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Cold acclimation of tomato plants is associated with differential expression of genes of the *SlLHC* family, carotenoid metabolism, and chlorophyll accumulation/degradation

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Abstract. Tomato (*Solanum lycopersicum* L.) is sensitive to cold stress. The plant's adaptive response to low temperatures is accompanied by cellular osmoregulation and a decrease in the rate of photosynthesis due to disruption of the antennae of photosystems I and II, including proteins of the light-harvesting complex (LHC) family that bind chlorophylls *a*/*b* and carotenoids. In this study, a transcriptome analysis of differential expression of *SlLHC* family genes, carotenoid and abscisic acid (ABA) biosynthesis genes, as well as *ORANGE* and *STAY-GREEN* genes associated with chlorophyll accumulation and degradation, was performed for the first time during two cycles (I and II) of "cold stress (12 h) – recovery (60 h)". The tomato cultivar Otradnyi was used for the analysis. 12 h before the end of the recovery period of the second cycle (II-48), the content of chlorophylls *a*/*b* and carotenoids in plant leaves was shown to be significantly decreased. It was revealed that 12 h of cold exposure in both cycles (I-12, II-12) suppressed the expression of most *LHC* family genes. At the same time points, a significant increase in the transcript levels of the *NSY*, *CCD-like*, *LeNCED1* and *NCED6* genes associated with ABA synthesis was detected. Genes, expression profile of which differed between the primary and repeated cycles of cold exposure, were identified. Based on these data, a list of candidate genes for cold stress memory markers in plants was compiled: 10 *SlLHC* family genes (*Cab-1A*, *Cab-1D*, *CAB12*, *CAB5*-LOC101257186, *SEP2*, *OHP2*-LOC101259223, *OHP1* and *LIL3-2*-LOC101247899) and five genes associated with ABA synthesis (*VDE*, *CCD4*, *CCD-like*, *LeNCED1* and *NCED6*). The obtained results are important for identifying and understanding the involvement of photosynthesis-related genes in the response to cold stress and determining cold tolerance of tomato plants, and can be used in breeding cold-resistant varieties.

Key words: *Solanum lycopersicum* L.; cold stress; LHC protein family; chlorophyll *a*/*b*-binding proteins; carotenoid biosynthesis; ABA; gene expression

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Холодовая акклиматизация растений томата связана с дифференциальной экспрессией генов семейства *SlLHC*, метаболизма каротиноидов и накопления/деградации хлорофиллов

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Аннотация. Растения томата (*Solanum lycopersicum* L.) чувствительны к воздействию холодового стресса, адаптационный ответ на который связан с клеточной осморегуляцией и снижением скорости фотосинтеза за счет нарушения работы антенн фотосистем I и II, включающих белки семейства Light-Harvesting Complex (LHC), связывающие хлорофиллы *a*/*b* и каротиноиды. В настоящей работе впервые проведен транскриптомный анализ дифференциальной экспрессии генов семейства *SlLHC*, генов биосинтеза каротиноидов и абсцизовой кислоты (АБК), а также генов *ORANGE* и *STAY-GREEN*, связанных с синтезом и деградацией хлорофиллов, в динамике двух циклов (I и II) «холодовой стресс (12 ч) – восстановление (60 ч)». Для анализа использован сорт томата

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Отрадный. Показано, что за 12 ч до завершения периода восстановления второго цикла (II-48) в листьях растения значительно уменьшается количество хлорофиллов *a*/*b* и каротиноидов. Выявлено, что 12 ч холодового воздействия в обоих циклах (I-12, II-12) подавляют экспрессию большинства генов семейства *LHC*. В тех же точках обнаружено значимое повышение уровня транскриптов генов *NSY*, *CCD-like*, *LeNCED1* и *NCED6*, связанных с синтезом АБК. Определены гены, профиль экспрессии которых различается между первичным и повторным циклами холодового воздействия. На основании этих данных составлен список генов-кандидатов в маркеры памяти растений о холодовом стрессе: десять генов семейства *SlLHC* (*Cab-1A*, *Cab-1D*, *CAB12*, *CAB5*-LOC101257186, *SEP2*, *OHP2*-LOC101259223, *OHP1* и *LIL3-2*-LOC101247899) и пять генов, ассоциированных с синтезом АБК (*VDE*, *CCD4*, *CCD-like*, *LeNCED1* и *NCED6*). Полученные результаты важны для определения и осмысления участия генов, связанных с фотосинтезом, в ответе на холодовой стресс и определении холодостойкости растений томата, и могут быть использованы в селекции холодостойких сортов.
Ключевые слова: *Solanum lycopersicum* L.; холодовой стресс; семейство белков LHC; хлорофилл *a*/*b*-связывающие белки; биосинтез каротиноидов; АБК; экспрессия генов

Introduction

The survival, productivity, and geographic distribution of plants are limited by unfavorable environmental conditions. Among the most serious factors, cold stress can have both less destructive (chilling) and more destructive (freezing) forms (Kidokoro et al., 2022). Unable to move, plants are forced to adapt to stressful conditions, including low temperatures (Browse, Xin, 2001), which is accompanied by physiological and molecular genetic changes (Ding et al., 2024). Primary stress triggers epigenetic modifications in the genome, thus improving plant adaptation to recurrent events and forming stress memory (Villagómez-Aranda et al., 2022). Genes that exhibit differences in expression patterns between priming and repeats, as well as during recovery periods, are considered markers of stress memory (Friedrich et al., 2019; Bäurle, Trindade, 2020; Jacques et al., 2021; Aina et al., 2024).

In the plant response to cold stress, one of the significant groups of genes is associated with photosynthesis, the reduction of which stimulates cellular osmoregulation (Yamori et al. 2014). The latter is achieved by stomatal closure under the action of abscisic acid (ABA) (Sreenivasulu et al., 2012; Agurla et al., 2018), formed during the oxidative cleavage of the carotenoid 9′-*cis*-neoxanthin (Nakashima, Yamaguchi-Shinozaki, 2013; Chen et al., 2020; Xu et al., 2023).

Cold-induced inhibition of photosynthesis is characterized by impaired light energy capture by the light-harvesting complexes (antennae) of photosystems I and II and electron transfer in thylakoids (Ashraf, Harris, 2013). The antennae include proteins of the Light-Harvesting Complex (LHC) family, which are embedded in the thylakoid membrane and noncovalently bind to chlorophylls *a* and/or *b*, altering their optical properties (Klimmek et al., 2006). The resulting set of different chlorophyll forms ensures the transfer of energy from absorbed quanta to the reaction centers of the photosystems (Li et al., 2025). Also, LHC proteins are involved in chlorophyll degradation by interacting with the STAY-GREEN (SGR) protein and chlorophyll catabolism enzymes (CCE) (Pietrzykowska et al., 2014). The LHC family includes chlorophyll *a*/*b*-binding proteins (CAB or Lhca/b), photosystem II protein psbS, Light-Harvesting-like proteins (LIL, also including Light-Inducible proteins (ELIP), One-Helix proteins (OHP), Stress-Enhanced proteins (SEP)), and ferrochelatases (FC or FeCh) (Jansson, 1999; Klimmek et al., 2006).

In addition to LHC proteins and chlorophylls *a*/*b*, antenna complexes include carotenoids (β-carotene, xanthophylls of the lutein and violaxanthin cycles), which are involved in the capture of light quanta in spectral ranges weakly absorbed by chlorophylls, followed by the transfer of excitation energy to chlorophyll molecules (Li et al., 2025). Furthermore, carotenoids (primarily violaxanthin, antheroxanthin, and zeaxanthin) protect the photosynthetic apparatus from excess excitation energy and prevent its destruction by neutralizing singlet oxygen and triplet chlorophyll (Li et al., 2025).

The ORANGE (OR) protein plays an important role in regulating chlorophyll and carotenoid levels in green plant tissues. For example, the expression level of *SlOR* and *SlOR-like* genes in the stems and leaves of tomato *Solanum lycopersicum* L. is positively associated with the levels of chlorophylls and carotenoids in leaf cells (Gao et al., 2025). However, overexpression of the *OR* gene in the model species *Arabidopsis thaliana* L. suppresses chlorophyll biosynthesis and thylakoid membrane development (Tee, 2019).

The *LHC* gene family has been identified in various plant species, including *A. thaliana* (30, 32, or 34 genes), poplar *Populus* spp. (36 or 39), cotton *Gossypium hirsutum* L. (86), and apple *Malus* × *domestica* Borkh. (27 or 42) (Klimmek et al., 2006; Zhao et al., 2020; Zhang et al., 2021; Lan et al., 2022).

Tomato plants (*S. lycopersicum*), an important vegetable crop, are sensitive to temperatures below 12 °C (Mesa et al., 2022) and are therefore grown primarily indoors in most regions of the Russian Federation due to the risk of sudden temperature fluctuations and recurrent spring cold spells or frosts. During the development of stress resistance, the parameters of photosynthesis undergo significant changes, including the expression of photosynthetic proteins (Neilson et al., 2011; Soualiou et al., 2023), so studying tomato *LHC* genes may be promising for the breeding of new varieties.

The number of *LHC* genes in *S. lycopersicum*, as in other plant species, is not precisely defined and depends on the specific study: 40 (Lan et al., 2022) or 39 (Meng et al., 2025). Most *LHC* genes contain stress-associated *cis*-regulatory elements in their promoters. These genes are differentially expressed in response to drought, cold, and salinity, may play an important role in many biological processes, and are shown to be regulated by circadian rhythms (Piechulla et al., 1998;

Lan et al., 2022; Meng et al., 2025). The transcript level of *SlLHC* genes increases during plant adaptation to salt stress (Ikuyinminu et al., 2023) and is positively related to the rate of photosynthesis (Yan et al., 2023).

The aim of this study was a transcriptome analysis of leaves of the cold-resistant tomato cultivar Otradnyi in terms of changes in the expression of *LHC* genes, genes of the carotenoid biosynthesis pathway, as well as genes associated with the synthesis of carotenoids and chlorophyll in chloroplasts, the formation of ABA and the degradation of chlorophylls, in response to two cycles of "cold stress – post-stress recovery". The results obtained are important for understanding the involvement of photosynthesis-related genes in the cold stress response and cold tolerance of tomato plants, with the potential to identify markers of cold stress memory among these genes.

Materials and methods

In the study, we used plants of the tomato *S. lycopersicum* cold-resistant cv. Otradnyi (code 8006741, https://gossortrf.ru/registry/) bred by the Federal Scientific Vegetable Center (FSVC, Moscow Region). This cultivar is early ripening and resistant to salinity (Filyushin et al., 2025; data from the originators).

Tomato cv. Otradnyi seeds (the 2024 harvest) were used to obtain seedlings at the 5–7 leaf stage (light/dark – 16 h/8 h, 23 °C/18 °C; dark phase 23:00–7:00; Photosynthetic Photon Flux (PPF) – 190 μM/(m^2·s)). Plants were subjected to two cycles of cold stress; controls were incubated under normal conditions (23 °C). Each cycle consisted of a cooling phase of 12 h at 3 °C (20:00–8:00) (I-12 and II-12) followed by a post-stress recovery period (memory phase) of 60 h under normal conditions (Fig. 1). The cycles followed one after another and in total took 144 h (12+60+12+60).

The use of two cycles was motivated by the signature of stress memory marker genes, implying differences in the expression patterns of such genes between priming and re-exposure (Friedrich et al., 2019; Bäurle, Trindade, 2020; Jacques et al., 2021; Aina et al., 2024). That is, if the expression profile of the genes we analyzed under two-cycle stress shows significant intercycle differences, such genes can become candidates for stress memory markers. In addition, we used a 60-h recovery period, since another characteristic of stress memory marker genes is the failure to return to normal expression values for a long time (more than 24 h) after stress (Friedrich et al., 2019; Bäurle, Trindade, 2020; Jacques et al., 2021; Aina et al., 2024).

Over the course of two cycles (a total of 144 h), no visible differences were found between the experimental plants and the controls. In both cold treatment cycles, no signs of leaf wilting were observed in the stressed samples (apparently due to the cold tolerance of the cv. Otradnyi). There were also no significant differences in plant height, leaf number and size between the control and experimental groups, as well as within them.

At the end of cold exposure (12 h, 8:00) and after 48 h of recovery (8:00) of the first (I) and second (II) cycles, leaf samples were collected from the experimental and control plants. In the recovery phase (60 h), a 48-h point (I-48 and II-48) was chosen, 12 h before the end of the post-stress period, so that sampling was carried out at the same time of day (8:00) both during stress and recovery. The choice was determined by the dependence of the expression of the analyzed photosynthesis genes on circadian rhythms (Piechulla et al., 1998; Meng et al., 2025). This temporal coordination, combined with a parallel cultured control for normalization, should improve the accuracy of interpretation of the resulting biochemical and transcriptomic data.

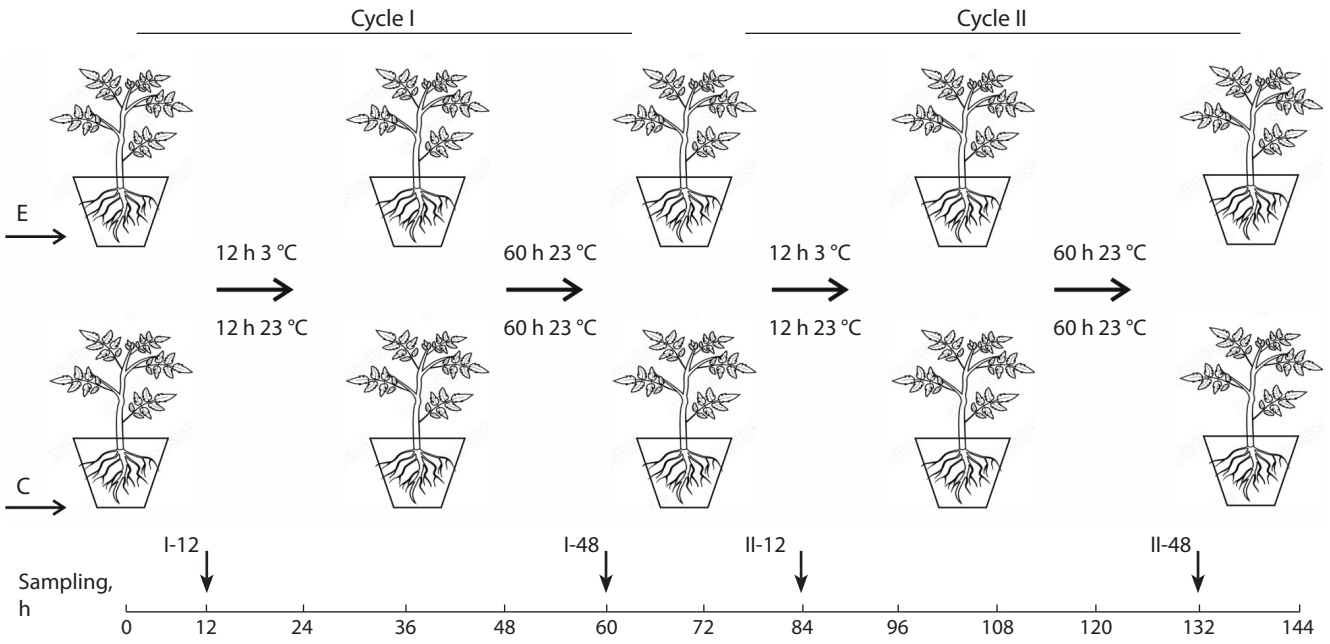


Fig. 1. Schematic representation of two-cycle (I, II) exposure to cold stress with subsequent recovery on tomato plants of the cv. Otradnyi.

The samples were analyzed for chlorophylls *a*/*b* and carotenoids (two biological and three analytical replicates); one biological replicate was a pool of leaves from two independent plants for each measurement point. The concentrations (mg/g fresh weight (FW)) of chlorophylls *a* and *b* and total carotenoids were determined in chloroform-methanol (3:1, v/v) extracts according to (Efremov et al., 2020), with absorption spectra recorded on an Eppendorf BioSpectrometer® basic (Eppendorf, Germany). The following formulas were used to calculate pigment concentrations:

Chlorophyll *a* (Chl*a*) =

$$11.47\times(A_{666} - A_{750}) - 2\times(A_{648} - A_{750}),$$

Chlorophyll *b* (Chl*b*) =

$$21.85\times(A_{648} - A_{750}) - 4.53\times(A_{666} - A_{750}),$$

Total carotenoids =

$$[1000\times(A_{480} - A_{750}) - 1.33\times\text{Chl}a - 23.93\times\text{Chl}b]/202,$$

where A_{666}, A_{648}, A_{750} and A_{480} are the absorption values at the indicated wavelengths.

The significance ($p < 0.05$) of differences between values was determined using Two-way ANOVA ("multiple comparisons, corrected with Bonferroni test") in GraphPad Prism v. 8 ("GraphPad Software Inc.", USA; https://www.graphpad.com/scientific-software/prism/).

For subsequent transcriptome analysis, total RNA was isolated from the same leaf samples (RNeasy Plant Mini Kit, Qiagen, USA) and used to obtain mRNA libraries (NEBNext® mRNA Library Prep Reagent Set for Illumina; New England BioLabs, USA), which were sequenced on the Illumina HiSeq2500 platform (Illumina Inc., USA). Based on the obtained reads, coding sequences were assembled using Trinity v3.5.13 (https://github.com/trinityrnaseq/trinityrnaseq/wiki) and TransDecoder v5.1.0 (https://github.com/TransDecoder/TransDecoder) and then annotated with NCBI-Blast (https://www.ncbi.nlm.nih.gov/). Relative transcript levels were estimated as RPKM (number of reads per 1 kb transcripts per million mapped reads).

Differentially expressed genes (DEGs) between the control and experimental plants in two cycles at the end of stress (I-12, II-12) and the recovery period (I-48, II-48) were identified using the DESeq2 method (Love et al., 2014). Differences in transcript levels were considered significant if the expression change was ≥2 (absolute log2FoldChange [FC] ≥ 1; padj (P-value adjusted, or false discovery rate) < 0.05). Next, the annotation was used to search for tomato *LHC* superfamily genes, carotenoid metabolism pathway genes, as well as *OR*, *OR-like*, and *SGR* genes, both among the primary gene list and among the DEGs. Changes in gene expression profiles during the experiment were visualized using heatmaps (Excel).

Results

In this study, we examined the effect of two "cold stress – recovery" cycles (I, II) on metabolite content and photosynthesis-related gene expression in leaves of the cold-tolerant tomato cv. Otradnyi. Leaves were collected from experimental plants (parallel to control samples) at two points in each cycle: at the end of the 12-h stress period (I-12, II-12); and 12 h before the end of the 60-hour recovery phase (I-48, II-48).

Tissue samples were used for chlorophyll *a* and *b* and total carotenoid content analysis. At point I-48, both in the control and the experimental group, the obtained values were significantly lower than at the other three points (II-48, I-12, and II-12). The exception was carotenoids: their content at point I-48 differed from I-12, but not from II-12, but was similar in the control and experimental groups (Fig. 2). In addition, in the experimental group, the amount of all analyzed pigments decreased significantly at the end of the recovery period of the second cycle (II-48) (Fig. 2).

The same leaf samples were used to obtain transcriptomes for subsequent analysis of the expression dynamics of photosynthesis-related genes. A preliminary list of genes of interest was compiled based on transcriptome annotations from SolGenomics, including studies (Lan et al., 2022; Meng et al., 2025), and NCBI (see the Table). The former study identified 24 *Lhca/b*, 10 *Lil*, one *Psbs*, and five *FCII* genes in tomato (Lan et al., 2022). The latter publication reported 29 *Lhca/b*, seven *Lil*, one *Psbs*, and two *FCII* genes (Meng et al., 2025).

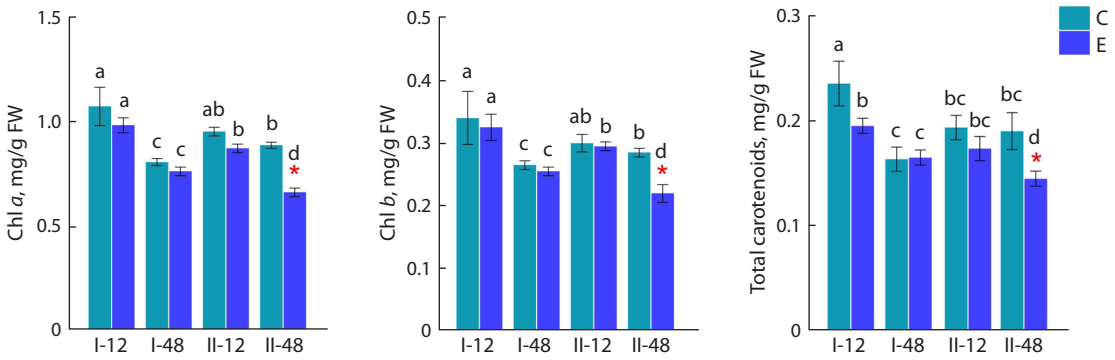


Fig. 2. Dynamics of the content (mg/g FW) of chlorophylls *a* and *b* and total carotenoids in the leaves of tomato cv. Otradnyi plants in response to cold stress and recovery.

In each of the two exposure cycles (I, II), measurements were taken at the end of stress exposure (I-12, II-12) and after 48 h of the recovery period (I-48, II-48). The value $^{a-d}$ $p < 0.05$ – for statistically significant differences between the experiment (E) and the control (C) at each of the points; a significant difference between the experiment and the control at point II-48 is additionally indicated by a red asterisk.

List of genes analyzed in the study

Superfamily *LHC*		
Solyc gene ID[1]	NCBI gene ID[2]	Annotation of the encoded protein (NCBI)
02g070940 02g070950 02g070970 02g071010 02g071030	LOC101264784	Chlorophyll *a*/*b*-binding protein Cab-1B (CAB1B)
02g070990	LOC101266182	CAB1B homolog, 100 %-cover, identity 99.25 %
02g070980	LOC104645884	Chlorophyll *a*/*b*-binding protein Cab-1A (Cab-1A)
02g071000	LOC104645883	Chlorophyll *a*/*b*-binding protein 1D (Cab-1D)
03g005760	LOC101267774	Chlorophyll *a*/*b*b-binding protein 1-like
03g005770	LOC101245729	Chlorophyll *a*/*b*-binding protein
03g005780	LOC108491835	Chlorophyll *a*/*b*-binding protein Cab-3C (Cab-3C)
12g006140	LOC543976	Chlorophyll *a*/*b*-binding protein 5, chloroplastic (CAB5)
07g063600	LOC101268123	Chlorophyll *a*/*b*-binding protein 13, chloroplastic-like
12g011450	LOC101243766	Chlorophyll *a*/*b*-binding protein 13, chloroplastic (CAB13)
09g014520	LOC101249002	Chlorophyll *a*/*b*-binding protein CP29.1, chloroplastic
06g063370	LOC101266527	Chlorophyll *a*/*b*-binding protein Cab9 (CAB9)
01g105030	LOC101256629	Chlorophyll *a*/*b*-binding protein CP24 10A, chloroplastic (CAB-10A)
04g082930 04g082920	LOC101246628	Chlorophyll *a*/*b*-binding protein 7, chloroplastic
01g105050	LOC101256131	Chlorophyll *a*/*b*-binding protein CP24 10B, chloroplastic-like (LHCP)
05g056050	LOC101253380	Chlorophyll *a*/*b*-binding protein 6A, chloroplastic
05g056070	LOC544310	Chlorophyll *a*/*b*-binding protein precursor (CAB6A)
10g006230	LOC101264376	Chlorophyll *a*/*b*-binding protein 7, chloroplastic-like (CAB7)
10g007690	LOC101253628	Type III chlorophyll *a*/*b*-binding protein (CAB-8)
12g011280	LOC101265617	Chlorophyll *a*/*b*-binding protein 8, chloroplastic-like
03g115900	LOC101268669	Chlorophyll *a*/*b*-binding protein CAB11 (CAB11)
06g069730	LOC101256006	Chlorophyll *a*/*b*-binding protein Cab12 (CAB12)
–	LOC101265886	Chlorophyll *a*/*b*-binding protein 1C, chloroplastic
–	LOC101263969	Chlorophyll *a*/*b*-binding protein
–	LOC101268491	Chlorophyll *a*/*b*-binding protein 3C, chloroplastic-like
–	LOC543975	Chlorophyll *a*/*b*-binding protein precursor (CAB4)
11g042640	LOC101267576	Uncharacterized (CAB-like)
07g022900	LOC101257186	Photosystem I chlorophyll *a*/*b*-binding protein 5, chloroplastic
12g009200	LOC101252151	photosystem I chlorophyll *a*/*b*-binding protein 6, chloroplastic
09g082690	LOC101257109	Early light inducible protein (ELIP)
09g082700	LOC138337022	ELIP homolog 97 %-cover, identity 84.74 %
01g107660	LOC101252927	Stress enhanced protein 1-like protein
04g053130	LOC101250395	Stress enhanced protein 2, chloroplastic
12g056620	LOC101259223	Light-harvesting complex-like protein OHP2, chloroplastic
04g071930	LOC101253996	Light-harvesting complex-like protein OHP2, chloroplastic
03g094000	LOC101247899	Light-harvesting complex-like protein 3 isotype 2, chloroplastic
08g007180	LOC101260362	Light-harvesting complex-like protein 3 isotype 1, chloroplastic
08g077880	LOC101248227	Light-harvesting complex-like protein 3 isotype 2, chloroplastic
09g011010	LOC101262449	Light-harvesting complex-like protein OHP1, chloroplastic
05g018650	LOC101247019	Ferrochelatase-2, chloroplastic
10g084140	LOC101248153	Ferrochelatase-2, chloroplastic
08g065480	LOC101263738	Ferrochelatase-2, chloroplastic
06g060340	LOC101260830	Photosystem II subunit S (psbS)

Table (end)

Carotenoid metabolism		
Annotation of the encoded protein (NCBI)	NCBI gene ID	Functional activity of the encoded protein
Phytoene synthase 1, chloroplastic (Psy1)	LOC543988	Carotenoid synthesis (ElShamey et al., 2025)
Phytoene synthase 2, chloroplastic (PSY2)	LOC543964	
Phytoene synthase 3	LOC101267734	
Phytoene desaturase (pds)	LOC544073	
15-*cis*-phytoene desaturase, chloroplastic/chromoplastic	LOC101244544	
Prolycopene isomerase, chloroplastic (CRTISO)	LOC101267857	
15-*cis*-ζ-carotene isomerase (Z-ISO)	LOC101253762	
ζ-carotene desaturase (zds)	LOC543629	
Lycopene-ε-cyclase (CrtL-e-1)	LOC544129	
Lycopene-β-cyclase, chloroplastic	LOC101267662	
Lycopene-β-cyclase (LCY1)	LOC544104	
Lycopene-β-cyclase, chromoplastic (NSY) = neoxanthin synthase, chloroplastic	LOC543649	
B-carotene isomerase D27, chloroplastic	LOC101266539	
B-carotene hydroxylase (CrtR-b1)	LOC544133	
B-carotene hydroxylase (CrtR-b2)	LOC544297	
Zeaxanthin epoxidase, chloroplastic	LOC101252420	
Zeaxanthin epoxidase, chloroplastic (ZEP)	LOC544162	
Violaxanthin de-epoxidase (VDE)	LOC543696	
Carotenoid cleavage dioxygenase 7 (CCD7)	LOC100313501	SGL and ABA synthesis (ElShamey et al., 2025)
Carotenoid cleavage dioxygenase 4, chloroplastic	LOC101250535	
Carotenoid cleavage dioxygenase 1A (LeCCD1A)	LOC543935	
Carotenoid cleavage dioxygenase 1B (LeCCD1B)	LOC544269	
Carotenoid 9,10(9',10')-cleavage dioxygenase-like	LOC101255364	
NCED1 9-*cis*-epoxycarotenoid dioxygenase (LeNCED1)	LOC544163	
NCED2 9-*cis*-epoxycarotenoid dioxygenase 2	LOC100316877	
9-*cis*-epoxycarotenoid dioxygenase NCED6, chloroplastic	LOC101246826	
Synthesis of chlorophylls and carotenoids in chloroplasts		
Annotation of the encoded protein (NCBI)	NCBI gene ID	Functional activity of the encoded protein
ORANGE protein-like	LOC101244466	Negatively regulate chlorophyll biosynthesis, thylakoid membrane development, and *ELIP* gene expression in *A. thaliana* (Tee, 2019). Positively influence the growth and stress resistance of tomato plants (Gao et al., 2025)
Protein ORANGE-LIKE, chloroplastic	LOC101247247	
Chlorophyll degradation		
Annotation of the encoded protein (NCBI)	NCBI gene ID	Functional activity of the encoded protein
G protein STAY-GREEN	LOC101260158	Interact with LHC proteins and chlorophyll catabolism enzymes (Pietrzykowska et al., 2014)
Protein STAY-GREEN homolog, chloroplastic-like	LOC101258237	
Senescence-inducible chloroplast stay-green protein 1 (SGR1)	LOC778212	

[1] Current Tomato Genome version SL4.0 and Annotation ITAG4.0, from September 2019 (https://www.solgenomics.net/organism/Solanum_lycopersicum/genome), using data of (Lan et al., 2022; Meng et al., 2025).
[2] SLM_r2.1 (GCF_036512215.1), assembly and annotation of the tomato genome, September 3, 2024 (updates October 2024–September 2025; https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_036512215.1/).

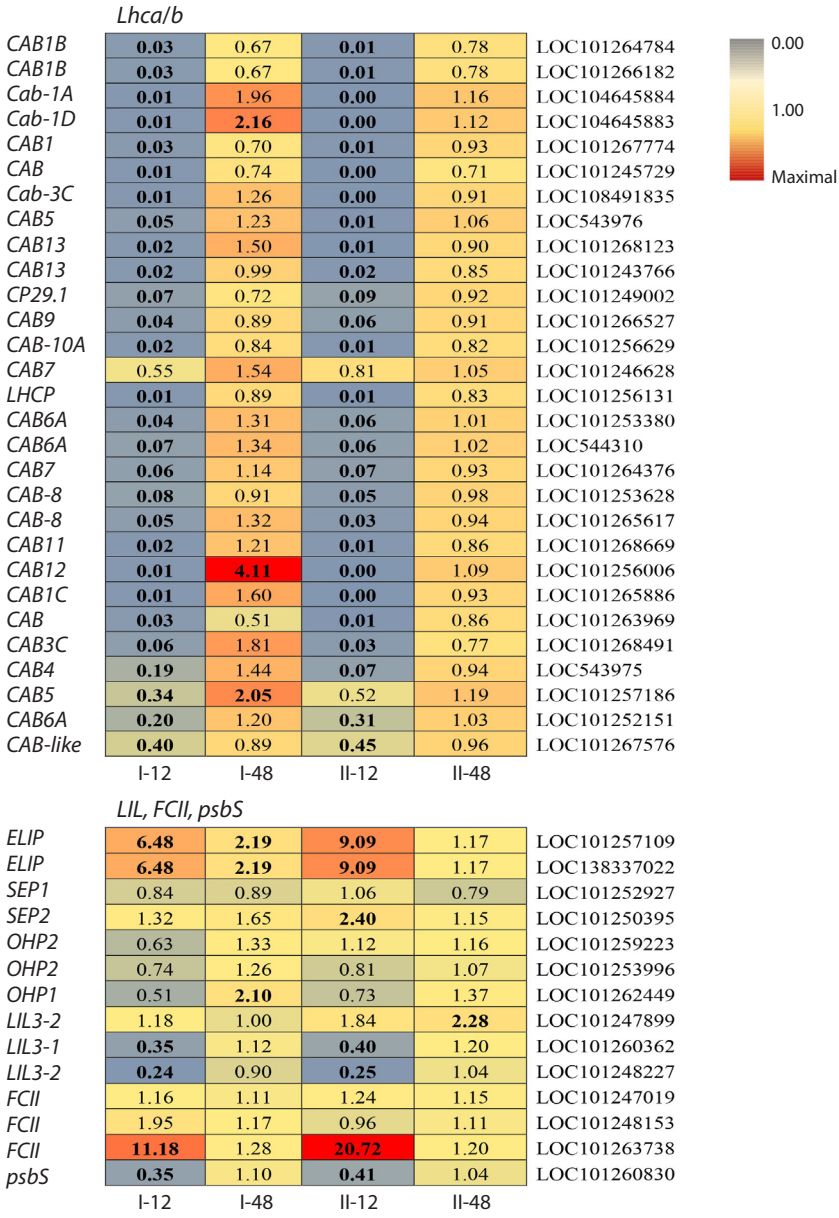


Fig. 3. Heatmap of changes in *LHC* superfamily gene expression in the leaves of the tomato cv. Otradnyi (*S. lycopersicum*) in response to two cycles (I, II) of "cold stress – recovery", constructed using our transcriptome data for points I-12, II-12 (end of stress) and I-48, II-48 (recovery period).

Here and in Figure 4: cells show RPKM values rounded to two decimal places; statistically significant differences from the control (taken as 1.00) are highlighted in bold. The cell color gradient corresponds to a decrease (shades of gray) or increase (shades of red) in gene expression compared to the control (1.00, yellow color).

We further relied on NCBI data, where the current version of the tomato genome is dated September 3, 2024, compared to SolGenomics (September 2019).

According to NCBI data, the tomato *LHC* family includes 43 genes (29 – *Lhca/b*, 10 – *Lil*, 1 – *psbS*, 3 – *FCII*) (see the Table). In addition, 18 genes of the carotenoid biosynthesis pathway, eight deoxygenase genes (catalyzing the conversion of carotenoids to the phytohormones strigolactones (SGL) and ABA), two homologous *OR* genes (synthesis of chlorophylls and carotenoids in chloroplasts) and three homologous *SGR* genes (chlorophyll degradation) were identified in tomato genome (see the Table).

The obtained transcriptome data were used to assess changes in the expression of the genes we selected (see the Table) in tomato leaves during two cycles of "cold stress – recovery" (Fig. 3 and 4).

It was shown that in both stress cycles after 12 h of cold exposure (I-12, II-12), the expression of *Lhca/b* genes decreased significantly (≥2-fold). The exceptions were *CAB7*-LOC101246628 (I-12, II-12) and *CAB5*-LOC101257186 (II-12), the level of transcripts of which also decreased at both points, however, less significantly (<2-fold). By the end of the recovery period of both cycles (I-48, II-48), the expression of *Lhca/b* genes returned to control values, with

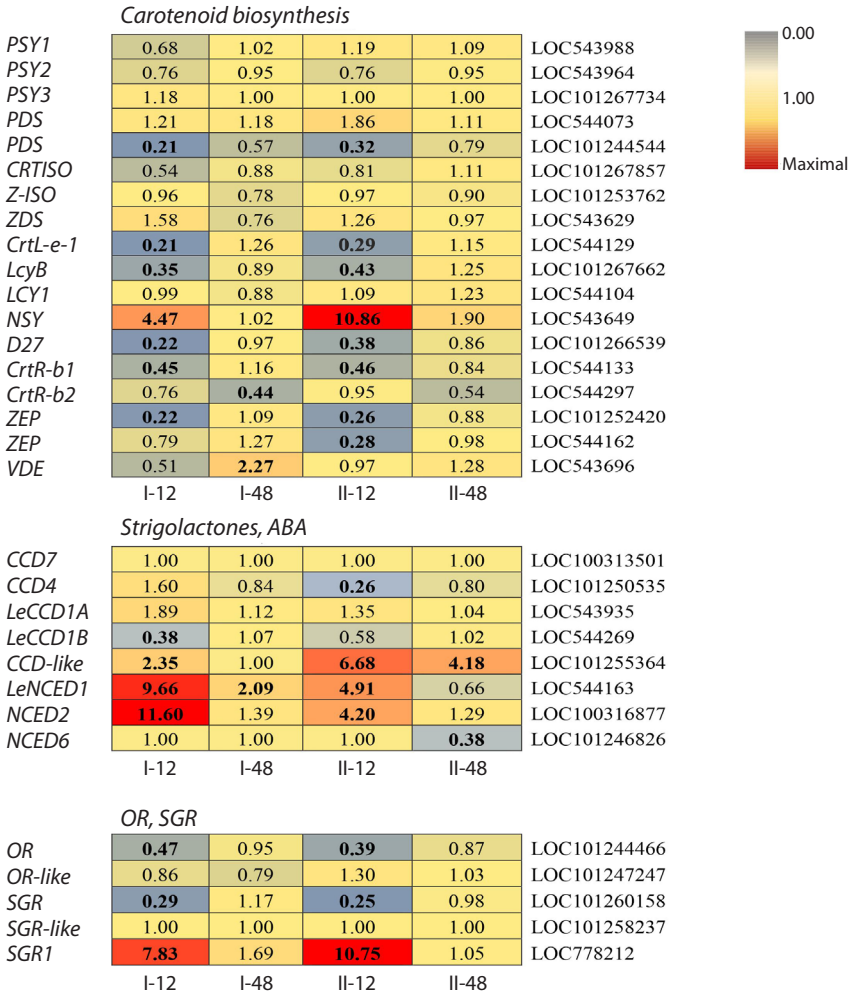


Fig. 4. Heatmap of changes in the expression of carotenoid, SGL and ABA biosynthesis genes, as well as *OR* and *SGR* genes, in the leaves of tomato cv. Otradnyi in response to two cycles (I, II) of "cold stress – recovery".

Constructed using the transcriptome data obtained for points I-12, II-12 (end of stress) and I-48, II-48 (recovery period).

the exception of an increased level of *Cab-1D*, *CAB12* and *CAB5*-LOC101257186 transcripts (I-48) (Fig. 3).

Among the other gene groups of the *LHC* family, the expression of both the *ELIP* genes and one of the ferrochelatases (LOC101263738) increases significantly towards the end of stress (I-12, II-12) and after recovery maintains an elevated value (I-48) or returns to the control level (II-48). The expression of two *LIL3* genes (LOC101260362, LOC101248227) and the *psbS* and *OHP1* genes, on the contrary, decreases in response to stress (I-12, II-12) and, upon recovery, returns to the control (I-48, II-48; except for the induction of *OHP1* in I-48). The remaining genes showed no significant changes in expression across the two stress – recovery cycles, with the exception of *SEP2* (upregulation, II-12) and one of *LIL3-2*-LOC101247899 (upregulation, II-48) (Fig. 3).

The previously formulated stress memory marker feature states that markers are genes, expression of which changes differently in response to the first and repeated stressful events, and to the recovery periods separating them (Friedrich et al., 2019; Bäurle, Trindade, 2020; Jacques et al., 2021). Given this, the *Cab-1A*, *Cab-1D*, *CAB12*, *CAB5*-LOC101257186, *ELIP*, *SEP2*, *OHP2*-LOC101259223, *OHP1* and *LIL3-2*-LOC101247899 genes, which showed intercycle differences in expression dynamics, may be considered candidates for such markers (Fig. 3).

In addition to the *LHC* family genes, transcriptome analysis of the carotenoid biosynthesis genes was performed. As a result, no significant response to both "cold stress – recovery" cycles was found for the genes of all three phytoene synthases (*PSY1–3*), one of the two *PDS*-LOC544073, *CRTISO*, *Z-ISO*, *ZDS*, and *LCY1* (Fig. 4). The second *PDS*-LOC101244544, *CrtL-e-1*, *LcyB*, *D27*, *CrtR-b1*, and one of the two *ZEP* genes showed the same expression dynamics: a decrease (I-12, II-12) and a return to the control level (I-48, II-48); the expression of the second *ZEP* gene had similar dynamics, except for a less significant decrease at point I-12 (Fig. 4). The level of *CrtR-b2* transcripts, on the contrary, decreased after the recovery periods (I-48, II-48). Two genes deserve special mention. The expression of the first one encoding neoxanthin synthase (*NSY*) was significantly increased in response to cold exposure (I-12, II-12) and returned to control levels after the recovery period (I-48, II-48). The level of transcripts of the second gene

encoding violaxanthin de-epoxidase (*VDE*) decreased in the first stress cycle (I-12), and then increased (I-48), and in the second cycle did not differ from the control (Fig. 4).

Among the genes encoding dioxygenases involved in the pathway for converting carotenoids into phytohormones (SGL, ABA), *CCD7* and *LeCCD1A* showed no significant response to two "cold stress – recovery" cycles. *CCD4* and *NCED6* expression differed from the control profile only by a decrease at points II-12 and II-48, respectively. The transcript levels of the *LeCCD1B* and *NCED2* genes significantly decreased and increased, respectively, after stress (I-12, II-12). The *CCD-like* and *LeNCED1* genes had transcript levels significantly higher than the control, except at points I-48 and II-48, respectively (Fig. 4).

Of the genes associated with chlorophyll synthesis and degradation, two (*OR-like*, *SGR-like*) showed no differences from the control, two (*OR*, *SGR*) had significantly reduced transcript levels after stress (I-12, II-12), and one (*SGR1*) had an increased level at the same points (I-12, II-12) (Fig. 4).

Given the definition of a stress memory marker (Friedrich et al., 2019; Bäurle, Trindade, 2020; Jacques et al., 2021), the following genes may be considered candidates: *VDE*, *CCD4*, *CCD-like*, *LeNCED1*, and *NCED6* (Fig. 4).

Discussion

The plant adaptive response to cold stress is closely linked to cellular osmoregulation mediated by ABA-induced stomatal closure (Sreenivasulu et al., 2012; Agurla et al., 2018). 9′-*cis*-neoxanthin serves as a precursor of ABA, and its increased synthesis in response to stress can be accompanied by a decrease in the content of violaxanthin cycle xanthophylls important for photosynthesis, leading to a decrease in the rate of photosynthesis (Nakashima, Yamaguchi-Shinozaki, 2013; Yamori et al. 2014; Chen et al., 2020; Xu et al., 2023). This disrupts the function of the antennae of photosystems I and II (Ashraf, Harris, 2013), which include proteins of the LHC family that bind chlorophylls *a*/*b* and carotenoids (Klimmek et al., 2006; Li et al., 2025). Studying *LHC* genes is therefore important for understanding their role in the cold stress response and determining plant cold tolerance.

Since the available information on the *S. lycopersicum LHC* genes varies (Lan et al., 2022; Meng et al., 2025), the first step was the identification of genes of this family using the above-mentioned publications and GenBank data (SolGenomics and NCBI). The results obtained differed from those of the studies (Lan et al., 2022; Meng et al., 2025) that used only the SolGenomics database, both in terms of the number and composition of the *LHC* family (see the Table). Our data are believed to be the most reliable since they are based on the latest version of the *S. lycopersicum* genome available in the NCBI database (from 2024 *vs*. 2019 in SolGenomics).

Considering the involvement of carotenoids in photosynthesis, the list of genes for analysis also included known genes of the carotenoid metabolism pathway (ElShamey et al., 2025), as well as *SGR* and *OR* genes associated with chlorophyll degradation (Pietrzykowska et al., 2014) and chlorophyll/carotenoid synthesis (Tee, 2019; Gao et al., 2025), respectively (see the Table).

Having selected the cold-resistant cv. Otradnyi for the study, we carried out two consecutive "cold stress – recovery" cycles (I, II), where each cycle included 12-h cooling (3 °C) followed by 60 h of normal conditions (Fig. 1). Since the level of *LHC* gene transcripts is regulated by circadian rhythms (Piechulla et al., 1998; Meng et al., 2025), we used control plants grown in parallel at normal temperature (23 °C), taking readings at the same points as the experiment.

Measurement of chlorophyll *a*/*b* and carotenoid contents in leaves collected at the end of the 12-h stress period (I-12, II-12) and during the recovery phase (I-48, II-48) showed that the amounts of all analyzed pigments decreased significantly at the end of the second cycle (II-48) (Fig. 2). This may suggest a more effective plant response to repeated cold stress due to a possible decrease in the rate of photosynthesis and an increase in osmoregulation, in accordance with data (Sreenivasulu et al., 2012; Agurla et al., 2018). That is, stress memory may have occurred in plants during the first cycle "cold stress – recovery", which could be reflected in transcriptomic data.

The same tomato leaf samples were used to obtain transcriptomes and analyze changes in the expression of genes of interest (see the Table): *LHC* family, carotenoid metabolism, *SGR* and *OR* genes. The observed decrease in transcript levels of most *LHC* genes and half of the carotenoid biosynthesis genes at the end of the stress exposure (I-12, II-12) (Fig. 3 and 4) may suggest a decrease in the rate of photosynthesis in tomato leaves in response to low temperature, since the rate of photosynthesis is positively associated with *LHC* gene expression (Yan et al., 2023). A sharp increase in the expression of the *SGR1* gene (Fig. 4), involved in chlorophyll degradation (Pietrzykowska et al., 2014), and a decrease in the expression of the *OR* gene (Fig. 4), positively associated with chlorophyll biogenesis and the development of thylakoid membranes (Tee, 2019), may also be associated with a decrease in photosynthesis. At the same time, a significant increase in the expression of the *NSY*, *CCD-like*, *LeNCED1*, and *NCED6* genes (Fig. 4) in I-12 and II-12 leaves may suggest increased ABA production (ElShamey et al., 2025), associated with stomatal closure in response to cold stress (Sreenivasulu et al., 2012; Agurla et al., 2018).

Previously, it was shown that the level of *S. lycopersicum LHC* gene transcripts increases during tomato plant adaptation to salt stress (Ikuyinminu et al., 2023). The discrepancy with our data (Fig. 3) can be explained both by the use of a different stress factor and by the use of the biostimulant PSI-475 by the authors before watering the plants with a saline solution (Ikuyinminu et al., 2023).

The publication (Meng et al., 2025) also presents data on the expression of *S. lycopersicum LHC* genes in response to a single-cycle 24-h cold stress. In particular, it was shown that the level of *SlLhcb1.11* (LOC108491835) gene transcripts decreases and *SlELIP1* (LOC101257109) increases (Meng et al., 2025), which is completely consistent with our results (Fig. 3). Silencing of *SlLhcb1.11* or *SlELIP1* in tomato plants leads to a decrease in chlorophyll content, impaired photosynthesis and cold resistance (Meng et al., 2025), indicating an important role of these genes in the adaptive response of *S. lycopersicum*. According to our data, the *SlELIP1* gene

may be a candidate for stress memory markers, since its expression profile differs between the first and second recovery periods (Fig. 3). In addition to *SlELIP1*, a number of genes correspond to the stress memory marker definition (Friedrich et al., 2019; Bäurle, Trindade, 2020; Jacques et al., 2021): *Cab-1A*, *Cab-1D*, *CAB12*, *CAB5*-LOC101257186, *SEP2*, *OHP2*-LOC101259223, *OHP1* and *LIL3-2*-LOC101247899, *VDE*, *CCD4*, *CCD-like*, *LeNCED1* and *NCED6* (Fig. 3 and 4).

Conclusion

Overall, in this study, we performed the first transcriptome analysis of the differential expression of *SlLHC* genes, carotenoid metabolism genes, and genes associated with chlorophyll synthesis and degradation in the leaves of a cold-tolerant tomato genotype during two consecutive cycles "cold stress – recovery". Signs of a possible decrease in the rate of photosynthesis were obtained: a decrease in the chlorophyll and carotenoid content at the end of the second cycle recovery (II-48) and a decrease in the transcript levels of most *LHC* family genes at the end of each chilling stage (I-12, II-12). At points I-12 and II-12, an increase in the expression of the *NSY*, *CCD-like*, *LeNCED1*, and *NCED6* genes was also detected, which may indicate a possible increase in ABA synthesis in response to low temperature. Based on the obtained expression dynamics, among the analyzed genes, candidates for the role of markers of tomato cold stress memory were identified, namely 10 *SlLHC* genes and five genes associated with ABA synthesis.

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