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Characteristics of Expression of Temperature-Regulated Genes in Winter and Spring Wheat Plants during Cold Adaptation[1]

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Abstract—The characteristics of the expression of genes *Wcs120*, *Wcor15*, and *Wrab17* during cold (4°C) adaptation, particularly slower changes in the expression of *Wcs120* and *Wcor15* in spring compared to winter wheat, are revealed in leaves of winter and spring wheat seedlings. It is concluded that the formation of increased cold tolerance both in winter and spring wheat is related to enhanced expression of the studied genes.

Keywords: winter and spring wheat, temperature-regulated genes, expression, cold adaptation.

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The problem of the formation and preservation of the yield of leading crops, among which is wheat, is first and foremost in today's plant industry, especially in regions of risky agriculture characterized by unstable climatic conditions. It is therefore important to investigate the mechanisms of plant tolerance to unfavorable environmental factors, including low temperatures. It has been established that an increase of low-temperature tolerance of plants is related not only to multiple structural and functional reorganizations [1] but also to a change in the expression of a rather large number of genes [1, 2]. Thus, induction of low-temperature expression of a number of *Cor* (Cold regulated) genes encoding COR proteins correlates positively with an increase of frost tolerance of plants [2]. Moreover, COR proteins of the WCS120 (Wheat Cold Specific Proteins) family have special significance in processes of cold adaptation of winter wheat and other cereals [2, 3].

The purpose of the present work was to study the expression of a number of low-temperature regulated *Cor* genes in winter and spring wheat plants under cold-hardening conditions.

METHOD

The investigations were conducted on seedlings of winter and spring wheat (*Triticum aestivum* L.) varieties Moskovskaya 39 and Leningradskaya 97, respectively. For this purpose they were grown for 7 days in rolls of filter paper on Knop's nutrient solution with the addition of trace elements (pH 6.2–6.4) in an artificial climate chamber at an air temperature of 22°C, relative humidity 60–70%, illuminance 10 klx, and photoperiod 14 h, and then subjected to the effect of a temperature of 4°C while keeping the other conditions the same.

Cold tolerance was evaluated on the basis of the temperature (LT_{50}) causing death of 50% of leaf palisade cells after 5-min test freezing in a TZhR-02/-20 microfreezer (Interm, Russia) [4]. Viability of the cells was determined by a Mikmed-2 light microscope (LOMO, Russia) on the basis of destruction of chloroplasts and coagulation of cytoplasm.

Total RNA was isolated by an AquaPure RNA Isolation Kit (Bio-Rad, United States). The quality and quantity of isolated RNA was determined by microchip capillary electrophoresis (Experion, Bio-Rad). To remove DNA residues, the RNA preparation was treated with DNase (Sileks, Russia). The level of gene expression was determined by the real-time PCR method. The intercalating stain SYBR Green was used as the fluorophore for detecting products. Amplification was carried out in an iQ5 iCycler (Bio-Rad) with the use of amplification kits combined with reverse transcription. The nucleotide sequences of the primers (Sintol, Russia) are given in the table. The arithmetic mean values and their standard errors are given in the figures.

RESULTS AND DISCUSSION

It was established that the dynamics of formation of cold tolerance of wheat seedlings under the effect of a 4°C temperature depends on the biological characteristics of the object. Thus, tolerance of winter wheat variety Moskovskaya 39 seedlings increased already 1 h

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Primers for conducting real-time PCR

Gene	Primer	Primer nucleotide sequence
Wcs 120	Forward	CACGGCACTGGCGAGAAGAAAGG
	Reverse	TGATGTTCTCCATGACGCCCTTC
Wcor 15	Forward	GGGAGCAACCTCTTCCATAGTGT
	Reverse	CCACCATCACAACCCTTCACTA
Wrab 17	Forward	CGG GATGGGAGAGACAAGTGAG
	Reverse	GGAATAGCGAAACAGAAGGAGGG

after the start of cold hardening, was close to the maximum value on days 2 and 3, and thereafter changed insignificantly (Fig. 1a). The cold tolerance of spring wheat variety Leningradskaya 74 seedlings increased more slowly, reached a maximum only after hardening for 6 days, and its increase compared to the initial level was noticeably less than that of winter wheat (Fig. 1b).

Along with a change in cold tolerance under the effect of the hardening temperature, the expression of *Cor* genes changed considerably. In particular, the level of *Wcs120* gene expression in var. Moskovskaya 39 seedlings increased markedly after only the 15-min effect of 4°C, its maximum was noted after 30 min, and then after 5-h hardening it decreased to the initial values and remained unchanged during the entire subsequent period of the effect of cold (Fig. 2a). Noticeable changes in the expression of this gene were not observed in var. Leningradskaya 74 seedlings during the first 5 h of cold hardening, and only after 24 was a pronounced and very considerable accumulation of its transcripts noted, which increased for 2 days and then decreased (Fig. 2b).

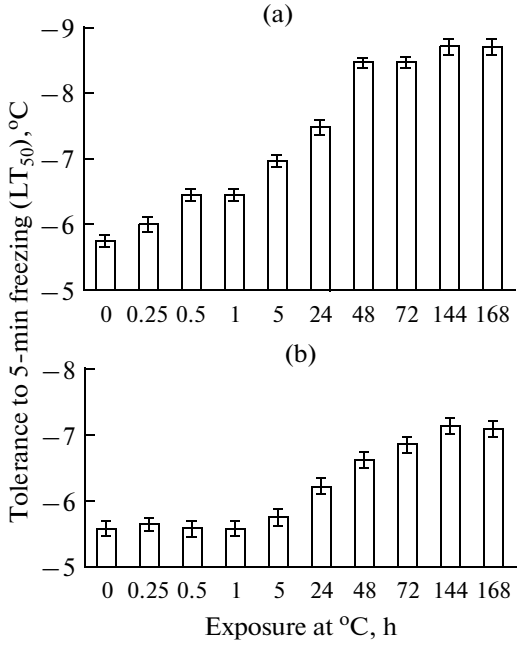

Fig. 1. Dynamics of cold tolerance of winter (a) and spring (b) wheat seedlings during cold adaptation.

The level of *Wcor15* gene expression in leaves of both winter and spring wheat seedlings increased 15 min after the effect of 4°C and then gradually increased and remained at a high level for 2 days. Further exposure of the seedlings under hardening conditions led to its decrease (Fig.2 c, d). Expression of the *Wrab17* gene also increased 15 min from the start of cold hardening of winter wheat, and its increased level remained almost during the entire low-temperature effect (Fig. 2e). At the same time, significant changes in *Wrab17* gene expression were not detected in spring wheat seedlings in the initial period of cold hardening, but the same multiple enhancement was noted for it as for the *Wcs120* gene during longer hardening (1–7 days), Fig. 2f.

The results obtained indicate the existence of a certain dependence between the dynamics of the level of expression of *Cor* genes of winter and spring wheat seedlings and formation of cold tolerance under conditions of the effect of low hardening temperatures. In winter wheat seedlings characterized by a stable yield, good grain quality, and high winter hardiness [5], the rate and magnitude of increase of low-temperature tolerance were substantially higher than in spring wheat. The expression of *Cor* genes in winter wheat increased also rather quickly (already at the start of cold hardening) and preceded or coincided in time with an increase of their cold tolerance. It is important that the slower increase of tolerance in spring wheat seedlings also correlated with enhanced expression of *Cor* genes, primarily *Wcs120* and *Wrab17*. It should be noted that induction of the expression of *Wrab17, Wrab19*, and *Wcs120* genes under cold-hardening conditions was observed earlier in spring wheat variety Mironovskaya 808 [6].

Thus, the detected considerable enhanced expression of *Cor* genes preceding or coinciding in time with an increase of cold tolerance allow speaking about their participation in the formation of increased tolerance of both winter and spring wheat plants in the initial period of their cold hardening.

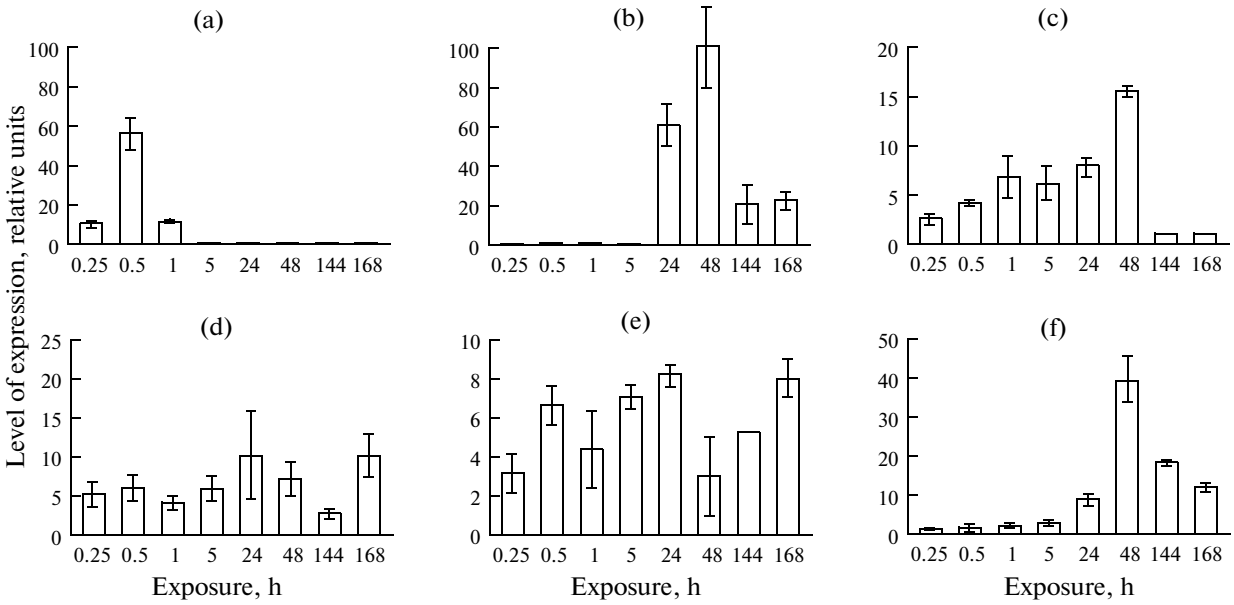

Fig. 2. Dynamics of the expression of *Wcs120* (a, b), *Wcor15* (c, d), and *Wrab17* (e, f) genes during cold adaptation of winter (a, c, e) and spring (b, d, f) wheat seedlings.

It is known that winter cereals compared with spring have more effective mechanisms of adaptation to low temperatures, which allows them to overwinter successfully and survive under these conditions. During evolution a number of protective adaptive mechanisms developed in them, including the expression of protective protein genes. They include, in particular, the *Wcs120* gene family characteristic of Poaceae, which encodes a group of proteins with molecular weight 12–200 kDa [3]. These genes are regulated by low temperature and encode the accumulation of WCS proteins, which correlates directly with the ability of plants to increase frost tolerance [3]. The given group of proteins belongs to desiccation-tolerant dehydrin proteins united into group II or D-11 family of the large class of LEA (Late Embryogenesis Abundant) proteins participating in increasing tolerance to various stress factors [3, 7]. Enhancement of the expression of dehydrin genes and the accumulation of their protein products are also important for increasing tolerance of plants to low-temperature stress [1, 4, 7].

It should be mentioned that the *Wcor15* gene encoding chloroplast proteins contains CRT/DRE *cis*-elements similar to *Wcs120* [8]. The activity of many genes is regulated not only by low temperature but also by abscisic acid (ABA), which is often called a stress hormone [9]. Furthermore, ABA-dependent gene *Wrab17* also belongs to the *LEA* gene group.

Thus, under low-temperature hardening conditions there exists a dependence between the level of expression of *Cor* genes *Wsc120, Wcor15*, and *Wrab17* in leaves of winter and spring wheat seedlings and their cold tolerance, which allows concluding the participation of these genes and proteins encoded by them in the formation of increased plant tolerance.

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