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CROP PRODUCTION

Features of the Photosynthetic Apparatus in Winter and Spring Wheat Plants with Different Cold Tolerance

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Abstract—It has been shown that winter wheat differs significantly not only in cold tolerance but also in the nature of changes in functional organization of the photosynthetic apparatus. Such changes occur already in the first hours of low temperature action, minimizing the adverse effects of cold on plants. They are of an adaptive nature and, along with others, permit the plants of winter wheat to survive under cold conditions. Accounting for these specific features may be useful for breeding to create cold-resistant varieties, as well for assessing the prospects of their introduction to regions that are characterized by frequent and significant lowering of temperature with long-term nature included in the period of active plant vegetation.

Keywords: winter and spring wheat, cold hardening, cold tolerance, photosynthetic pigments, nonphotochemical quenching of chlorophyll fluorescence

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INTRODUCTION

Centuries of experience of wheat cultivation [1] has led to a wide variety of varieties, and massive yield losses in regions with climate variability have necessitated the creation of varieties resistant to adverse environmental conditions, including low temperature. Simultaneously, there was an intensive study of phenomenology and mechanisms of formation of high cold tolerance of this crop [2, 3] using, in particular, a comparative analysis of vital activity features of winter and spring crops in the process of cold hardening. Despite considerable experimental material accumulated in this direction, the first hours and days of cold adaptation of plants were the least studied, although many important changes occur precisely in this period in their cells and tissues, and they predetermine to a large extent the final result of the adaptation process [2]. Previously, we have established [4] that the expression of some genes of cold response was observed in winter and spring wheat in the first minutes and hours of low temperature. However, it is obvious that, at the initial stage of plant hardening, major changes can occur not only at molecular genetic, but also at subcellular, cellular, and tissue levels, including structural and functional organization of the photosynthetic apparatus (PA), which is characterized, as we know, by an increased sensitivity to cold [3]. For a better understanding of the role of these changes in the formation of cold tolerance, it is necessary to study simultaneously the dynamics and stability of individual indicators of PA in plants of winter and spring wheat in the process of cold hardening from its first hours.

MATERIALS AND METHODS

The study was performed using equipment of the Center for collective use of scientific equipment of the Institute of Biology, Karelian Research Center, Russian Academy of Sciences. Experiments were carried out with seedlings of winter wheat (*Triticum aestivum* L.) variety Moskovskaya 39 and of spring wheat variety Leningradskaya 97 grown in rolls of filter paper on Knop nutrient solution in an environmental chamber at an air temperature of 22°C, relative humidity 60–70%, 10 klux illumination, and 14 hrs photoperiod. One week-old seedlings were exposed to 4°C temperature for 7 days, keeping other conditions unchanged.

Cold tolerance of seedlings was judged by the temperature (LT50) causing the death of 50% of palisade parenchyma cells of leaf cuttings after 5 min of freezing in thermoelectric microcoolers TZHR-02/-20 (Interm, Russia) with sequential changes in temperature with an interval of 0.4°C. Cell viability was determined using a light microscope (Micmed-2, LOMO, Russia lens 40x) based on chloroplast destruction and coagulation of cytoplasm [5].

Leaf area was calculated by the standard formula [6]. Chlorophyll content was determined on an SF-2000 spectrophotometer (Spectrum, Russia) in the alcoholic extract. Portion of chlorophylls in the light-harvesting complex (LHC) was calculated from

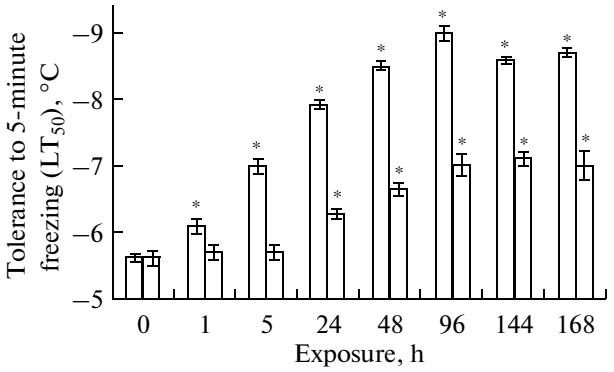

Fig. 1. Dynamics of cold tolerance of leaf cells in seedlings of winter (first column) and spring (second column) wheat during cold (4°C) hardening (* differences from baseline are significant at $P \le 0.05$).

their sum taking into account that all chlorophyll β is located in the LHC, and a ratio of chlorophyll α and β in LHC is 1, 2 [7]. Chlorophyll fluorescence parameters (relative electron transport rate and coefficient of nonphotochemical quenching of fluorescence) were measured as an aftereffect of cooling by a fluorometer MINI-PAM (Walz, Germany) on the leaves preadapted to the dark.

The tables and graphs show arithmetic mean values and their standard errors, and discussed values are significant at $P \le 0.05$.

RESULTS AND DISCUSSION

The tolerance of leaf cells in seedlings of winter and spring wheat increased under the influence of 4°C temperature, but a number of significant differences were revealed in its dynamics. In particular, the growth of tolerance of winter wheat leaf cells started within 1 hour from the beginning of hardening, whereas in spring wheat it started only 1 day later. At the same time, the tolerance of both varieties reached a maximum level on the fourth day of hardening in these conditions, but its growth in winter wheat was significantly higher than that of spring wheat (Fig. 1).

Table 1. Changes of leaf growth in plants of winter and spring wheat in the process of cold (4°C) hardening

Exposure, h	Leaf area, cm^2		Increased leaf area, % of initial	
	winter	spring	winter	spring
0	21.2 ± 0.5	25.3 ± 1.0	–	–
24	21.3 ± 0.6	25.6 ± 0.8	1	1
48	22.9 ± 0.9*	26.7 ± 0.8	8	6
96	23.2 ± 0.8*	27.6 ± 1.5	9	9
144	28.8 ± 0.9*	28.3 ± 0.8*	36	12
168	28.9 ± 1.0*	28.8 ± 0.9*	36	14

* Differences from baseline are significant at $P \le 0.05$.

An inhibition of leaf growth occurred in these varieties in the process of hardening along with an increase of cold tolerance, and its almost total termination was observed only in the first day of the experiment (Table 1). Leaf growth restored partly after 2 days of cooling. At the same time, an increase of leaf area relative to the baseline level in winter wheat at the end of the experiment (sixth to seventh day) was almost 2.5 times greater than that of spring wheat.

Total chlorophyll content in the leaves of winter wheat in the first hours of the action of cold decreased compared with the baseline (Fig. 2a), but then it began to increase due to their portion in the LHC (Fig. 2c). The amount of green pigments and a portion of chlorophyll in the LHC after 4–7 days of hardening were significantly higher than the baseline values (Figs. 2a, 2b). Total chlorophyll content decreased in spring wheat after 2 days of cooling, and this process was intensified by the end of the experiment (Fig. 2b), and the portion of chlorophylls in the LHC decreased to the seventh day of hardening (Fig. 2d).

A slight decrease in the relative rate of electron transport was observed in chloroplasts of winter wheat leaves after 5 hours from the beginning of hardening, and it was 15% of the initial level 2 days later; further, this indicator did not change (Table 2). In spring wheat, electron transport rate increased in the first several hours of cooling, and it decreased to 90% of the baseline on the fourth day. In addition, an increase in nonphotochemical quenching coefficient of chlorophyll fluorescence was observed in winter wheat seedlings after 5 hours from the beginning of hardening (Table 2). During the cooling of plants it gradually increased and it was above the baseline values by almost 34% on the fourth to seventh day. The value of this coefficient in spring wheat increased only at the end (seventh day) of the experiment.

Thus, winter wheat exceeds spring wheat not only in a magnitude of cold tolerance of leaf cells but also in the rate of its formation. It is important that an increase of cold tolerance of winter wheat begins after 1 hour from the beginning of hardening, and that of spring wheat begins only on the second day. At the same time, important changes in the functional organization of PSA occur in winter wheat at the initial stage of the action of low temperature: there is an increase in both chlorophyll content due to its portion in the LHC and the coefficient of nonphotochemical quenching of fluorescence.

It should be noted that the features of organization of PSA in winter and spring wheat were studied by many authors, but most of the known studies were carried out on the already hardened plants (when the process of tolerance formation is finished), and the detailed dynamics of parameter changes of PSA in the process of hardening was usually not monitored. For

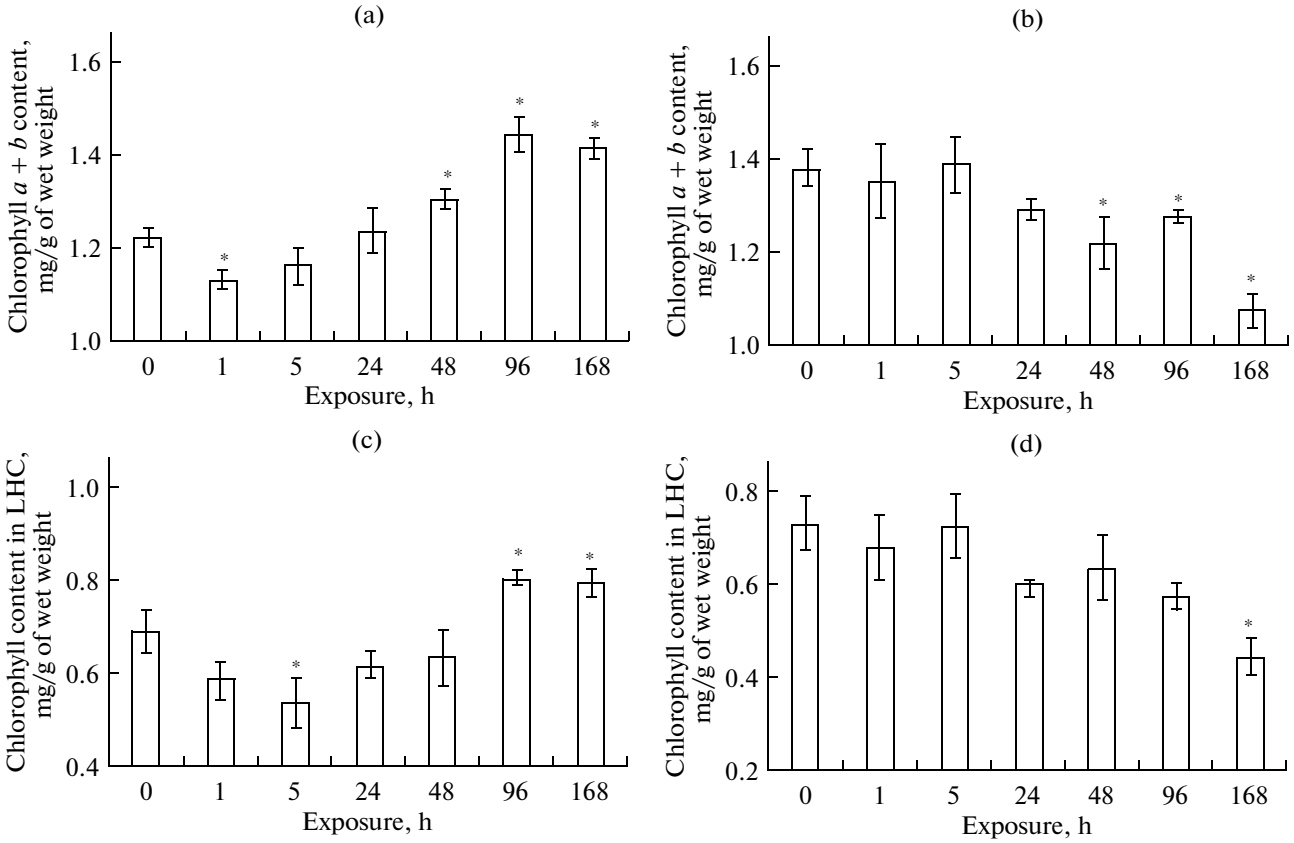

Fig. 2. Dynamics of (a, b) chlorophyll $a + b$ content and the (c, d) portion of chlorophyll in the LHC in the leaves of seedlings of (a, c) winter and (b, d) spring wheat during cold (4°C) hardening (* differences from baseline are significant at $P \le 0.05$).

example, total chlorophyll content of fully hardened (within 75 days) wheat plants of winter varieties Monipol and Kharkov was higher than the control values, and it did not change in spring wheat variety Glentea [8]. From our data on the dynamics of pigment content in leaves of winter wheat, it becomes clear that (1 h after the action of cold) chlorophyll content initially decreases somewhat, but then gradually restores due to the portion of chlorophyll in the LHC and exceeds the baseline at the end of the experiment. An increase in the total chlorophyll content due to its portion in the LHC is considered by researchers [9] to be an adaptive response of the pigment apparatus typical for the plants of the North; in our experiments, it was observed already at the initial stage of cold hardening.

Table 2. Changes in indicators of chlorophyll fluorescence in winter and spring wheat plants in the process of cold (4°C) hardening

Exposure, h	Electron transport rate		Coefficient of nonphotochemical quenching	
	winter	spring	winter	spring
0	103.8 ± 2.0	94.1 ± 1.9	0.56 ± 0.01	0.52 ± 0.01
1	105.0 ± 1.3	89.4 ± 4.9	0.59 ± 0.03	0.50 ± 0.02
5	96.71 ± 1.0*	100.7 ± 1.3*	0.63 ± 0.02*	0.51 ± 0.01
24	99.8 ± 2.2	104.7 ± 0.9*	0.62 ± 0.02*	0.53 ± 0.01
48	88.3 ± 1.9*	102.7 ± 2.3*	0.66 ± 0.03*	0.52 ± 0.02
96	86.3 ± 2.7*	88.3 ± 1.9*	0.70 ± 0.03*	0.50 ± 0.03
168	86.4 ± 2.8*	84.1 ± 0.9	0.75 ± 0.03*	0.64 ± 0.05*

* Differences from baseline are significant at $P \le 0.05$.

It was shown on wheat seedlings hardened for a week that the coefficient of nonphotochemical quenching of fluorescence in winter variety Sabalan is higher than that of spring variety Zagras [10]. Data are consistent with the results of our studies in which an increase in this coefficient was observed in winter wheat almost simultaneously with an increase in cold tolerance in the early hours of hardening and continued until the end of the experiment, whereas it was observed only on the seventh day in the spring. An increase in the value of coefficient under the action of cold is associated with the dissipation of excess light energy in the form of heat, which serves as one of the mechanisms of protection of photosystem II from hypothermia [10]. Obviously, in winter wheat, unlike spring wheat, this mechanism is activated in the first hours of cold hardening.

It should be noted that we detected an inhibition of growth of leaves under the influence of low temperatures as one of the necessary conditions for the adaptation of plants to cold [3, 8] for both wheat varieties. But they differ significantly in magnitude and rate of cold tolerance formation, as well as in the peculiarities of functioning of PSA. This allows us to suggest that, along with the inhibition of leaf growth, the features of functional organization of PSA play an important role in the process of cold adaptation; changes in them begin almost immediately with decreasing temperature.

Apparently, high ecological plasticity of winter wheat, manifested, in particular, in the ability to "reconfigure" PSA quickly to a new mode of functioning under the action of low temperatures, is explained by its origin. As is known, the evolution of wheat is closely related to its promotion from the southern to more northern latitudes, whereupon low temperature becomes a dominant factor of ontogeny control [1]. At the same time, winter forms that have originated from spring ones as a result of natural mutagenesis not only acquired the ability to withstand low temperatures but they even require them to move to the generative development [1].

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