Antarctic Science 15 (3): 345–352 (2003) © Antarctic Science Ltd Printed in the UK DOI: 10.1017/S0954102003001366

Accumulation of HSP70 in *Deschampsia antarctica* Desv. leaves under thermal stress

MARÍA ANTONIETA REYES[1,3], LUIS J. CORCUERA[2] and LILIANA CARDEMIL[1]*

[1]*Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile*
[2]*Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile*
**corresponding author: lcorcuer@udec.cl*
[3]*Present address: Departamento de Fruticultura, Instituto de Investigaciones Agropecuariarias (INIA), Casilla 439, Santiago, Chile*

Abstract : The aim of this study was to evaluate the hypothesis that *Deschampsia antarctica* Desv., the only grass of the Maritime Antarctic, responds to temperatures higher or lower than its optimum for photosynthesis (13ºC), with the accumulation of heat shock proteins. The LT_{50} was determined in plants acclimated at 4°C (cold-acclimated plants) and in plants grown at 13°C (control plants) by membrane damage and by the activity of respiratory dehydrogenases. The LT_{50} was 48.3ºC in both groups of plants when determined by membrane damage. When the LT_{50} was determined by the activity of respiratory dehydrogenases, this was, without significant difference, 47.8°C in cold-acclimated plants and 46.5°C in control plants. Western blot analyses were performed to investigate the accumulation of HSP70 at different temperatures. The optimal temperature for HSP70 accumulation was 35°C in cold-acclimated and control plants. Cold-acclimated plants subjected to a thermal stress of 35ºC accumulated HSP70 protein more than control plants subjected to 35ºC. After eight hours of continuous stress at 35ºC, the maximum relative content of HSP70 in cold-acclimated plants was 1.9 fold higher than control plants. The threshold temperature for HSP70 accumulation was lower in cold-acclimated plants than in control plants. It is concluded that *D. antarctica* is able to tolerate heat stress with an LT_{50} similar to those of heat tolerant plants and that the cold-acclimated plants accumulate higher levels of HSP70 than those grown at 13°C.

Received 16 September 2002, accepted 23 April 2003

Key words: Antarctic plants, cold acclimation, heat shock proteins, LT_{50}, thermotolerance

Introduction

Deschampsia antarctica Desv. (Poaceae) is the only grass that has naturally colonized the Maritime Antarctic (Moore 1970, Komárková *et al*. 1990, Alberdi *et al*. 2002). This species must withstand mean daily temperatures of 0°C in its habitat during the summer (Edwards & Smith 1988, Zúñiga *et al*. 1996). *Deschampsia antarctica* is also found at lower latitudes in cold areas in South America (Correa 1978). It has been suggested that this species has developed biochemical and physiological adaptations to low temperature (Casaretto *et al*. 1993). Among these adaptations are increases in the contents of lipids and soluble sugars (Zúñiga *et al*. 1994, 1996). An increase in leaf temperature with respect to ambient temperature has been observed in *D. antarctica*. The leaf temperature becomes higher than the optimum temperature for CO_2 assimilation which occurs at 13°C (Zúñiga *et al*. 1996). At 0°C, the CO_2 assimilation rate is reduced to 30% of the optimum (Edwards & Smith 1988, Xiong *et al*. 1999). Above 13°C, the rate is also reduced. Thus, *D. antarctica* is often negatively affected by high and low temperatures during the growth season (Edwards & Smith 1988, Zúñiga *et al*. 1996, Alberdi *et al*. 2002).

Membranes and proteins are among the most affected cell components during stress (Brold 1989, Palta 1990). Changes in these components can alter several processes, such as uptake of water and ions, translocation of solutes, photosynthesis and respiration, and produce inactivation of enzymes, accumulation of unprocessed peptides, and proteolysis. Thus, metabolic damage is produced and growth is, therefore, reduced (Cheng *et al*. 1982, Ferguson *et al*. 1990).

Plants living in extreme environments require a cell physiology able to compensate for stressful conditions (Brodl 1989). One common protective response in most organisms to temperature stress is the induction of genes that code for heat shock proteins (HSPs) (Lindquist & Craig 1988, Nieto-Sotelo *et al*. 1990, Goycoolea & Cardemil 1991, Li *et al*. 1999). HSP genes are induced by several types of environmental stress, such as an increase or decrease of 5–10°C with respect to the normal growth temperature of the organism (Vierling 1991, Guy & Li 1998). They accumulate also under water and saline stress, and are induced by several chemicals (Kimpel & Key 1985, Vierling 1991, Guy & Li 1998). The accumulation of these proteins is correlated with the acquisition of thermotolerance (Key *et al*. 1981, Lindquist & Craig 1988, Medina & Cardemil 1993, Schöffl *et al*. 1998, Ortíz &

Cardemil 2001). The intensity of the response to heat stress is determined by the rate, duration and magnitude of the change in temperature (Cheng *et al*. 1982). The environmental conditions, genotype, and state of acclimation of the organism determine the threshold temperature for the induction of HSP genes (Vayda & Yuan 1994). For example, *Plocamium cartilagineum* (L. Dix.), a sub-tidal red algae from the Antarctic Peninsula (sea water temperature is 0.3ºC in summer and -1.8ºC in winter), expresses HSP70 mRNA and poly-ubiquitine transcripts when the organism is incubated at 5°C. The maximum induction of HSP70 genes for the algae occurs at 10ºC (Vayda & Yuan 1994). The expression of HSP genes may also be elicited in plants at temperatures lower than normal growth temperature. Exposure of spinach to 5°C increases the synthesis of HSP70 (Neven *et al*. 1992, Guy & Li, 1998). The accumulation of HSP appears to be important in cold and heat acclimation (Cabané *et al*. 1993, Vayda & Yuan 1994, Li *et al*. 1999). HSP70 bind to denatured proteins, forcing their refolding to a native conformation and, thus, avoiding protein aggregation (Georgopoulos & Welch 1993, Schöffl *et al*. 1998, Feder & Hofmann 1999).

Since low and high temperatures induce protein unfolding, it may be suggested that in *D. antarctica,* the HSP genes could protect or prevent cellular damage by the accumulation of heat shock proteins, mainly HSP70, induced by heat or cold. The aim of this work was then to test the hypothesis that *D. antarctica* responds to temperatures higher or lower than its optimum for photosynthesis, with the accumulation of HSP70.

Material and methods

Site of collection and growth conditions of plant material

Deschampsia antarctica plants were collected in the Coppermine Peninsula, Robert Island, South Shetland Islands (62º22'S, 59º43'W), during the summer months of 1996–97. These plants were grown under laboratory conditions using plastic pots with peat moss and sterile soil in 1:1 proportion and were fertilized with a solution of 0.12g l^{-1} of phostrogen® (Solaris, Buckinghamshire, UK) and watered every three days. Control plants (non-acclimated) were maintained in a Heraphyt growth chamber (Heraeus Vötsch, Hanau, Germany) at 13°C, with a 12 hour-photoperiod under an intensity of 125 σmol/ m^2 s^{-1} and a relative humidity of 70%. A group of 40 plants homogeneous in size was acclimated for 15 days at 4°C (cold-acclimated plants).

Physiological experiments

Heat tolerance (LT_{50}) was determined by two methods:

1) measuring the lethal temperature of 50% of leaf tissue (LT50) by ion leakage using a modification of the method described by Martineau *et al*. (1979), or
2) measuring the temperature at which there is a 50% reduction of respiratory dehydrogenase activity. The dehydrogenase activity was determined by the reduction of triphenyl tetrazolium chloride (TTC) (Steponkus & Lanphear 1967).

A group of plants of *D. antarctica* was grown constantly at 13°C. Another group of plants was grown at 13°C and then acclimated to 4°C over 15 days. One hundred or 200 mg of foliar tissues were taken from both groups of plants and cut into pieces of 1 cm. The pieces were placed in flasks with deionized water (4 ml for ion leakage determinations and 1 ml for TCC experiments) and subjected for two hours to different temperatures.

For the ion leakage experiments, flasks with 200 mg leaf pieces suspended in 4 ml of deionized water were kept in a water bath at the desired temperature. After heat shock, the samples were cooled at room temperature and the volume of water was brought to 6 ml with deionized water. Each sample was incubated under agitation at 15°C for 18 h in the dark to allow diffusion of electrolytes from the plant tissues to the external solution. After this period, the flasks were maintained at room temperature and the electrical conductivity of the solution was measured. All samples were autoclaved afterwards at 121°C for 35 min to kill the foliar tissue. The flasks were cooled down at room temperature and the electrical conductivity of the solutions was determined again. The degree of damage caused by the thermal treatments was calculated by the following equation:

$$\%Injury = \left\{ \left[1 - \frac{1 - (T_1/T_2)}{1 - (C_1/C_2)} \right] \right\} \times 100$$

where T and C refer to treatment and control, respectively, and the subscripts 1 and 2 of T and C refer to initial and final conductivity, respectively. The ratio of initial conductivity/final conductivity of the treatment (T_1/T_2) is a relative measure of the amount of electrolyte leakage induced by the high temperature. It is assumed to be proportional to the amount of "injury" induced in cellular membranes (Martineau *et al*. 1979).

For the TCC experiments, 100 mg of pieces of leaves of both groups of plants were placed in 1 ml of deionized water in flasks under dark and treated at the desired temperatures for two hours. Control leaves were those maintained for two hours at 25°C. After the thermal treatment, the pieces of leaves were infiltrated under vacuum with a 0.6% (w/v) TTC in 0.05 M Na phosphate buffer, pH 7.4 for 5 min. After 20 hours of darkness at room temperature and constant agitation, the reduced TTC was extracted from the leaf tissue with 7 ml of 95% hot ethanol for 5 min. The extracts

were cooled down at room temperature and diluted with ethanol to a final volume of 10 ml. The extracts were quantified measuring A_{530}. Damage was calculated as follows:

$$\%Damage = 1 - (A_{530} \text{ treated leaves}/A_{530} \text{ control leaves}) \times 100$$

Thermal treatments

Groups of 10 plants grown at 13°C (non-acclimated plants or control plants) or acclimated at 4°C for 15 days (cold-acclimated plants) were exposed for 2 h to 25º, 30º, 35º, 40º and 45ºC. After temperature treatments, the total proteins of the plants were extracted and analysed by SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and by Western blot.

In other set of experiments, short-term and long-term kinetics of HSP70 protein accumulation were determined at 35°C, which was the optimal temperature of HSP70 protein accumulation. In the short-term kinetics, the plants were subjected at 35°C for 0 to 120 min taking samples at intervals of 10, 20 and 25 min. The amount of HSP70 was quantified densitometrically from the Western blots. In the long-term kinetics the plants were subjected to 35°C from 2 to 20 hours taking samples at intervals of 3 h and the amount of HSP70 was quantified as above.

Protein extraction and protein quantification

After temperature treatments, 100 mg of foliar tissue were macerated and homogenized with 3 volumes of extraction buffer at 5°C. The extraction buffer was 0.06M Tris-HCl, pH 6.8 containing 1% SDS, 2.5% of η-mercaptoethanol and 5% glycerol (Laemmli 1970). The extracts were boiled for 2 min and centrifuged for 7 min in a microfuge. The proteins were quantified in the supernatants using the colorimetric method of Bradford (1976). For protein quantification, a calibration curve was performed using BSA (Sigma Co.) as standard.

Protein analyses

The proteins of the extracts were separated and analysed in SDS-PAGE as described by Laemmli (1970). The stacking gel was made at 5% of polyacrylamide (w/v) and the resolving gel was made at 10% of polyacrylamide (w/v). Each gel well was loaded with 15 µg of proteins. The electrophoresis was run at 100 volts in the stacking gel and at 200 volts in the resolving gel using the Mini-Protean II chamber (Bio-Rad). Standard prestained proteins of known molecular weights were run in the same gel (Bio Labs). The protein bands of the mini gels were stained with Coomassie Blue.

Western blot analyses

For Western blot analyses the proteins were resolved in the SDS-PAGE. The gel was cut transversally in two halves. One half of the gel was electro transferred to nitrocellulose membranes to perform immunoblot analyses according to Guy *et al*. (1992). The other half was stained with Coomassie Blue to check and use it as an internal control of equal loading. The electro transference was performed using a semi-dry transfer chamber (Bio-Rad) for 40 min at 450 mA. The membranes were blocked with 10% non-fat powdered milk solution made in 20 mM Tris pH7.5 containing 500 mM NaCl (TBS buffer). The membrane was incubated with a mouse monoclonal antibody raised against the cytosolic HSP70 from bovine brain (Sigma Co) in a dilution of 1:500 in TBS buffer. The incubation was made overnight at 4°C. The membrane was washed for 20 min three times with TBS buffer containing 0.1% of Tween (TTBS buffer). The secondary antibody was an alkaline-phosphatase-goat-anti-mouse IgG (Sigma Co) diluted 1:10 000 in TBS. The membrane was developed with nitroblue tetrazolium and 5bromo-4chloro-3indolyl phosphate in 100 mM Tris-HCl, pH 9, containing 100 mM NaCl and 5 mM $MgCl_2$. The band intensities in the Western blot were densitometrically quantified using an Agfa 1212 scanner and the Kodak Digital Science 1D software (version 2.0.1). Immunoblots with serial dilutions of pure HSP71 from *Prosopis chilensis* were run for calibration curves. The results were expressed as relative amounts.

The analyses were performed three times. Because of the difficulties in comparing quantitatively blots performed at different dates, the results shown here correspond to the most representative of them.

Statistical analysis

A Kruskal Wallis test was performed to evaluate the significance of the differences between the LT_{50} of plants acclimated at 4∀C and plants grown at 13∀C.

Table I. LT_{50} of *Deschampsia antarctica.* Plants were grown at 13ºC and a group of plants was cold-acclimated at 4ºC for 15 days. Both groups of plants were treated at various temperatures for LT_{50} determinations. LT_{50} was measured by two procedures: conductivity assay and by reduction of TTC. Values are means of six replicates each ± standard error. Values of LT_{50} of plants acclimated at 4∀C and plants grown at 13∀C by TTC were not significantly different by the Kruskal Wallis test (P Ω0.05)

Growth temperature before stress (ºC)	LT_{50} by conductivity (ºC)	LT_{50} by reduction of TTC (ºC)
13	48.3 ± 0.1	46.5 ± 0.6
4	48.3 ± 0.3	47.8 ± 0.6

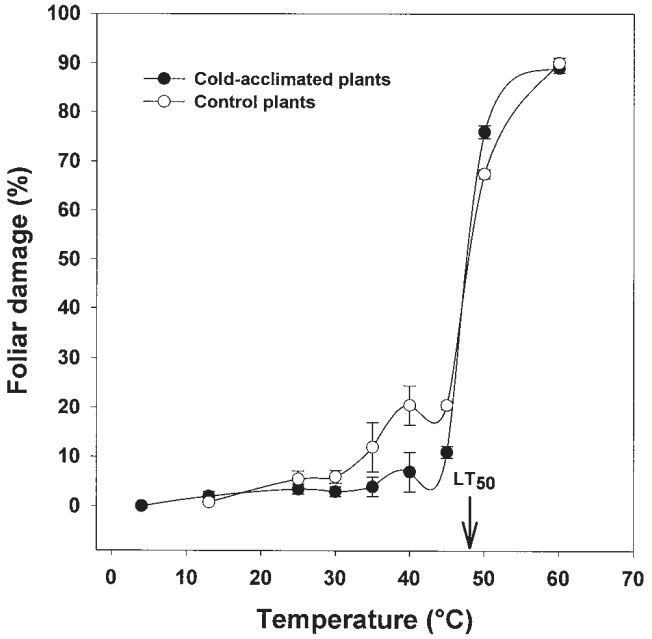

Fig. 1. Foliar damage determinations in *D. antarctica*. The damage was caused by 2 h of thermal treatments in plants acclimated at 4°C during 15 days (cold-acclimated plants) and plants grown at 13°C (control plants). The damage was determined by measuring the electrical conductivity in the external solution where the leaf tissue was incubated for the thermal treatments, as described in the material and methods. The arrow points the LT_{50} for cold-acclimated and control plants.

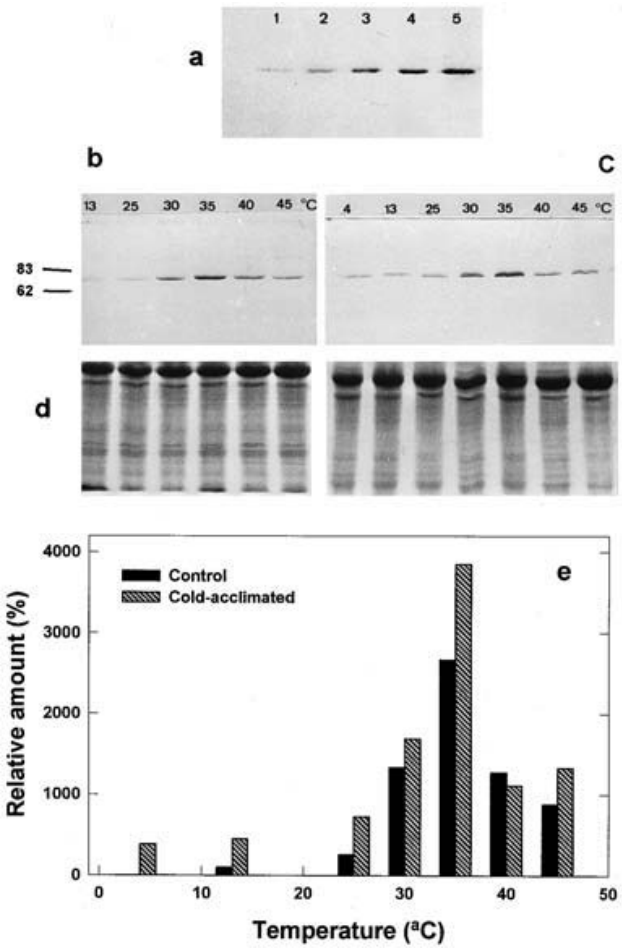

Fig. 2. Accumulation of HSP70 in *D. antarctica* leaves under heat treatment. To quantify the accumulation of HSP70 an immunoblot with serial dilutions of pure HSP71 from *Prosopis chilensis* was run (**a**). The protein amounts of pure HSP71 were: 1, 5 ng; 2, 15 ng; 3, 30 ng; 4, 50 ng and 5, 150 ng. For the experiment, plants were grown at 13ºC and a group of plants was cold-acclimated at 4ºC for 15 days. Both groups of plants were treated at various temperatures for 2 h to determine HSP70 accumulation. **b.** Western blot analysis for HSP70 accumulation in control plants, **c.** Western blot analysis for HSP70 accumulation in cold-acclimated plants, **d.** equal loading controls, **e.** relative amounts of HSP70 accumulation based on the above blots. The amounts are relative to the smallest band intensity shown by control plants treated at 13°C (100%).

Results

LT_{50} determinations

LT_{50} is defined as the temperature at which half of the tissue dies. It was measured originally by the decrease in the capacity of leaf segments to reduce triphenyl tetrazolium chloride (TTC) as described by Steponkus & Lanphear (1967). The LT_{50} determined by both methods were similar and were always between 46.5ºC and 48.3 (Table I). The LT_{50} determined by conductivity was the same in cold-acclimated plants at 4ºC and plants grown at 13°C (Fig. 1, Table I). The LT_{50} obtained by the activity of respiratory dehydrogenases with reduction of TCC was 46.5 and 47.8 in control and cold-acclimated plants respectively, without significant differences between them (Table I).

HSP70 accumulation in D. antarctica *plants subjected to different temperatures*

Figure 2a shows a Western blot of pure HSP71 of *Prosopis chilensis* run as a control illustrating the linearity between protein concentration and signal intensity.

Analyses of proteins accumulated in control and cold-acclimated plants subjected for two hours to different temperatures, detected one protein band of 70 kDa, which immuno-cross reacted with the antibody raised against the HSP72 from bovine (Fig. 2b & c). In both groups of plants, the band had its maximal accumulation at 35°C. Figure 2d shows loading controls for Fig. 2b & c. In the case of the plants acclimated at 4°C, the amount of HSP70 accumulated at 35°C was higher (1.4 fold) than the amount accumulated in control plants grown at 13°C (Fig. 2e). In addition, HSP70 started to accumulate at lower temperature in cold acclimated plants than in controls.

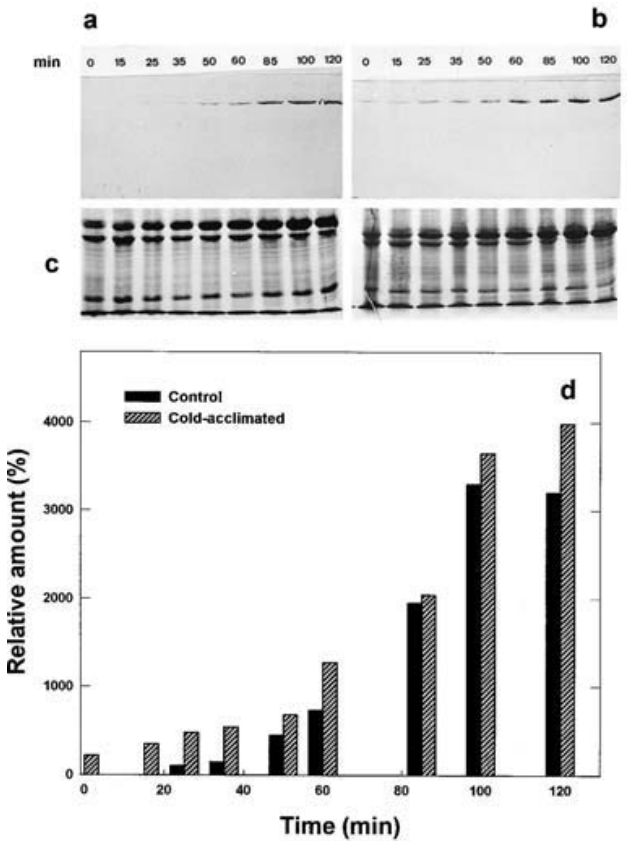

Fig. 3. Kinetics of accumulation of HSP70 in *D. antarctica* leaves under short term heat treatment. Plants were grown at 13ºC and a group of plants was cold-acclimated at 4ºC for 15 days. Both groups of plants were treated at 35ºC to determine HSP70 accumulation. **a.** Western blot analysis for HSP70 accumulation in control plants, **b.** Western blot analysis for HSP70 accumulation in cold-acclimated plants, **c.** equal loading controls, **d.** relative amounts of HSP70 accumulation based on the above blot. The protein amounts are relative to the smallest band intensity shown by control plants after 25 min treatment at 35°C (100%).

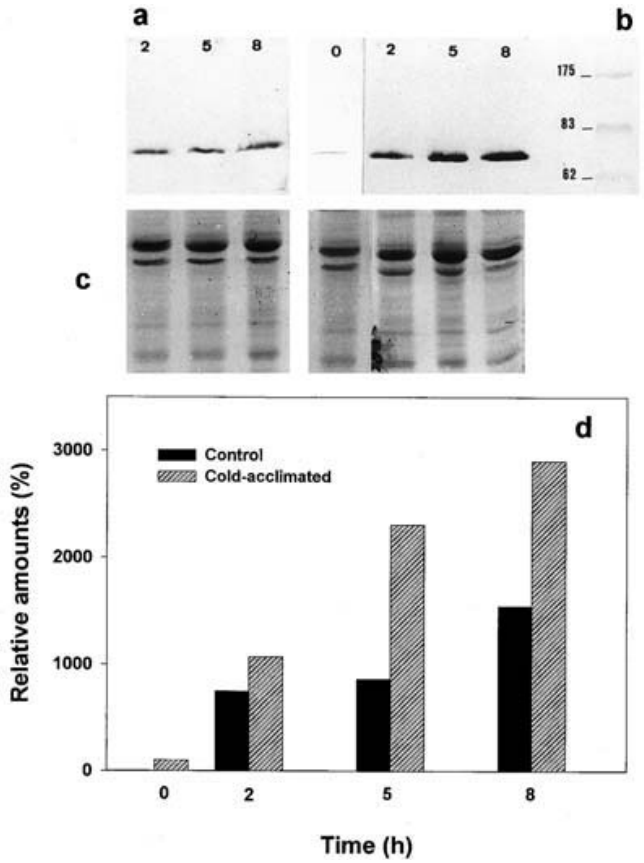

Fig. 4. Kinetics of accumulation of HSP70 in *D. antarctica* leaves under long term heat treatment. Plants were grown at 13ºC and a group of plants was cold-acclimated at 4ºC for 15 days. Both groups of plants were treated at 35ºC for 2, 5 and 8 h to determine HSP70 accumulation. **a.** Western blot analysis for HSP70 accumulation in control plants, **b.** Western blot analysis for HSP70 accumulation in cold-acclimated plants, **c.** equal loading controls, **d.** relative amounts of HSP70 accumulation based on the above blot. The amounts are relative to smallest band intensity shown by cold acclimated plants at 0 time (100%). Numbers above the protein bands are hours of heat treatment. Molecular weight markers are shown at the right of b.

Short-term kinetics of HSP70 accumulation

Kinetic studies of accumulation of HSP70 at 35°C allowed an estimate of the minimum time after heat shock for a significant increase in the basal level of the protein, the time for maximal accumulation of the HSP70, and the time at which its level begun to decline. These experiments were performed at the optimal temperature of 35°C for HSP70 accumulation, taking samples at various times after the onset of heat shock (Fig. 3a, b & d). Figure 3c shows loading controls for Fig. 3a & b. In control plants, HSP70 was detected 25 min after heat shock begun, reaching a maximum after 100 min (Fig. 3d). In cold-acclimated plants, HSP70 was detected even without treatment at 35°C, reaching a maximum at the longest times of treatment. Thus, the expression of HSP70 gene seems to be constitutive in cold-acclimated plants and inducible in control plants.

Long-term kinetics of HSP70 accumulation.

Long-term kinetic experiments were performed with control and cold-acclimated plants to determine the accumulation of HSP70 during eight or more hours of continuous heat shock at 35°C (Fig. 4a, b & d). Controls for equal loading are shown in Fig. 4c. Beyond 2 h of heat shock, HSP70 increased in both groups of plants. The accumulation of HSP70 was 1.9 fold higher in cold-acclimated plants than in control plants after 8 h of continuous heat shock (Fig. 4d). It was also possible to determine that the maximal level of the HSP70 reached at 8 h of treatment in cold-acclimated plants declining after 15 h of stress (data not shown). In control plants the maximum level was at 20 h of heat shock (data not shown). Thus, in contrast to other plants, *D. antarctica* is able to accumulate HSP70 well after 4 h of heat shock

(Cooper & Ho 1983, Goycoolea & Cardemil 1991).

Discussion

The plants living under extreme environmental conditions undergo physiological molecular and genetic changes, which may protect them from the harsh environment (Brodl 1989). *Deschampsia antarctica* is a freezing tolerant species, with an LT_{50} of -29ºC (Bravo *et al*. 2001). *Deschampsia antarctica* may suffer heat shock stress at temperatures over 13∀C which is the optimal temperature for its photosynthetic rate (Edwards & Smith 1988). Indeed, it has been reported that summer temperatures of 20∀C depress the photosynthetic rates of this plant, being these higher at 10∀C. (Xiong *et al*. 1999).

The LT_{50} at high temperature determined by membrane leakage was identical in both groups of plants (48.3°C ± 0.3). When viability was measured by the TTC reduction method, there was a small but not significant difference in the LT_{50} determinations of these two groups of plants ($P = 0.05$). Plants grown at 13°C showed a LT_{50} 1.3°C lower than the LT_{50} of cold acclimated-plants. As in the case of *D. antarctica*, a good correlation has been found in other organisms between the LT_{50} measured by membrane damage and measured by activity of respiratory dehydrogenases (Schaff *et al*. 1987).

The heat thermotolerance shown by cold-acclimated plants of *D. antarctica* may be related to the presence of HSP70, a chaperone which may be cold or heat induced, although, there are constitutive forms of the protein present in plants and other organisms. (Schöffl *et al*. 1998, Ortiz & Cardemil 2001) The HSP70 proteins seems to be partially responsible for the acquisition of thermotolerance, although they also play a protective role under other stress conditions. (Vierling 1991, Schöffl *et al*. 1998, Feder & Hoffmann 1999). Since *D. antarctica* grows at low temperature in its habitat with occasional heat stress, it is likely that HSP70 accumulation occurs in *D. antarctica* at lower and higher temperatures from its optimal photosynthetic temperature.

The temperature for maximal accumulation of the HSP70 was 35°C in both groups of plants. It has been reported that the temperature for maximal heat shock protein accumulation depends on the genotype, environmental conditions and growth and/or acclimation temperature (Vayda & Yuan 1994). Maximal accumulation of heat shock proteins in heat tolerant plants living in warm desert environments was reported to occur at 45°C (Medina & Cardemil 1993). Other plants living in warm environments, such as maize and soybean, have a maximal heat shock proteins accumulation at 40°C (Cooper & Ho 1983, Lin *et al*. 1984). Plants living in colder environments have a lower temperature for maximal HSP70 accumulation, as in the case of the sub-tidal red algae from the Antarctic Peninsula *Plocamium cartilagineum* (L. Dix) which was 10°C (Vayda & Yuan 1994) and in peas with a temperature of 37°C (Kimpel & Key 1985). In contrast to these examples, the acclimation and growth temperatures do not modify the temperature for optimal accumulation of HSP70 in *D. antarctica*, but modify the amount of the protein accumulated at 35°C, which was lower in control plants than in the cold-acclimated plants.

The threshold for temperature perception seemed to be also changed in the cold-acclimated plants with respect to non-acclimated ones. In cold-acclimated plants, HSP70 accumulation was detected at 4°C while in plants grown at 13°C a slight detection of HSP70 occurred at 13° and then at 25°C. The results of short-time and long-time kinetic experiments also demonstrated that the cold-acclimated plants respond sooner to heat shock conditions. However, HSP70 accumulated faster in control than in cold-acclimated plants within two hours. Therefore, the temperature of growth and/or acclimation modifies the threshold and the rate for thermal response, perhaps due to a faster transduction of the heat signal. It has been suggested that HSP70 may play the function of a signal transducer for the synthesis of other heat shock proteins (Sorger 1991). If HSP70 is a sensor protein for heat stress, the results of this work suggest that cold-acclimated plants of *D. antarctica* may sense the heat stress sooner and better than control plants just because the protein is present at 4°C (Ortiz & Cardemil 2001).

Long term kinetics experiments of HSP70 accumulation at 35°C in *D. antarctica* show that the plants accumulate this protein for many hours despite the literature reports that the heat shock proteins expression is transient after 3–4 h of continuous heat shock (Cooper & Ho 1983, Goycoolea & Cardemil 1991, Morimoto 1993). The long-time kinetic experiments indicated, as the previous experiments did, that the cold-acclimated plants of *D. antarctica* are better protected against heat stress than the plants at 13°C. The maximal level for HSP70 accumulation at 35°C in cold-acclimated plants was 1.9 times higher than in plants at 13°C after 8 h of thermal treatment.

Li *et al.* (1999) have identified HSP70 genes which are only expressed at low temperature. In our work with Western blot analyses, it is not possible to differentiate between the HSP70 genes that are expressed at low or at high temperatures. It may be that the protein detected at 4°C is the product of the expression of the HSP70 genes which protect the plant against cold, while the protein found in plants at 13°C and over is the product of the expression of HSP70 genes which protect against high temperature. The alternative being that HSP70 is the product of the same gene expressed under cold and/or under heat stress. HSP70 is encoded by a multigene family (Vierling 1991, Guy *et al*. 1994, Guy & Li 1998, Li *et al*. 1999) where there are constitutive and inducible genes by different environmental stress conditions (Vierling 1991, Neven *et al*. 1992, Schöffl *et al*. 1998, Li *et al*. 1999). In *P. chilensis* and in soybean

using a similar antibody, it was possible to detect two proteins expressed by heat stress, one constitutive and another inducible (Ortiz & Cardemil 2001). In spinach, 12 members of the multigene family have been identified (Guy & Li 1998, Li *et al*. 1999). If the protein of *D. antarctica* is the product of a single gene, the same protein accumulated under cold temperature may cross protect the plant against heat conditions. Since both conditions caused water losses in plants, probably HSP70 is also cross protecting the plants against dehydration. In any case, the HSP70 is probably protecting *D. antarctica* against cold and/or heat by the same type of molecular mechanism reported for many organisms. It is well known that the HSP70 proteins function as chaperones, recovering the structure of functional proteins which are denatured by cold, heat shock or other environmental stresses (Flynn *et al*. 1989, Boston *et al*. 1996, Schöffl *et al*. 1998, Feder & Hoffmann 1999).

If the protein expressed under cold temperature is a different protein from that accumulated under heat stress, cold-acclimated plants of *D. antarctica* are induced somewhat to express more and sooner the heat inducible HSP70 genes. From our results we cannot conclude if the higher accumulation of HSP70 shown by the cold-acclimated plants as compared with the control plants is due to a higher rate of HSP70 synthesis, or to a lower rate of HSP70 degradation, or to both. If the higher accumulation of HSP70 in cold-acclimated plants is due to a higher rate of synthesis, we can speculate that the threshold for thermal induction of the HSP70 gene or genes present in *D. antarctica* is a function of the temperature of growth or acclimation.

Acknowledgements

This research was supported with grants from FONDECYT 1940858 and Universidad de Chile (PG 020/96). We thank Luis González for his advice and help with the Western blot analyses. The assistances of Angélica Vega and Salvador Barahona are gratefully acknowledged. We thank the referees for their constructive comments.

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