Chlorophyll fluorescence of wheat leaves when infected with *Bipolaris sorokiniana*

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Abstract. For three spring wheat cultivars, characteristics of adaptive reaction to cereal root rot pathogen *Bipolaris sorokiniana* Shoem. infection are found by laboratory experiments using chlorophyll fluorecence parameter (ChlF) dynamics of 10-16 day old seedlings. Such ChlF parameters as *Y*(II), *ETR, qP, Fv / Fm, Fv / Fo, Y(NPQ), qN* and *Y(NQ)* are verified as informative, being reliable biomarkers for photosynthetic apparatus level pre-symptom estimate of cultivar resistance to the pathogen. For more resistant cultivars Novosibirskaya 29 and Sibirskaya 21, the reaction to the pathogen invasion appeared to be less expressed compared to less resistant cultivar Novosibiskaya 41. More resistant cultivars have the smallest ChlF parameter changes compared to the control ones. Sixteen day old seedlings infected with B. sorokiniana showed the largest intercultivar differences. The photosynthetic activity level during adaptive reaction to *B. sorokiniana* pathogenesis can be used as stress resistance criterion for selection material to accelerate its sampling and to increase its effectiveness by early non-perspective sample rejection.

1 Introduction

The common cereal root rot (the causative agent is fungus *Bipolaris sorokiniana* Shoem., abbr. *B. sorokiniana*) is a harmful wheat disease playing a significant role in the main world cereal growing areas including the region of Siberia. [1, 2] The infection affects almost all plant organs: primary and secondary roots, coleoptiles, stems, leaves and grains. The disease leads to seedling death, stem lack of growth and withering, head seedlessness and grain shriveling. The average yield losses are 15-50% due to the decrease of productive bushiness, grain content and grain mass. [3]

One of the ways to reduce the disease negative affect and to get high and stable spring wheat harvest is the reasonable selection of cultivars adaptive (resistant) to the disease. [4] In most selection programs, the cultivar resistance to the pathogen is estimated in the field conditions by yield diminishing, which appears to be laborious and time-consuming process. [5] In this regard, there is a necessity to find criteria for estimation and development of non-destructive selection methods providing acceleration of resistant to biotic stress genotype screening, especially at plant development early stages. These requirements conform to

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biophysical methods, including those based on analysis of photosynthesis, which is one of stress-sensitive processes in a plant cell. [6]

During photosynthesis process, all the light energy absorbed by chlorophyll molecules is spent on photochemical reaction (photochemical quenching), heat dissipation (non-photochemical quenching) and fluorescence – the processes competing in deactivation of photosystem II (PS II) pigment excited states [7]. The plant photosynthetic activity disorder can be estimated by chlorophyll fluorescence (ChlF) recording method which allows for a plant organism to determine its general bioenergetic status, i.e., its ability to transform photosynthetic energy [8]. Being non – destructive and high sensitive, this method allows to get information about photosynthesis efficiency and photosynthetic apparatus integrity at early stages of stress development [9]. In particular, the ChlF recording method is used to estimate wheat resistance to abiotic stresses such as temperature, drought, increased acidity, salinization and herbicides [6, 10–12].

The application of ChlF recording method is demonstrated in research of winter and spring wheat infection with the common cereal root rot. Photosynthesis stages are found for the most sensitive to B. sorokiniana infection plants [13]. Promising parameters for estimation of infected plant conditions are identified. It has been found, that maximal and effective quantum yields of primary photochemistry as well as electron transport efficiency and photochemical quenching coefficient decrease, minimal fluorescence absolute values as well as absolute values of regulated and unregulated non-photochemical quenching quantum yields increase, delayed fluorescence yield of infected plants until shoot visible degradation changes [14, 15]. However, the knowledge related to cultivar characteristics for adaptive reaction formation of the wheat plant is limited to estimate its stress resistance.

The goal of the work – is to investigate the formation of spring wheat cultivar adaptive reaction to the action of B. sorokiniana biostressor using ChlF kinetics parameter change monitoring to identify interaction between parameters and cultivar resistance.

2 Conditions, materials and methods

Experimental work was carried out in the laboratory for the study of physical processes in agrophytocenoses of the Siberian Physical and Engineering Institute, SFSCA RAS.

The research was conducted in greenhouse experiments (water cultures) under laboratory conditions on the seedling of three middle-early spring soft wheat zoned cultivars: Novosibirskaya 41, Sibirskaya 21 and Novosibirskaya 29 selected by Siberian Research Institute of Plant Production and Breeding – Branch of the Institute of Cytology and Genetics, SB RAS.

The experiment options:

a) control (no stressors);
b) infection with *B. sorokiniana* (5000 conidia per grain).

In specially conducted greenhouse experiments, the level of stress load - conidial suspension of B. sorokiniana 5000 conidia per grain – is determined as allowing to differentiate Siberian selection wheat cultivars by estimation of their resistance to the stress factor using biometric indices and cell membrane permeability [16].

Wheat seeds were presterilized with 96% ethanol during 2 min followed by triple distilled water rinsing. Then the seeds were placed in Petri dishes with moistened filter paper and germinated in the thermostat at 22 ºC for three days.

The seeds were infected with conidial suspension of medium pathogenic B. sorokiniana isolates mixture at germination stage (third day of cultivation). The suspension was prepared on 0.1% aqueous agar (one drop per grain). The seedlings were grown in “Biotron-7” climatic chamber in a roll culture with tap water at the "day-night" photoperiod with parameters of time 16 and 8 h, illumination 20 000 and 0 lux, temperature 22 and 18 ºC, respectively, and humidity 60%.

Daily ChlF kinetics and parameters were recorded by means of Dual-PAM-100/F fluorimeter (Heinz Walz GmbH, Germany) using amplitude-pulse modulation method in the mode of recording the slow kinetics of dark induction curves with saturation pulse analysis. Before fluorescence measurement, 10–16-day old wheat seedlings were placed in a sample chamber and adapted to darkness during 30 min. The following fluorescence parameters were obtained: Fo, Fm – minimal and maximal levels of ChlF induced by the light pulse after leaf adaptation to darkness; Fo', Fm' – minimal and maximal levels of ChlF induced by the light pulse after leaf adaptation to light; Fv / Fm – PS II maximal photochemical quantum yield; Y(II) – PS II effective photochemical quantum yield after leaf adaptation to light; Y(NPQ) – quantum yield of ChlF regulated non-photochemical quenching; Y(NO) – quantum yield of ChlF unregulated non-photochemical quenching; qP – ChlF photochemical quenching coefficient; qN – ChlF non-photochemical quenching coefficient; ETR – electron transport rate. The following values were calculated: ChlF variable Fv = Fm – Fo and PSII photochemical activity parameter Fv / Fo.

The cultivar reaction was determined by seedling fluorescence measured parameter relative change: parameters of seedling after exposure to the stressor and of control ones were compared. The smaller parameters change, the higher the resistance in the studied group of cultivars. Analytical and biological experiment repetitions are sixfold and triple, respectively. Twenty seedlings were estimated in each variant. Statistical data processing was performed in Microsoft Excel 2000 software with standard data analysis package. ChlF parameters recorded during 4 min were analyzed. Error of mean did not exceed 1,5-2,0%. Three series of experiments were performed. Student's t-test was used to determine the significance of differences in mean values.

3 Results and discussions

The research was carried out to confirm the possibility of ChlF parameter recording method application for three spring soft wheat cultivars to estimate their stress resistance.

In chloroplasts, there are two main target sites for biotic and abiotic stress: electron transport chain (ETC) and synthesis of chlorophyll and carotenoids. ETC with its electron carriers and enzymes takes part in phosphorylation and NADP photoreduction while chlorophyll and carotenoid synthesis can be associated with light-harvesting complex (LHC) and photosynthetic reaction center antennas [17].

The common cereal root rot causative agent B. sorokiniana and its toxins inhibit photosynthesis blocking electron transport between photosystems and dissociating phosphorylation, which leads to ATP synthesis rate decrease. Thus, both energy accumulation process and organism energy status are disordered [18, 19]. Inhibition of the main photosynthesis part – energy accumulation process – causes productivity decrease, which can be evidently watched at plant ontogenesis early stages (tillering – stem elongation) [20]. The pathogen tolerance level of the cultivar is defined by the reaction to stress during their growth and development.

For seedlings with grains infected by conidial suspension of B. sorokiniana isolates, the research of ChlF daily dynamics parameters showed that ChlF parameter changes on days 10 and 12 of seedling cultivation are not certain compared to the control ones.

Photosynthetic rate disorder related to the pathologic process is recorded for 14-day-old seedlings (Table 1).

Herewith, stressor reactions are related with heat dissipation increase: for all cultivars, regulated non-photochemical quenching parameters Y(NPQ) and qN increased certainly ($p \leq 0,05$) by the 24,9 to 55,3 % range compared to the control ones. For cultivars

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Novosibirskaya 29 and Sibirskaya 21, unregulated non-photochemical quenching parameter Y(NQ) related to free-radical oxidation generation increased certainly ($p \leq 0,05$) by 17,4% and 24,9%, respectively. For cultivar Sibirskaya 21 only, photochemical activity went down: values of parameters Y(II) and ETR decreased certainly ($p \leq 0,05$) by 18,1% and 18,6%, respectively.

Table 1. Daily dynamics of chlorophyll fluorescence parameters in the leaves of seedlings of wheat cultivars under *B. sorokiniana* infection.

Seedling age, days	**Option**	**Parameters**							
		Y(II)	*Y (NPQ)*	*Y(NO)*	*qP*	*qN*	*ETR*	F_V/F_m	F_V/F_0
		cultivar Novosibirskaya 41							
14	control	5.9	0.9	5.9	3.5	2.3	241.3	0.68	2.1
	B. sorokiniana	5.9	1.2	4.9	3.5	2.9	239.8	0.67	2.0
16	control	6.1	0.9	4.9	3.8	2.4	248.9	0.65	1.8
	B. sorokiniana	3.9	3.0	5.0	3.2	5.5	157.1	0.58	1.3
		cultivar Novosibirskaya 29							
14	control	5.9	0.85	4.3	3.6	2.1	226.6	0.72	2.5
	B. sorokiniana	5.6	1.32	5.1	3.2	3.0	224.0	0.71	2.4
16	control	5.2	1.19	5.5	2.9	2.6	210.5	0.74	2.7
	B. sorokiniana	5.1	1.51	5.3	3.1	3.2	205.7	0.71	2.4
		cultivar Sibirskaya 21							
14	control	6.4	0.76	4.7	3.5	2.1	263.5	0.71	2.4
	B. sorokiniana	5.3	0.75	5.9	3.2	1.9	214.6	0.63	1.7
16	control	6.4	1.11	4.5	3.6	2.9	260.6	0.70	2.4
	B. sorokiniana	5.5	0.84	5.6	3.4	2.1	225.1	0.66	2.0

On the 16th day of seedling cultivation, certain decreases of effective quantum yield Y(II) and electron transport rate ETR were discovered for cultivars Sibirskaya 21 and Novosibirskaya 41 by 13,1%, 13,6%, 24,8% and 36,9%, respectively, compared to the control ones. For cultivar Novosibirskaya 29, these parameters changed uncertainly. Light-dependent reaction inhibition went hand in hand with certain increase ($p \leq 0,05$) of non-photochemical quenching parameter values – coefficient qN, quantum yields Y(NQ) and Y(NPQ) – by the 21,8 % (cultivar Novosibirskaya 29) to 230,1% (cultivar Novosibirskaya 41) % range, compared to control.

Herewith, a cultivar specifics of adaptive reaction formation was observed. For cultivar Novosibirskaya 29, thermal scattering of excited chlorophyll energy decreased leading to decrease of values Y(NPQ), qN, Y(NQ) by the 1,8 to 4,7 times range with uncertain change of photochemical quenching parameters Y(II), ETR, qP on 14-16th days of seedling cultivation. Parameters Fv / Fo and Fv / Fm changed uncertainly.

Adaptive mechanisms of cultivar Sibirskaya 21 are directed against photochemical quenching depression: for parameters Y(II) and ETR, relative change values decreases by a range up to 1,4 times. Besides, relative change values decreased for parameters Fo, Fv / Fo by 1,5-1,7 times and for parameters Y(NPQ), qN by a range up to 27,5% on 14-16th days of the cultivation.

For cultivar Novosibirskaya 41 on 14-16th cultivation days, heavy increase of qN and Y (NPQ) values by 130,4% and 230,1% compared to the control ones was observed, i.e., fluorescence regulated non-photochemical quenching acts as defense mechanism against excess energy of excitation. But herewith, it was found, that values of parameters Y(II), ETR, qP, Fo, Fm, Fv, Fv/Fm, Fv/Fo decreased certainly ($p \leq 0,05$) by the 15,3 to 45,6 % range, i.e., photosynthetic activity was depressed.

At even stress load by B. Sorokiniana, the difference in photosynthetic activity parameter change between cultivars Novosibirskaya 29, Sibirskaya 21 and Novosibirskaya 41 indicates to different mechanisms of tolerance and strategy of transforming light energy into chemical one. Generally, plants react to stressors activating protective adaptive mechanisms in order to support photosynthetic activity for adaptation to a new background. Such mechanisms may include energy dispersion ability which is discovered by increase of non-photochemical parameters Y(NPQ) and qN, but without change of PS II maximal quantum efficiency parameters Fv/Fo and Fv/Fm. [21] One can observe this process for all three cultivars on 14th day of seedling cultivation. Herewith for cultivar Sibirskaya 21, there is a partial photoinhibition discovered by effective photochemical quantum yield Y(II) decrease. However, inhibition of photochemistry parameters Y(II) and ETR decreases by 1,4 times for this cultivar on 16th cultivation day.

Mounting stressor pressure on 16th cultivation day led to decrease of effective quantum yield Y(II), photochemical quenching coefficient qP and electron transport rate ETR, which means electron transport chain inhibition. For all three cultivars, light-dependent reaction inhibition went hand in hand with increase of Y(NPQ) and qN compared to the control ones. However, the was no serious irreversible PS II function disorder observed in our experiment options, otherwise ChlF parameters of photochemical and non-photochemical quenching could decrease, according to Pérez-Bueno et al. [21].

The chosen stressor load of B. sorokiniana (5000 conidia per grain) and its effect time duration in the experiment options have allowed discovering intercultivar differences. As a result of ranging taken on 16th day of seedling cultivation (the biggest intercultivar differences), the cultivar resistance to B.sorokiniana changed in descending order: Novosibirskaya 29 - Sibirskaya 21 - Novosibirskaya 41. For more resistant cultivar Novosibirskaya 29 under the pathogen effect, it was observed that only two parameter changed certainly: increase of Y(NPQ) and qN by 26,9% and 21,8%, respectively, with uncertain changes of the remaining parameters (Figure 1-3).

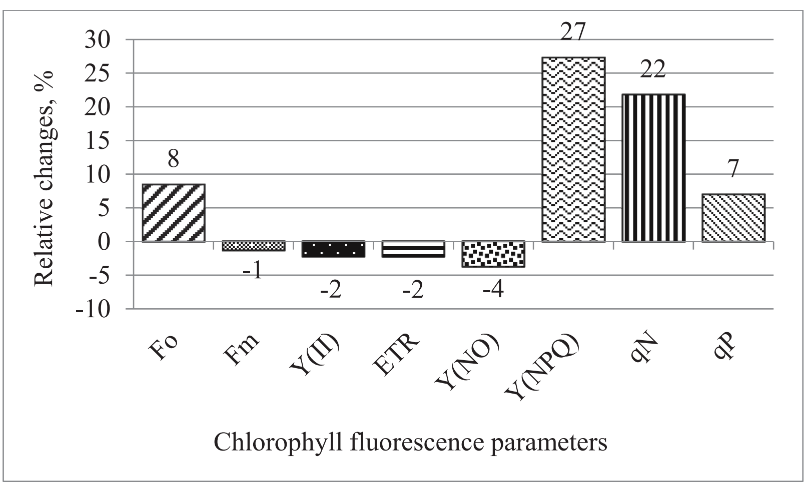

Fig. 1. Chlorophyll fluorescence parameters of 16-day-old Novosibirskaya 29 seedling leaves under B. sorokiniana infection (relatively resistant).

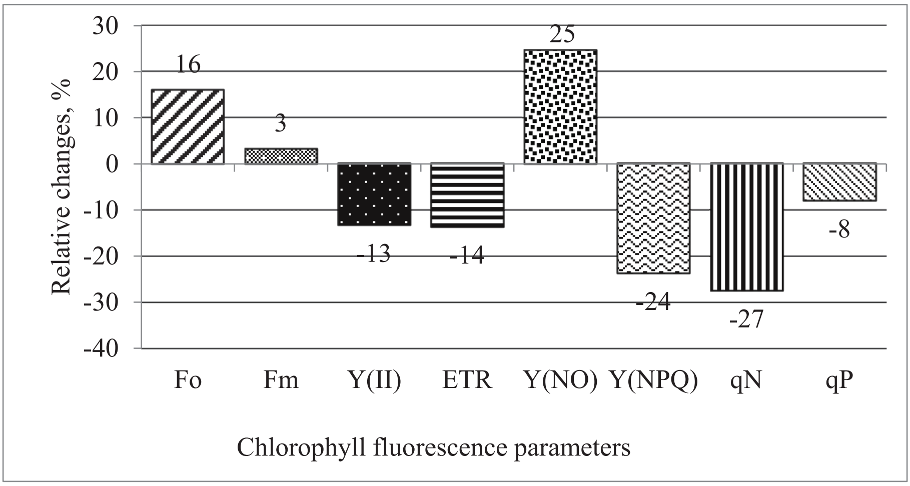

Fig. 2. Chlorophyll fluorescence parameters of 16-day-old Sibirskaya 21 seedling leaves under *B.sorokiniana* infection (relatively resistant).

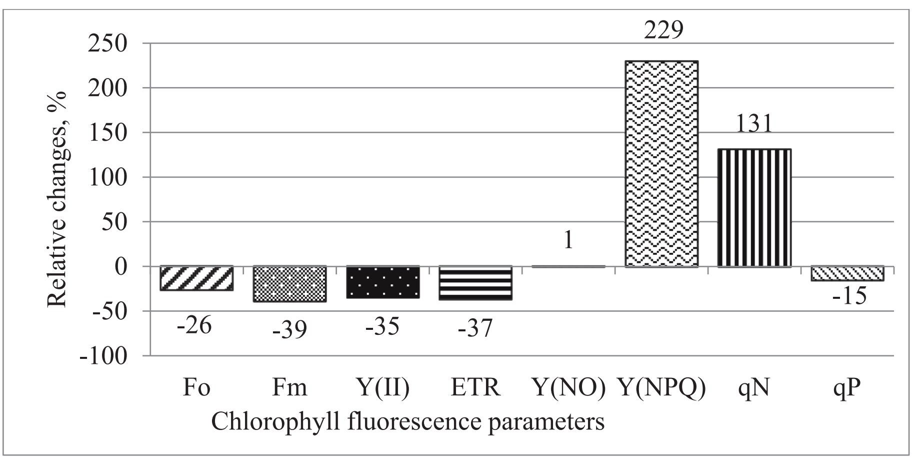

Fig. 3. Chlorophyll fluorescence parameters of 16-day-old Novosibirskaya 29 seedling leaves under *B. sorokiniana* infection (relatively unresistant).

Herewith, the reaction of cultivars Novosibirskaya 29 and Sibirskaya 21 more resistant to the pathogen invasion appeared to be less expressed showing the smallest or uncertain PhlF parameter changes compared to the control ones. In wheat leaves infected by B. sorokiniana, Rios et al. also showed a heavy disorder of photosynthetic activity by parameters Fv / Fm and Y(II) for more amenable cultivar compared to less amenable one. Similar results were obtained while researching cultivar reaction of wheat leaves infected by Blumeria graminis f., where PS II activity (Fv / Fm and ETR) decreased significantly for the amenable cultivar and did not change for the resistant one [22]. Katanić et al. [23] also report about discovered dependence of caused by Fusarium sp. head blight photosynthetic changes on winter wheat cultivar.

4 Conclusion

The performed research confirms that ChlF parameters Y(II), ETR, qP, Fv / Fm, Fv / Fo, Y(NPQ), qN and Y(NQ) are reliable biomarkers for photosynthetic apparatus level pre-symptom estimate of cultivar resistance to the causative agent of the common cereal root rot B. sorokiniana. Light parameters were more sensitive compared to dark parameters. Thus, the first ones may be preferable in tests of stress resistance. The most cited in the literature, parameter Fv / Fm has appeared to be less sensitive parameter compared to its analogue Fv/Fo. ChlF measurements have allowed detecting the pathogenesis of B. sorokiniana infection at seedling stage, prior to disease symptoms evident watching. The seedling age (16 days) and B. sorokiniana infection load (5000 conidia per grain) are found to range cultivars by their resistance to B. sorokiniana infection. In B. sorokiniana pathogenesis, photosynthetic activity level during adaptive reaction formation can be stress resistance criteria for selection material to accelerate its sampling and to increase its effectiveness by early rejection of non-perspective samples. The chlorophyll fluorescence recording method can be also applied for early detection of common cereal root rot detection.

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