PHASE PRESENTATION AND ACTION PROGRESS

PHASE 5.
TECHNIQUES INNOVANTES

PHASE 5. TECHNIQUES INNOVANTES

Action 5.1: Formation des partenaires.
Action 5.2: Elaboration de protocoles appropriés.
Action 5.3: Application des protocoles choisis.
Action 5.4: Validation des protocoles et mise en réseaux.

SCHEDULE

	2006	2007	2008
5.1			
5.2			
5.3			
5.4			

Action 5.1 – FORMATION DES PARTENAIRES

Schedule			
2006	2007	2008	
Initiated	Yes	Progress	95%

Action 5.1 – FORMATION DES PARTENAIRES

Action Detail	
ADDITIONAL TECHNIQUES FOR PLANT GERMPLASM CONSERVATION. An Introductory Seminar	Seminar held at launch of SEMCLIMED Project in Murcia, 26 September 2006.
Scientific training of partners	CCB: One month's stage (March 2007) of one of their researchers. Topic: micropropagation

Action 5.1 – FORMATION DES PARTENAIRES

Action Detail	
Scientific training of partners *(still in progress)*	CIEF: Two-week stage at the University of Alicante. Topic: Gain skills on the cryopreservation technique.

Action 5.1 – FORMATION DES PARTENAIRES

Action Detail	
Partnership advising *(still in progress)*	JBUV Topic: cryopreservation of recalcitrant seeds
	University of Mansoura Topic: Micropropagation and conservation of medicinal plants

Action 5.1 – FORMATION OF PARTENAIRE

Forthcoming formation or advising actions	
Scientific training of Partners	Still possible. 1-2 months at the University of Alicante
Partnership advising	CIEF - *Ulmus*
	AHUM – *Tetraclinis*

Action 5.2 – ELABORATION DE PROTOCOLES APPROPRIÉS

Schedule			
2006	2007	2008	
Initiated	Yes	Progress	98%

Action 5.2 – ELABORATION DE PROTOCOLES APPROPRIÉS

Species selection	
Tamarix boveana	*Tetraclinis articulata*
Helianthemum marminorense	*Astragalus nitidiflorus*
Cistus heterophyllus	

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Action 5.2 – ELABORATION DE PROTOCOLES APPROPRIÉS

Species	Micropropagation	Cryopreservation
Tamarix boveana	Propagation from apical and/or axillary buds	Encapsulation-Vitrification
Tetraclinis articulata		Vitrification
Helianthemum marminorense		Vitrification

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Action 5.2 – ELABORATION DE PROTOCOLES APPROPRIÉS

Species	Micropropagation	Cryopreservation
Astragalus nitidiflorus	Propagation from apical and/or axillary buds	Encapsulation-vitrification Vitrification
Cistus heterophyllus (still under revision)		

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IMPACT DU CHANGEMENT CLIMATIQUE SUR LA FLORE MEDITERRANEENNE ET ACTIONS DE CONSERVATION

Action 5.3 – APPLICATION DES PROTOCOLES CHOISIS

Schedule			
2006	2007	2008	
Initiated	Yes	Progress	45%

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Action 5.3 – APPLICATION DES PROTOCOLES CHOISIS

Action Detail				
Species	Mircropropagation stage			
	A	B	C	D
Tamarix boveana				
Tetraclinis articulata				

A: Sterilisation B: Multiplication C: Rooting D: Acclimation

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Action 5.3 – APPLICATION DES PROTOCOLES CHOISIS

Action Detail				
Species	Mircropropagation stages			
	A	B	C	D
Helianthemum marminorense	*			
Astragalus nitidiflorus				
Cistus heterophyllus				

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Action 5.3 – APPLICATION DES PROTOCOLES CHOISIS

Micropropagation of *Astragalus nitidiflorus*	
Explant	Shoots from seeds germinated *in vitro*
Sterilisation	Short immersion (60 s) in 70% ethanol + immersion in 20% commercial bleach with 0.05% Tween-20 (20 min) + Hot water bath (30 min).

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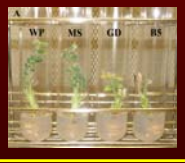
Action 5.3 – APPLICATION DES PROTOCOLES CHOISIS

Micropropagation of *Astragalus nitidiflorus*	
In vitro establishment	Each medium + 3% sucrose + 10 mg L^{-1} Fe-sequestrene + 7 g L^{-1} Plant Agar.



SELECTION OF CULTURE MEDIUM
Shoots (1.2-1.6 cm) without leaves were cultured on:
WPM (Lloyd and McCown, 1981).
MS (Murashige and Skoog, 1962).
GD (Gresshoff and Doy, 1972).
B5 (Gamborg et al., 1968).

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IMPACT DU CHANGEMENT CLIMATIQUE SUR LA FLORE MEDITERRANEENNE ET ACTIONS DE CONSERVATION

Action 5.3 – APPLICATION DES PROTOCOLES CHOISIS

Micropropagation of *Astragalus nitidiflorus*	
Multiplication	MS medium + 3% sucrose + 10 mg L^{-1} Fe-sequestrene + 7 g L^{-1} Plant Agar + 0.1 mg L^{-1} bencyladenine.

Action 5.3 – APPLICATION DES PROTOCOLES CHOISIS

Micropropagation of *Astragalus nitidiflorus*

Elongation	Double layer system: Lower layer: MS medium + 3% sucrose + 10 mg L^{-1} Fe-sequestrene + 6 g L^{-1} Plant Agar. Upper layer: 1 mL + 1/2 Knop salts + 3 g L^{-1} activated charcoal + 50 g L^{-1} sucrose.

Action 5.3 – APPLICATION DES PROTOCOLES CHOISIS

Micropropagation of *Astragalus nitidiflorus*

ELONGATION ON A DOUBLE LAYER SYSTEM

Effects of the sucrose present in the liquid upper layer on the elongation of shoots of *A. nitidiflorus*.

From the left to the right: Control (without double layer), 20 g L^{-1}, 30 g L^{-1} and 50 g L^{-1} sucrose.

The best results in terms of elongation, number of new leaves formed and spontaneous rooting were obtained with 50 g L^{-1} sucrose (in red)

Action 5.3 – APPLICATION DES PROTOCOLES CHOISIS

Micropropagation of *Astragalus nitidiflorus*

Rooting	Root induction medium: ½ MS medium + 3% sucrose + 10 mg L^{-1} Fe-sequestrene + 6 g L^{-1} Plant Agar + 0.5 mg L^{-1} indolacetic acid (IAA) in the dark. Root elongation medium ½ MS without IAA + 0.9 g L^{-1} activated charcoal.

Action 5.3 – APPLICATION DES PROTOCOLES CHOISIS

Micropropagation of *Astragalus nitidiflorus*

ROOTING OF EXPLANTS AFTER ELONGATION

The *in vitro* rooting strategy outlined gave near 50% of explants rooted

Action 5.3 – APPLICATION DES PROTOCOLES CHOISIS

Micropropagation of *Astragalus nitidiflorus*

Acclimatization	*In vitro*-rooted plants are transferred to a peat:vermiculite mixture and placed in a growth chamber. After two weeks RH is progressively reduced. Plant survival: 95%

Action 5.3 – APPLICATION DES PROTOCOLES CHOISIS

Forthcoming actions

- Test the micropropagation protocol for *Cistus heterophyllus*
- Apply the design cryopreservation strategy to all species

Action 5.4 – VALIDATION DES PROTOCOLES ET MISE EN RÉSEAUX

Schedule		
2006	2007	2008
Initiated	No	Progress -