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REVIEW

The Study of the Participation of Heat Shock Proteins in the Resistance to High and Low Temperatures with the Use of Thellungiella (*Thellungiella salsuguinea*) and Transgenic Lines of Arabidopsis (*Arabidopsis thaliana*)

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Transgenic lines of Arabidopsis with *HSP101* gene in sense and anti sense orientations acquired resistance to hard heat shock (50° C 10 min or 45-47° C 1 hour) and to freezing (-4° C 2 hours) due to the preliminary 2 hour's heating at 37° C. Thus, it was shown at the first time that the induction of the resistance to hard heat shock and freezing with mild heat shock is possible in the absence of HSP101 synthesis. Thellungiella with the genome to 95-97% identical to the genome of Arabidopsis did not have higher resistance to high temperature, but was significantly more resistant to freezing. It differed from Arabidopsis by several times higher contents of HSP101, HSP60 and HSC70. Contents of these HSPs in Arabidopsis increased as a result of hardening at 4° C what was accompanied by the increase of the resistance to freezing. It is supposed that the resistances to heat and cold shocks are dependent not only from HSP101, but also from other HSPs.

Key words: Arabidopsis, heat shock proteins, resistance to heat and cold, Thellungiella, transgenic plants

Living organisms developed different mechanisms of adaptation to environmental stresses during the evolution. The most studied mechanism of the adaptation is the induction of the appearance of heat shock proteins (HSPs) which include several evolutionary conserved protein families (Vierling, 1991). All these proteins have similar functions: they solve problems induced by protein denaturation. However, each family has exceptional action mechanism. Some of them accelerate degradation of denaturated proteins (ubiquitines and ubiquitin-conjugating enzymes), other ones prevent aggregation of intermediate denaturation products (HSP70 and HSP60), and third ones (HSP100) accelerate the reactivation of denaturated proteins (Parsell and Lindquist, 1993, 1994).

Plant ability for preadaptation to high temperatures was discovered firstly by scientists of Botanical Institute of the Academy of Sciences of the USSR in 1953 (Aleksandrov, 1975). They showed that small non-lethal rise of temperature above the optimal level induces the resistance to more high lethal temperatures. It was found later that the appearance of HSPs precedes to the induction of thermoresistance (Vierling, 1991). Simultaneous appearance of thermoresistance and HSP allows supposing the existence of cause-consequence dependence between them. Direct evidences that a just namely HSPs are responsible for the appearance of the resistance to high temperatures were obtained due to the use of transgenic plants in experiments. Quietch *et al*. (2000) showed that Arabidopsis transformants having *HSP101* gene in sense orientation under 35S-CMV promoter and due to that synthesized HSP101 constantly resisted to high temperature better than non-transformed plants and plants transformed with *HSP101* gene in the anti sense orientation. The latter ones could not acquire the resistance to high temperatures after mild heat treatment. Authors postulated that HSP101 plays a key role in the acquirement of thermoresistance.

Seeds of Arabidopsis transgenic lines used in experiments of Quietch *et al.* (2000) were presented kindly to us by S. Lindquist (University of Chicago) in 2006 year. Seeds were reproduced in our institute and used in experiments which are presented lower.

1. THE RESISTANCE OF ARABIDOPSIS TRANSGENIC PLANTS TO HIGH TEMPERATURES

The obtaining Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.) cell cultures and their use in experiments were described earlier (Rikhvanov *et al.*, 2007). Isolation DNA, RNA, proteins, their chromatographic separation, identification of genes, *HSP* transcripts and proteins were described in our previous publications (Gamburg *et al.*, 2011, 2014; Rikhvanov *et al.,* 2007).

It is shown in Fig. 1 that cultured *in vitro* Arabidopsis cells with sense orientation of *HSP101* gene contained protein corresponding to HSP101 even without mild heat treatment and its content increased due to 2 hour's heating at 37° C. Cells transformed with *HSP101* gene in anti sense orientation did not contain this protein without and with inductive treatment. HSP101 appeared in cells transformed with empty vector only after the inductive treatment. All this was in accordance with data of Queitch *et al.* (2000). HSP17.6 appeared in all cell cultures after heating at 37°.

Heat shock was given as 10 minute's heating at 50° C in our initial works (Rikhvanov *et al.*, 2007). The same shock we applied initially in our experiments with tissue and cell cultures of Arabidopsis transgenic lines. As shown in Table 1, callus tissue of S-line with *AtHSP101* in sense orientation and A-line with *AtHSP101* in anti sense orientation did not differ after this treatment with the duration of 8-10 min due to total growth inhibition. Distinct difference between these lines were observed only at the duration of 2 and 4 minutes where S-line was significantly more resistant. It may be concluded that this difference was caused by HSP101 presence in S-line. However, degree of its synthesis was not sufficient for the resistance to 8 and 10 minutes of heating, whereas preliminary 2 hour's heating at 37° C induced resistance of cultured Arabidopsis (Columbia strain) cells even to 10 minutes of heating at 50° (Rikhvanov *et al.*, 2007). It may be supposed that treatment with 37° C induced other changes necessary for thermoresistance in addition to the induction of HSP101 synthesis one of which may be the induction of HSP17.6 synthesis (Fig. 1).

To work with 10 and less minutes of heating was not convenient. Heating at 45-47° C with longer durations was applied in following experiments. Data presented in Table 2 shows that the ability of callus tissue of S-line for TTC (2,3,5-triphenyl-tetrazolium chloride) reduction and fresh weight of tissue increased 2 and 7 days after 2 hour's heating at 37° C. TTC reduction ability of A-line was also increased 2 days after this heating, but diminished after 7 days. Fresh weight was also slightly decreased. Heating of S-line at 46º C for 1 hour caused decrease of TTC reduction and fresh weight 2 and 7 days after treatment, but this decrease was statistically not significant. The same heating of A-line caused decrease of TTC reduction on 85% after days 2 what was significant, and the ability for TTC reduction disappeared totally 7 days after the treatment. It means that cells were dead. Tissue fresh weight was 2.5 times less than without thermal treatments. Heating of S-line at 37º C given before heating at 46º C caused no increase of TTC reduction in comparison with heating at 46° only 2 days after thermal treatments, but alleviated negative effect of hard heat shock on the ability for TTC reduction and fresh weight after 7 days. Preliminary to 46º C treatment heating at 37º C of A-line prevented cell death and restored the ability for TTC reduction.

Data obtained with tissue cultures were conformed in experiments with seedlings. As shown on Fig. 2, seedlings of S-line retained chlorophyll after heating at 47º for 1 hour, whereas seedlings of A-line and O-line (transformed with empty vector) lost it. However preliminary heating at 37º C removed this negative effect. Data in Table 3 coincided with these results. Most of seedlings of A-line became dead after heating at 47º 1 hour and could not grow. Therefore their fresh weight was less than without this heating, but preliminary mild heating restored growth of A-line (Table 4). **Thus, A-line was very less resistant to high temperatures, but it could acquire this resistance due to the mild heat shock treatment what was different from data of Queitch** *et al.* **(2000).**

2. THE RESISTANCE OF ARABIDOPSIS TRANSGENIC PLANTS TO FREEZING.

It is known that HSPs participate in the resistance to abiotic stresses apart from heat (Vierling, 1991; Larkindale and Vierling, 2008). We tried to estimate the participation of HSP101 in freezing tolerance using transgenic lines of Arabidopsis. Seeds of S- and A-lines were sown in plastic vials with soil and grown at 23/17º C (day/night 12/12 h) for 1 month. Then half of vials were transported to 4º C for 1 week (hardening) and then were frozen at different temperatures for 2 hours. Survival of plants was determined 6 days after the freezing. Data of one of three experiments are presented in Table 5. Unhardened plants of both lines sustained freezing at -5º C. The hardening increased the resistance of both lines to the freezing at -6º C, whereas unhardened plants became dead. Hardened and unhardened plants of both lines could not resist to more strong frosts at -7 and -8º C. It may be concluded that difference in the HSP101 synthesis between S- and A-lines is not important for the resistance to the freezing. But leaves of both lines increased the resistance to the freezing when plants were heated preliminary at 37º C (Figure 3). This meant that acquired resistance to frost induced by 37º C is conditioned by some changes which are not related to HSP101.

3. COMPARISON OF THE RESISTANCE TO HIGH TEMPERATURE OF ARABIDOPSIS AND THELLUNGIELLA (Gamburg *et al.*, 2011).

Thellungiella salsuginea (Pall.) O.E. Schulz, other member of *Brassicacae* family, is selected as a model organism for the study of the resistance to abiotic stresses (Amtmann, 2009). It can survive at 500 mM NaCl, freezing to -19º C, resistant to water deficit and low content of nitrogen in soil. The genome of Thellungiella is to 92-95% identical to the Arabidopsis genome. However, it was not known, if it resistant to high temperatures.

We obtained cell cultures of Arabidopsis and Thellungiella and underwent them to the heating at 45º C. As can be seen in Table 6, the ability for TTC reduction decreased accordingly to the increase of heating duration from 10 to 60 minutes in both cell cultures with some faster decrease in Thellungiella. Heating at 45º C for 1 hour caused significant decrease of staining with TTC and growth of fresh cell mass in

both cultures and Thellungiella cells dyed due to this treatment. Preliminary heating at 37º C did not caused negative effects on the viability and growth of both cultures, but totally removed the inhibition induced by hard heat shock. So, cell cultures of Arabidopsis and Thellungiella acquired thermoresistance in similar manner.

Antibodies to HSPs of Arabidopsis were applied for the detection of them in cell cultures of Arabidopsis and Thellungiella (Figure 4). It may be seen that HSP101 and HSP17.6 were not observed in both cultures at 26º C, they appeared only after heating them at 37º C. The amount of HSP101 in Thellungiella was somewhat less than in Arabidopsis, but Thellungiella had significantly more HSP17.6. Thus, it may be supposed that acquirement of the resistance to hard heat shock due to heating with 37º C is caused by HSP101 and HSP17.6 in both cultures.

Amplification of Thellungiella's DNA with primers specific for *HSP* genes of Arabidopsis induced the appearance of amplification products in all cases, but number and length of fragments were different: instead of one fragment in Arabidopsis there were 2-5 fragments in Thellungiella *HSP* genes (Table 8). Only *HSP17.6*(II) gene had 1 from 4 fragments which was similar to the fragment of Arabidopsis. It may be supposed that Thellungiella had several locuses which are complemental to primers of Arabidopsis. Referent genes (β-*Tub* and *Act2*) as evident were similar in both plant species.

Results of OT_PCR with primers for *HSP* genes of Arabidopsis are shown on Figure 5. Transcripts of *HSP101* gene were not observed in Arabidopsis at 26° C, but slight signs of its presence were seen in Thellungiella. Treatment with 37° C caused the appearance of very bright spot of *HSP101* transcript in Arabidopsis and an increase of it in Thellungiella, but it remained significantly smaller than in Arabidopsis. Treatment with 37° C caused also the increase of *HSP17.6CI* in Arabidopsis and its appearance in Thellungiella and the appearance of *HSP17.6CII* in both species. Transcripts of *HSP60* were presented constantly in Arabidopsis and appeared after heating at 37° C in Thellungiella. There may be seen some coincide between HSPs visualized by immunoblotting (Fig. 4) and transcripts of *HSPs* visualized by OT_PCR (Fig. 5).

Thus, Thellungiella does not have greater resistance to high temperature in comparison with Arabidopsis and heating with 37º C caused similar changes in HSPs synthesis and induction of thermoresistance. However, it may be supposed that genomic structures of Thellungiella *HSPs* had more complicated structures.

4. COMPARISON OF THE RESISTANCE TO FREEZING OF ARABIDOPSIS AND THELLUNGIELLA (Gamburg *et al.*, 2014).

It is know that Thellungiella has high resistance to low positive and negative temperatures (Inan *et al.,* 2004; Griphith *et al*., 2007; Amtmann, 2009). Inan *et al*. (2004) showed that Thellungiella plants (ecotype Shandong) endured 24 hour's freezing at -15° C after weekly hardening at 4° C whereas Arabidopsis plants dyed at these conditions. We compared resistance to the freezing of Arabidopsis (race Columbia) and Thellungiella (ecotype Shandong) plants and its relation with HSPs contents in them.

2 week's plants of Arabidopsis and 4 week's plants of Thellungiella cultured at 23° C received hardening at 4° C for one week. Then hardened and unhardened plants were frozen 2 hours at different temperatures, thawed at 4° C 8-12 hours and cultivated 7 days at 23° C. HSPs were detected with antibodies to HSP101 (AS07 253, "Agrisera", Sweden), HSP60 (SPA_807, "StressGen Bioreagents", Canada), common HSP70 (HSP70/HSC70; SPA_820, "StressGen Bioreagents"), HSC70 (constitutive cytoplasmic HSP70; SPA_817, "StressGen Bioreagents"). Staining intensities were measured with the program Gel Analysis (Russia).

Arabidopsis and Thellungiella plants were hard and fragile at once after the freezing what proved that they were iced. Leaves damaged with frost differed from undamaged ones by loss of turgor after thawing. Most parts of these leaves dyed during following cultivation at 23° C. Unhardened Arabidopsis plants were damaged severely after the freezing at -10° C (data not shown) and -15° C (Fig. 6), whereas hardened plants withstanded successfully to this freezing. Thellungiella

plants had no need for hardening for remaining alive after the freezing (Fig. 7). These data conformed that Thellungiella plants resist to the freezing at -15° C significantly better than Arabidopsis plants.

It is shown in Fig. 8 that unhardened Arabidopsis plants contained only trace amounts of HSP101, HSP60 and HSP70, whereas unhardened Thellungiella plants possessed significant amounts of them. Hardening caused significant increase of the contents of these HSPs in Arabidopsis, but did not cause visible influence on them in Thellungiella plants. There were no differences in HSC contents between unhardened and hardened Arabidopsis and Thellungiella plants. Results of semi quantitative estimation of HSPs contents (Table 9) coincide with data presented in Fig 8. These data allowed supposing that the increase of HSP101, HSP60 and HSP70 contents is necessary for the induction of the frost resistance in the course of hardening in Arabidopsis, but it was not necessary for Thellungiella because it contained sufficient amounts of these HSPs for the frost resistance even before the hardening. However, they did not give the possibility to estimate specific role in the frost resistance of each HSP separately.

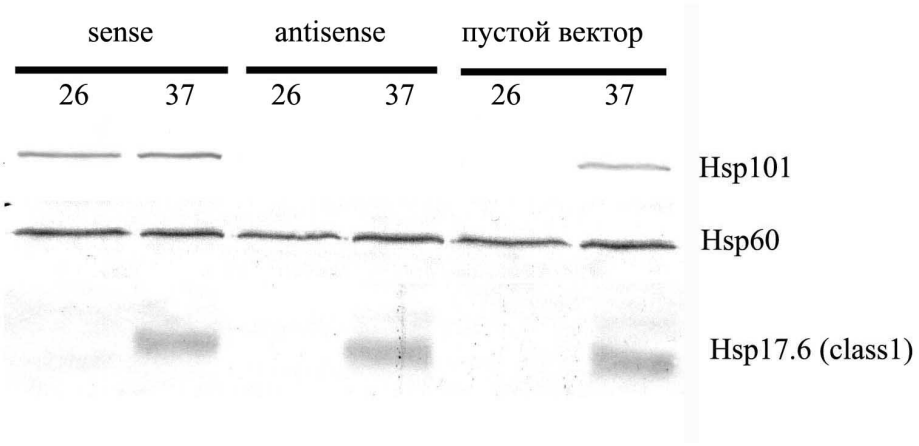


Figure 1. Immunoblotting of proteins isolated from callus tissue cultures of transgenic Arabidopsis lines S, A and O with antibodies to with and without 2 hour's heating at 37° C.

* HSP101 and HSP17.6 – the gift of E. Vierling and M. Escobar, University of Arizona, USA.

** HSP60 – SPA 807, StressGen.

Table 1. The effect of heating at 50º C on the growth of calli of Arabidopsis transgenic lines.

Duration of heating at 50º C, (minutes)	Fresh weight of calli, (mg per 1 dish)			
	S-line		A-line	
	mg	%	mg	%
0	410 ± 2	100	410 ± 21	100
2	427 ± 64	104	233 ± 11	57
4	271 ± 14	66	82 ± 10	20
6	109 ± 3	27	76 ± 0	19
8	84 ± 6	20	93 ± 17	23
10	97 ± 14	24	96 ± 0	23

Table 2. The effects of temperature treatments on the growth and viability (estimated with TTC reduction) of calli of Arabidopsis transgenic lines.

Lines	Tempe-rature	2 days after treatments		7 days after treatments			
		TTC, light absorption per 1 mg fw		Fresh weight, mg per 1 callus piece		TTC, light absorption per 1 mg fw	
S	25º	2.65 ± 0.66	100	96.1 ± 6.8	100	1.61 ± 0.26	100
	37º 2 h	4.12 ± 1.03	155	120.2 ± 20.4	125	2.24 ± 0.20	139
	46º 1 h	1.90 ± 0.47	72	71.6 ± 5.6	74.5	1.43 ± 0.12	89
	37º→46º	1.87 ± 0.47	71	112.8 ± 21.1	117	2.15 ± 0.35	133
A	25º	5.04 ± 0.76	100	111.3 ± 10.2	100	8.32 ± 0.65	100
	37º 2 h	7.37 ± 1.69	146	98.9 ± 15.4	89	5.30 ± 0.48	64
	46º 1 h	0.77 ± 0.23	15.3	44.6 ± 2.7	40*	0.09±0.026	1*
	37º→46º	3.80 ± 1.27	75.3	101.0 ± 16.3	91	5.86 ±0.44	70

* dead cells

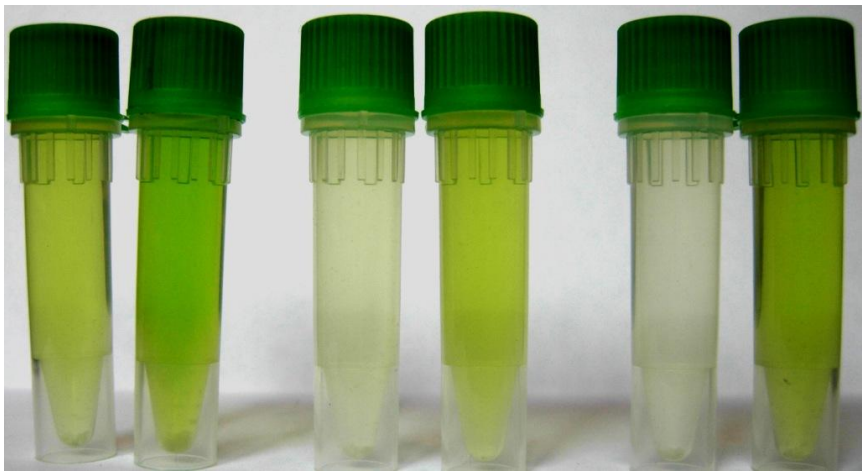


Figure 2. Effects of temperature treatments on chlorophyll contents in seedlings of Arabidopsis transgenic lines. Seedlings were extracted with 80% acetone proportionally to seedling's fresh weights.

S – line transformed with *HSP101* in sense orientation, **A** – line transformed with *HSP101* in anti sense orientation, **O –** line transformed with empty vector. Designation of flacons from left to the right: **S45 -** seedlings heated 1.5 hours at 45° C, **S37-45 –** seedlings were heated 2 hours at 37º C before heating 1.5 hours at 45° C, **A45 –** the same as **S45, A37-45 –** the same as **S37-45. O45** – the same as **S45, O37-45–** the same as **S37-45.**

Table 3. Contents of chlorophyll in seedlings of Arabidopsis transgenic lines after treatments with different temperatures (µg per 1 g fw).

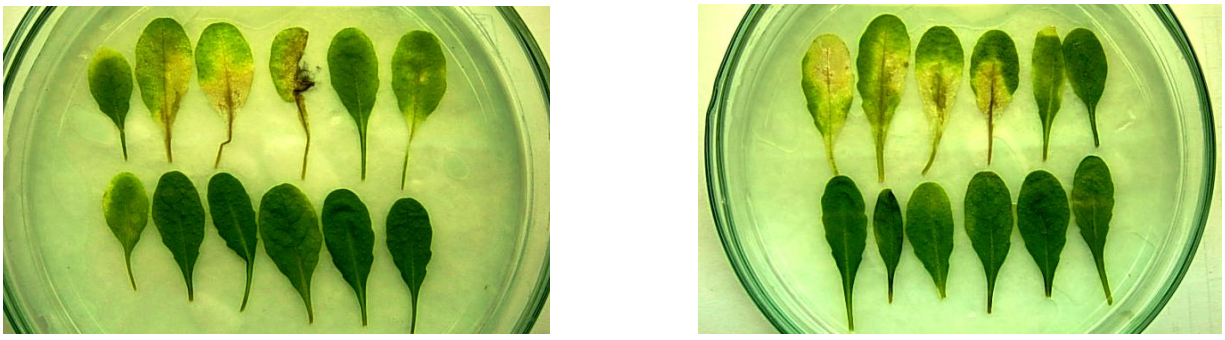
Lines	Temperatures, °C			
	23º	37º 120 min	47º 90 min	37º – 47º
S	560 ± 70	610 ± 17	570 ± 70	480 ± 70
A	498 ± 45	570 ± 10	120 ± 12	440 ± 10

Table 4. Effects of temperature treatments on the growth of seedlings of Arabidopsis transgenic lines (fresh weight, mg per 1 seedling).

Lines	Temperature treatments							
	23°	%	37° 2 h	%	47° 1.5 h	%	37°→47°	%
S	9.81 ± 0.69	100	10.10 ± 0.58	102	9.09 ± 0.22	94	10.49 ± 1.11	107
A	11.93 ± 0.48	100	8.95 ± 1.38	77	6.36 ± 0.82	57	8.97 ± 0.46	81

Table 5. The effect of hardening (8 days at 4° C) of seedlings of Arabidopsis transgenic lines on the resistance of leaves excised from them to the 2-hour's freezing at different temperatures. (Balls: turgescent = 2, yellow = 1, dead = 0, the sum of balls of 6 leaves).

t°	S		A	
	Not hardened	Hardened	Not hardened	Hardened
-5	9	12	12	12
-6	0	6	0	9
-7	0	2	0	0
-8	0	2	0	1



A S

Figure 3. The action of preliminary 2 hour's heating at 37º C of seedlings of Arabidopsis S and A transgenic lines (see Fig. 2) on the damage of leaves excised from them induced with the 2 hour's freezing at -4º C. Upper rows – leaves were not heated at 37º C, lower rows – leaves heated at 37º C.

Table 6. The effect of heating at 45° C on the viability of Arabidopsis and Thellungiella cell cultures (staining with TTC, E_{495} per 1 g fresh weight, %) (Gamburg *et al*., 2011).

Cell cultures	Duration of heating, min				
	0	10	20	40	60
Arabidopsis	100 ± 4	63 ± 5	46 ± 2	32 ± 0	20 ± 1
Thellungiella	100 ± 3	53 ± 11	33 ± 3	20 ± 1	10 ± 0

Table 7. Effects of the preliminary 2 hour's heating at 37° C and subsequent heating at 45° C 1 hour on the viability (staining with TTC 2 days after second heating, E_{495}/1g fw) and fresh weight of cell biomass (mg/ 10 ml suspension, 6 days after second heating) of cell cultures of Arabidopsis and Thellungiella (Gamburg *et al*., 2011).

Temperatures	Arabidopsis	Thellungiella	Arabidopsis	Thellungiella
	E_{495} на 1 г	E_{495} на 1 г	мг/10 мл	мг/10 мл
26º C	10.0 ± 0.3	8.4 ± 0.3	367 ± 71	474 ± 23
37º C	7.5 ± 0.1	8.3 ± 0.1	531 ± 122	423 ± 15
45º C	2.7 ± 0.01	2.6 ± 0.2	104 ± 7	27 ± 7*
37º → 45º C	9.4 ± 0.01	9.6 ± 1.0	489 ± 79	485 ± 20

* - dead cells

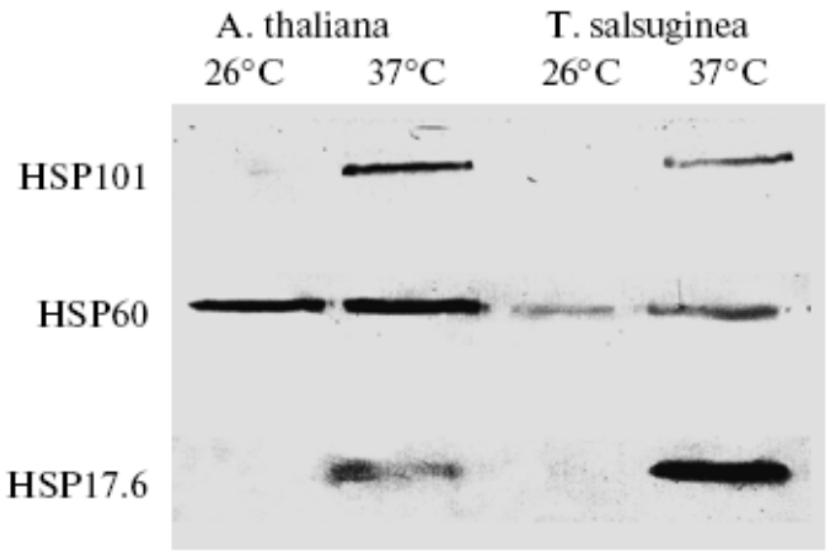


Figure 4. Immunoblotting of proteins isolated from cultured *in vitro* cells of *A. thaliana* and *T. salsuginea* with and without heating at 37° C with antibodies to AtHSPs*. (Gamburg *et al.,* 2011).
*see Fig. 1

Table 8. Amplification products of Arabidopsis and Thellungiella genomes (cell cultures) with primers to HSPs, β-tubuline and actine2 of Arabidopsis (length of amplification fragments, t. p. n.) (Gamburg *et al.*, 2011).

Genes	Locus in *A. thaliana* genome	*A. thaliana*	*T. salsuginea*
HSP101	1g74310	1.5	0.3; 0.4
HSP60	3g18780	0.89	0.6; 0.8; 1.0; 1.4
HSP17.6(I)	1g53540	0.65	0.3; 0.6; 0.7; 1.0; 1.3
HSP17.6(II)	5g56120	**0.3***	**0.3***; 0.45; 0.7; 1.25
β-*Tub*	4g20890	**0.968***	**0.968***
Act2	3g18790	**0.426***	**0.426***

* fragments with similar length in Arabidopsis and Thellungiella.

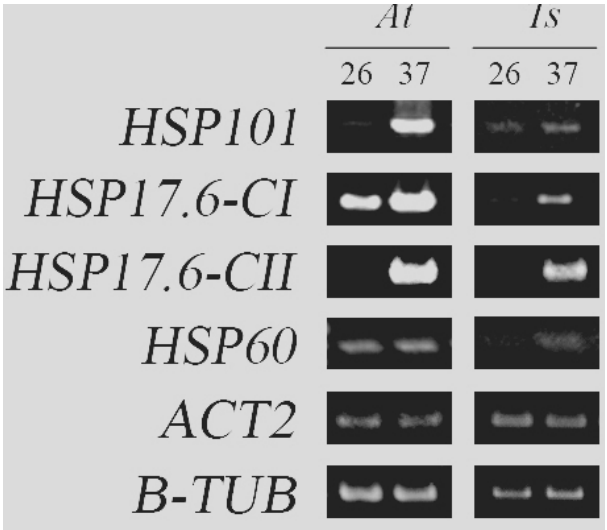


Figure 5. OT_PCR of control genes isolated from cultured *in vitro* cells of *A. thaliana* and *T. salsuginea* with and without heating at 37° C with primers to the genes of *A. thaliana* (oligodT18 was used for the construction of first chains) (Gamburg *et al.,* 2011).

Figure 6. The effect of freezing at -15° C on Arabidopsis plants not obtaining (upper row) and obtaining (lower row) preliminary hardening 7 days at 4° C (Gamburg *et al.,* 2014).

Figure 7. The effect of freezing at -15° C on Thellungiella plants not obtaining (upper row) and obtaining (lower row) preliminary hardening 7 days at 4° C (Gamburg *et al.,* 2014).

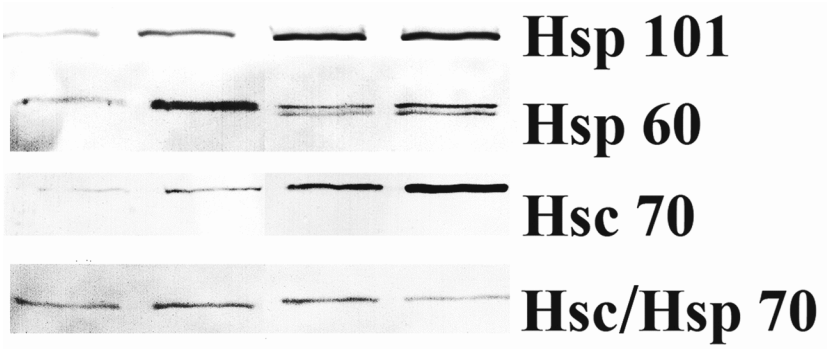


Figure 8. Immunoblotting of proteins isolated from plants of *A. thaliana* and *T. salsuguinea* with antibodies to HSP101 (AS07 253, “Agrisera”, Sweden), HSP60 (SPA_807, “StressGen Bioreagents”, Canada), common HSP70 (HSP70/HSC70; SPA_820, “StressGen Bioreagents”), HSC70 (constitutive cytoplasmic HSP70; SPA_817, “StressGen Bioreagents”)(Gamburg *et al.,* 2014).
First left line - *A. thaliana* without hardening (control), second line - *A. thaliana* with 7 days hardening at 4º C, third line - *T. salsuguinea* without hardening (control), fourth line - *T. salsuguinea* with 7 days hardening at 4º C.

Table 9. The change of HSPs relative contents (spot's intensity) in Arabidopsis and Thellungiella plants as affected by the hardening (7 days at 4° C) according to immunoblotting data (Gamburg *et al.,* 2014). Measurements of staining intensity have been made on the typical membrane.

HSP	Without hardening	Hardening	
	Thellungiella *	Arabidopsis **	Thellungiella **
HSP101	6.8	3.0	1.1
HSP60	2.1	3.7	1.1
HSC70	5.6	1.5	1.6
HSP/HSC 70	0.7	1.1	0.6

* Intensity of corresponding spot of Arabidopsis has been taken as 1.
** Intensity of corresponding spot of not hardened plants has been taken as 1.

DISCUSSION

It was shown in our works that preliminary moderate heating at 37° C prevented damage and decay of Thellungiella and **all** transgenic lines of Arabidopsis induced by subsequent heating at 45-47° C. These data contradicted to the results obtained by Quietch *et al.* (2000). They postulated that heating transgenic line with *HSP101*gene in antisense orientation at 38° did not prevent the harmful action of subsequent hard heat shock (47° 2 hours). It may be supposed that this duration of hard heat shock was too long in order to discover the acquired resistance induced with the mild heat shock. In their experiments, even the transgenic line with *HSP101*gene in sense orientation dyed at this duration of hard heat shock. This coincides with our data presented in Table 1 where growth of both transgenic lines was inhibited severely and equally at 8 and 10 minutes of the heating at 50° C. Larkidale and Vierling (2008) observed that transformed with *HSP101* and wild Arabidopsis plants differed in the response to heating at 45° C from 100 to170 min, but both dyed at the duration of 190 minutes.

Heating at 37° C removed unfavorable action of hard heat shock on all transgenic lines and untransformed plants even at 10 minute's duration in our experiments (Rikhvanov *et al.*, 2007), whereas S-line could not endure this shock without preliminary heating at 37° C. Apparently, constitutive synthesis of HSP101 in S-line was not enough for the resistance to this heat shock and something else must happen at 37° C. It may be the induction of additional HSP101 synthesis due to the activation of the transcription of own *HSP101* gene and some other genes one of which may be *HSP17.6* gene. The induction of the transcription of this gene occurred also in A-line and this coincided with the appearance of the acquired resistance in it (Fig. 1 and 2, Tables 2, 3 and 4). The appearance of small HSPs due to the mild heat shock was observed also by other authors (Malik *et al.*, 1999; Sabehat *et al.*, 1998). This HSPs form multimeric complexes (200-800 kD) which reconstruct natural conformation of denaturated proteins (Lee *et al.*, 1995; Hendrick and Hartl, 1993).

Our data concerning to the relation between the resistance to freezing and HSP101 were contradictory. On the one side, constant presence of HSP101 in S-line and its absence in A-line did not cause any difference in their reaction on the freezing (Table 5). But on the other side, preliminary heating at 37° C increased the resistance to freezing in both lines. So, acquired resistance was induced in spite of the absence of HSP101 appearance in A-line. It is possible that favorable action of the mild heating on the frost resistance may be related to the appearance of HSP17.6. Sabehat *et al.* (1998) reported that preliminary heating of unripe tomato fruit at 38° C neutralized unfavorable action of two week's storage of them at 2° C. This effect was connected with the appearance of small HSPs during the heating.

It was established in our work (Gamburg *et al.*, 2014) that Arabidopsis plants needed hardening in order to resist to freezing at -15° C, whereas Thellungiella plants had no need of it (Fig. 6 and 7). Higher resistance to frost of Thellungiella coincided with several times higher contents of HSP101, HSP60 and HSC70 without the hardening (Table 9). The rise of the resistance to

freezing of Arabidopsis plants due to the hardening coincided also with the increase of contents of the same HSPs. It is possible to suppose on the ground of these data that these HSPs participate in regulation of frost resistance. However, they do not give the possibility to elucidate the role of each HSP separately.

Comparison of electrophoregrams of HSPs presented on Fig. 4 and Fig. 8 revealed significant differences between them. Antibodies to HSPs of Arabidopsis obtained from Vierling discovered HSPs of Thellungiella which had the same mobility on electrophoregrams. However, primers to the genes of Arabidopsis discovered different sites in Thellungiella genome with different length of amplification fragments. Some of them had the same length in both species (*HSP17.6*(II), β-*Tub, Act2*). It may be supposed that genomic structure of *HSPs* of Thellungiella is more complicated than that one in Arabidopsis. It is possible also that those antibodies to HSPs obtained from E. Virling visualized in Thellungiella only HSPs identical to HSPs of Arabidopsis (Gamburg *et al.*, 2011), but antibodies bayed from biotechnological firms (Gamburg *et al.*, 2014) had higher specificity and revealed in Thellungiella additional HSPs which are absent in Arabidopsis. This can explain the difference between our data published in 2011 and 2014 concerning HSPs contents in Arabidopsis and Thellungiella.

Our data on the participation of HSPs in the regulation of the resistance to high and low temperatures coincide with data of other authors showing that their role is not limited to the regulation only of the resistance to high temperatures (Vierling, 1991; Larkindale and Vierling, 2008; Swindell *et al.*, 2007). The induction of thermoresistance is dependent on many changes, and the appearance of HSP101 is only one of them.

REFERENCES

Alexandrov V.Ya. (1975) Cells, Molecules and Temperature. “Nauka”, Leningrad Division, Leningrad, USSR. 330 P. (in Rus.).

Amtmann A. (2009) Learning from evolution: Thellungiella. *Mol. Plant*. **2**: 3–12.

Bartels D., Sunkar R. (2005) Drought and Salt Tolerance in Plants. *Crit. Rev. Plant Sci.* **24**: 23-58.

Gamburg K.Z., Varakina N.N., Rusaleva T.M., Tauson E.L., Rikhvanov E.G., Borovskiy G.B., Voinikov V.K. (2011) Comparison of the resistance to high temperature of suspension cultures of Arabidopsis (*Arabidopsis thaliana*) and Thellungiella (*Thellungiella salsuginea*). *Dokl. PAN* **439**: 421-424. (in Rus.).

Gamburg K.Z., Korotaeva N.E., Baduev B.K. Borovskiy G.B., Voinikov V.K. (2014) Interrelation between differences in the resistance to freezing of Arabidopsis and Thellungiella and contents of heat shock proteins and dehydrins. *Fisiologia rasteniy* **61**: 343-349. (in Rus.).

Griffith M., Timonin M., Wong C.E., Gray G.R., Akhter S.R., Saldanha M., Rogers M.A., Weretilnyk E.A., Moffatt B. (2007) Thellungiella: an Arabidopsis-related model plant adapted to cold temperatures. *Plant Cell Environ*. **30**: 529–538.

Inan G., Zhang Q., Li P., Wang Z., Cao Z., Zhang H., Zhang C., Quist T.M., Goodwin S.M., Zhu J., Shi H., Damsz B., Charbaji T., Gong Q., Ma S., Fredricksen M., Galbraith D., Jenks M.A., Rhodes D., Hasegawa P.M., Bohnert H.J., Joly R.J., Bressan R.A., Zhu J.-K. (2004) Salt cress: a halophyte and cryophyte Arabidopsis relative model system and its applicability to molecular genetic analyses of growth and development of extremophiles. *Plant Physiol*. **135:** 1718–1737.

Larkindale J., Vierling E. (2008) Core Genome Responses Involved in Acclimation to High Temperature. *Plant Physiol.* **146:** 748–761,

Malik, M.K., Slovin, J.P., Hwang, C.H., Zimmerman, J.L. (1999). Modified expression of a carrot small heat shock protein gene, HSP17.7, results in increased or decreased thermotolerance. *Plant J.* **20:** 1–11.

Pareek A., Singla S.L., Grover A. (1995) Immunological evidence for accumulation of two high-molecular-weight (104 and 90 kDa) HSPs in response to different stresses in rice and in response to high temperature stress in diverse plant genera. *Plant Molecular Biology* **29**: 293-301.

Parsell, D.A., Lindquist, S. (1993) The function of heat-shock proteins in stress tolerance: Degradation and

reactivation of damaged proteins. *Annu. Rev. Genet*. **27**, 437–496.

Rikhvanov E.G., Gamburg K.Z., Varakina N.N., Rusaleva T.M., Fedoseeva I.V., Tauson E.L., Stupnikova I.V., Stepanov A.V., Borovskii G.B., Voinikov V.K.. (2007) Nuclear-Mitochondrial Cross-Talk during Heat Shock in Arabidopsis Cell Culture. *Plant J.* **52**: 763–778.

Sabehat A., Lurie S., Weiss D. (1998) Expression of Small Heat-Shock Proteins at Low Temperatures. A Possible Role in Protecting against Chilling Injuries. *Plant Physiol*. **117**: 651–658

Swindell WR, Huebner M, Weber AP. (2007) Transcriptional profiling of *Arabidopsis* heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways. *BMC Genomics* **8**: 125

Vierling E. (1991). The roles of heat shock proteins in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **42**: 579–620.

Zhang X., Guo S.-L., Yin H.-B., Xiong D.-J., Zhang H., Zhao Y.-X. (2004) Molecular Cloning and Identification of a Heat Shock Cognate Protein 70 Gene, Thhsc70, in Thellungiella halophila *Acta Botanica Sinica* **46**: 1212-1219.