₉₀ K ₉₀	3.85	3.10	6.62	4.84	2.255	2.64	81.63	2.19	0.736	0.856	72.284	156.5
N ₂₁₀ P ₉₀ K ₉₀	3.95	3.30	6.74	3.95	2.265	2.57	81.36	2.05	0.690	0.781	68.607	140.12
LSD ₀₅	0.19	0.09	0.96	0.97	0.022	0.20	0.33	0.35	0.024	0.067		

Experimental design* of data measurements was as in **Table 4.

Table 7. Effect of fertilizer dose on tomato fruit average chemical components concentrations, total square of leaves surface and yield*.

Doze	Quantity in % of dry weight					Assimilation area in sq. m	Yield in centner per ha	Yield per assimilation area unit in rel. unit	S _{total} in rel. unit	S _{total} /A in rel. unit
	N _f	P _f	S _f	K _f	VitC					
N ₀ P ₀ K ₀	4.46	1.02	53.71	5.54	3.587	14.15	445.6	31.491	23933	1691
N ₉₀ P ₉₀ K ₉₀	4.89	1.28	60.94	7.38	4.457	15.96	510.6	31.992	31116	1950
N ₁₅₀ P ₉₀ K ₉₀	4.84	1.5	58.64	8.00	4.648	23.64	598.9	25.334	35119	1486
N ₂₁₀ P ₉₀ K ₉₀	5.34	1.15	64.33	8.16	4.292	20.79	559.0	26.888	35960	1730
LSD ₀₅	0.76	0.43	8.24	2.85	1.02	5.28	42.7			

Experimental design* of data measurements was as in **Table 4.

an increased dose of nitrogen fertilizer. It was previously shown that an inhibition of carbohydrate synthesis and an activation of synthesis of amino acids was caused at a high nitrogen amount (similar to the mentioned variant (**Tables 5 and 6**)) [18]. It was also detected that the activation of nitrate reductase was significantly affected by the intensity of metabolically active mineral nitrogen inflow to the leaf, which increased as the nitrate dose growth in soil [19,20].

Earlier it was showed also that in the case of M_s deficit, inhibition of protein synthesis and a reduction in N_p, as well as an increase in N were observed [21-23]. During the experiment a significant M_i decrease was obtained among leaves of variants (apart from the variant N₂₁₀P₉₀K₉₀), which, however, had a significant correlation with the changes of M_s in only fertilized variants (d_{M_iM_s} = 59-64%) (**Figure 2(d)**, **Table 5**). In case of the increase in the nitrogen fertilizer dose M_i determinacy from M_s change was also raised. However, in all variants the reduction of N_p, N and nonprotein nitrogen did not reliably correlate with M_s change. Significant correspondence between N and M_i values was registered only in second group variants of N₉₀P₉₀K₉₀ and N₂₁₀P₉₀K₉₀ (**Table 5**). This shows that the M_s and M_i could not be regarded as key factors causing N_p reduction, as well as P_i, K_i and S_i changes also did not depend generally on the M_s changes (**Figure 2**). At the same time, in variant

N₉₀P₉₀K₉₀, showing the highest degree of M_i reduction (**Table 4**), a reliable correspondence between changes of S_i and ratio S_i/N_p with M_i could be induced as a result of significant correlations of both N, P_i with M_i changes, peculiar only to this variant (**Tables 5 and 8**). This results show that watering plants on July 19 could be only a trigger mechanism promoting substantial changes in metabolism of plants (**Figure 2**).

It was obtained that the productivity Y/A of variants was decreasing in comparison with the increase in nitrogen fertilizer dose and in reliable correspondence with the S_i/N_p changes (**Tables 7 and 8**). At the same time, the S_{total} was increasing in parallel with the enhanced dose of nitrogen fertilizers and significantly correlated with the reduced concentration of S_i (**Figure 2(e)**, **Tables 7 and 8**). Consequently, the S_i accumulation reduction in variants was caused by the increase in requirement of assimilates by fruits. However, in N₀P₀K₀ variant, which had lower productivity, and in N₁₅₀P₉₀K₉₀ variant, which had higher productivity, the total accumulation of soluble sugars in fruits per unit of A (S_{total}/A) was less, as compared with the second group of two other variants (**Table 6**). It was obtained that these two groups of variants had differences by their N_p, P_i, P_i/N_p, S_i/N_p, K_i/N_p, N_f, and S_f (**Tables 6 and 7**). These results are consistent with previous findings that the nitrogen fertilizer, effect on yield growth was achieved mostly due influence on the assim-

Table 8. Correlation Correspondences between Tomato Average Quantities of Chemical Compounds, DF Parameters and Yield*.

Coefficient Description (leaf)	Correlation Coefficient in rel. units	Coefficient Description (leaf-fruits)	Correlation Coefficient in rel. units	Coefficient Description (with yield)	Correlation Coefficient in rel. units
$rN_p M_I$	0.841	$rN N_f$	0.929	rAY	0.950
$rN_p P_I$	0.970	$rN_p N_f$	0.909	$rS_I Y$	-0.987
$rK_I S_I$	0.946	$rN_p S_f$	0.937	$rK_I Y$	0.982
$rS_I M_I$	0.876	$rK_I vit C$	0.975	$rN_p Y$	0.304
$rSt M_I$	0.963	$rN_p S_{total}$	0.604	rIY	0.454
$rI \tau$	0.780	$r S_I S_{total}$	-0.977	$rS_I A$	0.957
$rI N$	-0.829	$rSt S_{total}$	0.896	$rN_p A$	0.175
$rI N_p$	-0.941	rIS_f	0.929	$rS_I Y/A$	0.886
$rI S_I$	0.829	$r\tau S_f$	0.767	$rS_I/N_p Y/A$	0.962
$rI P_I$	0.831	rIN_f	-0.971	$rSt Y/A$	-0.915
$rI M_I$	0.974	$rI S_{total}$	0.739	$rN_p Y/A$	-0.110
$r\tau N_p$	0.574	$r\tau S_{total}$	0.925	$r S_{total} Y$	0.943
$r\tau S_I$	0.929			$r\tau Y$	0.864
$rI K_I$	-0.302			$r\tau A$	0.877
$r I_{total} K_I$	0.985			$r I_{total} Y$	0.939
$r I_{total} S_{total}$	0.780				

**Experimental design* of data measurements was as in tables 4 and 7. At the significance level of 5%, the critical value of correlation coefficient is 0.950.

lating surface square increase [24].

During the experiment, I reduction was registered in all fertilized variants (**Figure 3(a)**, **Table 9**) and substantially correlated with the N decrease (apart from $N_{90}P_{90}K_{90}$ variant) (**Table 9**). I reduction reliably correlated with N_p decrease in variants of the first group – $N_0P_0K_0$ and $N_{150}P_{90}K_{90}$ (**Table 9**), the average ratios of I and I/N_p of which were higher (**Figures 3(a)** and **(b)**, **Table 10**) as well as N_p and S_{total}/A lower (**Table 6**) compared to the second group of variants. In the mean time, in the course of the experiment only the first group variants showed a reliable I/N_p ratio growth, which was reliable correlated with N_p decrease (**Figure 3(b)**, **Table 9**). According to the chemical kinetics laws, the reduction in concentration of interacting centers, as well as the decrease in efficiency of LR products used in DR (as a result of ribulose-1,5-diphosphate carboxylase concentration reduction) should lessen the effectiveness of coupling of LR and DR and increase of the back reaction probability between QA^- and Z^+ . Thus must promote certain growth of counter interaction between primary photo-oxides and photo-reducing products. *Is it possible that high levels of first group I generally determined decrease coupling between LR and DR compared to the second group?*

Over and above, it should be pointed out that Y did

not significantly correspond with concentrations N and N_p (**Table 8**). That is why the amount of average values of I variants was not significantly correspondent with Y (**Table 8**). Taking into account the fact that I indicates the activity of PSII reactions per unit of A, a calculation

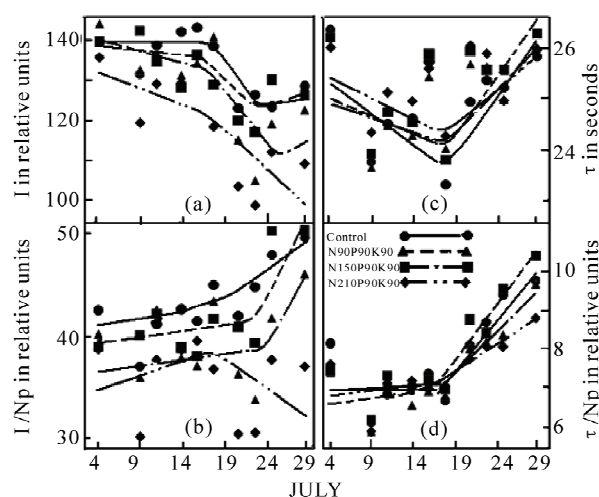


Figure 3. Temporary variation of maximal amplitude of leaf delayed fluorescence (a), I/N_p ratio (b), DF amplitude half decrease time (c) and τ/N_p ratio (d).

Table 9. Temporary variation of DF parameters' and fertilizer dose affect on correlative correspondence between DF parameters' and concentrations of chemical compounds*.

Coefficient Description	Correlation Coefficient in rel. units			
	N ₀ P ₀ K ₀	N ₉₀ P ₉₀ K ₉₀	N ₁₅₀ P ₉₀ K ₉₀	N ₂₁₀ P ₉₀ K ₉₀
rIT	-0.625	-0.729	-0.712	-0.669
r(I/N _p)T	0.780	0.227	0.675	-0.148
rτT	0.118	0.262	0.327	0.233
r(τ/N _p)T	0.702	0.765	0.820	0.708
rIN	0.779	0.567	0.823	0.797
rIN _p	0.756	0.595	0.655	0.498
r(I/N _p)N _p	-0.885	-0.507	-0.847	-0.216
rτN	-0.293	-0.668	-0.286	-0.343
rτN _p	-0.302	-0.323	-0.314	-0.276
rIP _i	0.368	0.781	0.796	0.610
r(I/N _p)(P _i /N _p)	0.802	0.626	0.923	0.420
rτP _i	0.235	-0.077	-0.112	0.003
r(τ/N _p)(P _i /N _p)	0.906	0.897	0.922	0.852
rIM _i	0.708	0.879	0.582	0.672
rτM _i	-0.175	-0.468	-0.510	-0.390
rτS _i	-0.016	0.224	-0.147	0.115
rIK _i	0.497	0.600	0.662	0.711
rτK _i	-0.507	0.085	-0.638	-0.201
r(τ/N _p)K _i	-0.451	-0.401	-0.767	-0.504
rIτ	-0.103	-0.275	-0.298	-0.189
bIT	-0.630	-1.174	-0.733	-1.216
b(I/N _p)T	0.351	0.112	0.384	-0.070
bτT	0.0143	0.0304	0.0387	0.0204
b(τ/N _p)T	0.0572	0.0565	0.0728	0.0381
bI N _p	17,15	22.59	13.99	24.46
bI M _i	3.3059	6.727	3.1207	5.6734

Experimental design* was as in **Table 4. At the 5% significance level the critical value of the correlation coefficient is 0.632.

was made for total I produced by all PSII of A per variants (**Table 10**). In this case I_{total} of variants highly determined their Y (d I_{total} Y = 88.2%) and simultaneously significantly correlated with average K_i values (**Table 8**). On the other hand, average K_i did not significant affect the changes of I average levels per unit of assimilating surface between the variants (**Table 8**). These results allowed to exclude the general influence of K_i on I as

Table 10. Fertilizer dose influence on tomato DF parameters average values*.

Doze	I in rel.units	τ in seconds	I/N _p	τ/N _p	I _{total} in rel. units
N ₀ P ₀ K ₀	133.1	23.90	43.68	7.90	1883.365
N ₉₀ P ₉₀ K ₉₀	127.9	24.06	39.91	7.56	2041.284
N ₁₅₀ P ₉₀ K ₉₀	130.1	24.42	42.39	8.02	3075.564
N ₂₁₀ P ₉₀ K ₉₀	119.3	24.60	36.23	7.52	2480.247
LSD ₀₅	5.7	0.48	2.47	0.32	

Experimental design* of data measurements was as in **Table 4.

direct result of K_i induced changes on an ECP of chloroplast membrane. Despite it during the experiment a significant correspondence was obtained between I and K_i changes in N₁₅₀P₉₀K₉₀ and N₂₁₀P₉₀K₉₀ variants (**Table 9**). A significant decreasing of K_i in leaves was obtained exclusively for variant N₁₅₀P₉₀K₉₀ (**Figure 2(f)**, **Table 4**), which reliably correlated with the N_p reduction again in only this variant (**Table 5**), depending, presumably, on higher K_i amount among variants at the initial period of the experiment (**Figure 2(f)**). It may stimulate an intensive carbohydrate metabolism and assimilate transport in the earlier period, versus other variants. As it is well known, the increase of K_i promotes the phloem transport raising [24]. These factors could cause accelerated senescence of N₁₅₀P₉₀K₉₀ variant, proved also by the VitC accumulation degree in fruits of variants (**Tables 6 and 7**). Significant correlation between average values of K_i and VitC was also registered between variants (**Tables 6, 8 and 10**). It is well known that VitC increase in Y correlated with intensive decrease of N_p in senescent leaves [15,25]. Consequently, the binding force between I_{total} and K_i was, generally, stipulated by the K_i affect on metabolism of plants. On the other hand, the high values of I in first group variants, compared to second group of variants, could not result solely from the decrease coupling between LR and DR generally due to dark reaction productivity deactivation, since in case of elimination of photosynthetic efficiency influence per unit of A, a reliable dependence on the I increase persisted in case of N_p reduction (rIN_p (S_{total}/A) = -0.995, rIN_p (Y/A) = -0.981). This shows that the high average values of I in variants could principally stipulate a high concentration of active PSII per unit of A.

During the experiment, the difference in N_p between two groups of variants was visualized as well by the fact that in the first group variants of N₀P₀K₀ and N₁₅₀P₉₀K₉₀ registered lower values of I regression coefficient and a significant increase I/N_p in concurrently higher N_p regression coefficient, versus the second group variants (**Tables 4 and 9**). The similar changes of I/N_p in variants (**Table 9**), confirmed that N_p reduction was probably caused generally by carbohydrate metabolism enzyme

quantity decrease, because as previously reported, at the end of the vegetation N_p decrease is basically caused by the reduction in ribulose-1,5-diphosphate carboxylase amount, leading also to a reduction of photosynthesis intensity [21,26-29]. Furthermore, the fact that the changes in phosphate status have only small effect on the photochemical apparatus of leaves was also previously confirmed [30]. Our data also shows that in case of nitrogen fertilizer affect the total concentration of LR centers is likely to constitute a negative feedback with Y of variants per unit of assimilating surface square ($r_{I_{total}} Y/A = -0.944$).

Theoretically, it was assumed that the τ (**Figure 1**), in general, depended on the activation of phosphorylation reaction of sugars in Calvin cycle, which led to proton gradient reduction as a result of ATP use and described the intensity of LR and DR of photosynthesis coupling [4-6]. The obtained results showed that the τ values of variants were not in significant correspondence with N_p , M_1 , S_1 , but in contrast to I, were determined mostly by the level of carbohydrate metabolism end products per unit of A ($R_{\tau(S_{total}/A St)} = 1.000$) (**Figure 3(c)**, **Table 8**). The average values τ of variants were highly determined by the S_{total} in fruits ($d_{\tau S_{total}} = 85.6\%$) (**Tables 7, 8, 10**). This is why in the event of elimination of effect of starch formation processes the values τ significantly correlated with quantity of S_{total} ($r_{\tau S_{total}(St)} = -0.997$), which was generally determined by the Y of variants (**Table 8**), being previously caused by the reliable binding force $r_{\tau Y/A(St)} = -0.995$. Besides, in case of the increase S_{total} , a significant reduction in S_1 and S_t raise in leaves was registered between variants (**Table 8**). This clearly shows the interaction between the Y formation and the photosynthetic production process in leaves and its affect on τ values.

Thus, I and τ parameters of tomato variants were stipulated substantially by different key factors. Therefore, there was not a reliable correspondence between their average values (**Table 8**) and within those parameters in the stages of fructification up to harvesting (**Table 9**). During the experiment, reduction in LR/DR coupling efficiency in case of N_p decrease was confirmed by the increase in ratio τ/N_p and its reliable correlation with ratio P_1/N_p changes in all variants (**Figure 3(d)**, **Table 9**), as well as by the fact that in the first group with a low protein amount (**Table 4**), the average τ/N_p ratio was higher compared to the second group variants (**Table 10**). However, it is important to point out that during the experiment significant increases of τ was showed only per unit of N_p and, on the other hand, temporal significance change of τ of variants was not obtained (**Table 9**). This could be caused by τ high dependence on carbohydrate metabolism end products accumulation in fruits.

The data also shows that during the experiment I decrease generally correlates with M_1 reduction of variants

(**Table 9**), which appears to be the key generator for reliable correlation between their averaged values (**Table 8**). It is well known that nitrogen fertilization affects on the osmotic adjustment of tomato plants [31]. Water deficit stimulated both leaf relative water amount decrease, osmotic potential, as well as increase in glycinebetaine and sugar leaf amounts [32]. Simultaneously, a marked increase in electrolyte leakage, decrease in amounts of chlorophylls a and b, and inhibition of PSII activity were observed. Thus, the previously analysis data showed that the amounts of major PSII proteins, including D1 and D2 proteins in PSII reaction centre and light-harvesting Chl *a/b*-protein complex (LHC2) in periphery, declined in case of water stress increasing [33,34]. Therefore, it is possible that M_1 changes were not a key factor stipulating degradation of the main part of N_p pool (**Table 5**), but in case of PSII, had their own input in deactivation of those centers. Indeed, in case of elimination of M_1 affect on I the binding force between I and N_p was increased, I also reliably determined changes of both N_p and M_1 ($r_{IN_p}(M_1) = -0.995$, $RI(N_p, M_1) = 1.000$). In that event, during the experiment, the high degree of I reduction especially in second group variants of $N_{90}P_{90}K_{90}$ and $N_{210}P_{90}K_{90}$ (**Figure 3(a)**, **Table 9**) was determined by water stress influence increase as a result of higher concentrations of N compared with plants of first group variants (**Table 6**) and more intensive M_1 reduction in $N_{90}P_{90}K_{90}$ variant (**Table 4**). This assumption is also confirmed by the significant correlation between N and M_1 , which is obtained only in second group of variants (**Table 5**). Previously, it was shown that in case of dehydration of cut-off leaves of potato both I growth and τ reduction were seen at the initial stage, which was caused by the influence of osmotic or ionic potentials on an ECP change [35]. This study showed that more than 3.2-3.8 % leaf moisture loss induced I reduction resulting from PSII deactivation. In the present experiment on tomato, the differences between average initial and end levels of M_1 variants are 4% (apart from the variant $N_{150}P_{90}K_{90}$ (around 3%)) (**Figure 2(d)**). Therefore, this assumption has certain reasons. Some input in I reduction via changes of ionic potential during water loss which may be caused by the disturbance of interaction between weakly associated proteins and membranes in a case of salt concentration increase [12] or through explicit effects of osmotic potential on genetic apparatus was possibly made in vivo moisture decrease (northern hybridization indicated that progressive water stress remarkably reduced amounts of the chloroplast gene *psbA* and *psbD* and nuclear gene *cab* transcripts) [34].

It is well known that in a number of events the level of Y may be stipulated by the resistance of metabolism and photosynthetic apparatus toward unfavourable environmental impacts [7,36,37]. Therefore, the next experiment was carried out to study the potato production processes

in the period between bud and harvesting stages. The resistance of plants to drought was modified by various doses of phosphorus and potash fertilizers in water deficit conditions (Table 11). During this experiment, it was observed that P_1 concentration did not depend on P_s , so N was determined by phosphorus amount in leaves and soil ($rN(P_1P_s) = 0.974$) (Tables 11 and 12). Besides, N concentration increased in case of P_s amount growth ($rNP_s(P_1) = 0.955$), and, in contrast, during P_1 increase, N amount reduced accordingly ($rNP_1(P_s) = 0.968$). At the same time, K_s increment reliably increased K_1 , which significantly correlated with P_1 increase (Table 12).

$P_{120-150}$ doses are heightening for potato plants [9]. In case of such doses, a decrease in drought resistance of plants and yield falls were obtained [7,22,23,36], which probably depended on the decrease in free water concentration in cells [31,37]. The above-mentioned is also proved by I dependence on the dose of phosphorus anion in nutrient medium (Table 1). Previous studies have proved that water deficit induced the N increase that accompanied with N_p reduction as a result of inhibition of protein synthesis [22,23]. Evidently, these were dominant cause for productivity decrease in potato variants of $N_{150}P_{120}K_{45}$ and $N_{150}P_{120}K_{60}$, showing increased N (Table 11). As described previously K_s stimulates increase of photophosphorylation efficiency, assimilates transport intensity and nitrogen metabolism [6,25,38]. In addition, it was assumed that the favourable effects of K_1 on plants in droughty conditions may be promoted via affection on exchange of cytoplasmic K^+ and H^+ cations of stroma [39]. Generally, while eliminating the effects of leaves moisture changes caused by the unfavourable impact of increased P_s doses on plants, the K_s increase significant correlated with the N decrease ($rNK_s(M_1) = -0.906$). This result confirmed that the increased dose of potash fertilizer induced optimization of nitrogen metabolism, and drought resistance enhancement among variants. A further proof of this was also the increased P_1

amount and yield (Tables 11 and 12). This assumes harmony with previously studies results [38].

In the end of vegetation it was obtained that both I and τ values of potato variants were highly determined by N ($d_{IN} = 87.6\%$, $d_{\tau N} = 76.0\%$), so the values of both DF parameters reliably correlated (Table 12), as opposed to the experiment on tomato (Table 8). In the meantime, the I of variants was substantially growing, and the τ was reliably decreasing in case of K_s increase (Table 12). The average values of both I and τ values of variants significantly correlated and each of these parameters reliably correlated with the Y of variants (also, as opposed to the experiment on tomato variants) (Table 12). As known, in droughty conditions PSII deactivation and electron transport inhibition were confirmed [40,41]. Although the positive correlation between I and Y of variants indicated that the Y increase mostly depended on nitrogen metabolism optimization, which probably resulted in the increase of photosynthesis primary reaction centers concentration.

4. CONCLUSIONS

The raised quantity of potash fertilizer induced increase in the efficiency of nitrogen fertilizers effect resulted by enhancement of protein production ($rK_1 N_{p-total} = 0.954$), ripening A ($rN_{p-total} A = 0.985$, $rK_1 A = 0.983$), Y ($rN_{p-total} Y = 0.973$, $rK_1 Y = 0.982$) and the total amount of starch accumulated in leaves ($rN_{p-total} S_{total} = 0.970$, $rK_1 S_{total} = 0.979$) of tomato plants. However, nitrogen fertilizer induced falling of the yield per unit of A ($rN_{p-total} Y/A = -0.952$). Besides, K_1 had a significant impact on the yield generation per unit of A ($rK_1 Y/A = -0.915$).

Generally, accumulation of soluble sugars in tomato fruits determined Y of variants, however, total N_p and K_1 of plant did not significantly correlate with the S_{total} and the efficiency of its formation per unit of A ($rN_{p-total} S_{total} = 0.933$, $rN_{p-total} S_{total}/A = -0.549$, $rK_1 S_{total} = 0.863$, $rK_1 S_{total}/A = -0.615$). As for potato, the data shows that in

Table 11. Fertilizer doze affect on chemical components quantities, yield and DF parametres of potato*.

Fertilizer Doze	Quantity in leaves in % of dry weight				Quantity in soil in % of dry weight		Yield in centner per ha	I in rel. units		τ in seconds	
	N	P_1	K_1	M_1	P_s	K_s		$I_{average}$	I_{end}	$\tau_{average}$	τ_{end}
$N_0P_0K_0$	0.28	0.28	5.7	83.7	67.4	108.2	256.3	95.91	73.1±2.4	21.9	17.28±1.26
$N_{150}P_{90}K_{45}$	0.28	0.30	5.9	79.6	69.2	112.3	316.9	110.87	80.1±3.2	20.16	15.36±1.62
$N_{150}P_{120}K_{60}$	0.34	0.25	5.6	81.0	65.6	110.2	305.3	112.17	75.0±3.2	19.56	15.9±1.38
$N_{150}P_{150}K_{75}$	0.28	0.30	6.2	81.5	68.0	113.4	339.8	112.91	75.73±3.7	18.96	14.88±1.02
$N_{150}P_{120}K_{45}$	0.84	0.25	5.4	79.0	69.4	106.1	294.7	102.33	61.18±3.13	21.36	19.5±1.44

*The leaf DF parameters of leaves were measured since bud stage, per 5 dates, and concentration of chemical compounds of leaves and soil at the end of vegetation. The deviation of average values of DF parameters' did not exceed 3-5% of its average value.

Table 12. Correlative correspondence between chemical compounds amounts in leaves and soil, DF parameters' and yield of potato*.

Coefficient Description (leaves)	Correlation coefficient In rel. units	Coefficient Description (leaves-fruits-yeild)	Correlation coefficient In rel. units
rN M _l	-0.608	rN _p P _s	0.457
rN P _l	-0.655	r P _l P _s	0.339
rN K _l	-0.706	rK _l K _s	0.910
rK _l P _l	0.888	rN P _s	0.457
rP _l M _l	0.223	rN K _s	-0.754
rK M _l	0.260	rP _l K _s	0.757
rI N	-0.936	rM _l P _s	-0.540
rτ N	0.872	rI K _s	0.927
rI τ	-0.929	rτ K _s	-0.966
rI K _l	0.629	rI P _s	-0.294
rτ K _l	0.838	rI _{average} Y	0.915
rI P _l	0.690	rτ _{average} Y	-0.902
rI _{average} τ _{average}	-0.909		

**Experimental design* of data measurements was as in table 11. At the significance level of 5%, the critical value of correlation coefficient is 0.950.

frames of moisture deficit, aggravated and implemented by unbalanced increased doses of phosphorus fertilizer, the optimization of metabolism and yield of variants were ensured by the increase in potash fertilizer dose.

During both field experiments, I mostly depended on the activity of nitrogen metabolism and probably determined changes of active PSII concentrations per unit of A. The amounts of I average values of variants did not have a significant correlation with Y for tomato plants, as their productivity did not depend from N_p and on production process intensity per unit of A. Simultaneously, total I from full A of tomato plant significant correlated with the productivity of variants (**Table 8**). In case of potato, the production process mostly depended on the degree of water deficit influence on plants' through affect on nitrogen metabolism, thus leading to the correlation between I and τ as well as their both correspondence with Y of variants as opposed to tomato experiment.

It was observed that the values τ predominantly depended on the level of carbohydrate metabolism in the whole plant system, including the quantity of its end products, therefore resulting in correlation with Y of both tomato and potato variants as opposed to their I.

Thus, the results showed that the DF parameters are closely intertwined with metabolism and formation of yield. This is a biological base triggering the use of these

parameters in system analysis of production process. The combined use of the both DF parameters allows generating complete information about key affects on the production process. Fast measurement of DF parameters, the availability of portable devices designed for this goal, as well as the possibility of saving of resources and time versus the chemical-analytical method, allow to recommend the use of DF parameters for the system analyses of production process and the diagnosis of physiological state of plants.

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Abbreviations:

A – total square of leaves surface of plants	M_s – soil humidity,
DF – delayed fluorescence of chlorophyll	N – leaf nitrogen
I – maximum amplitude of leaves DF	N_p – leaf protein
I_{total} – total I from A emitting surface	N_f – fruit nitrogen
τ – time of half decrease of I	$N_{p-total}$ – total protein of leaves
LR – light reactions of photosynthesis	P_l – leaf phosphorus
DR – dark reactions	P_f – fruit phosphorus
ECP – electrochemical potential	P_s – soil phosphorus
r – correlation coefficient	K_l – leaf potassium
b – regression coefficient	K_f – fruit potassium
d – determination coefficient	K_s – soil potassium
R – multiple correlation coefficient	S_l – leaf soluble sugars
NPK – added amount of fertilizers in N:P ₂ O ₅ :K ₂ O in kg/ha	S_f – fruit soluble sugars
T – vegetation period	S_{total} – total quantity of soluble sugars accumulated in yield ($S_f \times Y$)
Y – yield	St – leaf starch
Y/A – productivity per unit of A	VitC – fruit vitamin C
M_l – leaf humidity	