Herramientas biotecnológicas para la producción de planta forestal en el futuro escenario de cambio climático

Paloma Moncaleán
BIOECONOMIA, Argentina 2015

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Intergovernmental Panel of Climate Change (IPCC)

↑ Temperaturas ↓ Disponibilidad de agua

↓

↓ Supervivencia ↓ Productividad

↓

1º Alternativa: mejora de la producción forestal

Plan Nacional de Investigación

"… Análisis y mejora de los sistemas de producción agrícola y forestal, actuando sobre la mejora genética y la biotecnología de especies vegetales, la fisiología vegetal y el manejo de cultivos..."

El protocolo de Kyoto insta a los gobiernos tanto a incrementar la superficie forestal como a mejorar la eficiencia de los sistemas forestales.

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CULTIVO DE TEJIDOS

La mejor alternativa de producción de planta de alta calidad es la multiplicación vegetativa de individuos seleccionados que hayan expresado características fenotípicas de interés.

Inconvenientes:

Evaluar características deseables en fases maduras, mientras que su multiplicación se limita a las fases juveniles.

PROGRAMAS DE MEJORA

MEJORA CLÁSICA

Polinización controlada, establecimiento de huertos semilleros, etc.

+

NUEVAS HERRAMIENTAS BIOTECNOLÓGICAS: CULTIVO *IN VITRO*

Organogénesis, embriogénesis somática

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FORESTRY

GEORGE H. WEYERHAEUSER JR., Weyerhaeuser Co.

BIOTECHNOLOGY in FORESTRY: The promise and the economic reality

Forestry biotechnology promises major benefits for the forest products industry. Getting

toward sustain
the Weyerhaeu
era of conserva
people in the p
many Weyerha
were skeptical
reforesting la

New $3.2 million research lab to be constructed in Sussex

Media

Premier David Alward, ACOA Minister Rob Moore, Dr. Yil Sung Park, Minister Bruce North
Research Manager, Jim Irving, Minister Bruce Fitch, Blake Brunsdon, JDI Chief Forester at
$3.2 million research lab will be built. (CNW Group/J.D. Irving, Limited)

SUSSEX, NB, May 16, 2014 /CNW Telbec/ - Today federal, provincial, and J
announced a new $3.2 million state-of-the-art laboratory in Sussex. The facili
Limited, a division of JDI. Building of the 7200-square-foot facility will begin i
indirect construction jobs. Once completed, the new laboratory will create tw
addition to the current three) and at least five additional jobs over the next fou
a more than $5 million investment initiated in 2011 to commercialize new tre
this total is a direct investment of $2.1 million by the company.

"Over the past 20 years, J.D. Irving, Limited has invested over $20 million in f

ORGANIZATION PROFILE

J.D. Irving, Limited

Irving Shipbuilding's Partnership with Women Unlimited Provides Commu...

Through Bioforest, our own scientific and technological research center, we apply leading-edge biotechnology to the development of new ways to increase the quality, productivity and performance of our plantations, to improve the production process of pulp, and safeguard the rich biodiversity that exists within our forest holdings.

The research center, a leading initiative in South America, has been operating for 21 years with a highly qualified staff using top line equipment, labs, nurseries and greenhouses. Bioforest develops applied forestry technology, covering a broad range of research areas such as phitosanitary protection, land productivity and studies on the properties of wood.

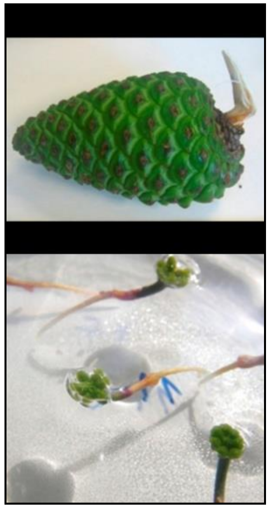
In addition, Bioforest operates under an open collaboration model with national and international organizations, leading technological innovation in forestry sciences and enriching this industry's practices.

CULTIVO DE TEJIDOS

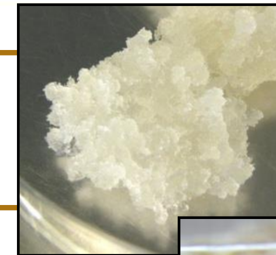
- **Obtención de plantas libres de enfermedades.**
- **Disminución del tiempo para la obtención de un gran número de individuos genéticamente idénticos.**
- **La producción de plantas no está condicionada por las estaciones del año, ni por la climatología dentro de las mismas.**
- **Conservación del germoplasma (especies en peligro de extinción, relictas, etc….).**
- **Almacenamiento de gran cantidad de material vegetal en un espacio pequeño.**
- **Disponibilidad de material vegetal a demanda del mercado.**
- **Posibilidad de propagar plantas que no tienen semillas viables.**
- **Superar las dificultades de la propagación vegetativa tradicional en algunas especies.**

NEIKER experiences: *Pinus* spp.

Juvenile Material



Organogenesis
De Diego et al. 2011
Montalbán et al. 2011a

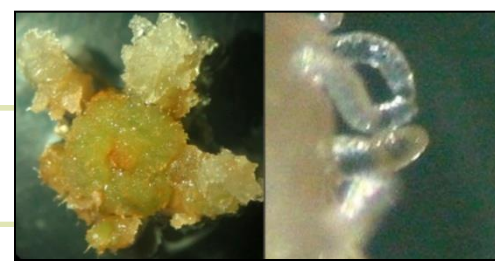


Somatic embryogenesis
Montalbán et al. 2010; 2011b; 2012; 2013; 2014

Adult Material

Organogenesis
De Diego et al. 2010
De Diego et al. 2008
Cortizo et al. 2009

Somatic embryogenesis
Montalbán et al. 2011c



NEIKER experiences: Seed organogenesis



Pinus radiata D. Don:
Montalbán et al. 2011. Forestry 84: 363-373.


Pinus pinea L.:
Moncaleán et al. 2005. Tree physiology 25 (1):1-9.
Alonso et al. 2006. Annals of forest Science 63: 879-885.


Pinus pinaster Ait.:
De Diego et al. 2011. Scandinavian Journal of Forest Research 26 (3): 201-211.

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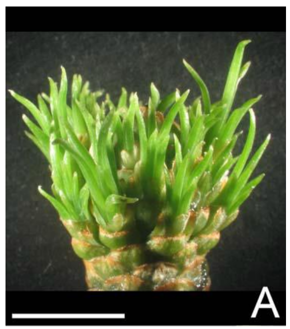
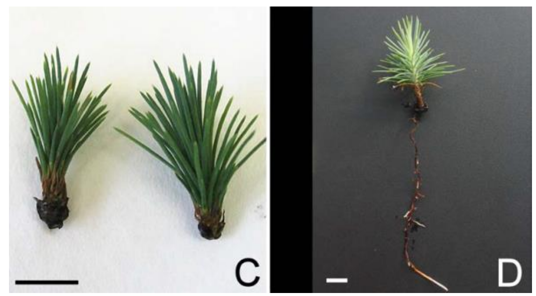
NEIKER experiences: organogenesis from adult trees


Pinus pinaster Ait.

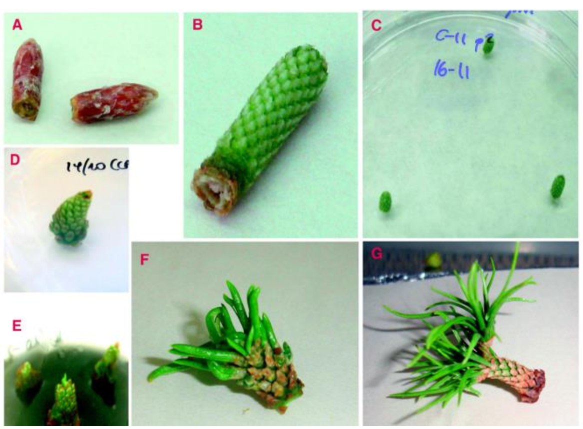
De Diego et al. 2008.
Can J For Res 38(10): 2607-2615.



Pinus pinea L.

Cortizo et al. 2009.
Trees-Struc Fun 23(4): 835-842.



Pinus sylvestris L.

De Diego et al. 2011.
Journal of Forest Research 76 (1):158-162.

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NEIKER experiences:Embriogénesis somática

EMBRIOGÉNESIS SOMÁTICA

- **PRODUCCIÓN DE PLANTA SELECCIONADA TOLERANTE A ESTRÉS BIÓTICO Y ABIÓTICO**
- **PRODUCCIÓN MASIVA A LA CARTA**
- **SILVICULTURA CLONAL DE ALTO RENDIMIENTO.**
- **CLONACIÓN DE MATERIAL CON FINES DE INVESTIGACIÓN**

NEIKER experiences:Embriogénesis somática

EMBRIOGÉNESIS SOMÁTICA

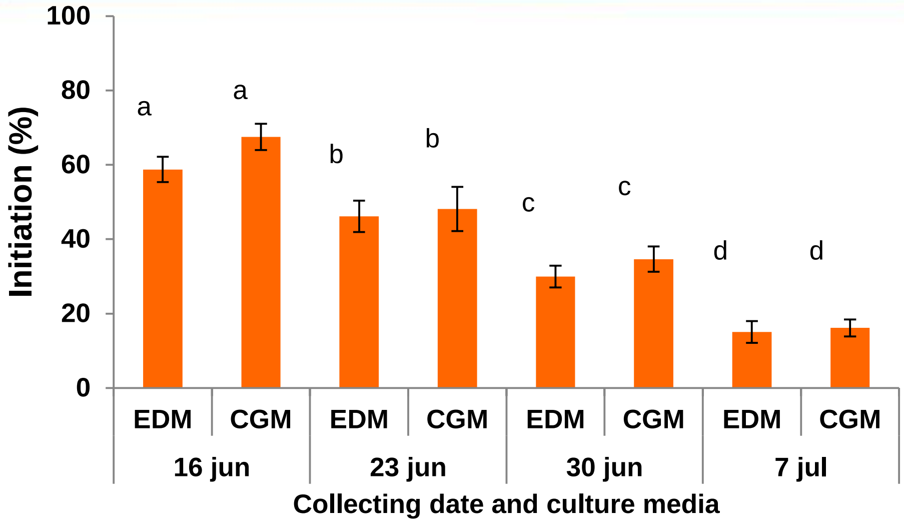
- PRODUCCIÓN DE PLANTA SELECCIONADA TOLERANTE A ESTRÉS BIÓTICO Y ABIÓTICO
- PRODUCCIÓN MASIVA A LA CARTA
- SILVICULTURA CLONAL DE ALTO RENDIMIENTO.
- CLONACIÓN DE MATERIAL CON FINES DE INVESTIGACIÓN

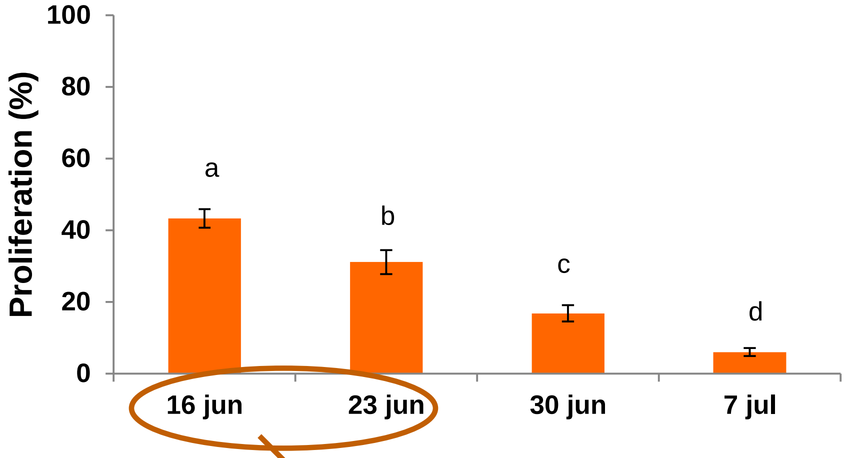
↓ **INICIATION AND PROLIFERATION PERCENTAGES**

↓ **MATURATION SUCCESS**

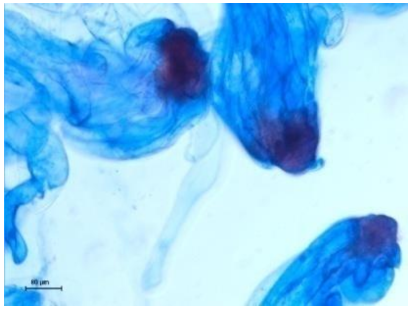
↓ **GERMINATION RATES**

Somatic embryogenesis juvenile material: initiation and proliferation

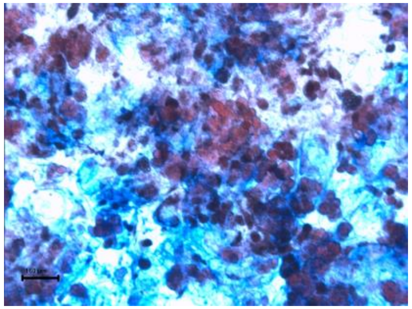


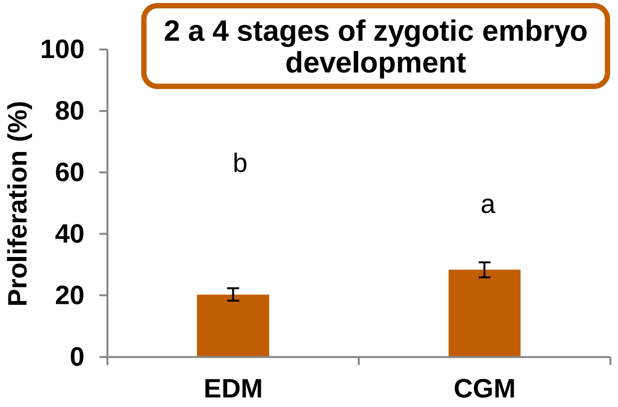


EDM



CGM





Pinus radiata D. Don:
Montalbán et al. 2012. Acta physiologia plantarum 34 (2): 451-460.

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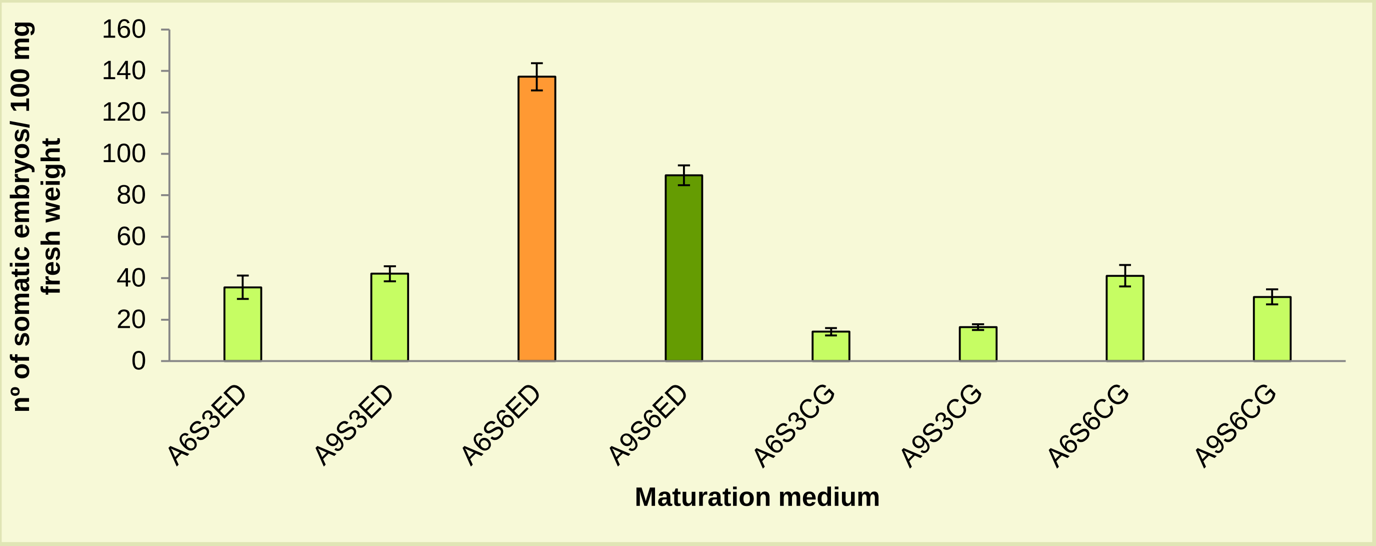
Maduración del tejido embriogénico: el cuello de botella



Mejor dato publicado hasta la fecha en el género *Pinus* spp.

Testado en más de 30 lineas

AMINOACIDOS

MEDIO	ABA	SACAROSA
A6S3ED	60 µM	30 g L^{-1}
A9S3ED	90 µM	30 g L^{-1}
A6S6ED	60 µM	60 g L^{-1}
A9S6ED	90 µM	60 g L^{-1}
A6S3CG	60 µM	30 g L^{-1}
A9S3CG	90 µM	30 g L^{-1}
A6S6CG	60 µM	60 g L^{-1}
A9S6CG	90 µM	60 g L^{-1}

EDM

- **L-glutamine (550 mg L^{-1})**
- **asparagine (525 mg L^{-1})**
- **arginine (175 mg L^{-1})**
- **L-citruline (19,75 mg L^{-1})**
- **L-ornitine (19 mg L^{-1})**
- **L-lysine (13,75 mg L^{-1})**
- **L-alanine (10 mg L^{-1})**
- **L-proline (8,75 mg L^{-1})**

CGM

- **Casein hydrolysate (1 gL^{-1})**
- **L-glutamine (500 mgL^{-1})**

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Montalbán et al. 2010. Trees-Structure and Function 24 (6): 1061-1071.

Then, what's the problem?

When … immat… eeds for SE initiation, the competence window is narrow…

When m… are …ed as i…al explant, low frequencies of initiation hav… been found in some *Pinu…* species.

Often, low frequencies of ma…ation of …aluable embryogenic lines.

Sometimes, low regeneration capacity of …yopreserv… tissue after clonal test.

Some embryogenic lines show recalcitrance to …e cryop… …rved.

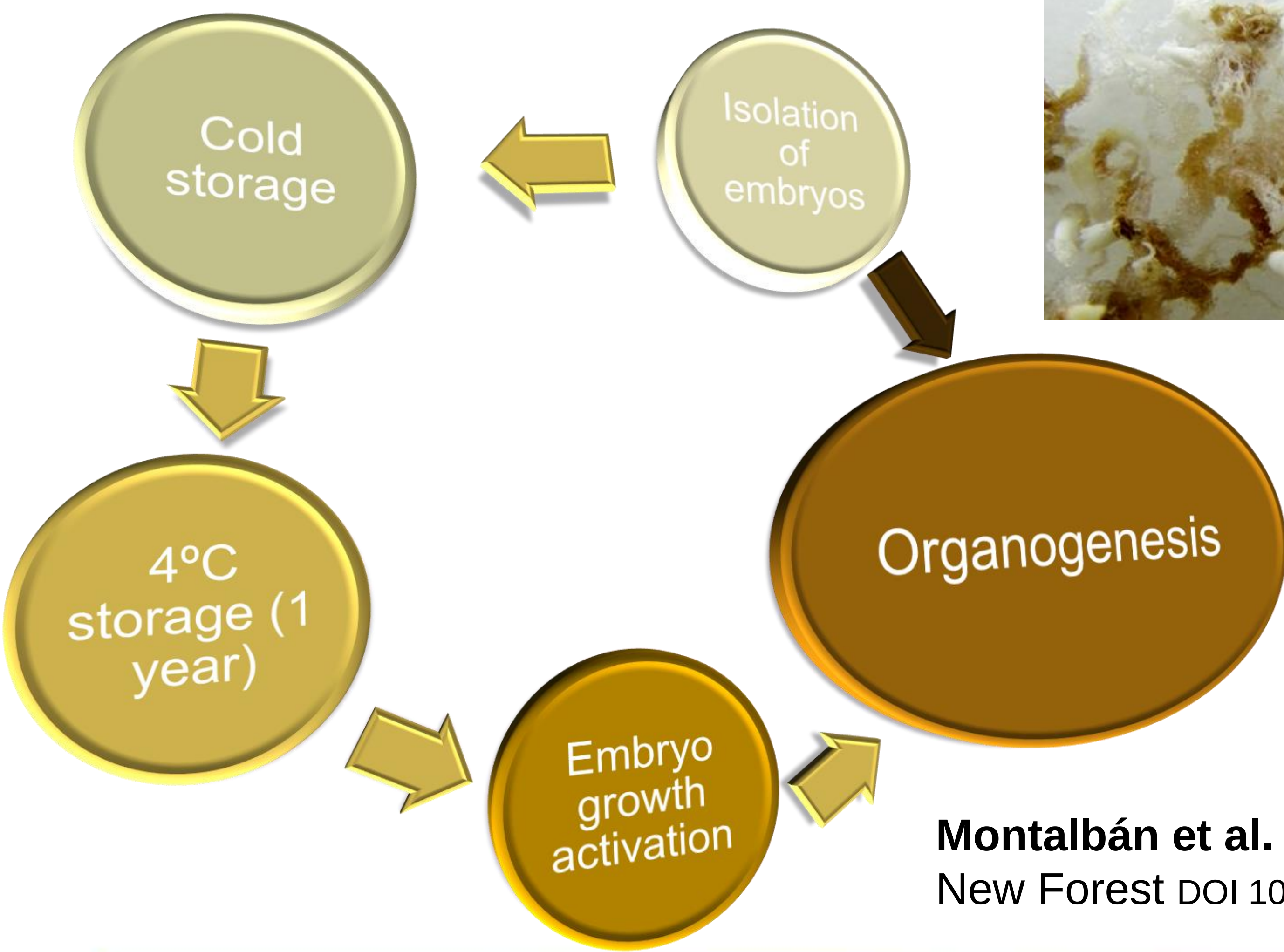
ORGANOGENESIS

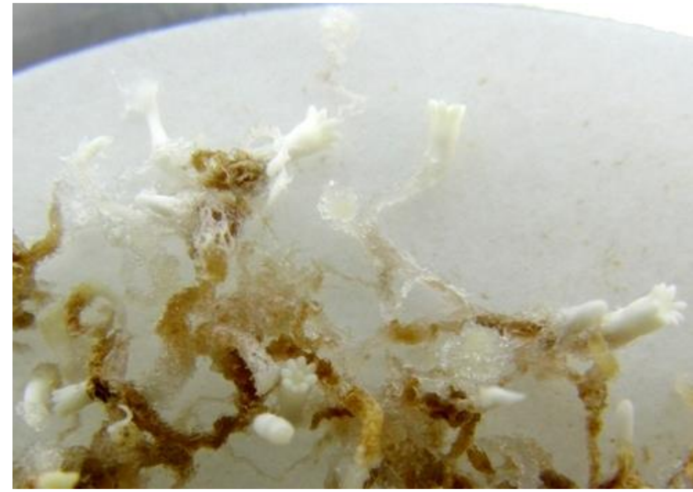
COLD STORAGE

COLD STORAGE and/or ORGANOGENESIS

Development of a combined somatic embryogenesis and organogenesis protocol including a cold storage step.

Development of a combined somatic embryogenesis and organogenesis protocol





Montalbán et al. 2014.
New Forest DOI 10.1007/s11056-014-9457-1.

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Combined somatic embryogenesis and organogenesis protocol

17,000 shoots / 1g ET

$$\frac{17{,}000 \text{ shoots}}{1\text{g ET}}$$

60 % rooting

>19 shoots/embryo

150 embryos/100 mg ET

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Cambio Climático (IPCC 2007- actualidad)

↑ Temperaturas ↓ Disponibilidad de agua

↓

↓ Supervivencia ↓ Productividad

↓

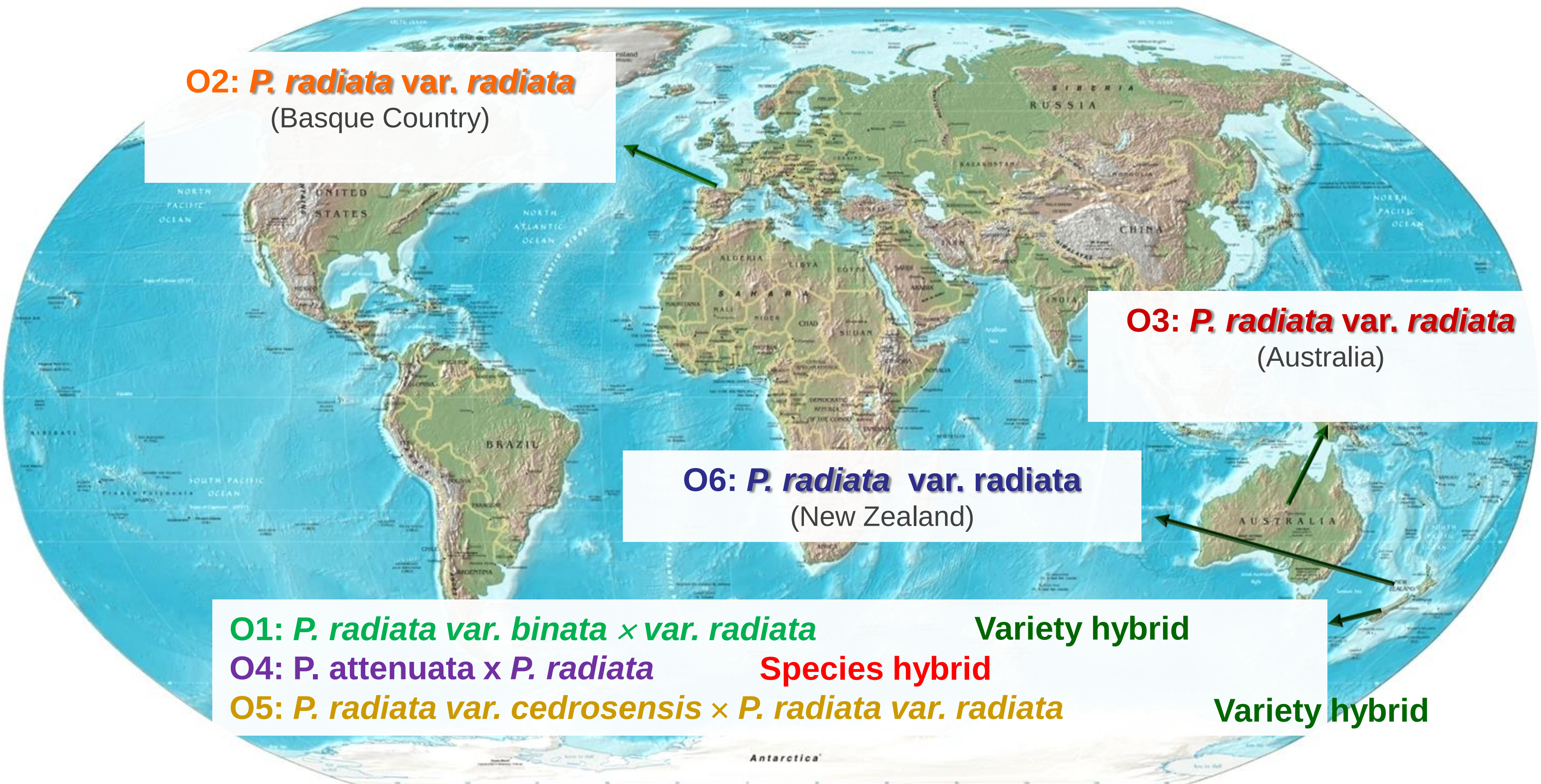
2ª Alternativa: búsqueda de especies y/o genotipos tolerantes al estrés

ESTUDIO DE PROCESOS FISIOLÓGICOS

BUSQUEDA DE MARCADORES DE TOLERANCIA

Plant Material

Six *Pinus radiata* ecotypes



O2: *P. radiata* var. *radiata*
(Basque Country)

O3: *P. radiata* var. *radiata*
(Australia)

O6: *P. radiata* var. radiata
(New Zealand)

O1: *P. radiata var. binata × var. radiata* **Variety hybrid**
O4: P. attenuata x *P. radiata* **Species hybrid**
O5: *P. radiata var. cedrosensis × P. radiata var. radiata* **Variety hybrid**

Híbridos *Pinus* spp.

O5- var. *Cedrosensis x P. radiata* var. *radiata*

O5 ≅ O4 but ↑ Biomass

O4: P. attenuata x P. radiata

	Physiology	Morphology	Phytohormones
Tolerance	$\cong\Psi_{leaf}$, Ψ_t, RWC	↓Heigh	= JA and ACC
	$\downarrow\Psi_\pi$ ↑Active OA	=Diameter	–SA
	↑ε < 4MPa	↓Substomatal chamber	–ABA and IAA
	↑Put, Spd, Spm		
Hardening	↑Active OA		
	↑Pro, GABA, Glu		

O1- *P. Radiata var. Binata X P. Radiata*

Tree Physiology 33, 69–80
doi:10.1093/treephys/tps125

Research paper

Solute accumulation and ela
pine breeds exposed to dro

N. De Diego[1,2], M. C. Sampedro[3], R. J. Bar

[1]NEIKER-TECNALIA, Department of Biotechnology, Vitoria-Gasteiz E-010

Tree Physiology 33, 537–549
doi:10.1093/treephys/tpt033

Research paper

Immunolocalization of IAA and ABA in roots and
pine (*Pinus radiata*) during drought and rewaterin

N. De Diego[1,2], J.L. Rodríguez[3], I.C. Dodd[4], F. Pérez-Alfocea[5], P. Moncaleán[1

[1]Departamento de Biotecnología, NEIKER-TECNALIA, Vitoria-Gasteiz E-01080, Spain; [2]Departamento de Biología Vegetal y Ecolo

Tree Physiology 32, 435–449
doi:10.1093/treephys/tps029

Research paper

Physiological response to drought in radiata pine: phytohormone
implication at leaf level

N. De Diego[1,2], F. Pérez-Alfocea[3], E. Cantero[3], M. Lacuesta[2†] and P. Moncaleán[1,4†]

[1]Department of Biotechnology, NEIKER-TECNALIA, Vitoria-Gasteiz E-01080, Spain; [2]Department of Plant Biology and Ecology, Faculty of Pharmacy, University of Basque Country

Organisation for Economic Co-operation and Development (OECD) (2014):Development of productive contingency species for forestation in Spain and New Zealand may be better adapted to predicted changes in climate due to global warning.

P. attenuata x P. radiata

SCION (Nueva Zelanda) NEIKER

↓

Desarrollo de protocolos de embriogénesis somática de híbridos de Pino

Estudio de procesos organogénicos de embriones somáticos

Cambio Climático (IPCC 2007............)

↑ Temperaturas ↓ Disponibilidad de agua

↓ Supervivencia ↓ Productividad

3ª Alternativa: Modular la tolerancia al estrés

Somatic embryogenesis

Cell lines → High number of somatic embryos → *Ex vitro* adaptation, clonal plants

Montalbán et al. 2010, 2011, 2012, 2013, 2014

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Are we able to modulate the response of somatic pines to drought stress?

Antecedentes

Semillas de hortícolas sometidas a tratamientos con NaCl, mostrataban en su etapa productiva mayor tolerancia a suelos con elevada salinidad.

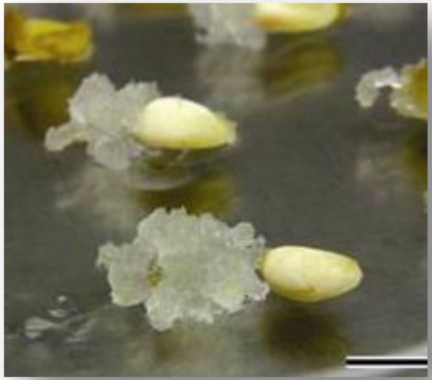
Plantas de Picea procedentes de semillas originadas en ambientes fríos, mostraban mayor tolerancia a las heladas

↓

CAMBIOS EPIGENÉTICOS

Objective

The aim of this work was to analyse the effect of physical and chemical conditions at initial stages of radiata pine SE in order to evaluate if the environment of cultures at initiation stage is critical to modulate the tolerance of somatic plants to drought stress months later.



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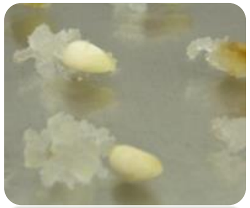
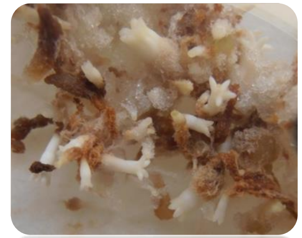
Material and methods

Initiation	Proliferation	Maturation	Germination
			

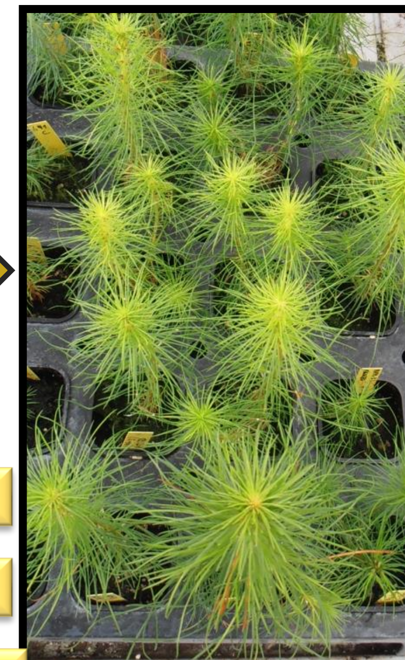
18 ºC: 2 g/L, 3 g/L, 4 g/L

23º C: 2 g/L, 3 g/L, 4 g/L

28º C: 2 g/L, 3 g/L, 4 g/L

2880 megagametophytes
124 embryogenic cell lines maturated

Same culture conditions



EDM

23ºC

4.5 g/L — 9 g/L — 7 g/L

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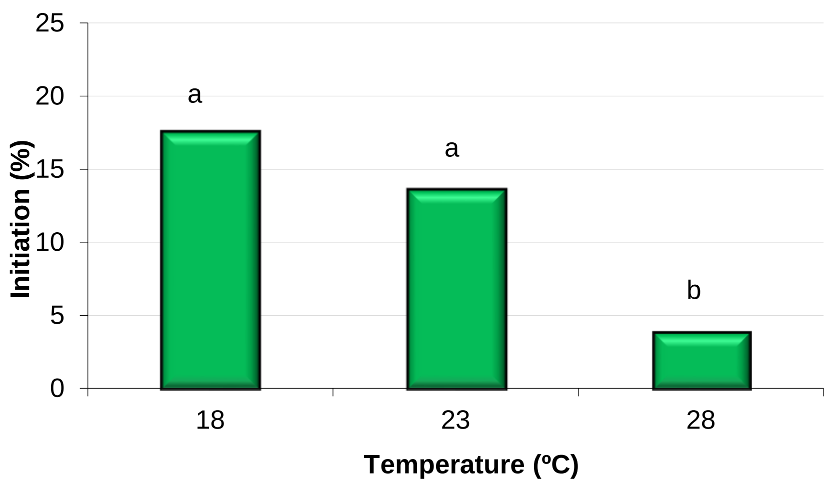
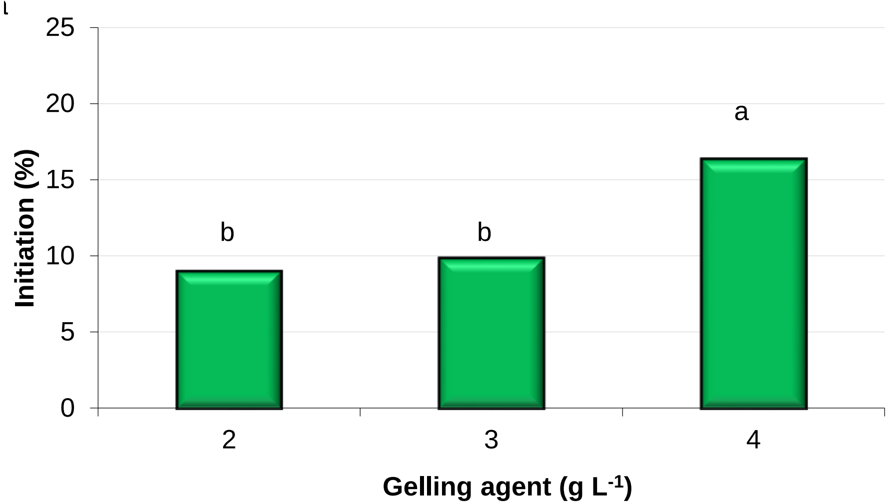
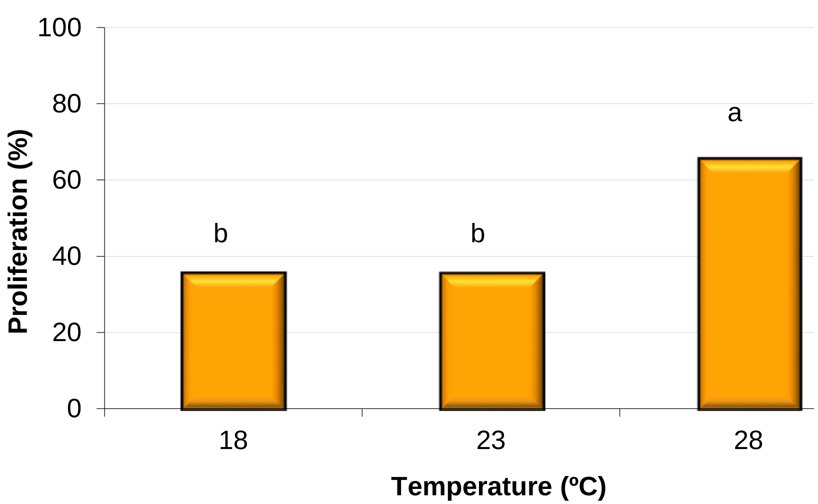
Results



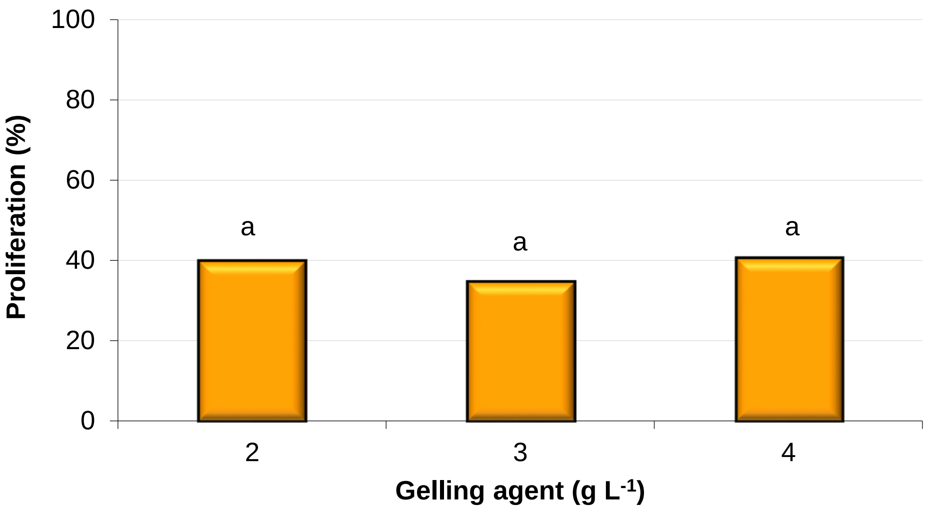
Initiation (%)
25
20
15
10
5
0
a
a
b
18
23
28
Temperature (ºC)



Initiation (%)
25
20
15
10
5
0
b
b
a
2
3
4
Gelling agent (g L-1)

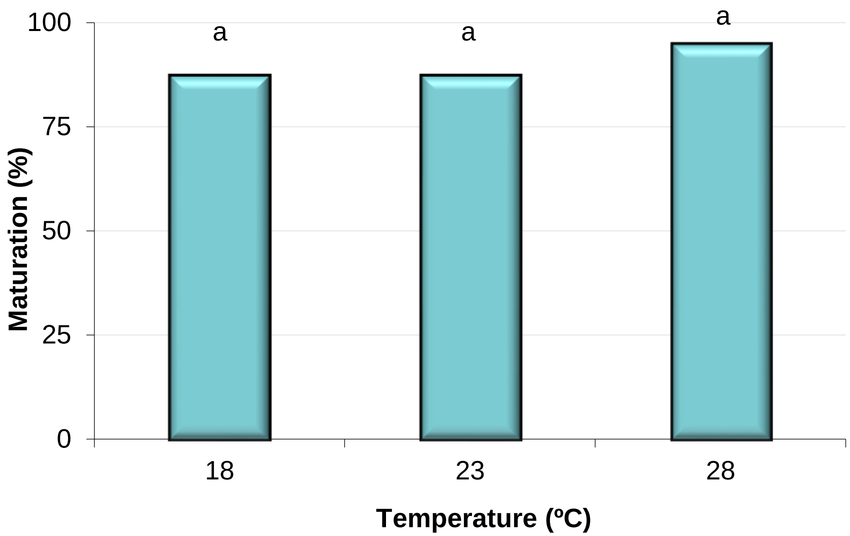


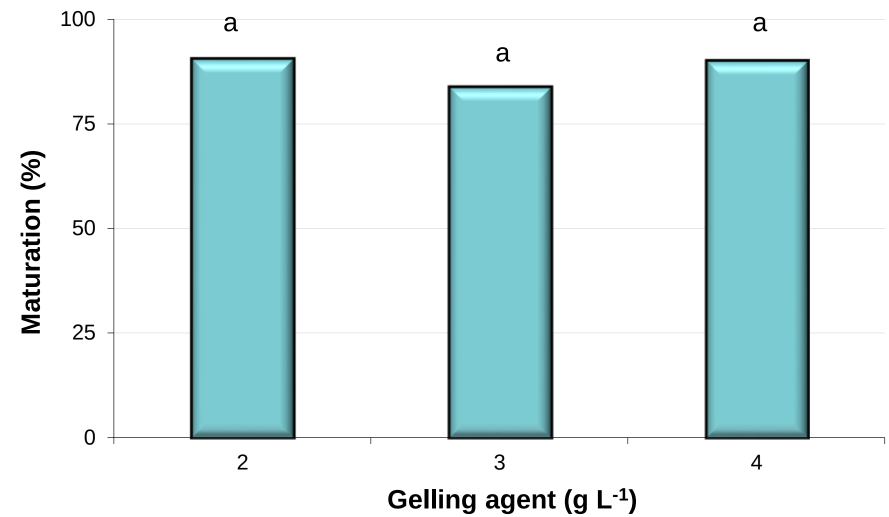
Proliferation (%)
100
80
60
40
20
0
b
b
a
18
23
28
Temperature (ºC)

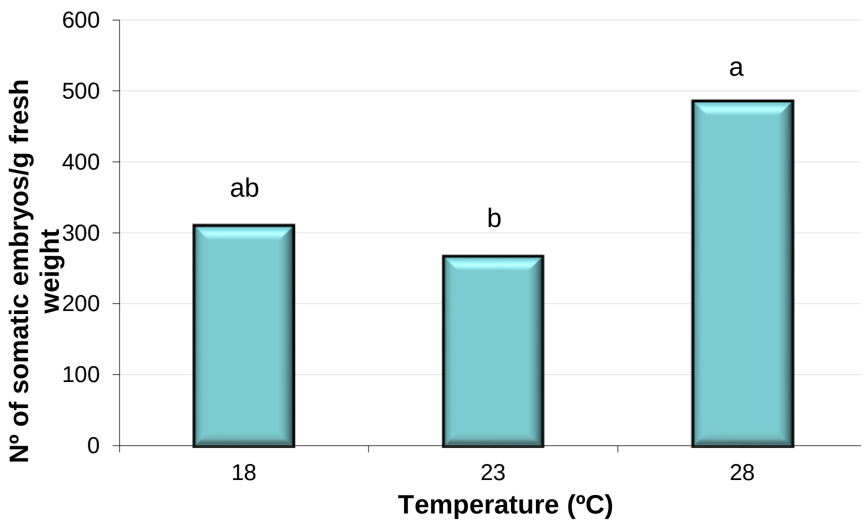


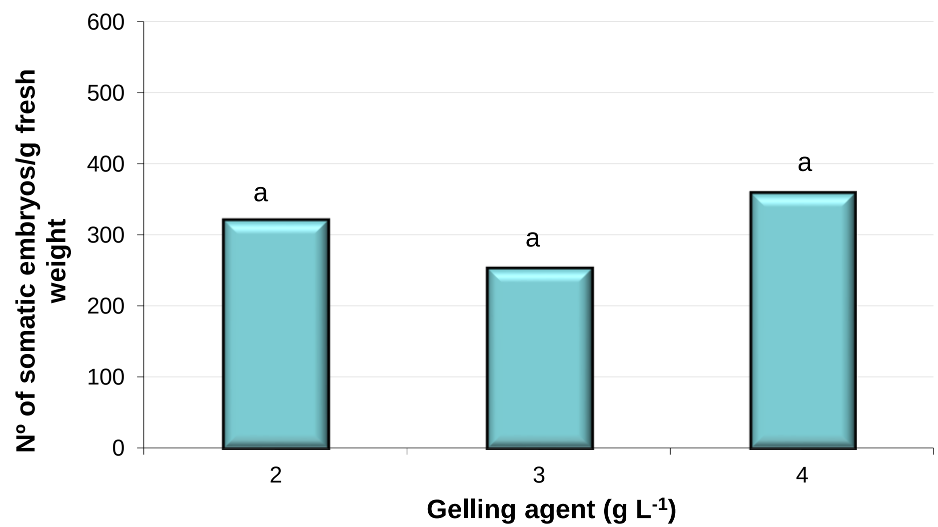
Proliferation (%)
100
80
60
40
20
0
a
a
a
2
3
4
Gelling agent (g L-1)

Results









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Scheme of Drought Experiment

Initiation

18º C

23º C

28º C

Proliferation, Maturation and Germination

Same culture conditions

Control plants

Stressed plants

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Parameters analyzed

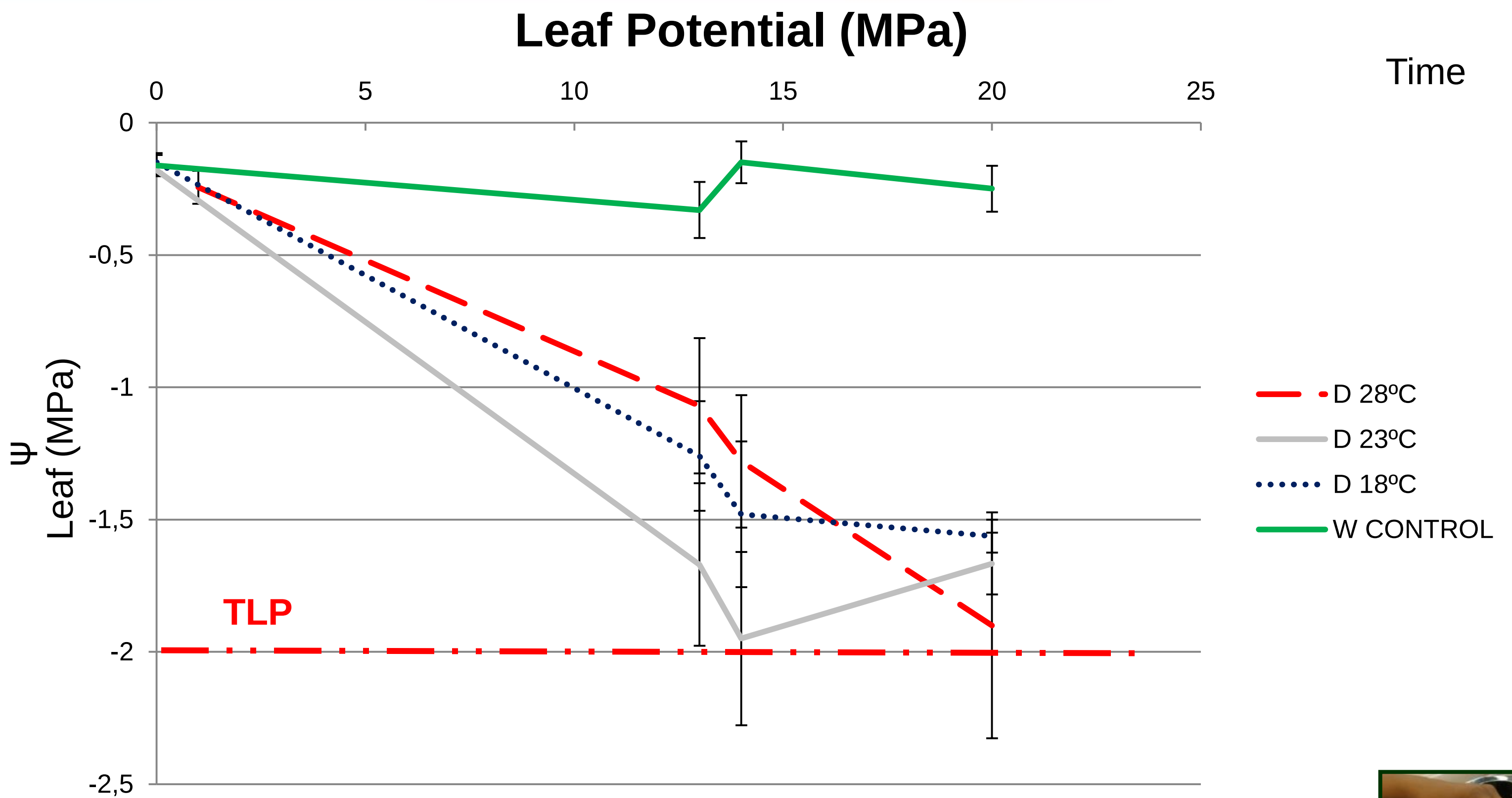
Analysis of survival and quality

Somatic plants

Physiological characterization

- Water status determination
- Gas exchange parameters
- Phytohormone concentration
- Protein profile

Water potential measurements



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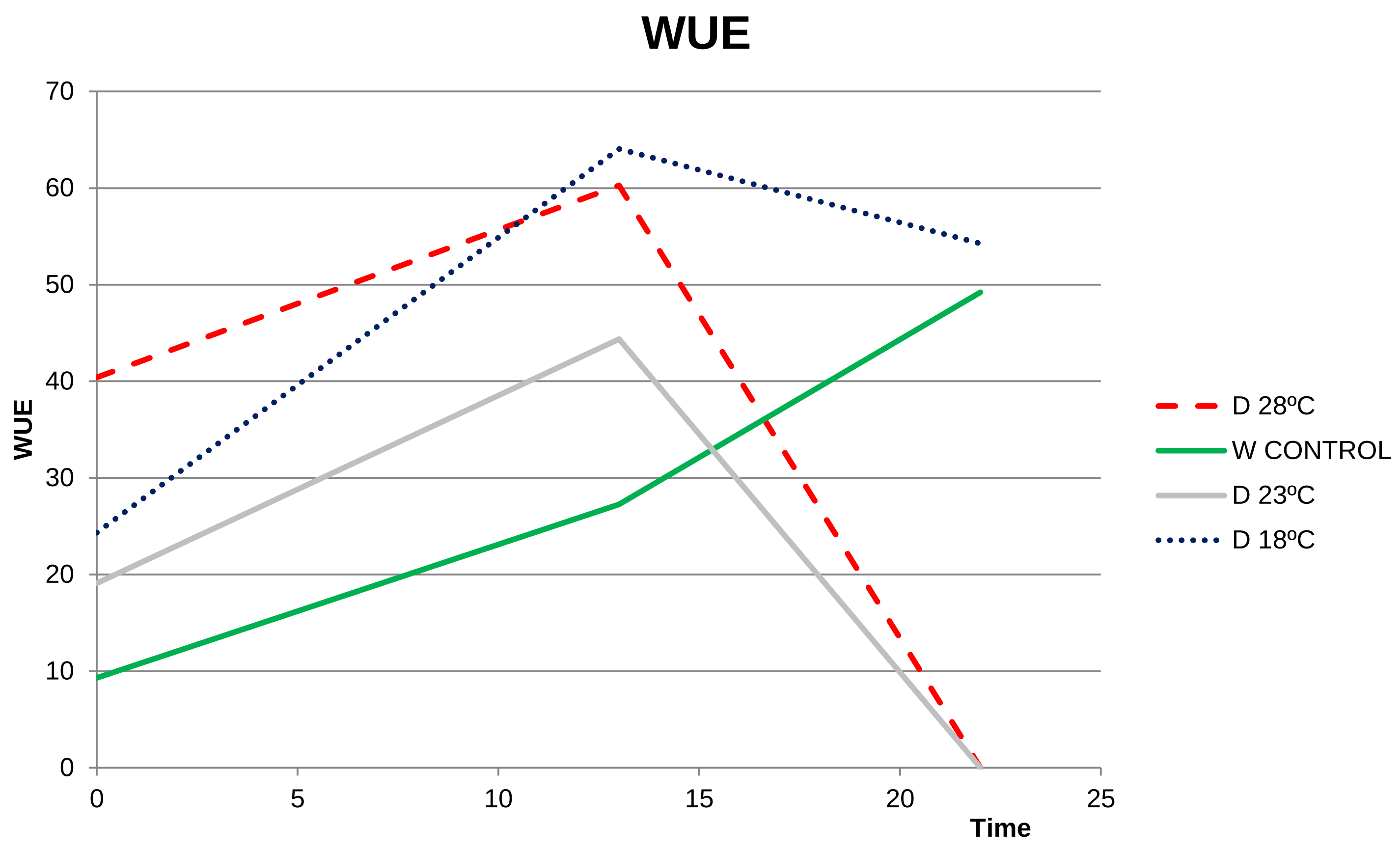
Water Use Efficiency



Conclusions

•Stressful conditions during initiation of the embryogenic tissue affect the quantity and quality of plantlets produced at the end of maturation stage.

•It seems that the embryogenic tissue has a “memory” to remember after 15 months the conditions in which it has been initiated.

•It seems possible to modulate the tolerance to water stress by changing environmental conditions in the initiation phase of embryonal masses.

My tresure

- Krystyna Klimaszewska (Canadian Forest Services, Canada)
- Cathy Hargreaves (SCION, New Zealand)
- Jorge Canhoto (Univ. Coimbra, Portugal)
- Mirek Strnad (Palacky University, República Checa)

Funding:

Basque Government
(DAPA)

Spanish Government
(Ministerio de Ciencia y Tecnología)

OECD (Organisation for Economic Co-operation and Development)

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2008 Quebec
2010 Corea
2012 Brno (República Checa)
2014 Vitoria (España)
2016 Argentina

- Yill-Sung Park, Natural Resources Canada – Canada
- Jean-François Trontin, FCBA Technological Institute, Francia
- Mariano Toribio, IMIA, España
- Heung-Kyu Moon, Korea Forest Research Institute, Corea
- Jana Krajnakova, Forestry and wood technology. Brno, Czech Republic.
- Paloma Moncaleán, Neiker, España

IUFRO

III International Conference of the Iufro Working Party
Somatic Embryogenesis and Other Vegetative Propagation Technologies
Vitoria-Gasteiz, Spain. September 9-12, 2014

130 investigadores de 60 países

Woody Plant Production Integrating Genetic and Vegetative Propagation Technologies

The aim of the conference

To join together vegetative propagation specialists, tree breeders, biotechnologists and industrial practititioners to exchange latest advances in scientific knowledge and technology.

Conference topics

1. Somatic embryogenesis (SE) of woody plants.
2. Physiology and genetics of SE and other vegetative propagation technologies.
3. Advances in tree breeding.
4. Implementation of SE and other vegetative propagation methods in tree breeding.
5. Development and application of in vitro vegetative propagation.
6. Large-scale propagation and deployment of new tree varieties and development of automated industrial production systems.
7. Development of complementary technologies.
8. Social and regulatory issues of deploying new tree varieties.

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Campus Agroalimentario de Arkaute.
Vitoria-Gasteiz. SPAIN.
www.neiker.net

www.iufro20902.org

We will meet again in

Argentina 2016

Sandra will Organize the conference i

¡¡Contamos con vuestra ayuda¡¡

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