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Scenario Development for Sustainable Land Use
in the Greater Caucasus, Georgia

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In vitro Technologies for Propagation and Exchange of Plant Biodiversity

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The flora of Georgia numbers approximately 13 300 plant species

- 4 200 angiosperms
- 380 species (9%) endemics of the country
- 600 species (14%) endemics of the Caucasus

56 threatened species are included in the Red List

- 36 vulnerable (VU)
- 18 endangered (EN)
- 2 critically endangered (CR)

*according to the criteria of the International Union for Conservation of Nature (IUCN)

The main threats impacting the biodiversity

- Devastation of habitats
- Excessive exploitation of biological resources
  - Irregular cuttings of forests
  - Degradation of water ecosystems
  - Intensive grazing

Conservation of plant biodiversity

- *in situ* strategies - maintenance of plant species in their natural habitats
  - conservation of domesticated and cultivated species on the farm
- *ex situ* strategies - maintain the biological material outside their natural habitats
  - storage in seed banks
  - field gene collections
  - *in vitro* collections
  - botanical gardens

In vitro technology - one of the most efficient methods of ex situ conservation

- The reproduction of plants growing in wild nature is impossible by traditional methods
- Seeds of plants are ‘recalcitrant’
- Do not store in dry form or low temperature conditions

OBJECTIVES

- Conservation of rare and endangered plants of the Red List of Georgia using tissue culture technology

  - Optimization of *in vitro* protocols for micropropagation of several threatened woody perennials included in the Red List of Georgia;
  - Evaluation of the physiological viability of explants as a source for establishment of aseptic cultures and approaches for their large-scale propagation.

Taxon	IUCN category	Endemism
Amygdalus georgica **Desf.**	**EN** B2a(I,ii)	Endemic
Betula medwedewii **Regel.**	**VU** B1b (i,ii,iii)	Endemic/Relict sp.
Betula megrelica **Sosn.**	**VU** B1a	Endemic
Betula raddeana **Trautv.**	**VU** B2a(i)b	Endemic
Castanea sativa **Mill.**	**VU** A2	-
Populus euphratica **Oliv.**	**CR** B1a+2a	Relict

Amygdalus georgica Desf.

Georgian almond
IUCN category EN
IUCN criteria - B2a(I,ii) – extremely small distribution range

Endemic of Georgia

Betula medwedewii Regel.

Medvedev crooked birch

IUCN category VU

IUCN criteria - B1b (i,ii,iii)

Distribution area extended in upper and subalpine belt at 1900-2250m altitude ASL, in some cases reaching 2350 m height.

Betula megrelica Sosn.

Colchis endemic birch

An extremely rare birch known only from collections from Mt. Migaria in Georgia.

IUCN category VU

IUCN criteria - B1a

Endemic of Georgia

Betula raddeana Trautv.

Rades birch

IUCN category VU

IUCN criteria - B2a(i)b

Relic

Endemic of Caucasus

Castanea sativa Mill.

Sweet chestnut

Distributed continuously along the southern slope of the Caucasus Mountains near the Black Sea

Has not yet been assessed for the IUCN Red List, but is in the Catalogue of Life;

Included into the Red List of Georgia due to the decreased distribution range and habitat fragmentation;

Main threat-Chestnut blight.

Populus euphratica Oliv.

Transcaucasian poplar

IUCN category CR

The only site of occurrence of *P.euphratica* is Vashlovani State reserve, in extreme east of Georgia

Establishment of an in vitro cultures

- Axillary buds
  - *P. euphratica*, *C. sativa* and *A. georgica*
- Zygotic embryos
  - *C. sativa and A. georgica*
- *In vitro* seedlings
  - *B. megrelica, B. raddeana and B. medwedewii*

Shoot Initiation and Rooting

- Two basal medium were used as initiation cultures for all explants:
  - 1) Murashige and Skoog medium, MS
  - 2) Woody plant medium, WPM
- Shoot initiation and multiplication:
  - N^6-6-Benzylaminopurine (BAP) at the concentrations of 0.4, 1.1, 2.5 or 4.4 µM
  - Kinetin at the concentrations of either 0. 05 µM or 1.16 µM
- Development of Rooting:
  - Indole-3-butyric acid (IBA) at the concentrations of either 1 µM or 15 µM
  - 45.7 µM indole-3-acetic acid (IAA)

Culture conditions

- Cultures were maintained at 24±0.5°C day/night with 16h photoperiod with an irradiance of 40-42 µmol m^{-2} s^{-1}

- Shoot production was accomplished every 4-5 weeks by sequential subculture of an individual shoots with significant acceleration in the proliferative rate

- The rooted shoots were relocated for adaptation in controlled chambers over the following few weeks and maintained to 60±5% moisture content at 23±1°C.

Summary of successful culture media and growth regulators used for shoot multiplication

	Explants source	Culture media[1]	Number of new shoots[2]
Betula medwedewii	Bud, in vitro seedlings	MS, WPM	
		a)BAP 4.4	3.8±0.5
		b)BAP 2.5	1.7±0.6
		c)KIN 1.16	0.8±0.2
Betula megrelica	Bud, in vitro seedlings	MS, WPM	
		a)BAP 4.4	3.6±0.5
		b)BAP 2.5	1.3±0.4
		c)KIN 1.16	0.6±0.3
Betula raddeana	Bud, in vitro seedlings	MS, WPM	
		a)BAP 4.4	3.9±0.4
		b)BAP 2.5	2.2±0.6
		c)KIN 1.16	1.2±0.5
Populus euphratica	Bud	WPM	
		a)BAP 2.5	4.4±1.1
		b)BAP 1.1	11.5±0.8
		c)BAP 0.4	1.6±0.6
Amygdalus georgica	Zygotic embryo, bud	WPM	
		a)BAP 2.5	9.6±1.1
		b)BAP 1.1	6.3±0.8
		c)KIN 0.05	1.4±0.5
Castanea sativa	Zygotic embryo, bud	WPM	
		a)BAP 4.4	0.6±0.3
		b)BAP 2.5	0.9±0.2
		c)BAP 0.4	1.8±0.6

[1]Culture media: Basal media plus growth regulators (µM), [2] mean ±SD.

Effect of growth regulators on rooting and plantlet survival

	Culture media[1]	Rooting %	Survival %
Betula medwedewii	MS, WPM		
	a) IBA 1	95	97
	b) IBA 15	33	92
Betula megrelica	MS, WPM		
	a) IBA 1	93	95
	b) IBA 15	35	86
Betula raddeana	MS, WPM		
	a) IBA 1	98	100
	b) IBA 15	43	94
Populus euphratica	WPM		
	a)IBA 0.5	65	40
	b)IBA 1	38	30
Amygdalus georgica	WPM		
	a)IBA 1	36	40
	b)IBA 15	46	53
	MS		
	c) IAA 45.7	21	37
Castanea sativa	WPM		
	a)IBA 1	17	-
	b) IBA 15	50	20

[1]Culture media: Basal media plus growth regulators (µM)

*Survival percentages are for the rooted shoots

Results

- Timing of collection and physiological condition of plant material was important to the success of *in vitro* cultures
  - Seasonal changes
  - Risk of contamination
- Different responses on initiation culture media and growth regulators were observed in general for all specimens
  - Almost all specimens showed the better initiation and multiplication rates in WPM medium
  - Among cytokines BAP was most effective in shoot multiplication and IBA for rooting

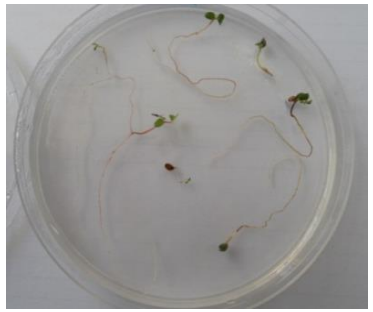
Betula spp.

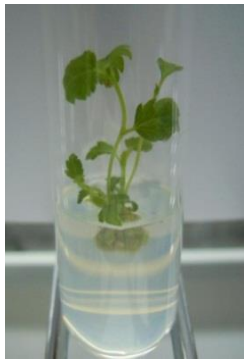
Explant source:

in vitro seedlings

Shoot multiplication:

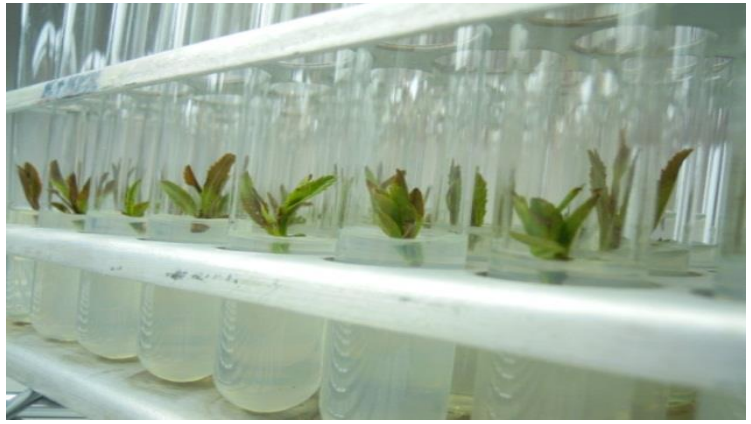
MS, ½ MS

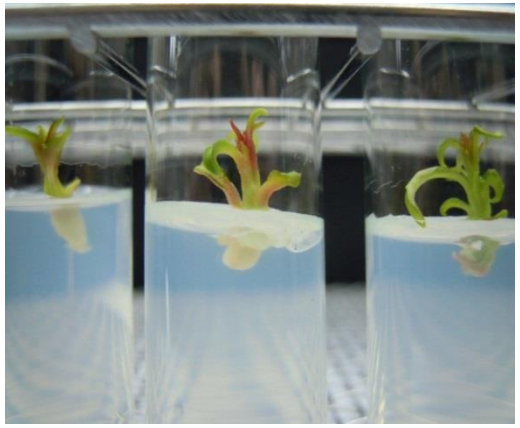


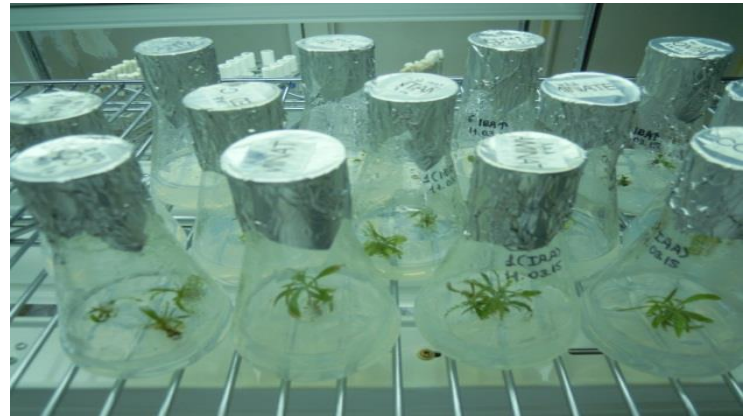


Amygdalus georgica Desf.

Explant source: zygotic embryo, bud ; *Culture:* WPM; *Shoot*: BAP2.5; *Rooting:* IBA15



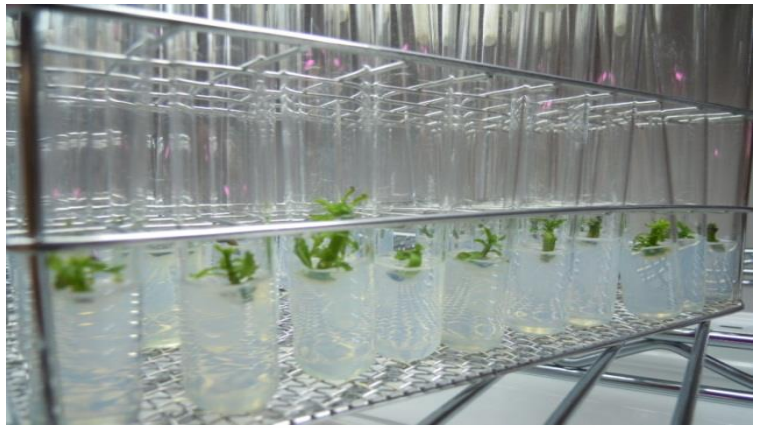




Castanea sativa Mill.

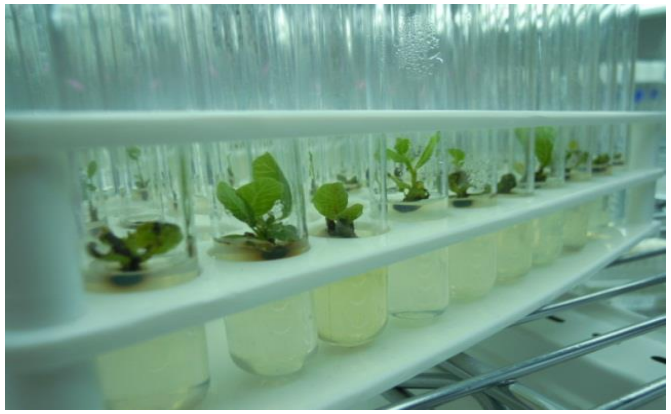
Explant source: zygotic embryo, bud ; *Culture:* WPM; *Shoot*: BAP0.4; *Rooting:* IBA15






Populus euphratica Oliv.

Explant source: bud ; *Culture:* WPM; *Shoot*: BAP1.1; *Rooting:* IBA0.5



Conclusions

- Six threatened species were successfully propagated displaying diverse response to culture conditions and explants sources;

- Further optimization of feasible *in vitro* protocols would have the great value to forest ecosystems for maintaining genetic resource diversity and offsetting the pressure on the natural populations.