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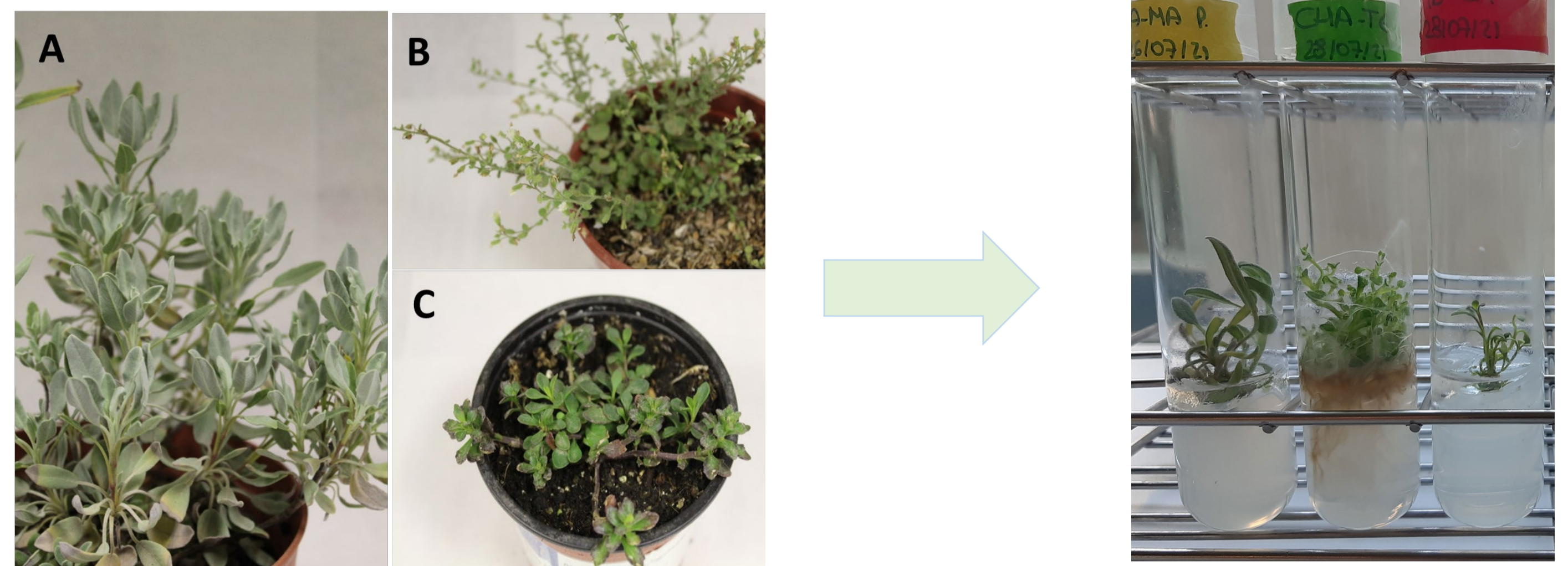
## INTRODUCTION

*In vitro* culture includes several methodologies useful for plant breeding, micropropagation, and germplasm preservation which can be carried out by maintaining *in vitro* plants under standard or low growing conditions, or by cryopreservation of explants. Therefore, it is a useful and complementary tool for other *ex-situ* conservation strategies. The Valencian endemism *Salvia blancoana* subsp. *mariolensis* Figuerola, and two other endemic plants present at the Valencian Community (Spain), *Iberis carnosa* subsp. *hegelmaieri* (Will) Moreno and *Chaenorhinum tenellum* (Cav) Lange, have been *in vitro* established. The development of micropropagation protocols to increase the multiplication rates in these species that are not easily propagated or could be in risk of extinction has been started. From these endemisms, only *Ch. tenellum*, that inhabits in calcareous rocks and preferably in caves or on rocky ledges, is currently protected by the List of Monitored Flora Species of the GVA (DOGV, 2013). *Salvia blancoana* subsp. *mariolensis*, does not produce to many seeds, and its vegetative multiplication is interesting. The other two endemisms have no problem regarding seed formation, although recalcitrance or erratic germination has been found. *Salvia blancoana* subsp. *mariolensis*, as other species of this genera, has a great value due to their medical features and synthesis of essential oils and other secondary metabolites (Wu et al. 2012; Chemical Reviews, 112: 5967-6026). In addition, it is used, along with other aromatic plants in the Alcoià-Comtat county (where it is common at Mariola and Aitana mountains in altitudes over 700 m), to elaborate a traditional liqueur named 'herbero'. All the three endemisms are of interest as ornamental plants for rockeries in gardens.

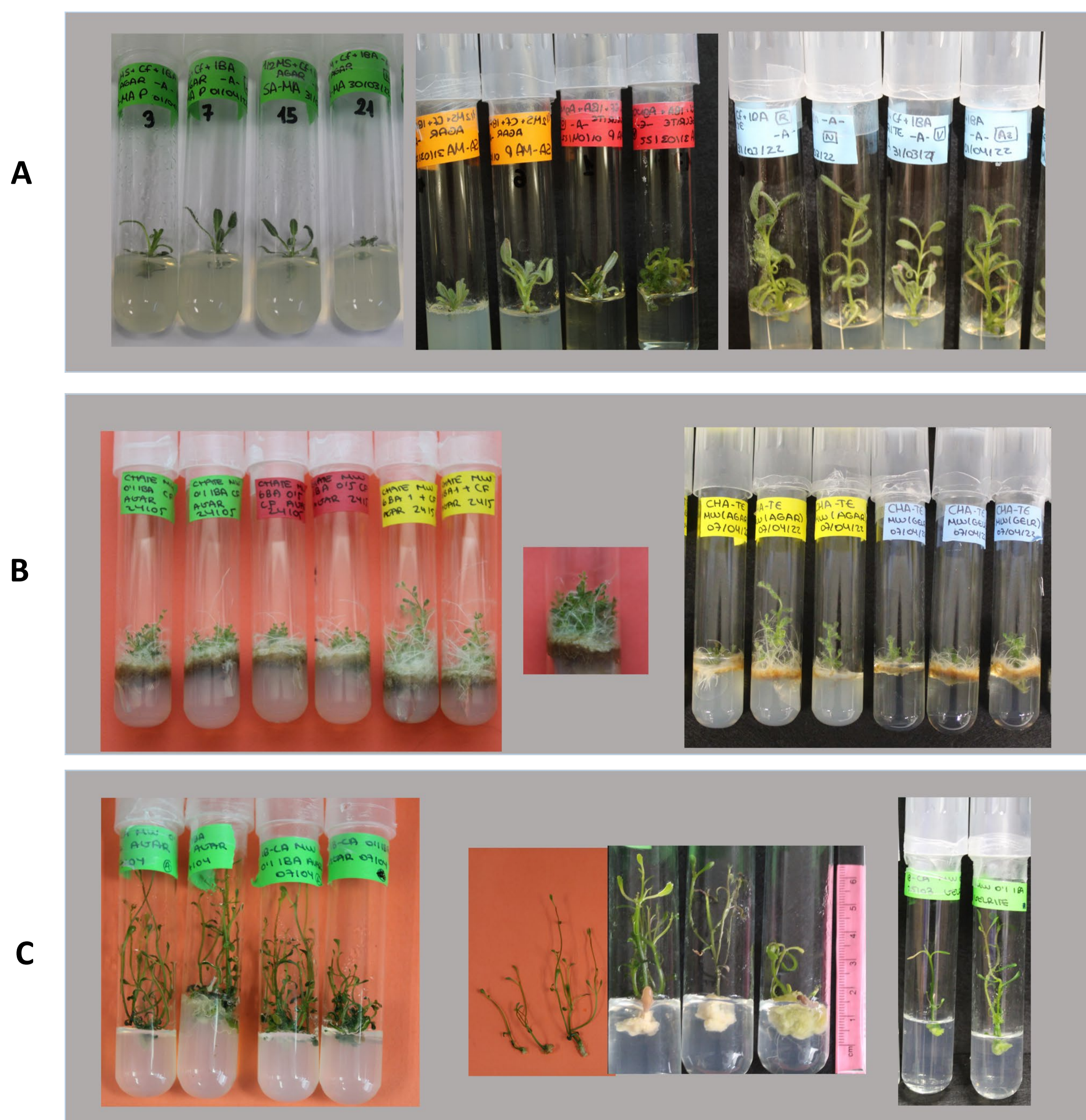
## MICROPROPAGATION

### 1. In vitro establishment

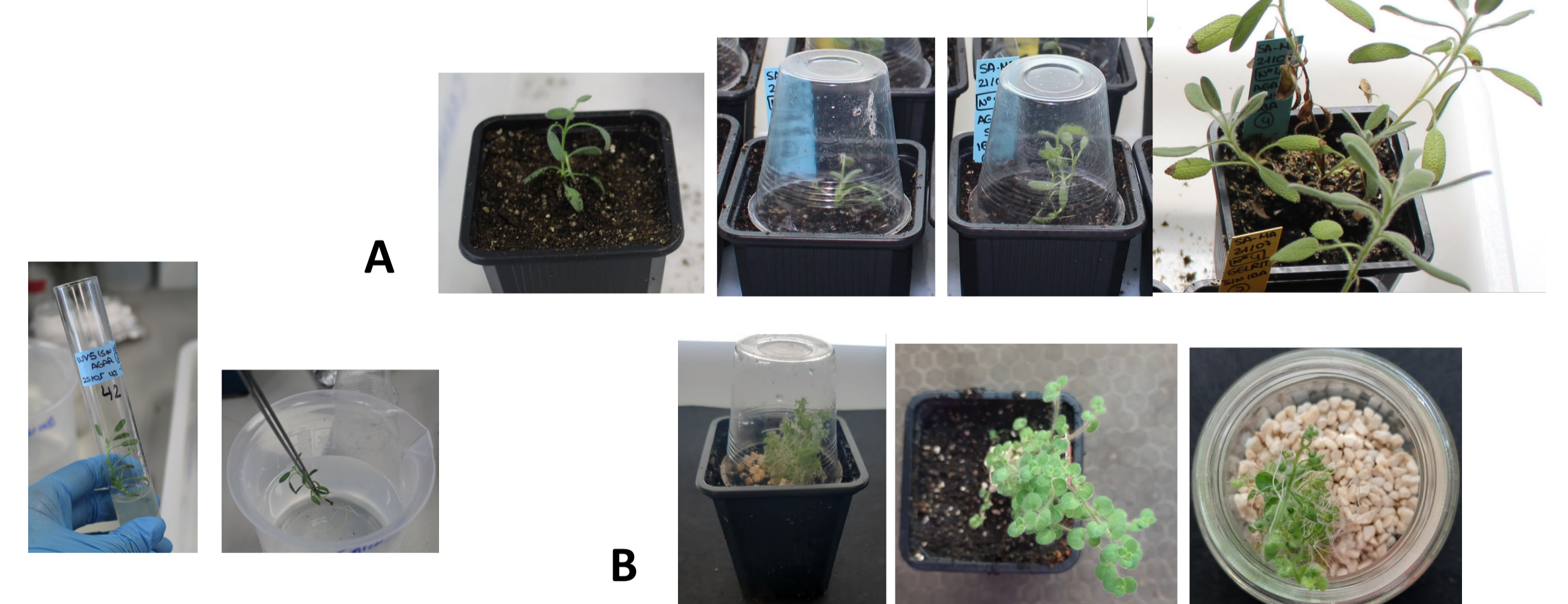
Plants of *Salvia blancoana* subsp. *mariolensis* (A), *Chaenorhinum tenellum* (B) and *Iberis carnosa* (C) have been provided by the CIEF (Centre for Forestry Research and Experimentation, Generalitat Valenciana). For disinfection, several concentrations of bleach have been evaluated. Treatments with a fungicide, made previously to the bleach application, have increased the percentages of disinfested plants cultured on the MW medium (Gisbert et al. 2018; InTechOpen, 68059).



### 2. Multiplication and rooting

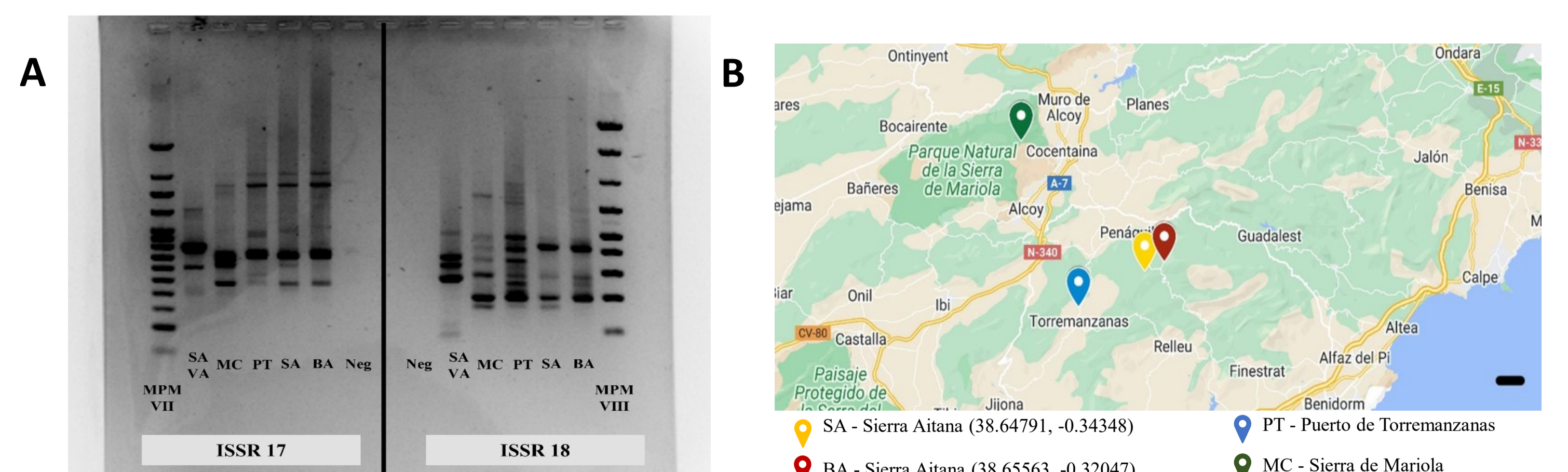


### 3. Acclimatization



Agar is removed previously to transplant *in vitro* plants to soil conditions. The gradual reduction of humidity has resulted in a successful transference of *S. blancoana* subsp. *mariolensis* (A) and *Ch. tenellum* (B).

## ANALYSIS OF GENETIC VARIABILITY



A. Electrophoretic analysis of PCR product from iPBS markers (17 and 18). B. Location of *S. blancoana* subsp. *mariolensis* sampled.

Multiplication of *S. blancoana* subsp. *mariolensis* (A), *Ch. tenellum* (B), and *I. carnosa* (C) was made by using apical and internode sections obtained from the plants *in vitro* established. Good growth, multiplication, and rooting was obtained for *Ch. tenellum* on MW. Several experiments which include modifications on salts minerals, gelling agents, and/or growth regulators have been carried out for obtaining multiplication and/or rooting in the *S. blancoana* and *I. carnosa*. The effect of adding silver nitrate to reduce basal necrosis in *S. blancoana* subsp. *mariolensis* has been also tested. Rooting is being improving in *I. carnosa*.

## CONCLUSIONS

*In vitro* establishment and micropropagation protocols have been developed for *Salvia blancoana* subsp. *mariolensis*, *Chaenorhinum tenellum*, and *Iberis carnosa*, with successful acclimatation rates in the first two. Selected ISSR have been validated in populations of *S. blancoana* subsp. *mariolensis* from the Alcoià-Comtat county with the aim to detect plants carrying genetic variability that could be micropropagated and used in future reintroductions.