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Research article

Early establishment response of different *Pinus nigra* ssp. *salzmanii* seed sources on contrasting environments: Implications for future reforestation programs and assisted population migration

K. Taïbi [a, b, d, **], A.D. del Campo [a, *], A. Aguado [c], J.M. Mulet [d]

[a] Research Group in Forest Science and Technology (Re-ForeST), Institute of Water and Environment Engineering, Universitat Politècnica de València, Cami de Vera, Valencia 46022, Spain
[b] Faculty of Life and Natural Sciences, Ibn Khaldoun University, BP 78, Karman Campus, Tiaret 14000, Algeria
[c] Centro Nacional de Recursos Genéticos Forestales de Alaquàs, Ministerio de Agricultura, Alimentación y Medio Ambiente, Joaquín Ballester, 39, Valencia 46009, Spain
[d] Instituto de Biología Molecular y Celular de Plantas (IBMCP), Universitat Politécnica de Valencia-Consejo Superior de Investigaciones Científicas, Avd. de los Naranjos s/n, Valencia 46022, Spain

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ABSTRACT

Forest restoration constitutes an important issue within adaptive environmental management for climate change at global scale. However, effective implementation of these programs can only be achieved by revising current seed transfer guidelines, as they lack inherent spatial and temporal dynamics associated with climate change. In this sense, provenance trials may provide key information on the relative performance of different populations and/or genotypes under changing ecological conditions. This study addresses a methodological approach to evaluate early plantation performance and the consequent phenotypic plasticity and the pattern of the adaptation of different seed sources in contrasting environments. To this end, six seed sources of Salzmann pine were tested at three contrasting trial sites testing a hypothetical assisted population migration. Adaptation at each site was assessed through Joint Regression and Additive Main effect and Multiplication Interaction (AMMI) models. Most of the observed variation was attributed to the environment (above 90% for all traits), even so genotype and genotype by environment interaction (GxE) were significant. Seedlings out-planted under better site conditions did not differ in survival but in height growth. However, on sites with higher constraints, survival differed among seed sources and diameter growth was high. The adaptation analyses (AMMI) indicated that the cold-continental seed source 'Soria' performed as a generalist seed source, whereas 'Cordilleras Béticas', the southernmost seed source, was more adapted to harsh environments (frost and drought) in terms of survival. The results supported partially the hypothesis that assisted migration of seed sources makes sense within limited transfer distances, and this was reinforced by the GxE results. The present study could be valuable to address adaptive transfer of seedings in ecological restoration and to determine the suitable seed sources for reforestation programs and assisted population migration under climatic changes. The reported results are based on 3 years' data and need to be considered in this context.

1. Introduction

Climate change is one of the major challenges for forest ecosystems and biodiversity in the near future. It is expected to drastically affect growing conditions for trees (Herrero et al., 2013; Rittenhouse and Rissman, 2015). Great impact is expected in the Mediterranean region, a transitional climatic zone between arid and humid regions of the world, where temperature is expected to

* Corresponding author. Research Group in Forest Science and Technology (Re-ForeST), Institute of Water and environment engineering, Universitat Politècnica de València, Cami de Vera, Valencia 46022, Spain.
** Corresponding author.
E-mail addresses: khaledtaibi@hotmail.com (K. Taïbi), ancamga@upv.es (A.D. del Campo).

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increase between 2 and 5 °C while precipitation is expected to decrease by 30% over the next century (A1B scenario), mainly in summer and autumn (Lindner et al., 2014). Nevertheless, climate change impacts on plant species may vary significantly depending on the species' ability to tolerate to the future conditions (Sánchez-Salguero et al., 2013). Plant species respond to these new conditions through phenotypic plasticity, adaptation through natural selection or migration to follow more suitable conditions. These options are not mutually exclusive (Williams and Dumroese, 2013).

The rapidity of climate change, however, is expected to exceed natural migration rates and the expected northward migrations of plant populations could be subjected to growth decline and mortality events (Lindner et al., 2014). Moreover, many tree species reach their southern distribution limit in the Mediterranean basin where drought constraints their ecosystem structure and function (Herrero et al., 2013). In forestry in particular, adaptive silviculture (reforestation and human-assisted population migration) is proposed to counteract long generation cycles and modest dispersal abilities of forest trees through selection of tolerant genotypes and ecotypes to expected abiotic stresses in forest restoration programs (Williams and Dumroese, 2013; Taïbi et al., 2014). However, there is an important knowledge gap for the development of operational programs in adaptive silviculture as highlighted by international organizations (EFI, 2009; FAO, 2013).

Pine species have been extensively used for reforestation and land restoration in many parts of the world because they are stress tolerant, have pioneer features, and facilitate the long-term development of late-successional hardwoods. Black pine (*Pinus nigra* Arnold) is one of the most important pines for mid to high elevation sites in southern Europe and especially the Mediterranean basin. The Salzmann pine (*P. nigra* ssp. *salzmannii*) is an endemic subspecies of black pine native to the Western Mediterranean basin and covers extensive areas of 350 000 ha in Spain (Rubio-Moraga et al., 2012). However, the species is expected to be severely affected by the impacts of climate change (temperature increase, irregularity of precipitation, drought, fire, etc.), so its regeneration, growth and mortality are seriously jeopardized in many ecological contexts (Banquét et al., 2013; Tíscar and Linares, 2011), especially when carrying out forest restoration programs. Salzmann pine exhibits a high genetic diversity among populations most probably due to the geographic isolation and the fragmented (sub-) Mediterranean distribution of populations (Rubio-Moraga et al., 2012). This genetic diversity has to be considered in reforestation programs in the new context of climate change as genetic material based on local seed sources may become ineffective in the near future (Aitken et al., 2008; Breed et al., 2013; Williams and Dumroese, 2013). Provenance trials could reveal disparate performance of provenances from various geographical and climatic origins (Varelides et al., 2001; Seho et al., 2010) regarding plantation survival and early growth performance, thus indicating differences in phenotypic plasticity, adaptation and GxE (Taïbi et al., 2014, 2015). These traits should be considered for managing reproductive materials, as both their magnitude and their pattern have deep implications for breeding, screening, and seed deployment, particularly when using a broad range of genotypes and environments (Potter and Hargrove, 2012). These issues need to be addressed in order to develop effective reforestation policies and to cope with the new environmental cues from climate change (EFI, 2009; United Nations, 2011; FAO, 2013).

In this context, studies focussing on better understanding of the optimal choice of plant material (different provenances) to changing environmental conditions as key factor for improving forest restoration programs and assisted population migration may be desirable. In this study, we hypothesized that Salzmann pine seed sources covering the geographical and climatic diversity of the species in Spain, will differ in their performance (survival and growth), thus indicating capabilities of local adaptations and/or ecotypic differentiation when planted beyond their original region on contrasted trial sites reflecting different ecological constraints. Six seed sources of Salzmann pine (i.e. plant material from which forest reproductive material is derived) were tested in three contrasting trial planting sites within the distribution range of the species over three years. The specific goals of this work were (1) to evaluate inter and intra-site seed sources variation in their survival and early growth performance as well as (2) to elucidate the phenotypic plasticity and the pattern of adaptation (for survival and early growth) by using the joint regression (Finlay and Wilkinson, 1963) and AMMI (Additive Main effect and Multiplication Interaction model, Gauch, 1992) models, taking into account both the transplant shock (one year after planting) and the final establishment of seedlings (three years of out-planting). A similar strategy was previously tested on *Pinus halepensis* with successful results (Taïbi et al., 2014, 2015).

2. Materials and methods

2.1. Plant material

Six seed sources (basic material) of *P. nigra* ssp. *salzmannii* covering most of the wide natural range of this species in Spain were selected. These seed sources correspond to five Spanish seed source zones specifically defined for the species (Alía et al., 2009) and one seed orchard (Table 1; Fig. S1). The later being improved material from the seed source 'Sistema Ibérico Meridional' and considered here to compare its performance to that of the natural populations. The six seed sources can be grouped within three main provenance groups according to Alía et al. (2009): Mediterranean warm sub-humid (Alto Maestrazgo), cold continental (Sistema Ibérico Meridional, Serranillo and Soria) and warm sub-dry (Béticas). Seeds were collected as open-pollinated bulk populations from selected stands and grown as one year old container stock in a public nursery located in Centro Nacional de Mejora Genética Forestal de Alaquàs, Valencia (Spain) in 2009 using the standard conditions for the species (shade-house, 33 weeks, 200 cm^3 container, sphagnum peat and a 15-4-7.5 slow release controlled fertilizer added at 7 g l^{-1}). During nursery culture, the different basic materials were placed following a randomized block design with four replicates in order to avoid important differences in stock quality associated with microsite differences in the nursery benches.

2.2. Trial sites

Seed sources were tested on three contrasting trial sites in Eastern Spain (good quality site, drought-prone, high altitude) along a south-north gradient reflecting different climatic regions (Table 1, Fig. S1). The northernmost site is in Berga (Barcelona); the soil is deep and mainly limestone, and the climate is considered a kind of transition between typical Mediterranean and sub-Mediterranean (humid Mediterranean with continental tendency). The vegetation is mainly composed of forest of *P. nigra* ssp *salzmanii* with the presence of *Quercus humilis*, *Quercus ilex*, and *Buxus sempervirens*. The site belongs to the cool sub-humid provenances group (Alía et al., 2009) i.e., none of the seed sources tested belonged to this site, and can be considered a good quality site for this species (Gandullo and Sánchez-Palomares, 1994).

The site Xert (Castellón, eastern Spain) is situated in one terraced hillside on a southeast-facing slope characterized by marl-clay soil parent material. The climate is typically Mediterranean with hot summers and mild winters that favour forests of *P. nigra*, *P.*