

### **Ex-situ genetic conservation of *Pinus nigra salzmannii* endangered populations from the Spanish Central Range.**

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Despite being an abundant species as a whole, some subspecies and populations of the European black pine (*Pinus nigra* Arnold) are particularly threatened due to climate change and alterations of fire regimes across the Mediterranean basin. This is the case of westernmost populations of *P. nigra salzmannii* in the Spanish Central Range (mostly in Sierra de Gredos mountains). Scattered and aged stands thrive in acidic soils, frequently surrounded by Maritime pine and oaks in mid and lower altitudes. The main challenges for the conservation of stands are wildfires –to which Maritime pine is much better adapted– and general lack of regeneration due to low seed viability, putatively deriving from a combination of ageing and inbreeding. In addition, planted stands of allochthonous subspecies can be hybridizing with some neighbouring natural stands.

All factors considered, the regional forest administration (pertaining to *Junta de Castilla y León*) led an initiative aimed both at conserving these genetic resources and ensuring the supply of genetically diverse reproductive material. A highly cooperative work among institutions (JCyL, CIFOR-INIA, UCLM and CITA) culminated with the plantation of a clonal seed orchard devoted to the ex-situ genetic conservation of these *P. nigra salzmannii* populations.

Scions of 98 ortets (donor trees) were collected in 9 natural stands of *P. nigra salzmannii* in Gredos mountains. Scions were tip-grafted in two-years-old rootstocks of the same species. Simultaneously, ISSR molecular markers were analyzed in needles of the donor trees. Using SoftSog software and relying on molecular relationships, the optimum design fitted to the plantation size and shape was determined. This design maximized the genetic distance among neighbour plants by minimising the global coancestry between trees weighted by the distance between them; therefore, ensuring minimum inbreeding for future seed orchard crops even with open pollination. The plantation was carried out successfully in a former farmland, far enough from any undesired pollen source.

We further compared the levels of inbreeding and genetic diversity of the natural stands with those expected from seeds originated in the conservation seed orchard to evaluate the utility of the latter for increasing the fitness of these endangered black pine populations.