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Additional Information

Early establishment response of different *Pinus nigra* ssp. *salzmanii* seed sources on contrasting environments: an approach to improve reforestation programs and assisted population migration towards climate change

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Abstract

Forest restoration constitutes an important issue within the proactive adaptive measures for climate change on a global scale. However, effective implementation of these programs can only occur if target transfer guidelines are developed because current seed transfer guidelines 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 in ecological restoration and conservation of genetic resources. This study addresses a methodological approach to evaluate early plantation performance, phenotypic plasticity and the pattern of the adaptation of different seed sources in contrasting environments. To this end, six seed sources of Salzman pine were tested in three contrasting trial sites testing a hypothetical northward migration. Adaptation in each site was assessed by comparing the efficiency of 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 (GEI) were significant. Seedlings out-planted in the better site conditions did not differ in survival but in height growth; however, in the sites with higher constraints, survival differed among seed sources and diameter growth was high. Phenotypic plasticity was higher for survival than for early growth. The adaptation analyses 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. The results supported partially the hypothesis that northward migration of seed sources makes sense within limited transfer distances, and this was reinforced by the GEI results. The AMMI models have provided details to analyse complex GEI since they have been able to reveal the nature of interaction through the biplot. The present study could be valuable to address seedling adaptive transfer in ecological restoration and to determine the suitable seed sources for reforestation programs and assisted population migration under climatic changes.

Key words

Adaptive silviculture, survival, growth, phenotypic plasticity, Genotype by Environment Interaction, adaptation analysis.

1. Introduction

Climate change is one of the major challenges to forest ecosystems and biodiversity in the next future. It is expected to drastically modify growing conditions for trees (Herrero et al., 2013; Rittenhouse and Rissman, 2015). Greatest impacts are expected in the Mediterranean region, a

transitional climatic zone between arid and humid regions of the world, where temperature is expected to increase between 2 and 5°C while precipitation is expected to decrease until 30% over the next century, mainly in summer and autumn (Garrido et al., 2012; Lindner et al., 2014). Nevertheless, climate change impacts on plant species may vary significantly depending on the species' ability to adapt 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 (Linares and Tíscar, 2011; Lindner et al., 2014). Moreover, many tree species reach their southern distribution limit in the Mediterranean basin where drought constrains 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 (Pausas et al., 2004). 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 (*Pinus 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 global change (temperature increase, irregularity of precipitation, drought, fire...), so its regeneration, growth and mortality are seriously jeopardized in many ecological contexts (Banquet et al., 2013; Tiscar and Linares, 2011), especially when carrying out forest restoration programs. Salzman 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, phenotypic plasticity, adaptation and genotype by environment interaction (Taïbi et al., 2014). 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 et al., 2011; FAO, 2013).

In this context, studies focusing on a better understanding of the use of plant material (different provenances) to changing environmental conditions as background for improving forest restoration programs and assisted population migration may be desirable. In this study, we hypothesized that Salzman pine seed sources covering the geographical and climatic diversity of the species in Spain, will differ in their performance (survival and growth) and/or adaptive capabilities to exhibit local adaptations and/or ecotypic differentiation when planted beyond their original region on contrasted trial sites reflecting different ecological constraints,. Six seed sources of Salzman 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, early growth and phenotypic plasticity as well as (2) to elucidate 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).

2. Experimental methodology

2.1. Plant material

Six seed sources (basic materials) of *Pinus 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 sources zones defined for the species (Alía et al., 2009) and one seed orchard (Table 1; Fig. 1), which is improved material from ‘Sistema Ibérico Meridional’ and was considered here in order to compare its performance to that of the natural populations on the considered traits. 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 for one year in container stocks 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 cm³ 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 located following a randomized block design with four replicates in order to avoid important differences in stock quality associated to microsite differences in the nursery benches.

2.2. Trial sites selection and characterization

The study was performed on three different sites in Eastern Spain (Table 1, Fig. 1). The objective in this selection was to test the seed sources in contrasting sites (good quality site, drought-prone site and high altitude site) trying to keep a south-north gradient reflecting different climatic regions. The northernmost site is in Berga (Barcelona), located in a valley between the Central Depression and the Catalan Pre-Pyrenees at an altitude of 670 m.a.s.l where it is protected from the north winds by the mountains. In this area, the soil type is mainly limestone while the climate is considered as a kind of transition between typical Mediterranean and sub-Mediterranean. The site is subjected to humid Mediterranean climate with continental tendency. Annual rainfall is comprised between 700 and 900 mm. The vegetation, grown on deep soils is mainly composed of forest of *Pinus nigra* ssp *salzmanii* with the presence of *Quercus humilis*, *Q. ilex*, *Buxus sempervirens*, *Genista scorpius* and *Rubus ulmifolius*. The site belongs to the cool sub-humid provenances group (Alfá 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 Sanchez-Palomares 1994).

The site Xert (Castellón, eastern Spain) is situated in one terraced hillside on a southeast-facing slope at an altitude of 600 m.a.s.l. The site is characterized by marl-clay soil parent material. This area is the transition between two geologic periods; so the interface acts as a waterproof layer just below the limestone-dolomite dominating the higher parts. The climate is typically Mediterranean with hot summers and mild winters that favour vegetation of Mediterranean forest. The annual rainfall is around 600 mm. This hillside was occupied by *P. nigra*, *P. halepensis* and *Quercus ilex*. After the fire of 2002, there was a great natural regeneration of *P. halepensis*, *Rosmarinus officinalis*, *Ulex parviflorus* and *Cistus monspeliensis*. The site is located in the Mediterranean warm sub-humid group and hence, Alto Maestrazgo is the local seed source. However, the increasing frequency of summer droughts, together with its low altitude, is turning this site towards marginal for the species (Gandullo and Sanchez-Palomares 1994).

Tramacastiel is the southernmost site, located in south of Teruel's province (central-eastern Spain). The area belongs to the Jurassic and presents limestone-clay mother rocks with some sandstone presumably belonging to the surface as a result of quaternary glaciations and landslides. The soil is poor chalky-clay with little or low humus. The site is characterized by a continental mountain climate where frosts are common from November to April. Annual rainfall is around 531 mm and summer drought is mitigated by altitude (1200 m) and storms. Before the 1994 fire, the area was occupied by a natural stand of *P. nigra* in the north and in the ravines and *P. pinaster* in the rest although, on many occasions, both species overlapped. Since the fire, little regeneration of both species has occurred. Other species on the area are *Rosmarinus officinalis*, *Juniperus thurifera* and *J. oxycedrus*. The site is located in the cold continental group (Alía et al., 2009) and hence several of the seed sources tested can be considered as suitable local provenances. Main ecological constraints of the site are severe frosts, precipitation irregularity and shallow soils.

2.3. Site preparation, planting works and experimental layout

The trials were established in the same year (February 2010) on plots considered as suitable for forest field experimentation. Seedlings were out-planted using the techniques for operational reforestation programs. Before out-planting, sites were disked and each planting hole (0.5 x 0.5 x 0.4 m) was opened with a backhoe excavator. On all sites the experimental layout was a randomized complete block design with three blocks. Each seed source was represented by thirty-three trees per block for a total of ninety-nine trees per experimental site (594 seedlings per site). Seedlings were out-planted in rows and spaced by 2.5 m apart.

2.4. Plantations monitoring and measurements

Survival and growth variables (growth in height, H, and stem diameter, D) were measured three times during the first year of plantation: after out-planting during the spring growth, during summer drought period (July, after shoot elongation), and after summer (September-October, once the late growth period concluded). These measures were also done in the spring of the third

year of plantation (May) for all sites. By this way, the first three years of growth and the first two summer periods were registered, which is considered enough to evaluate early plantation performance (Landis et al., 2010). Appending 1, 2, 3, or T to the variables indicates the measurement period. Seedling height was measured to the nearest 0.5 cm from soil to the terminal bud and stem diameter at 1.0 cm above the soil surface. Survival was assessed by determining the status (alive, dead) of each individual.

2.5. Data analysis

Geographic and climatic variables with recognized influence on ecotypic variation of Mediterranean pines (Voltas et al., 2008) were selected (Table 1) to calculate the transfer distance from the trial site to the corresponding seed source native site (Aitken et al., 2008; Benito-Garzón et al., 2011). The Kaplan-Meier method (Kaplan and Meier, 1958) was used to compare the survival functions of the different seed sources on a site basis. The statistics of LogRank, Breslow and Tarone-Ware were used to detect significant differences either among overall seed sources or for pair wise comparisons when significant differences appeared. Differences among seed sources were tested also on H and D. For this purpose, a multivariate analysis of variance (MANOVA) using the Wilks' Lambda statistic was performed on all growth variables (for height or for diameter) as dependent variables and block and seed sources as factors. Follow-up analyses (ANOVA) were performed to assess whether differences among groups on the population means for each dependent variable were significant. Post-hoc comparisons of means were made using Tukey's test. In all cases, data were examined to ensure normality and homogeneity of variances, indicating in the text when these assumptions are violated.

Analysis of *phenotypic plasticity and adaptation* was addressed both on the post-planting shock stress of the first year (from plantation to the post-summer growth/survival of the first year of out-planting) and the final establishment growth/survival (after three years). This was done because post-planting response can be independent from that of the following years because it is

affected by multiple factors such as stock quality, works execution, precipitation following plantation, etc. Consequently, it resulted in six environments (3 sites x 2 assessments) for the analysis.

The *phenotypic plasticity* of each trait (x : Survival, height and stem diameter) and seed source was estimated from the absolute difference (distance) in the values of x between two individuals of the same seed source grown in different environments using the relative distance plasticity index (RDPI) from Valladares et al. (2006). A RDPI ranging from 0 (no plasticity) to 1 (maximal plasticity) can be obtained for each seed source as:

$$RDPI = \frac{\sum \frac{d_{ij \rightarrow i'j'}}{(x_{ij} + x_{i'j'})}}{n}, \quad (1)$$

where n is the total number of distances. Each relative distance d was calculated as the absolute value of the trait distance between each two selected individuals i and i' of the same seed source belonging to different environments j and j' , divided by the sum of their trait values. The overall standardized RDPI value, one per seed source and trait, is obtained by summing all relative distances obtained and dividing by the total number of distances.

Regarding *Genotype-Environment Interaction (GEI)*, the commonly statistical methods applied to GEI were performed to analyze adaptation patterns of the six black pine seed sources across the trial sites since it is recommended to use two or more approaches together (Chahal and Gosal, 2002). Here, (1) analysis of variance, (2) concurrence or joint linear regression (Finlay and Wilkinson 1963), and (3) the additive main effects and multiplicative interaction (AMMI) model (Gauch, 1992) were performed. The percentage of variation explained by each one of the three factors (seed sources, environments and their interactions) was calculated with the variance components, assuming all the factors were random.

The *analysis of variance* (ANOVA) model is

$$y_{ij} = \mu + G_i + E_j + G_i E_j + \varepsilon_{ij}, \quad (2)$$

where y_{ij} is the mean of seed source i in environment j ; μ is the general mean; G_i is the mean of the i seed source; E_j is the mean of the j environment, G_iE_j is the seed source by environment interaction (GEI) component and ε_{ij} is the residual variation.

The *joint regression analysis* is a simple linear regression that provides a conceptual model for genotype adaptation. In this approach, the components of GEI are linearly related to environmental effects, which are represented by the average performance of all the seed sources tested for the trait under consideration (Finlay and Wilkinson, 1963). The linear regression model is

$$y_{ij} = \mu + G_i + E_j + b_iE_j + \varepsilon_{ij}, \quad (3)$$

where y_{ij} is the mean of seed source i in environment j ; μ is the general mean; G_i is the mean of seed source i over all environments; E_j is the environmental index for environment j ($Y_{.j} - Y_{..}$); b_i is the slope of the regression specific for seed source i ; and ε_{ij} is the residual variation, which is assumed to be zero for the values averaged over replications. According to Finlay and Wilkinson (1963), the slope of regression was plotted against the mean of the measured variable of the seed sources to determine the wide adaptation of each seed source. When the regression coefficient of the seed source is 1.0 and its measured variable mean above the overall average, the seed source is well adapted to all the tested environments. If the mean measured variable is below the overall mean and the regression coefficient is 1.0, the seed source is poorly adapted to all environments. Regression coefficients above 1.0 indicate seed sources with a response above average to high performing environments. Regression coefficients below 1.0 provide a measure of below average responses to high performing environments and/or above average response to low performing environments. Because the regression is performed on the environmental mean, an ANOVA can be constructed according to Hardwick and Wood (1972) (for the joint regression analysis), where SS_{GEI} is partitioned into two components related, respectively, to the heterogeneity of seed source regressions and the residual term.

The *additive main effects and multiplicative interaction (AMMI)* method uses the standard ANOVA procedure, separates the additive variance from the multiplicative variance (interaction), and then applies PCA to the interaction (residual) portion from the ANOVA analysis to extract a new set of coordinate axes which account more effectively for the interaction patterns following the model:

$$y_{ij} = \mu + G_i + E_j + \sum_{k=1}^n \lambda_k \alpha_{ik} \gamma_{jk} + \varepsilon_{ij}, \quad (4)$$

where y_{ij} is the observation on the i^{th} seed source in the j^{th} environment; μ is the overall mean; G_i is the i^{th} seed source effect; E_j is the j^{th} environmental main effect; n is the number of PCA axes considered in the model, λ_k is the eigen value of the n^{th} PCA axis and α_{ik} and γ_{jk} are scores for the i^{th} seed source and the j^{th} environment on the n^{th} PCA axis and ε_{ij} is the residual term which includes the experimental error (Gauch, 1992). The SS_{GEI} was divided into n singular main components of interaction axes (IPCA) that described the standard portion (effects of seed sources and environments) and the noise portion, which is the additional residue of unpredictable and not interpretable responses. After selecting the AMMI model, the interpretation of wide or/and specific adaptation was accomplished by plotting IPCA1 on abscissa and IPCA2 on ordinate for each seed source and site scores. The lower the IPCA absolute values, the less plastic is the seed source. The ideal seed source is one with high performance and IPCA values close to zero suggesting wide adaptation to the tested environments. The undesirable one has high plasticity associated with low performance (Gauch, 1992). A large seed source' IPCA reflects more specific adaptation to environments with IPCA score of the same size, the reverse sign of IPCA value depicts negative interaction i.e. poor performance of seed sources in such environment (Chahal and Gosal, 2002).

Correlations between the measured (survival, growth traits, the studied indices) and the seed sources origin (geographic coordinates and climatic variables) variables were determined based on the coefficient of Pearson. The seed orchard 'H.S. Serranillo' was excluded in these analyses

because it is grown from various sources. All statistical analyses were performed using the computing environment R (R Development Core Team, 2013).

3. Results

Seedlings growth and survival were different among sites. The highest final survival rate and height growth were observed in Berga while the highest diameter growth was in Xert.

Tramacastiel, the site with highest mortality rates along the monitoring period.

3.1. Survival response

We observed a very variable survival rate among sites (Fig. 2). The most favourable site for the seedlings was Berga (78%) with a mean survival time of 22.8 months over 28 in the Kaplan-Meier analysis. Differences between different seedlings were not significant (Fig. 2). In Xert, the survival rate was 97% during the first year (2010) but decreased in the spring of 2012 (third year) to an average of 53%; the mean survival time in this site was 18.4 months out of 28 months. In this site we could observe significant differences among seed sources in the third year ($p < 0.01$): seedlings from 'Sistema Ibérico Meridional a and b' showed the highest survival rate (59.6% and 58.6% respectively) while those from 'Cordilleras Béticas' manifested the lowest survival (45.0%) (Fig. 2). As expected the highest mortality was found in Tramacastiel although the rate did not differ among seed sources. After the first summer of plantation survival rate was 56% and drop to 33% between September 2010 and May 2012. The mean survival time was about 13 months out of 28 months. (Table 3).

3.2. Growth response

We could observe significant growth differences (Fig. 3). In general, height growth, either seasonal or total, was higher in Berga (except during the first spring of out-planting, being higher in Tramacastiel). Diameter (D) growth in Xert and Tramacastiel presented the highest values

during the first spring of out-planting, whereas Berga showed more diameter growth during and after the first summer. At the end of the monitoring, D growth was higher in Xert (Fig. 3).

In the intra-site comparison of growth among seed sources (MANOVA), Wilks' Lambda statistic was significant for H and D in Xert and Tramacastiel but only for H in Berga (Table 2, Fig. 3). The differences in diameter were due mainly to the higher performance of 'Alto Maestrazgo' comparing to the other seed sources in Xert and Tramacastiel during the first year of out-planting (D1, D2 and D3) (Table 2, Fig. 3). Regarding height growth, during the first summer (H2) and the first autumn (H3), differences among seed sources were due to the highest performance of 'Soria' in Berga and 'Soria' and 'Alto Maestrazgo' in Tramacastiel comparing to the other tested seed sources. At the end of the monitoring, differences in Berga and Xert were due to 'Cordilleras Béticas' showing the lowest final growth (HT) among the other seed sources (Table 2, Fig. 3).

Significant correlations were found between geographic and climatic variables of the seed sources origin (Table 1) and growth on the three sites. In Berga, height growth (H2 and HT) showed positive correlation with latitude and negative with the altitude, the annual precipitation and the T_{MW} ($p < 0.01$). Diameter growth was correlated positively with latitude (D1 and D3, $p < 0.05$) and summer precipitation (D2 and D3, $p\text{-value} < 0.05$) and negatively with T_{MC} (D2 and D3, $p < 0.05$). In Xert, there were negative correlations of total height growth (HT) with altitude ($p < 0.05$) and T_{MW} ($p < 0.05$) and positive correlation with latitude ($p < 0.05$). However, diameter (D1, D2 and D3) was negatively correlated with T_{MC} ($p < 0.05$) and positively with summer precipitation ($p < 0.05$). In Tramacastiel, height was correlated positively with latitude and summer precipitation and negatively with T_{MW} ($p < 0.05$). Diameter (D1, D2 and D3) was negatively correlated with T_{MC} ($p < 0.05$) and positively with latitude and summer precipitation ($p < 0.05$).

3.3. Phenotypic plasticity

The tested seed sources showed low plasticity index (Table 3). After the first year of out-planting, survival plasticity ranged from 0.14 (Sistema Ibérico Meridional b) to 0.25 (Sistema Ibérico Meridional a) while growth plasticity ranged from 0.03 (Alto Maestrazgo) to 0.05 (H.S. Serranillo) for diameter and from 0.11 (Cordilleras Béticas and Sistema Ibérico Meridional a) to 0.15 (H.S. Serranillo) for height. After the final establishment of seedlings, 'H.S. Serranillo' manifested the lowest survival plasticity while 'Alto Maestrazgo' manifested the highest one. 'Alto Maestrazgo' and 'Cordilleras Béticas' manifested the lowest growth plasticity for both growth traits.

3.4. Genotype by environment interaction (GEI)

Analysis of variance showed that the most observed variation was attributed to the environment, explaining around 90% of the observed variability for survival, 92% for height growth and 97% for stem diameter growth, which indicates that the tested environments were extremely diverse (Table 4). The genotypic contribution was higher for height growth (5.1%) than for survival and stem diameter growth (2.4% and 1.2% respectively). The GEI effect was significant for all the measured traits. The interaction term (GEI) explained up to 7% of the total variance in survival, 3% of height and 2% of diameter growth.

The regression term explained around 38% of the SS_{GEI} for survival and stem diameter growth variability and about 42% for height growth (Table 4). The residual GEI was attributed to random deviations. The AMMI models have provided more detailed partitioning of the SS_{GEI} than linear regression since it accounted for at least 80% for height and about 90% for survival and diameter growth with the two IPCA components (sum of IPCAs with significant differences) while the regression model accounted no more than 42% of GEI terms, less than IPCA1.

3.5. Adaptation analysis

The joint regression analysis for survival (Fig 4a) showed that 'Cordilleras Béticas' and 'Sistema Ibérico Meridional b' presented regression coefficients $b_i < 1$ (less responsive to the changing

environments), although the former showed lower survival and the latter was above the average. Slope coefficients $b_i < 1$, indicated better response in relative terms, of the seed source in extreme environments (Tramacastiel-T/-P and Xert-T in this case) than seed sources owning $b_i > 1$. On the contrary, 'Alto Maestrazgo' survived above the average, showing better relative performance in better environments ($b_i > 1$; in this case: Berga-P/-T and Xert-P). 'H.S. Serranillo', 'Sistema Ibérico Meridional a' and 'Soria' were poorly adapted to all the environments (survival below average and slope near the unity).

Regarding growth, 'Cordilleras Béticas' showed similar survival, being less responsive to the changing environment and growing less in height. In diameter, these seedlings would be considered well adapted to the unfavorable environments since they showed an average growth and less plasticity to the environmental changes. 'Sistema Ibérico Meridional b' and 'Alto Maestrazgo' showed values above the average for height and diameter, especially in favourable environments ($b_i > 1$) (Table 3, Figs 4b,c). 'Soria' and 'H.S. Serranillo' showed height values indicating they were well adapted to all the tested environments, but the opposite trend was true for diameter in 'Soria'.

The AMMI results complement the adaptation analysis and they were graphically interpreted by plotting the IPCA1 scores against the IPCA2 scores to explore also wide and/or specific adaptation (Fig. 5). In general, the tested environments were more separated than the seed sources in the plots, indicating their higher influence on the variation of seedlings' performance. Moreover, sites had high contribution to the GEI as they were plotted on the opposite side of each other. In terms of survival, 'Soria' seedlings manifested a wide adaptation to all environments with their position closer to zero with less contribution to the GEI. However, 'Alto Maestrazgo' and 'Sistema Ibérico Meridional a' seedlings showed specific adaptation to both Xert environments. By the same, 'Cordilleras Béticas' was specifically adapted to Tramacastiel conditions. 'Sistema Ibérico Meridional b' seedlings, with high contribution to the GEI, were in an intermediate position between Xert-T and Tramacastiel-P manifesting their affinity to such

conditions. ‘H.S. Serranillo’ was well adapted in the post-planting period in the three sites, but only to Tramacastiel at the end of the study. Regarding height growth, a wide adaptation was observed for seedlings from both ‘Sistema Ibérico Meridional’. However, ‘H.S. Serranillo’ and ‘Soria’ seedlings were specifically adapted to the conditions of Berga. ‘Alto Maestrazgo’ seedlings manifested a specific adaptation to Xert-T and Tramacastiel-P conditions. As regards diameter, ‘Soria’ seedlings manifested a wide adaptation to all the tested environments. Specific adaptation was manifested by ‘Sistema Ibérico Meridional a’ and ‘Alto Maestrazgo’ to Tramacastiel-T and by ‘Sistema Ibérico Meridional b’ to Xert-T conditions. In addition, ‘H.S. Serranillo’ seedlings were more suited to Berga conditions. ‘Cordilleras Béticas’ seedlings showed a large IPCA of the same sign to post-planting conditions in the three sites (TR-P, XT-P and BG-P) for both growth traits indicating also their wide adaptation (Fig. 5).

4. Discussion

We have analyzed the variability in survival and growth among six Salzmänn pine seed sources along with their phenotypic plasticity and adaptation to three different environments in eastern Spain. The obtained data will be useful for future reforestation programs and assisted migration taking climate change in consideration. As expected. During the period of the study, the sites exhibited contrasting environmental conditions, with Berga acting as a core habitat, while Xert and Tramacastiel showed constraints related either to summer droughts or frost occurrence (Table 1). The results evidenced a significant variation among seed sources, revealing that matching genotypes, environments and their interactions in assisted migration programs may require the study of their phenotypic plasticity and adaptation analyses.

Seedlings out-planted in Berga showed the highest final survival (78%), without significant differences between seed sources. Similar results were reported by Perez et al. (2005) in the sub-humid north-western Spain, with survival rates above 85% and no differences among 22 provenances, confirming that was an ideal location to be used as control site. In contrast,

seedlings grown at Xert and Tramacastiel showed higher mortality (53% and 33%, respectively) and differences among seed sources' performance. Summer drought is a key factor affecting Salzman pine seedlings survival (Tiscar-Oliver, 2007; Tiscar and Linares, 2011), especially in the dryer regions of Spain, and this can explain the high decrease of survival in Xert after 2010 (with less than 50% of the expected summer rain, see Table 1) as well as in the second and third years in Tramacastiel. Summer precipitations below 52 mm fall out of the ecological range of this parameter for this species in Spain (Gandullo and Sanchez-Palomares, 1994). However, there was also an important survival decrease in the spring of 2010 in Tramacastiel, which was attributed to the minimal temperatures recorded after outplanting in February (average: -3.2°C; range -7.8 to 4.8°C) and March 2010 (average: 0.16°C; range -7.2 to 4.1°C). Kreyling et al. (2012) suggested that low temperatures may pose a serious threat which naturally determines the poleward range limits of black pine since that extreme winter temperatures cause frequent freeze-thaw events, which induce xylem embolism and frost drought (Mayr, 2007). This dual stress (drought and frost) made that survival patterns among seed sources were not consistent along time (eg. 'Sistema Ibérico Meridional a, b' and 'Cordilleras Béticas').

Growth also differed among seed sources according to site and time, and the ranking varied among the sites, as reflected in the strong interaction component in the analysis (Table 4). Among sites, seedlings from 'Soria', 'Alto Maestrazgo' and 'Sistema Ibérico Meridional b' showed higher growth. Differences in early growth performance among *Pinus nigra* provenances of different geographic origins have been observed in trials with different results depending on the sites (Varelides et al., 2001 and references therein). Height differed more among seed sources than diameter growth within each site. This can be explained by the fact that seedlings diameter, being frequently more sensitive to change in environmental conditions, is further fixed than height in establishing seedlings (Baquedano et al., 2008). However, there were specific differences among sites, with height growth being higher in Berga while in Xert diameter growth was the highest and height growth rates were the lowest. Higher diameter growth, which is

related to root growth (Mexal and Landis, 1990), can be considered as a response to water stress on this site, as this is essential to ensure survival after transplanting (Grossnickle, 2005). Several studies have shown that black pine growth is sensitive to water deficit during spring and summer and to high summer temperatures (Martin-Benito et al., 2010; Linares and Tiscar, 2010).

Seedlings may significantly reduce height and diameter growth as an adaptive strategy (Voltas et al., 2008). This is known to occur also in other pine species, e.g. *P. sylvestris* and *P. taeda* (Cregg and Zhang, 2001) and *P. halepensis* (Taïbi et al., 2014).

It can be drawn from the survival and growth response that under good site conditions (Berga in our case), seed sources differences were mainly related to the height growth, and ‘Soria’ and ‘H.S. Serranillo’ (which is improved from ‘Sistema Ibérico Meridional’) showed the highest growth, whereas those seedlings associated to large transfer distance from their site origin to the trial (‘Cordilleras Béticas’) performed worse. This trade-off between tolerance of provenances from low and high latitudes and growth performance has been also reported in other conifer species such as *Pinus taeda* (Schmidting, 1994) and *Pinus contorta* (Rehfeldt et al., 2002). On the opposite, when environmental constraints operate, survival (and growth) differences came up, and the hypothesis of northward migration became supported: seed sources from the warm sub-dry group (‘Cordilleras Béticas’) survived more in the upper-north continental site (especially after the drought of years 2011-2012) than local provenances (Sistema Ibérico Meridional), and the seed sources from this region (Tramacastiel belongs to Sistema Ibérico Meridional) survived more in the upper-north warm sub-humid site. Again in this case, long transfer distances and frost tolerance appear as interacting factors affecting survival of the southernmost provenance.

Tramacastiel site acted as a marginal site where a compromise between frost and drought tolerance has determined establishment success. Therefore, referring to the early pine performance, seed sources not belonging to the target site could be selected for facing current climate irregularities in different environments (Taïbi et al., 2014). Similar clinal trends were

exhibited by *P. sylvestris* (Persson, 1994) and *P. halepensis* (Agúndez et al., 1997; Climent et al., 2008; Taïbi et al., 2014).

Regarding phenotypic plasticity, and according to RDPI range established by Valladares et al. (2006), the tested Salzman pine seed sources showed low phenotypic plasticity for both growth traits and survival, which concords with previous results of pine species such as *Pinus halepensis* (Baquedano et al., 2008) or on other Mediterranean species such as *Quercus coccifera* (Balaguer et al., 2001), *Quercus robur* and *Fagus sylvatica* (Valladares et al., 2000, 2002). Some traits might vary in their sensitivity to the environment being more plastic than others (Aitken et al., 2008; Benito-Garzón et al., 2011) as was the case in our experiment where height growth plasticity was higher than of that diameter among sites. The facts that higher plasticity values were correlated positively with cooler temperatures and summer precipitation and negatively with warmer temperatures corroborates the theory that limited phenotypic plasticity is beneficial in stressful environments (Chambel et al., 2005).

Either from the joint regression analysis or the combined ANOVA with AMMI, the environmental effect has shown a great influence on seedlings performance variation (Table 4). This means that the tested environments were extremely diverse and confirms findings that environment is the major factor affecting Salzman pine growth performance (Amodei et al., 2012). However, the significance of the GEI term justifies the need for investigating the nature of differential response of the seed sources to the experienced environments. Significant genotype by environment interactions (GEI) have been frequently reported in *Pinus nigra* provenance trials and were attributed to the broad natural distribution of the species in a wide range of environments leading to distinct races (Varelides et al., 2001 and references therein), although other studies did not report significant interactions (Perez et al., 2005). The removal of the additive parameter from the multiplicative component in the AMMI model overcomes one major statistical deficiency that is the dependency of the site and population indicators encountered in joint regression (Gauch, 1992; Wu and Ying, 2001) and thus the AMMI models

have provided more detailed partitioning of the SS_{GEI} than linear regression since they accounted for at least 80% for height and 90% for survival and diameter growth with the two significant IPCA axes while the joint regression model accounted no more than 42% of GEI terms.

Therefore, the AMMI models have been recommended to analyse complex GEI since it have been able to reveal the nature of interaction mainly if it captures more SS_{GEI} than joint regression (Nachit et al. 1992). Even with this, the joint regression analysis remains valuable for determining genotypic behaviour over environments since it demonstrates comparable results to the AMMI analysis and allows for the prediction of performance in untested environments (Barnes et al., 1984).

The differential responses of the seed sources selected from a broad geographic area have reflected their differential adaptation strategies to natural environments of Salzmänn pine, which reinforces the need of cautious selection of the seed source according to the target environments (Wu and Ying, 2001). In this sense, the joint regression and the AMMI results refined those obtained in the Figures 2 and 3. An attempt to synthesise the overall results of this work is presented in Table S1. Survival is likely to be the most important trait to focus at this time of early establishment. It can be observed that ‘Soria’ showed a wide adaptation to all the environments, since it is specifically appointed just once. On the other hand, ‘Alto Maestrazgo’ and ‘H.S. Serranillo’ seedlings manifested a specific adaptation to good site conditions (Berga and Xert-P), whereas ‘Cordilleras Béticas’ seedlings (from the lower latitude in the South of Spain, where drought is more frequent) manifested specific adaptation to the poor conditions encountered in Tramacastiel of shallow soil and adverse climate by performing high survival in the site. Similar results were found for *Pinus halepensis*, where Southern provenances performed better in marginal habitats (Taïbi et al., 2014). Besides, ‘Sistema Ibérico Meridional a and b’ seedlings showed disappointing results except for survival in Xert, indicating that the ecotypic variation in this seed source could make advisable a deeper subdivision. Moreover, the general

performance of 'H.S. Serranillo' (improved material from 'Sistema Ibérico Meridional') can be considered as average or above the average in most cases.

It should be stressed that early growth assessments for analysing phenotypic plasticity and seed sources adaptation' may not be reliable for assessing GEI at maturity, which implies the need to evaluate GEI at mature ages in forest trees (Chambel et al., 2005). As well, studies at the molecular level on the relationship between adaptive traits and environments are required to define seed zones and achieve advanced understanding for more Salzmänn pine populations' reforestation and restoration in specific environments in the context of assisted population migration toward climate changes. However, the transfer of seed sources to the tested environments should be carried out with caution and carefully managed from the genetic conservation point of view.

Conclusions

This work has shown a multi-analysis approach to address the early performance of six Salzmänn pine seed sources outplanted in three environments in eastern Spain regarding their variability in survival and growth along with phenotypic plasticity and adaptation for subsequent reforestation and assisted migration toward climate change.

Seedlings out-planted in better site conditions did not differ in survival performance but in height growth; however, under climate constraints, survival differed among seed sources and diameter growth was higher (Xert). Seed sources manifested higher plastic response for survival and height than for diameter, though phenotypic plasticity was low in general. The differences in survival and early growth performance among seed sources support the hypothesis that northward migration of seed sources makes sense within limited transfer distances. However, the genotype by environment interaction survey carried out in this work refines and complements the performance of the different seed sources. Either from the joint regression analysis or the combined ANOVA with AMMI, the environmental effect showed a great influence on seedlings

performance variation at early growth stage. The differential responses of the tested Salzmänn pine seed sources from a broad geographic area have reflected their differential adaptation strategies to the experienced environments and thus, the justification to further investigate the nature of these differential responses to the assisted migration strategy. The present study makes a contribution towards reforestation and breeding research programs in the species, with practical implications for abiotic stress tolerance, assisted population migration and adaptive silviculture for climate change. Furthermore, the genomic approaches are becoming necessary to confirm our findings in order to select for more resistant genotypes at the molecular level.

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