

CONSERVATION OF ENDANGERED NATIVE PLANTS AT HANBURY BOTANIC GARDENS

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Introduction

Hanbury Botanic Gardens (GBH), with the Germplasm bank, are partners of the *LIFE20 Project NAT/IT/001468 - LIFE SEEDFORCE*, using seed banks to restore and reinFORCE the endangered native plants of Italy, whose main objective is to improve the conservation status of species of European Union interest currently in unfavourable conditions, through propagation and translocation.

GBH activities are focused on three species endemic to the Maritime and Ligurian Alps: *Acis nicaeensis* (Ardoino) Lledó, A.P.Davis & M.B.Crespo, *Campanula sabatia* De Not. and *Gentiana ligustica* R.Vilm. & Chopinet; all of them are listed in Appendix II and/or IV of Habitats Council Directive 92/43/EEC. For each species, in collaboration with the Research Centre for Vegetable and Ornamental Crops (CREA) of Sanremo, we assessed the germination capacity of seeds, developed specific protocols for *in vitro* propagation and produced new individuals for *in situ* translocations within six Special Area of Conservation.

Acis nicaeensis germination and propagation



Propagation techniques used

1 - **Lab incubator germination**: test on sterile MS + agar 0,8 g/L in sterile petri dishes pretreatment: 30 days dark +23°C, then +15°C, photoperiod 8/16 L/D
code ACINICA210601 1, n° germinated seeds 131/138: 95%
code ACINICA220601 1, n° germinated seeds 265/427: 62%
code ACINICA220527 1, n° germinated seeds 33/37: 89,2%
code ACINICA220601 2, n° germinated seeds 15/15: 100%

2 - **in vitro propagation** in MS + IBA 0,5 mg/L substrate, propagation start 14 September 2022, 23°C, 8/16 L/D
code ACINICA210601 1, n° clones in propagation 131, 2,212 bulbs
code ACINICA220601 1, n° clones in propagation 99, 454 bulbs
code ACINICA220527 1, n° clones in propagation 33
code ACINICA220601 2, n° clones in propagation 15

3 - **In vivo acclimatization** in greenhouse: at least 1 year old *in vitro* rooted bulbs were transferred into plastic alveolar pots filled with a substrate composed of peat and perlite in a 30:70 (v:v) ratio.
Total number of **bulbs 2,688**

Campanula sabatia germination and propagation



Propagation techniques used

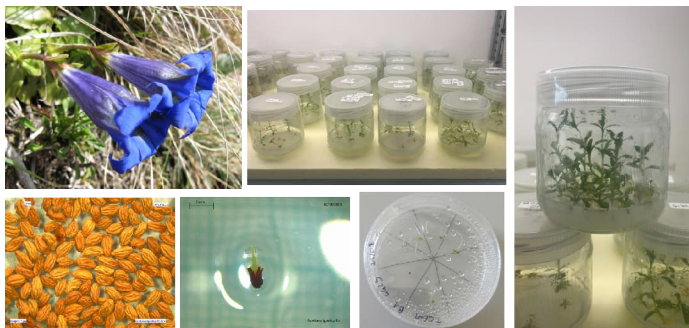
1 - **Induction** with GA3. 100 ml of a solution of 0.5 g/L GA3; 22°C for 24 h, dark.

2 - **Lab incubator germination**: test on sterile distilled water/agar 6 gr/litre in sterile petri dishes pretreatment: 30 days dark +20°C, then photoperiod 16/8 L/D, +20°C
code 21-CB210813, n° germinated seeds 23/41: 56,1 %
code 21-CRB210813, n° germinated seeds 13/24: 54,1 %
code 21-GT210815, n° germinated seeds 48/222: 21,6%
code 21-Vend210825, n° germinated seeds 182/215: 84,6 %
code 20-08081, n° germinated seeds 91/100: 91%
code 21-PredB, n° germinated seeds 21/100: 21 %

3 - **in vitro propagation** in MS + BA 0,2 mg/L substrate,
code 21-CB210813, n° 6 clones; n° plants 61
code 21-CRB210813, n° 5 clones; n° plants 85
code 21-GT210815, n° 8 clones; n° plants 47
code 21-Vend210825, n° 80 clones; n° plants 244
code CAMSABA2008081B, n° 66 clones; n° plants 1076
code 21-PredB, n° 14 clones, 14; n° plants 74

4 - **In vivo acclimatization** in greenhouse of clones rooted *in vitro* in cell trays, Kasmann substrate + perlite (30:70 V:V), spraying 10" every hour (from 9 to 17) current running
Total number of **plants 1,645**

Gentiana ligustica germination and propagation



Propagation techniques used

1 - **pretreatment**: seed sterilization with EtOH 70% for 30" NaClO 1% + Tween 20 for 20" and rinsed 2 times with sterile deionized H₂O for 10"
2 - **lab incubator germination**: test on sterile MS + 30 g/L sucrose + 8 g/L agar 0,8 g/L in sterile petri dishes 60 days 24 Dark +4°C
960 seeds code GENLIGU220711 1, n° germinated seed 16/960: 1,7 %
3 - **in vitro propagation** in MS + BA 0,3 g/L + 30 g/L sucrose + 8 g/L agar (23°C ± 1), 16/8h, PPFD 30 µE m⁻² s⁻¹ substrate, propagation start 28 March 2023
code GENLIGU220711 1, number clones in propagation 16;
Total number of **plants 252**

Translocation activities

Taxa	Sites	Type of translocation	N° of individuals to be translocated	Date of translocation	Donor accession	N° of clones	N° of individuals translocated	% survival
<i>Acis nicaeensis</i>	IT1315717 Monte Grammondo - Torrente Bevara	reinforcement	200	18/12/2023	ACINICA 2106011	6	16	
<i>Acis nicaeensis</i>	IT1316118 Capo Mortola	reinforcement	200	18/12/2023	ACINICA 2106011	1	4	April 2025 100 %
<i>Acis nicaeensis</i>	IT1316118 Capo Mortola	reinforcement	200	01/04/2025	ACINICA 2106011	24	215	
<i>Acis nicaeensis</i>	IT1315717 Monte Grammondo - Torrente Bevara	reintroduction	100	scheduled for autumn 2025				
<i>Campanula sabatia</i>	IT1324896 Lerrone - Valloni	reintroduction	200	07/12/2023	21-CB210813 21-CRB210813 21-GT210815 21-Vend210825	4	161	June 2025 27,3 %
<i>Campanula sabatia</i>	IT1324896 Lerrone - Valloni	reintroduction	200	13/12/2024	CAMSABA2008081	11	100	
<i>Campanula sabatia</i>	IT1323202 Isola di Bergeggi - Punta Predani	reinforcement	100	13/12/2024	21Bergeggi-Punta Predani	6	74	
<i>Campanula sabatia</i>	IT1323202 Isola di Bergeggi - Punta Predani	reintroduction	50	scheduled for autumn 2025	21Bergeggi-Punta Predani			



1-2-3 IT1324896 Lerrone - Valloni; 4- IT1316118 Capo Mortola

Our results indicated a good propagation capacity for *C. sabatia* with about 1,600 individuals produced, and for *A. nicaeensis* with 2,688 bulbs. Differently, *G. ligustica* showed poor capacity for germination and subsequent propagation; currently, only 252 seedlings are available *in vitro*.