Washington
WASHINGTON·UNIVERSITY·IN·ST·LOUIS

Department of Biology

PN-ABD-199
7.068

August 28, 1989.

Dr. Mike Benge
Project Officer
AID/S&T/Energy
Room 515 SA-18
Washington, D.C. 20523

Ref. Grant# DPE-5542-G-SS-8073-00
Project # 936-5542
Project # 7.068 (Office of Sc.Ad.)

Dear Dr. Benge:

I am mailing the Report # 2 of the above grantee, even that I was never acknowledged for Report # 1, that, I hope is in your hands.

So far, most of the funds I received at the end of December 1988, has been spent and the amount of $ 13,820 that was requested in Report # 1, is in its way to Chile, as I understood from Dr. John Daly when I phoned him a week ago from St. Louis, MO. Indeed, I am mailing this report to you from Washington University, St. Louis, Missouri, where I am staying for a month of work in the project.

As you can see from the disbursements of the budget, we have spent in reagents almost two times the original amount requested in the original project, but, we have spent less in salaries due to I have hired less graduate students to work in the project, for the year 1989. Besides, we have to buy a small equipment, a UV transilluminator (from VWR Scientific) with a cost of $ 1,431 that we need for DNA and RNA examination on agar gels.

I have talked about these changes with Paul Fritz, in Chile, since I could not get any reply of my FAXes and letters concerning the budget. So, I hope you do not mind about these changes in the items of it.

For the 2nd year of the project, I would like to have more money for reagents and supplies since molecular molecular biology chemicals are a lot more expensive now from the time I proposed the

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Rec'd in SCI SEP 19 1989

budget, back in 1986.

The cash advance requested for the next reporting period is $ 30,070. This includes the $ 13,820 that I did not received for the period May 5 to August 30 of the present year. The additional funding is for 6 months of salaries ($ 6,000), 6 months of travel ($ 5,250) and reagents ($ 5,000).

I hope this report will be of entire satisfaction to AID.

Sincerely yours,

Dra. Liliana Cardemil O.
Principal Investigator
Grant# DPE-5542-G-SS-8073-00

RESEARCH REPORT # 2

Grant # : DPE-5542-G-SS-8073-00

Period covered: May 5, 1989
to
August 30, 1989

PRELIMINARY REMARKS.-

The research proposal supported by this grant was submitted in August 1986. At the beggining of November 1988, the grant agreements were received in Chile (by slow mail) and the funds were granted by the the middle of December, 1988.

For this late start of the grant, we submitted the Research Report # 1 on May 5 of the present year. In this first report, we requested the amount of US $ 13,820 that corresponded to ½ of the salaries and ½ of the travel and reagents of the past semester. However, this amount has not been deposited yet in the account of the Universidad de Chile, Banco de Chile, Dep. del Ext. Sec. Contabilidad, account # 51, Casilla 151-D, Santiago Chile.

At this point, in order to have cash available for reagents and supplies, we have to spend from "other costs", funds that we urgently need for reinforcement of electricity and installation of air conditionning of the Laboratory.

This report is for the period May 5 to August 30. A detailed report of the expenses made during this period, is enclosed.

The results obtained from May 5 to August 30 are briefly described in this report.

DISBURSEMENTS OF BUDGET GRANT # DPE 5542-G-SS-8073-00

CUMULATIVE DETAILED REPORT FROM MAY 5 TO AUGUST 30, 1989.

	US$
Salaries	2,424
Equipment	1,431
Travel	1,794
Reagents	4,174
Other costs	12,473
Total disbursements	22,296

LIST OF EQUIPMENT PURSHASED

	US$
1.- UV Transilluminator, VWR Scientific, OS-21475-243	1,431

TOTAL DISBURSEMENTS OF BUDGET

CUMULATIVE REPORT FOR THE FIRST YEAR OF THE PROJECT
AUGUST 30. 1988 TO AUGUST 30, 1989.

	From : August 30, 1988 to May 5, 1989	From: May 5, 1989 to August 30, 1989	Total of year
	US$	US$	US$
Salaries	1,440	2,424	3,864
Equipment	45,755	1,431	47,186
Travel	5,876	1,794	7,670
Reagents	4,190	4,174	8,364
Other csts	1,230	12,473	13,703
Total	58,491	22,296	80,787

A. TEMPERATURE STRESS EXPERIMENTS

PREVIOUS RESULTS UNDER AID GRANT ON ARAUCARIA ARAUCANA SYSTEM.

We detected 8 heat shock proteins by unidimentional gel electrophoresis in SDS polyacrylamide, when the embryo axes are incubated for 30 minutes or two hours in a buffer containing a precursor of protein synthesis, that was ^{35}S-methionine. The incubation was performed at the sublethal temperatures of 32°,36°and 40°C, and the total proteins were extracted and electrophoresed. After electrophoresis, the new bands of proteins were detected by fluorography followed of exposure on a X ray film. The molecular weights of the heat shock proteins (hsp) are 126,106,89,77,44,24,19 and 17 KD. The optimal temperature for hsp synthesis and accumulation is 36°C. At this temperature Araucaria araucana seedlings aquire thermotolerance.

RESULTS OBTAINED UN HEAT SHOCK EXPRESSION DURING THIS PERIOD.

Kinetics experiments of appearnce of the hsp, reveal that as soon as 30 minutes after heat shock begins, the new proteins are present. Some of them disappear after 5 hours of continuous exposure at 36°C. The maximal accumulation of hsp occurs from 3-4 hours at 36°C.

The accumulation of mRNA for two hsp was studied by Northern hybridization analysis. We used probes for two proteins that are highly conserved among living organisms. These two hsp are the ubiquitine and the 70 KD protein. The probe for ubiquitine was a cDNA probe from chicken embryo cloned in the pBR 322 vector and the other was a maize cDNA for the 70 KD hsp cloned in a derived plasmid from pUC 19 , pMON 19.

Our results show that messengers for ubiquitine increase 6 folds in the embryo axis and 3 folds in the cotyledons during two hours of exposure at 36°C. The messenger for ubiquitine is also present at 28°C (control temperature). However, it increases considerably under heat shock. The messengers for hsp 70 KD are almost no present at 28°C and at 32°C, but the mRNA synthesis for this protein turn on under exposure at the optimal temperature for hsp expression, 36°C, Figures 1 and 2.

Our results also show that that there are one population of mRNA for ubiquitine and two populations of mRNA for the 70 KD hsp.

The *Araucaria araucana* research, performed on heat shock protein expression and mRNA synthesis for ubiquitine and hsp 70 KD, constitutes the Master dissertation of Mr. Claudio Goycoolea from whom, a copy of the dissertation abstract is enclosed,in english and in spanish, plus a copy of the print of the cover of his dissertation.

PREVIOUS RESULTS UNDER AID GRANT ON PROSOPIS CHILENSIS SYSTEM.

Seeds of Prosopis chilensis collected in Chacabuco, Peldehue, were scarified and germinated at 25°, 35°, 40°, 45° and 50°C. The germination was 100% from 25° to 35°C and more than 80% at 40°C. A few of the seeds germinate at 45° and none at 50°C. The optimal growth rate for the seedling was 35° declining at temperatures higher than 35°C. At 45° the roots stop growing, thus 45° was considered the lethal temperature for the seedling, Figure 3, A and B. Seedlings originated from seeds that have been germinated at 35°C, have the optimal growth rate at 40°C and can grow at 45°, suggesting that at 35°C the seeds acquire thermotolerance, Figure 4.

As in Araucaria araucana case, sections of embryo axes and roots of Prosopis chilensis were incubated in the presense of ^{35}S-methionine, as precursor of protein synthesis, at temperatures of 25°, 30°, 35°, 40°, 45°, and 50°C. The proteins were extracted and run in SDS-polyacrylamide gel electrophoresis. The electrophoresis was followed by fluorography and exposure to X ray film, to detect the radioactive bands of proteins.

The heat shock proteins in Prosopis chilensis are 9. One of the new proteins is very prominent and its estimated molecular weight is 62 KD. The intensity of other protein is extremely increased under heat shock. This protein has a molecular weight of about 71 KD. Figure 5, and Table I . Table I is a summary of the proteins synthesized and accumulated under temperature stress.

Kinetic experiments on hsp appearance, show that the proteins are present in the tissue as soon as 30 minutes after heat shock starts. After 4 hours of continuous heat shock, some of the heat shock proteins disappear, Figure 6.

FUTURE WORK PLANNED FOR HEAT SHOCK PROTEINS.

We are planning to perform a kinetic experiment on mRNA appearance in embryo axis of *Araucaria araucana*, to see how early after heat shock starts, the genes for ubiquitine and hsp 70 KD are transcribed. Northern hybridization analysis will allow us to study this kinetic, and the expression of mRNA for ubiquitine and the hsp 70 KD in *Prosopis chilensis*. A kinetic experiment on mRNA appearance in embryo axes of *Prosopis chilensis* will be performed as well.

B. INJURY STRESS EXPERIMENTS.

PREVIOUS RESULTS UNDER AID GRANT ON *ARAUCARIA ARAUCANA* SYSTEM.

Western analysis, PAS (periodic acid Schiff reaction) and cationic neutral gel electrophoresis of native proteins suggested that three proteins present in the cell wall extracts are similar to

extensin proteins isolated from carrots roots and soybean seed coats.

The manuscript written on this subject, enclosed in the Report #1 has been sumitted to Plant Physiology.

RESULTS OBTAINED ON INJURY STRESS DURING THE PERIOD MAY 5 TO AUGUST 30, AND FUTURE WORK PLANNED.

Two of the three proteins present in the cell walls of Araucaria araucana tissues, have purified by chromatofocusing using a PBE 118 gel of Pharmacia Fine Chemicals. The proteins have an isoelectric point between 10.6 and 10.4. We are traying at the present to separate one of the protein from the other by centrifugation in CsCl gradient.

The characterization of similar proteins in the cell walls of tissues from Prosopis chilensis just started.

ENCLOSURES:

1.- Summary of Caludio Goycoolea's dissertation.

2.- Abstract of the presentation on "Change in the pattern of protein synthesis in two trees of Chile, Araucaria araucana and Prosopis chilensis" at the VI Inter Congress of the Pacific Science Association.

3.- Figures: 1 and 2, from Araucaria araucana.
3, 4, 5, 6, and Table I, from Prosopis chilensis.

" PROTEINAS DE ESTRES TERMICO JUEGAN UN ROL FISIOLOGICO EN LA ADQUISICION DE TOLERANCIA A ALTAS TEMPERATURAS EN PLANTULAS DE *Araucaria araucana* (MOL)KOCH."

Tesis
presentada a la
Universidad de Chile
en cumplimiento parcial a los requisitos
para optar al grado de
Magister en Ciencias Biológicas
Mención en Botánica

por

CLAUDIO ALBERTO GOYCOOLEA PRADO

Prof. Patrocinante: Dra. Liliana Cardemil O.

SANTIAGO. Agosto, 1989
CHILE

RESUMEN

El objetivo de esta tesis fue investigar sobre la respuesta fisiológica que presentan semillas y plántulas de Araucaria araucana al estrés provocado por el calor y para comprobar la hipótesis de que: "Proteína de estrés térmico (PET) juegan un rol fisiológico en la adquisición de tolerancia a altas temperaturas en Araucaria araucana"; en razón de que Araucaria araucana constituye bosques relictuales desde el Terciario donde existen diferencias de temperaturas de 20°C entre día y noche y entre Invierno y Verano. Además, porque las semillas caen y germinan, y las plántulas crecen y se establecen en el suelo volcánico de estos bosques, donde la temperatura alcanza hasta 50°C a medio día en Verano.

El test del cloruro de trifenil tetrazolico permitió medir la viabilidad de las tres partes de la semilla quiescente de Araucaria araucana que son eje embrionario, cotiledones y megagametofito cuando fueron sometidos a 28°C (control) y a 32, 36, 40, 44 y 48°C. Se determinó que los ejes embrionarios pierden viabilidad después de 5 horas de estar expuestos a temperaturas de 44°C. Los cotiledones demostraron ser un tejido más sensible al calor, ya que pierde viabilidad después de 3 horas de exposición a 44°C. El megagametofito, en cambio, fue el tejido más resistente, pierde viabilidad después de 7 horas de exposición a 44°C. La pérdida de viabilidad de los cotiledones podría, sin embargo, ser una condición experimental artificial, porque los cotiledones están protegidos y aislados del medio ambiente por el tejido megagametofítico.

La tolerancia de las plántulas al estrés de calor fue determinada por la capacidad de recuperar su crecimiento a 28°C después de una exposición por 2 horas a temperaturas de 32, 36, 40 y 44°C. Plántulas expuestas a 44°C no recuperaron el crecimiento una vez vueltas a 28°C. Por lo tanto 44°C fue considerada la temperatura letal. Plántulas expuestas a 32, 36 y 40°C por 2 horas, recuperaron su crecimiento una vez vueltas a 28°C y por lo tanto fueron consideradas temperaturas subletales. La temperatura subletal que permitió mejor recuperación del crecimiento a 28°C fue la temperatura de 36°C.

Tratamiento a temperaturas subletales induce en ejes embrionarios una tolerancia para resistir una exposición de 2 horas a 44°C la temperatura letal, siendo la temperatura óptima para adquisición de termotolerancia la de 36°C, siempre que se otorgue por más de 30 minutos.

Estudios del patrón de proteínas sintetizadas y acumuladas durante el estrés, fue realizado por marcación de las proteínas usando ^{35}S metionina en el medio de incubación donde ejes embrionarios fueron sometidos al tratamiento térmico. Después que las proteínas fueron extraídas del tejido, se analizaron por electroforesis SDS en geles de poliacrilamida seguida de fluorografía del gel y exposición de éste en un film de rayos X. Se encontraron 8 nuevas proteínas no presentes a 28°C. Además 7 proteínas mostraron un aumento considerable en su expresión durante el estrés térmico.

Las 8 proteínas "de novo" sintetizadas fueron de 126, 106, 89, 77, 44, 24, 19 y 17 KD de peso molecular, mientras que aquéllas que aumentaron su expresión durante el estrés térmico

fueron de 101, 98, 94, 84, 80, 69 y 20 KD de peso molecular. La temperatura óptima tanto para la síntesis "**de novo**" de las proteínas PETs como para la acumulación de ellas fue 36°C.

Cinética realizada a 36°C demuestra que la expresión de la mayoría de las PETs de ejes embrionarios ocurre a los 30 minutos de iniciado el tratamiento a la temperatura subletal, perdurando la mayoría de ellas después de 5 horas de exposición del tejido a esta temperatura.

Análisis de hibridización Norte, de los RNA mensajeros para 2 de los PETs, usando sondas de cDNA para ubiquitina y proteína 70 KD, evidencian que el mayor acúmulo de RNA mensajero ocurre a 36°C, detectando una población de RNA mensajero para ubiquitina y dos para proteína de 70 KD.

El RNA mensajero para ubiquitina está presente a 28°C pero aumenta considerablemente durante la exposición del tejido embrionario a 36°C, llegando a ser entre 6 y 7 veces más que a 28°C en ejes embrionarios. Los RNAs mensajeros para la proteína 70 KD están muy poco expresados a 28° y 32° pero la expresión de mensajeros se enciende a 36°C.

Se concluye que las plántulas de <u>Araucaria</u> <u>araucana</u> responden bien al estrés térmico con síntesis y acumulación de PETs y de los mRNA para las PETs ubiquitina y la proteína 70 KD.

SUMMARY

The objective of this dissertation was to investigate the physiological responses of seeds and seedlings of *Araucaria araucana* to high temperature stress and with the purpose to test the hypothesis that:"Heat shock protein (hsp) play a physiological role in the acquisition of tolerance to high temperature in *Araucaria araucana*", since *Araucaria araucana* constitutes relictual forests from the Tertiary times, where there are differences of temperatures of 20°C between day and night and between Winter and Summer. Besides, the seeds are shed and germinate, and the seedlings grow and establish in the volcano soil of these forests where the temperature reaches 50°C at noon of Summer days.

The triphenyl tetrazolium chloride reagent was used to test the viability of embryo axis, cotyledon and megagametophyte tissues of quiescent seeds of *Araucaria araucana* when exposed to 28°C (control) and to 32, 36, 40, 44 and 48°C. The embryo axis loose viability after 5 hours of exposure to 44°C. The cotyledons are more sensitive to heat since they loose viability after 3 hours of exposure at 44°C.

The megagametophyte tissue was the most resistant because it looses viability after 7 hours of exposure at 44°C. The lost of cotyledons viability could be, however, an experimental condition, since in the natural system, the cotyledons are isolated and protected from the environment by the megagametophytic tissue.

The tolerance of the seedlings to heat stress was determined by the capacity to recover growth at 28°C, after 2 hours exposure

at temperatures of 32, 36, 40 and 44°C. Seedlings do not recover growth after exposure at 44°C. Therefore, 44°C was considered the lethal temperature for Araucaria araucana seedlings. Seedlings exposed for 2 hours at 32, 36 and 40°C recover growth when they are back at 28°C and therefore, they were defined as sublethal temperatures. The sublethal temperature that allows a better growth recovery of seedlings at 28°C, was the temperature of 36°C.

Treatment with sublethal temperatures induces thermotolerance to embryo axis to resist a two hours exposure of lethal temperature, being the optimal temperature for acquisition of thermotolerance 36°C if given for more than 30 minutes.

The pattern of proteins synthesized and accumulated during heat stress was determined by labelling the proteins with ^{35}S-methionine, used as precursor of protein synthesis, which was added to the incubation medium where the embryo axis were subjected to thermal treatment. After protein extraction, they were analized by SDS-polyacrylamide gel electrophoresis followed for fluorography and exposure to X ray film.

There were found 8 new proteins non present at 28°C. Besides, 7 proteins increase considerably their expression during thermal stress.

The 8 proteins "de novo" synthetized were of 126, 106, 89, 77, 44, 24, 19 and 17 KD molecular weight while those having a higher expression during thermal stress were of 101, 98, 94, 84, 80, 60 and 20 KD molecular weight. The optimal temperature for synthesis and accumulation of the hsp, was 36°C.

Kinetic experiments performed at 36°C showed that the

expression of heat shock proteins (hsp) in embryo axis can be detected as soon as 30 minutes after the thermal stress begins at this sublethal temperature, remaining most of the hsp after 5 hours of continuous exposure of the tissue at 36°C.

Northern hybridization analysis of mRNA for two hsp, using cDNA probes one for ubiquitine and one for hsp 70 KD, showed that the higher accumulation of mRNA takes place at 36°C, detecting one population of mRNA for ubiquitine and two populations of mRNA for the hsp 70 KD.

The mRNA for ubiquitine is present also at 28°C, but increases considerably during tissue exposure at 36°C, being 6 or 7 folds the amount of mRNA present in embryo axis at 28°C. The mRNAs for the 70 KD protein are very low expressed at 28°C and 32°C but the expression is turn on at 36°C.

Conclusions are made that <u>Araucaria araucana</u> seedlings efficiently respond to thermal stress with synthesis and accumulation of hsp and of mRNA for ubiquitine and hsp 70 KD.

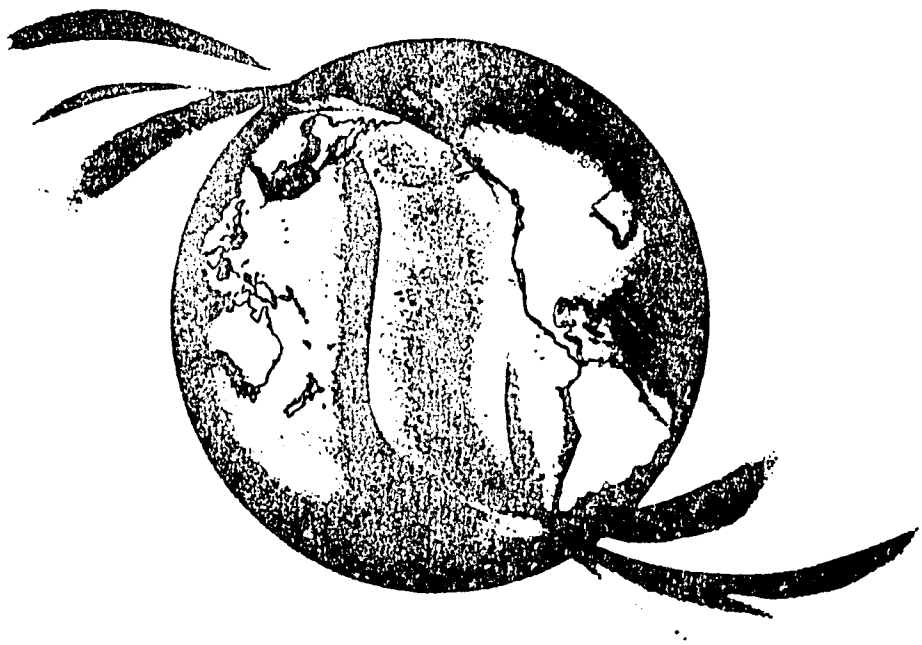
PACIFIC SCIENCE ASSOCIATION

VI INTER CONGRESS

VALPARAISO - VIÑA DEL MAR, CHILE, AUGUST 7-10, 1989

THE PACIFIC, BRIDGE OR BARRIER?

ABSTRACTS



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THE INSTITUTE OF INTERNATIONAL STUDIES
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INFORMATION: CONDELL 249 SANTIAGO 9 - CHILE. P.O. BOX 14187 SUC. 21
SANTIAGO, CHILE, CABLES: INTERACADEMIC:

16:30 - 16:50 Hrs. Convergences and non-convergences between Central Chile and California: What is the role of human disturbance?
Professors E. Fuentes, J.A. Simonetti and M.R. Muñoz, Pontificia Universidad Católica de Chile. Santiago, Chile.

16:50 - 17:10 Hrs. The response of communities of small mammals to fire: comparisons between Australia, California and Chile
Professor B. Fox, University of New South Wales, Australia.

17:10 - 17:30 Hrs. Summing up: Are there more differences than similarities?
Doctor Mary T. Kalin de Arroyo and J.Armesto, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

17:45 - 18:30 Hrs. GENERAL SYMPOSIUM II.

WEDNESDAY AUGUST 9, 1989:

8:30 - 9:20 Hrs. -GENERAL SYMPOSIUM I.

SPECIAL INTEREST SYMPOSIUM

PHYSIOLOGY OF LAND PLANTS OF THE PACIFIC BASIN: Plant Physiological Responses to Stress conditions in Ecosystem of the Pacific Basin.

CHAIRMAN : Professor Liliana Cardemil
SECRETARY : Professor Jon Keeley

9.30 - 9:50 Hrs. Metabolic Consequences of Drought Stress in Plants.
Doctor Leslie Paleg, Department of Plant Physiology, Waite Agriculture Research Institute, University of Adelaide, Australia.

9:50 - 10:10 Hrs. Woody Plant Responses to Environmental Stress in South Central Chile,
Doctor Mirem Alberdi, Instituto de Botánica, Universidad Austral de Chile. Valdivia, Chile.

10:10 - 10:30 Hrs. Woody Plant Responses to Environmental Stress in South Central Chile,
Doctor Mirem Alberdi, Instituto de Botá-

	nica, Universidad Austral de Chile. Valdivia, Chile.
10:30 - 10:50 Hrs.	Change in the Pattern of Protein Synthesis in Two Trees of Chile, Araucaria araucana and Prospis chilensis, Induced by High Temperature. Doctors Liliana Cardemil, Claudio Goycolea and Consuelo Medina, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.
10:50 - 11:15 Hrs.	Coffee Break.
11:30 -14:25 Hrs.	Boat trip along the Bay of Valparaíso. (Including snacks).
14:30 - 14:50 Hrs.	Environmental Stress and Resistance of Barley to Aphids. Doctor Luis Corcuera, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.
14:50 - 15:10 Hrs.	Photosynthetic Diversity in Vernal Pool Aquatics. Doctor Jon Keeley, Occidental College, Los Angeles, California, U.S.A.
15:10 - 15:30 Hrs.	Seasonal changes in Acetylene reduction Activiy (Nitrogen Fixation) of the Actinorizal Members of the Rhamnaceae Species Growing in the chilean matorral. Doctor Orlando Balboa, Universidad de La Pampa, Santa Rosa, La Pampa, Argentina.
15:30 - 15:50 Hrs.	Coffee Break.
15:50 - 16:10 Hrs.	Seed Longevity and strength relationship with seed dispersal and Plant distribution in Tropical Forest Ecosystems. Doctor Carlos Vásquez Yañez, Centro de Ecología, Universidad Autónoma de México, México.
16:10 - 16:30 Hrs.	Seed germination and life history characteristics of Californian Chaparral Shrubs. Doctor Jon Keeley, Occidental College, Los Angeles, California, U.S.A.
17:45 - 18:30 Hrs.	GENERAL SYPOSIUM II
19:30 - 20:30 Hrs.	Folklor dance and music. Municipal Theater, Viña del Mar.

CHANGES IN THE PATTERN OF PROTEIN SYNTHESIS IN TWO TREES OF CHILE, ARAUCARIA ARAUCANA AND PROSOPIS CHILENSIS, INDUCED BY HIGH TEMPERATURE.

Cardemil, L.; Goycoolea, C. and Medina, C. Departamento de Biologia, Facultad de Ciencias, Universidad de Chile.

Araucaria araucana constitutes temperated relictual forests in the high mountains of Chile Austral, with 20ºC differences between days and nights and between seasons. Prosopis chilensis instead, is a leguminous, nitrogen-fixing tree of the arid and semi arid regions of Chile with temperatures reaching 60ºC or higher, in Summer time. We have investigated how seeds and seedlings of these two plant trees, respond to high temperature exposure, performing heat shock experiments, and how seeds and seedlings acquire thermotolerance when exposed to sublethal temperatures. Indeed, the main idea of this research has been to test the hypothesis that "Native plants are adapted to their natural environments because they show a higher degree of expression of those genes encoding proteins related to plant protection during environmental harsh conditions". The higher degree of expression may be due either to regulatory mechanisms of gene function or to the number of copies of the genes involved. SDS-gel electrophoresis of proteins synthesized during heat shock experiments and Northern hybridization analysis to detect the mRNA of these proteins, using probes for heat shock genes, allow to evaluate this hypothesis. Proyecto FONDECYT 0160/88 and AID grant # DPE-5542-G-SS-8073-00. Universidad de Chile Proyecto de Desarrollo # 178501-4.

FIGURES FROM *ARAUCARIA ARAUCANA* HEAT SHOCK EXPERIMENTS.

Figure 1.- X ray film of a Northern blot hybridization of total RNA extracted from cotyledons (C) and embryo axes (E) of *Araucaria araucana* seedlings exposed for 2 hours at different temperatures (28°, 32° and 36°C). The hybridization was performed with a probe containing the cDNA information for the ubiquitine protein fron chick embryo."Cont." is a positive control where 3 ug of maize RNA extracted from plants subjected to 42°C, was run. The 25S and 18S mark the points where the ribosomal RNA for the large and the small subunits run in the gel and were used for size estimation of RNA. The two channels marked as " C 28°C* " were overloaded with 3 times the RNA amount present in the extracts of the cotyledons exposed at 28°C and with 5 times the amount of RNA present in the embryo axes exposed at 28°C, thus to estimate how much more mRNA for ubiquitine was present in tissues exposed at 36°C.

Figure 2.- X ray film of Northern blot hybridization ot total RNA extracted from megagametophyte (M) and cotyledons (C) of *Araucaria araucana* seedlings exposed for two hours at different temperatures (28°, 32° and 36°C). The hybridization was performed with a probe containing the cDNA information for hsp 70 KD form Maize."Cont." is a channel for a positive control where 3 ug of maize RNA extracted from plants subjected at 42°C. 25S and 18S mark the points where the ribosomal RNA for large and small subunits run in the gel, and was used for size estimation of RNA.

FIGURES FROM PROSOPIS CHILENSIS, HEAT SHOCK EXPERIMENTS

Figure 3.- Germination and seedling root growth of Prosopis chilensis at different temperatures.

A. Percent of germination of seeds after imbibition at different temperatures.

24 hours after imbibition

48 hours after imbibition

B. Growth rate of seedling roots at different temperatures.

24 hours after imbibition

48 hours after imbibition

Figure 4.- Growth rate of seedling roots of Prosopis chilensis when seeds are germinated at 35°C. Arrows point that, after 18 hours of imbibition seedling were transferred to the indicated temperature.

Figure 5.- X ray film of SDS-polyacrylamide gel electrophoresis of proteins present in embryo axis of Prosopis chilensis when the tissue is incubated for two hours at different temperatures in the presence of ^{35}S-methionine. The methionine was added to the medium in the last 30 minutes of two hours of incubation. Numbers at the left, are the molecular weight markers run in the same gel. Complete arrows point those bands of proteins non present at 35°C. Arrow heads point those bands of proteins which increase intensity at temperatures higher than 35°C.

Figure 6.- Kinetic experiments of appearance of the main bands of proteins that are new or increase at 45°C. ^{35}S-methionine was added the last 30 minutes of the incubation time. Experiments were terminated at 0.5, 2.0 and 4.0, hours after the beginning of the incubation. Numbers at the left are the molecular weight markers run in the same gel. Arrows indicate the main bands of proteins that are new or increase at 45°C compared with those present at 35°C.

Table I.- Summary of the proteins synthesized and accumulated in embryo axis of *Prosopis chilensis*, incubated for 2 hours at temperatures in the presence of ^{35}S-methionine added in the last 30 minutes of incubation.

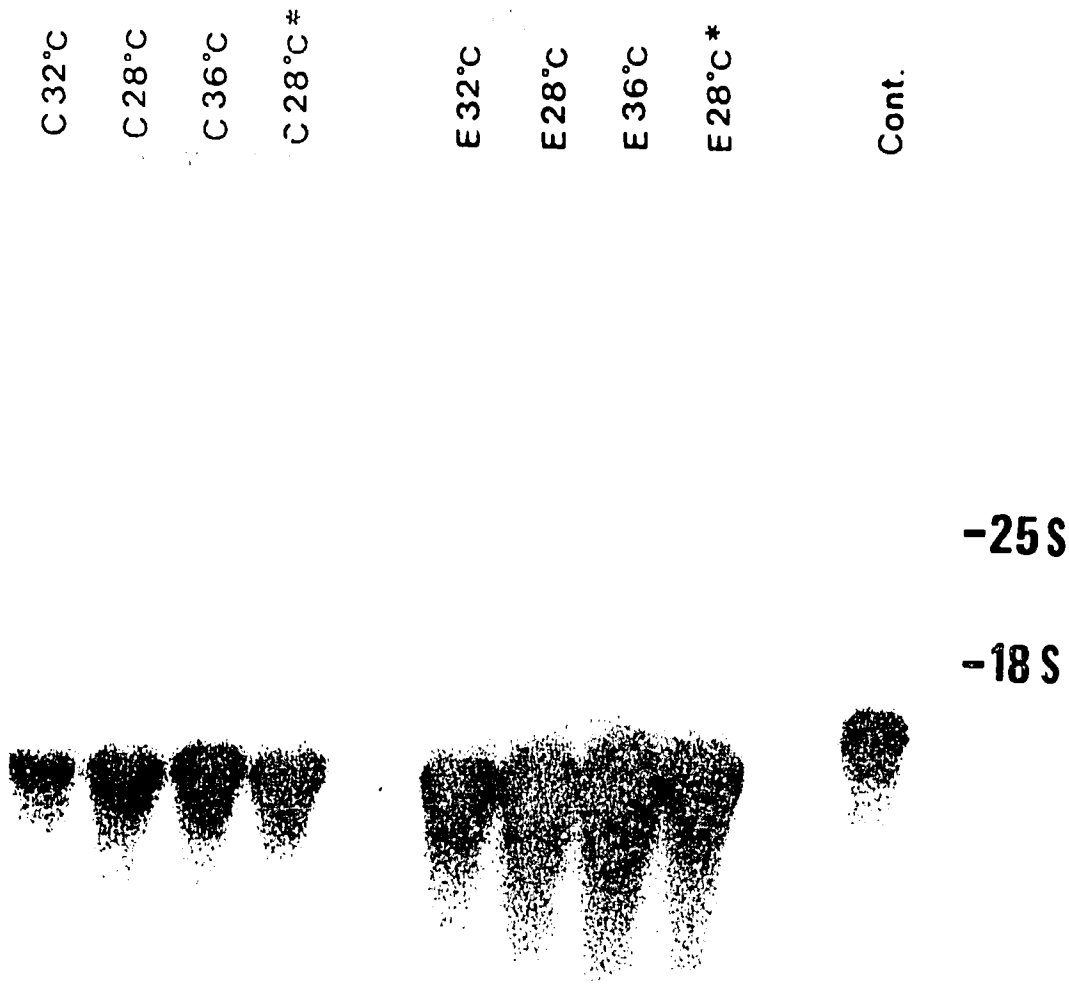

Figure 1

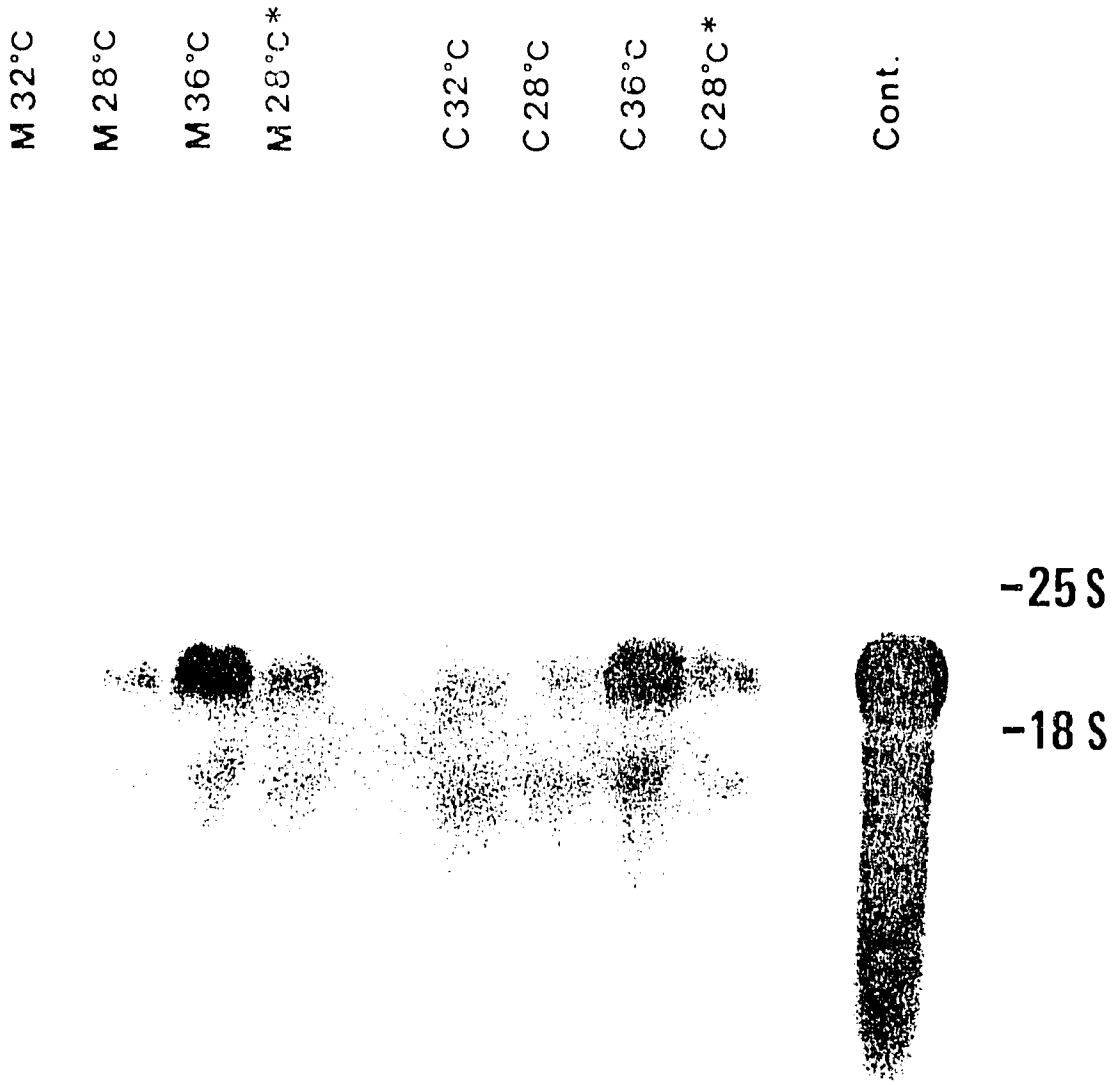

Figure 2

GERMINACION Y CRECIMIENTO RADICULAR DE *Prosopis chilensis* A DIFERENTES TEMPERATURAS

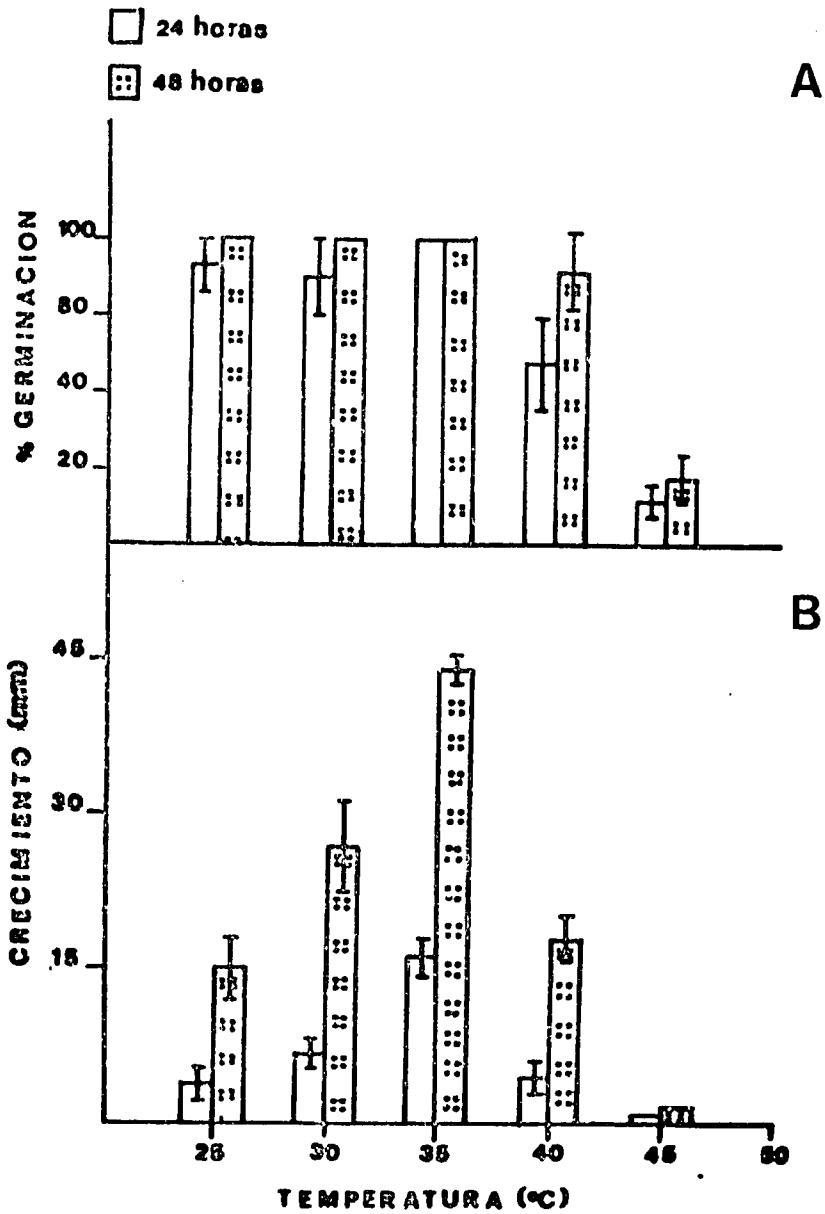

Figure 3

CRECIMIENTO RADICULAR DE *Prosopis chilensis* A DIFERENTES TEMPERATURAS LUEGO DE GERMINACION A 35°C

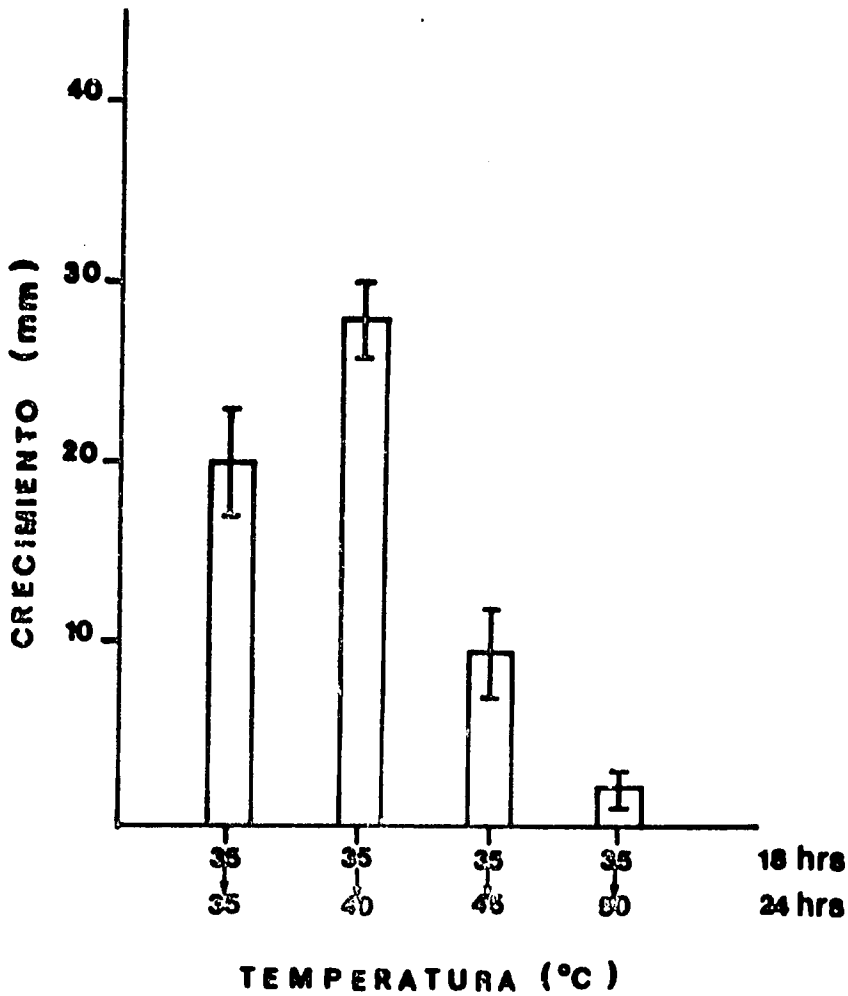

Figure 4

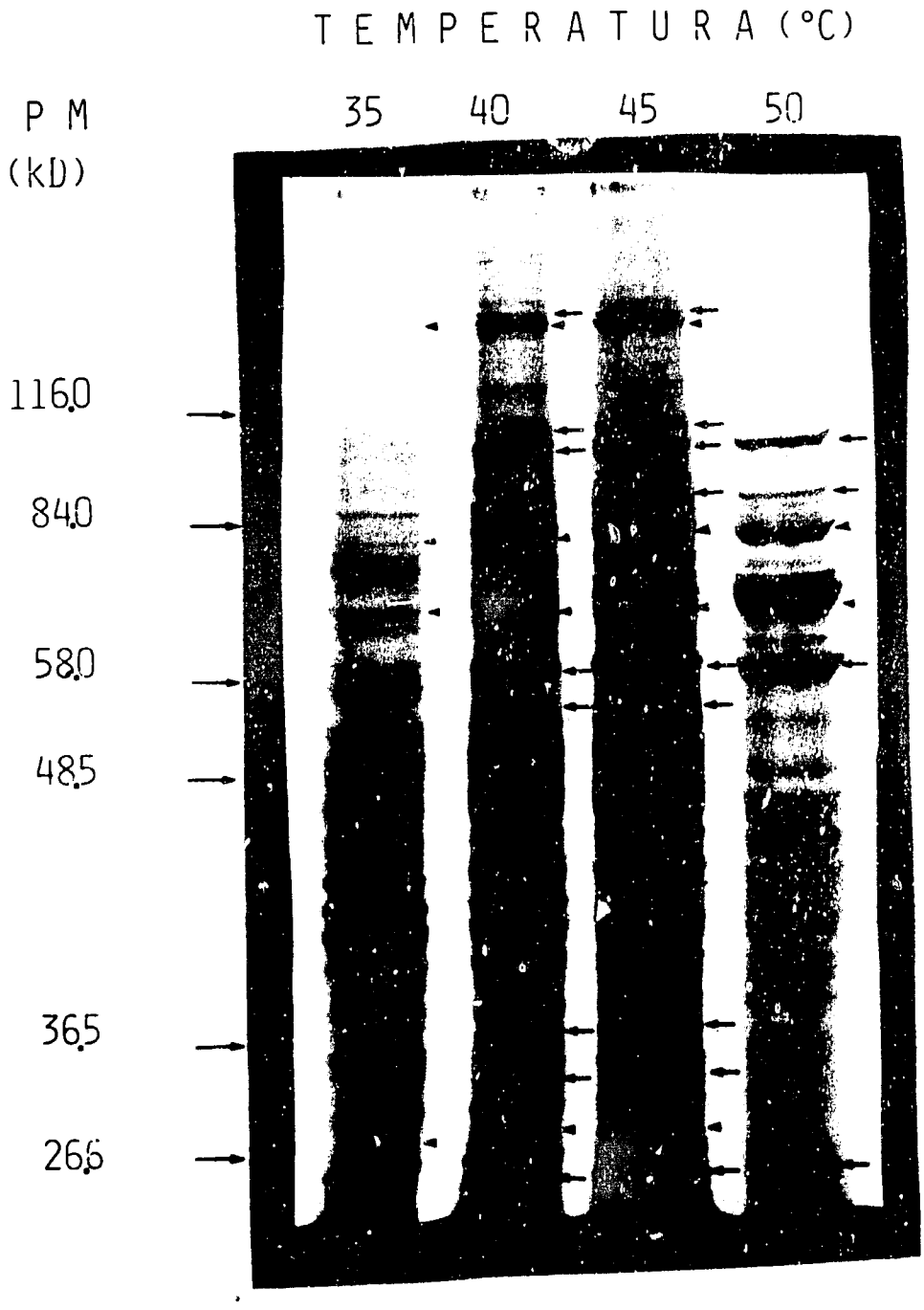

Figure 5

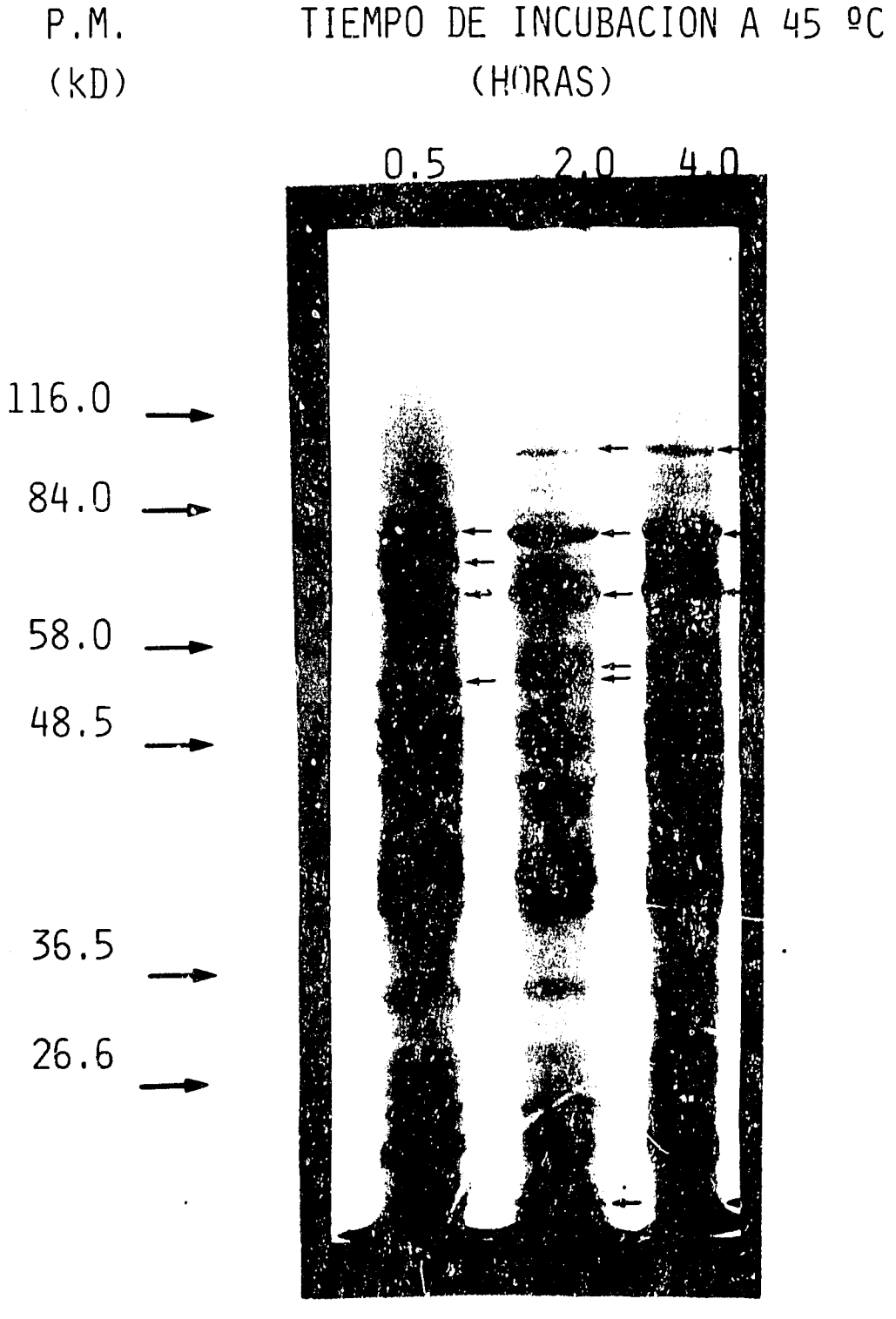
P.M.
(kD)
TIEMPO DE INCUBACION A 45 ºC
(HORAS)
0.5
2.0
4.0
116.0
84.0
58.0
48.5
36.5
26.6

Figure 6

TABLA RESUMEN DE SINTESIS DE PROTEINAS EN RADICULAS DE *Prosopis chilensis* INCUBADAS POR 2 HORAS A DIFERENTES TEMPERATURAS, EN PRESENCIA DE MET-S^{35} DURANTE LOS ULTIMOS 30 MINUTOS.

NUEVAS PROT.	TEMPERATURA DE INCUBACION (°C)			
(kD)	35	40	45	50
123	-	+	+	-
122	+	++	++	+
100	-	+	+	-
95	-	++	++	++
87	-	-	+	+
71	+	++	++	+++
62	-	++	+++	+++
59	-	+	+	-
33	-	+	++	-
31	-	+	++	-
28	+	+	++	-
25	-	++	+++	+

Table I