

# HEAT SHOCK PROTEINS HSP70 AND HSP90 IN PEA SEEDLINGS UNDER CLINOROTATION OF DIFFERENT DURATION

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## ABSTRACT

We have previously shown an increase in the Hsp70 and Hsp90 level in pea seedlings (cv. Damir) in response to clinorotation. In this work, Western-blot analysis of the Hsp70 and Hsp90 under clinorotation of different duration – from hours to days - was carried out with pea seedlings (cv. Intensive) with more intensive seed germination and seedling growth. Under clinorotation, the higher Hsp70 and Hsp90 amounts in the germinating seeds seem to be caused by some deceleration of their hydrolysis that correlated with common slowing down of seedling growth. Time-dependent changes in response to clinorotation were determined: some temporary increase in the Hsps level with the maximum for the Hsp70 at 2 h of clinorotation and for the Hsp90 – at 6 h and their subsequent returning to the control level.

## 1. INTRODUCTION

Heat shock proteins (Hsps) are known to take a part in plant response to different stresses and be a component of the multiple stress resistance mechanism [1, 2]. They being molecular chaperones determine protein folding, oligomeric assembly, transport to a particular subcellular compartment, protein and mRNA protection or degradation [2].

Microgravity (real and simulated) causing changes in metabolism and ultrastructure of cells [3] has been considered as a stress factor. A number of studies with human and animal cells has shown different changes of Hsp expression in response to microgravity from up- to down-regulation as well as absence of changes [4-7]. As Hsps are highly conserved and perform similar functions in different organisms [1, 2] so results of the experiments with animal cells can be also important for plant cells. Possible causes for the differences between the results can be species- and tissue-specificity of Hsp gene expression as well as different duration of the exposures to microgravity. In each of these experiments, only one or two periods of exposure were analyzed. However, Hsp gene expression induced by a stress factor seems to have itself dynamics.

Earlier, we determined for pea (cv. Damir) that Hsp70 and Hsp90 being abundant in dry seeds and decreasing significantly during seed germination, as it is

characteristic for seeds [1], were higher in the seedlings grown on the slow horizontal clinostat for 3 d [8]. The analyzed period coincided with the period of Hsp hydrolysis in seedlings. So it was difficult to answer whether the higher Hsp70 and Hsp90 level could be caused by a delay of hydrolysis or activation of their expression under clinorotation. Then some increasing in the Hsp70 and Hsp90 in response to short (2-10 h) clinorotation was determined [9]. In this work, pea (cv. Intensive) with more intensive seed germination and seedling growth were used to verify that results. Hsp70 and Hsp90 expression in response to clinorotation of different duration – from hours to days – was analyzed.

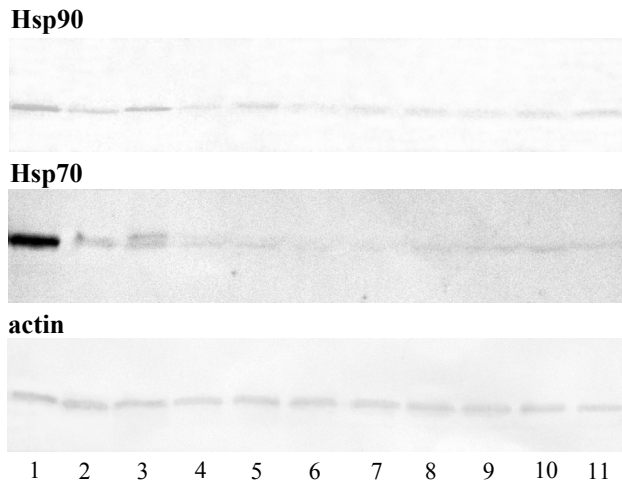
## 2. MATERIAL AND METHODS

Pea (*Pisum sativum* L., cv. Intensive) seedlings grown in the dark at  $22 \pm 1^\circ\text{C}$  were used for two variants of experiments: 1) seeds germinated and seedlings grew under slow horizontal clinorotation (2 rpm) for 1, 2, 3, 5 and 7 d; 2) 3-d seedlings grown in the stationary conditions were subjected to 2-, 4-, 6-, 16- and 24-h clinorotation. Seedlings grown in the stationary conditions served as a control. Hsp70 and Hsp90 were analyzed by Western-blot analysis after 10% SDS-PAGE electrophoresis of total soluble proteins of the seedlings. Monoclonal antibodies against Hsp70, Hsp90, actin and secondary anti-mouse antibodies coupled to biotin (Sigma) were used [8]. Quantification of the blots was done by measuring of the spot integrated density values corrected for background using ImageMaster™ TotalLab, 2.00 (Amersham). The experiments were carried out at least 3 times.

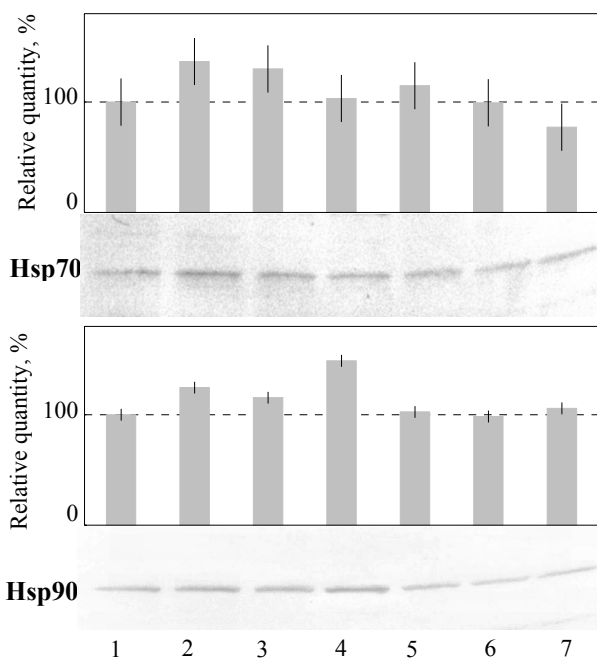
## 3. RESULTS AND DISCUSSION

Abundance of Hsp90 and especially Hsp70 in dry seeds and their reduction for 2 d of germination and seedling growth were shown (Fig. 1). Under clinorotation, Hsp70 and Hsp90 levels were higher for the first 2 d of seedling growth, but then did not differ from the control. Thus, the higher Hsp level in clinorotated seedlings was characteristic for the periods of Hsp hydrolysis for cv. Intensive as well as for cv. Damir [8] that correlated with common slowing down of seedling growth under clinorotation (data not shown). It may be explained rather by some deceleration of metabolism including Hsp hydrolysis under clinorotation at least during seed

germination and earliest seedling growth. But clinorotation prolonged up to 7 d did not change appreciably Hsp70 and Hsp90 amounts.



**Fig. 1.** Immunoblots of Hsp90, Hsp70 and actin (as an internal control of protein loading) of the embryo axes from dry pea seeds (1), 1-d (2, 3), 2-d (4, 5), 3-d (6, 7), 5-d (8, 9), 7-d (10, 11) old seedlings: control (2, 4, 6, 8, 10) and grown on the clinostat (3, 5, 7, 9, 11).



**Fig. 2.** Immunoblots of Hsp90 and Hsp70 of the pea seedlings grown for 3 days in the stationary growth conditions and then subjected to 2-, 4-, 6-, 16- and 24-h clinorotation (2, 3, 4, 5, 6) as well as 3- and 4-d control seedlings (1, 7, correspondently). Relative protein quantity was calculated as a ratio of the integrated density value of every spot to the control one. Mean values  $\pm$  SE ( $p=0.95$ ) from three experiments are shown at the graphs.

Under short-term clinorotation, we determined some temporary increasing in the Hsps level with maximum for the Hsp70 at 2 h of clinorotation and for the Hsp90 – at 6 h (Fig. 2) and their subsequent decreasing to the control level by 16-24 h. It agrees with the results for cv. Damir [9].

These data indicate development- and time-dependent changes in the Hsp70 and Hsp90 level in pea seedlings under simulated microgravity. At first, clinorotation resulted in some temporal increasing in the Hsp level for several hours following by recovery of the normal one. Apparently, clinorotation disturbs some processes, causes dysfunction of some proteins and their maintenance may temporarily need straining of Hsp functions. These events seem to be compensated during following adaptation.

#### 4. REFERENCES

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