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Substrate effect on the growth of seedlings of four provenances of Atlas cedar (*Cedrus atlantica* M.) in plant nursery

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Abstract
In Morocco, scientific research on the Atlas cedar (*Cedrus atlantica* M.) has helped to understand its ecology and to characterize its behavior in response to drought. However, few studies have dealt with the analysis of its appearance in relation to the diversity of habitats and different ecological situations. The aim of this study is to assess, in plant nursery, the relative magnitude variation in seeds germination and seedling growth among four cedar provenances: Occidental Middle Atlas = OMA, Eastern Middle Atlas = EMA, Eastern High Atlas = EHA and Occidental Rif = OR. The results showed that seeds collected from Occidental Rif and Eastern Middle-Atlas have the ability to germinate earlier than the two provenances. This observation was not significantly maintained after two weeks throughout the germination period. Nevertheless, the growth expressed by the average height and collar diameter of cedar seedlings showed that, over time, the OMA provenance was significantly more efficient in the first year of culture. This result was maintained even after transplantation of the different provenances in the two types of substrate (basalt and limestone). The growth of the OMA seedlings in basaltic soil was significantly better compared to the limestone soil. These findings are beneficial for the reforestation programs to safeguard the cedar ecosystem ensure the continuity of the cedar forest ecosystem. It emphasizes the importance of using suitable seed provenances on appropriate plantation sites.

Keywords: Cedrus atlantica, provenances, seed germination, growth seedling.

1. Introduction
In Morocco, cedar Atlas woodlands represent approximately 2.8% of the total forest cover [1]. This ecosystem is conventionally divided into four biogeographical regions [2, 3]: Rif mountains region with an area of 15,000 ha, the central Middle-Atlas 120 000 ha, the Eastern Middle Atlas region 23,000 ha and Eastern high-Atlas 26,000 ha. Over all their natural range, the cedar forests are found in altitudes between 1500 and 2600 m [4].
The Atlas cedar grows in different types of substrates [4, 6]. The Rif mountains schist and the central Middle-Atlas basalt offer a suitable water balance due to the nature of substrates constituted from limestone and dolomitic limestones in the Eastern Middle Atlas or High-Atlas [7]. The annual rainfall in these regions ranges from 500 mm to 2000 mm per year [1,8] and the cedar woodlands are observed in bioclimates per-humid, humid and sub-humid where they organize different plant groupings [9]. These pedo-climatic conditions have a great influence on cedar natural or assisted regeneration [10]. It has been noticed that the germination of cedar seeds is easy on basaltic substrates, doleritic and quartzite sandstone, but more difficult on limestone and dolomite compact [11].
The altitudinal heterogeneity of regeneration between the warm slopes and bottomlands to precipitation, type of substrate and temperature which should reach 9 to 10°C in a period of 9 to 10 days for the onset of seeds

germination[10]. Besides these factors, social impacts in these eco-regions are important and exceed their potential production and their ability for any natural regeneration [9]. Although cedar woodlands are of high ecological and socio-economical interests, they remain, however, subjects to an intense degradation and to a decline phenomenon [12, 13]. In the context of climate change expressed by the increase of temperatures and the reduction of rainfall this could get much worse [14,15].

Numerous studies have established great variations in seedlings growth in response to different environmental conditions (light regime, water availability and nutrient status) [16,17]. In Morocco, scientific research on the Atlas cedar helped to understand its ecology and to characterize its reaction in response to water stress both in the laboratory and under natural conditions [15,18,19,24]. Some authors have shown that cedar tree has a high phenotypic plasticity and is well adapted to drought [25-27]. However, few studies have dealt with the analysis of the phenological behavior in relation to cedar habitats and different ecological situations. The geographic variability within the genus of cedar especially that of Morocco shows a fairly genetic variability which explains the high risk of reforestation failures [28]. Therefore, new investigations on genetic characterization of Atlas cedar are crucial in order to determine its genetic variability. This allows the selection of provenances with high adaptation potential to be used in national reforestation programs.

Although the artificial regeneration based on plants production in nurseries was undertaken a long time ago, the choice of genotypes has not taken into account the potential growth of seedlings, which can vary depending on the seed origins. A trial conducted in Italy on three Moroccan seed origins has shown the importance of the provenances. In Fact, 7 years after plantation, the seeds originated from the Middle -Atlas and the High-Atlas have shown to be more vigorous than those of the Rif mountain region [29,30].

In this context, the aim of this study is to evaluate, in the plant nursery of Azrou (Middle Atlas), the relative magnitude of variation in seeds germination and seedling growth among four Atlas cedar provenances cultivated in two types of soil (basalt and limestone) extracted from a cedar stand in Moroccan Middle Atlas. The results will have a practical value for improved and successful of the regeneration programs by determining the best adapted provenance for the breeding of planting materials.

2. Materials and methods

2.1. Origin of provenance

The present study focused on four Atlas cedar provenances: Tizi Ifri (Occidental Rif), Sidi M'guild (Occidental Middle-Atlas), Bouadel from the Eastern High-Atlas and the Tamtroucht (Eastern Middle-Atlas. The habitats of these provenances are summarized in table 1.

Table 1: Cedar seed stand sources by region (According to [2,3,31])

Provenance region		**Occidental Middle-Atlas**	**Eastern Middle-Atlas**	**Eastern High-Atlas**	**Occidental-Rif**
Name of provenance		Sidi M’guild	Tamtroucht	Bouadel	Tizi Ifri
Area (ha)		340	25	202	229
Altitude (m)		1880	1800	2090	1820
Latitude N		33°12’	33°48’	32°27’	34°51’
Annual rainfall mean		884	765	415	1103
Source substrate		Limestone Dolomite	Silty-Schist	Limestone	Schist and Quartzite
Soil texture		silty clay	Silty, clay sand	Clay	Silt Clay and sand
Bioclimatic stage		subhumid with cold winter	subhumid with cold winter	humid with cold winter	humid with cold winter
T°C	**Maximum**	30.9° - 27.6°	28.7° - 26.5°	29.6° - 23.2	28.8° - 23.7
	minimum	-4.7° - -0.5°	-6.4° - -3	-8.3° - -3.1°	-5.6° - -0.4°

2.2. Production of cedar seedlings

2.2.1 Seed features

All seeds used in this experiment were provided by the Regional Forest Seed Centers of Chefchaouen (Rif) and Azrou (Middle-Atlas). Qualitative and quantitative features of these provenances are given in table 2.

Table 2: Cedar seed characteristics of each studied provenance

Provenance	Woodland nature	Altitude (m)	Germination (%)	Purity (%)	Number of living germs /kg	Weight of 1000 seeds (g)
Sidi M'Guild	Natural	1880	65	98	8450	79.6
Tamtroucht	Natural	1800	70	95	9100	79.9
Bouadel	Natural	2090	70	98	9100	75
Tizi Ifri	Natural	1600	77	90	5000	80

2.2.2. Seed stratification

To break dormancy, seeds of different provenances were sorted and disinfected by soaking them in sodium hypochlorite solution at 20 % for 10 min, then rinsed three times with a sterile distilled water. These latter were soaked a second time in 33 of hydrogen peroxide for 10 minutes, followed by three rinses with a sterile distilled water. At the end, they were submerged in distilled water and placed in the refrigerator at 4 °C for 15 days, by changing the water every 48 hours [32].

2.2.3. Germination of seeds

The experiment was conducted at the Azrou forest nursery located in the Middle-Atlas (X: 514.920; Y: 314.514). Stratified seeds were sown in March 2010, in containers with capacity of 300 (cubic centimeter). Three seeds per cell filled with breeding ground usually used in the nursery. The substrate consists of a mixture of forest soil, sand and compost. The physico-chemical composition of this substrate is reported in table 3.

Table 3*:* Physicochemical composition of forest soil

pH	Electrical conductivity (mmoh/cm$^{3)}$	Organic matter (%)	Carbone (%)	Nitrogen (‰)	Ratio C/N	water retention capacity (%)
7.03 ± 0.05	263 ±7	20.7± 0.5	10.3 ± 0.5	0.7±0.05	185	207.6 ±3.2

To ensure roots pruning, the containers were supported on benches of 50 cm height. After germination, the seedlings were removed in order to keep only one seedling per cell. In parallel to this test, the kenitic germination of seeds was assessed in the laboratory in CRF, under controlled temperature (10°C), humidity (80-90%) and brightness (55.000lux). This experiment aimed to better promote the germination of different sources by reducing the interference of climatic factors. Cultures were watered as needed and monitored weekly for germination lifting during a month.

2.3. Growth of plants

Two dependent experiments were carried out in this study (two phases):

In the first year, the plants were grown in breeding ground usually used in the nursery and fertilized by a commercial fertilizer NPK (20-20-20). These plants were also treated in June with a curative antifungal against damping-off (Previcur: 2cc/L in the first and the second week and Simuscles (Taschigarine) to 2cc / L in the third week).

In the second year, the plants were transplanted in polyethylene bags with a volume of 20L, filled with two types of substrates shipped from the Middle-Atlas cedar forests: limestone and basaltic substrate. After transplantation, all plants were amended by a commercial liquid fertilizer, whose chemical composition is shown in table 4.

Table 4: Chemical composition of the fertilizer

Free amino acid	10	Polysaccharids	5
L-Glutamic acid	5.5	Bore(B)	0.2
Glycine	2.5	Iron (Fe)	4.5
L-Méthionine	2.5	Manganèse (Mn)	1
Organic nitrogen	3	Molybdène (Mo)	0.05

The kinetic growth was evaluated monthly using the measurement of the shoot height and the collar diameter by a caliper (150 mm^3 volt caliper accuracy ± 0.02 mm). The experiment was conducted in a randomized block design with 3 repetitions, for a total of 216 seedlings per provenance. The plants were watered twice every day.

2.4. Statistical analyses

Analyses of data were performed in two steps. The first step concerned the first year study in 2010 and in 2011 has been done the second one. Regarding 2010, the germination was analyzed by using a repeated-measure ANOVA (General Linear Models, GLM), after checking for homoscedasticity (Levene's test) and for sphericity (Mauchly's test). Because of following the same blocs (randomly chosen, n = 3), germination was analyzed by using a repeated-measures ANOVA (General Linear Models, GLM), after checking for homoscedasticity (Levene's test) and for sphericity (Mauchly's test). The number of newly germinated seeds per week (in March) was included as a within-subject factor, and the provenance (OR, OMA, EHA and EMA) as a between-subject factor.

In the same way, the monthly height of cedar seedlings and the basal diameter have also been included as repeated measures (within-subject factor), with the provenance as the sole between-subject factor. In 2011, because the height of Cedar seedlings and the basal diameter were found to be highly correlated ($rp = 0.778$, $p < 0.001$), we decided to take into account only the height of seedlings in analysis knowing that the same results can be assigned for the basal diameter. Just like the first step, we analyzed growth of cedar seedlings changes by using repeated-measures ANOVA (GLM).

The height of cedar seedlings data per month in the period between April-November, was considered as a within-subject factor, whereas the nature of substrate (basaltic versus limestone) and the provenance as between-subject factors. For both stages of analysis, we used Tukey's significant difference (HSD) *post-hoc* tests to know which of the four provenances provides a higher number of newly germinated seeds and a better growth of Cedar seedlings. All statistical analyses were conducted using SPSS ver. 20. We report mean ± SE and two-tailed 5 significance values throughout the paper.

3. Results and discussion

3.1. First year of experimentation

3.1.1. Seed germination

In the first year of the experimentation, the number of newly germinated seeds increases progressively according to weeks (Repeated measures ANOVA, F = 198.82, ddl = 3, $P < 0.001$), however it does not vary according to the provenance ($F = 0.689$, ddl = 3, $P = 0.584$). The interaction between the provenance and the week was significant (F = 18.36, ddl = 3, P < 0.001). Indeed, within the first two weeks, the Occidental Rif and Occidental Middle-Atlas seeds germinated more and faster than those of the Eastern High-Atlas and Occidental Middle-Atlas (figure 2), whereas, in the remaining weeks, the reverse effect was recorded (figure 1).

3.1.2. Cedar growth

The height of Cedar seedlings has gradually increased according to months (Repeated measures ANOVA, F = 3288.45, ddl = 7, $P < 0.001$) (figure 2a). It has also varied depending on the provenance (F = 6.593, ddl = 3, $P < 0.001$). Throughout the eight study months, the Occidental Middle-Atlas provenance has been the most prolific (F = 5.846, ddl = 21, $P < 0.001$, *Post-hoc* Tukey test) (figure 2a). Similarly, the basal diameter variable has also varied according to the month (repeated measures ANOVA, F = 2096.14, ddl = 6, P < 0.001), the provenance (F = 11.611, ddl = 3, P < 0.001), and their interactions (F = 5.738, ddl = 18, P = 0.001) (figure 2b).

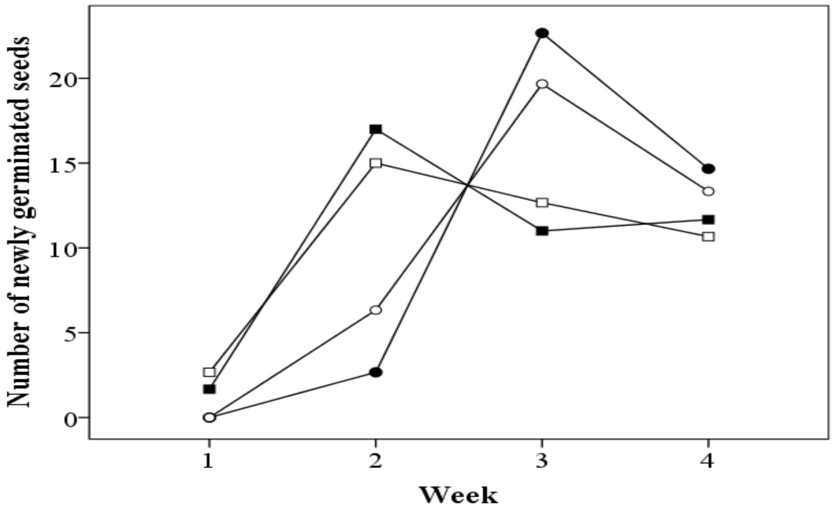

Figure 1: Kinetics of cedar seeds germination (● = OMA; ○ = EMA; ■ = EHA; □ = OR)

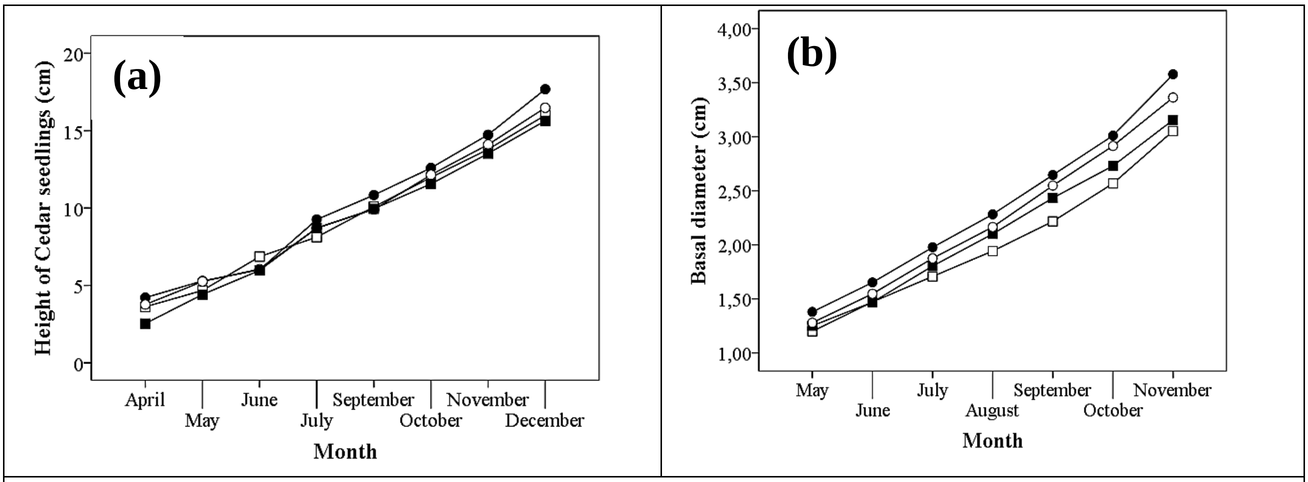

Figure 2: Height (a) and Basal diameter (b) of Cedar's seedlings *Cedrus atlantica* depending on provenances ((● = OMA; ○ = EMA; ■ = EHA; □ = OR)) in 2010.

3.2. Cedar growth during the second year of experimentation

Throughout the two year of the present study and after transplantation of the four provenances to basaltic and limestone substrates, the height of cedar seedlings differed depending on nature of soil and provenance: the growth of seedlings from basaltic soil and from the OMA provenance was significantly better compared to limestone soil and to three other provenances (*Post-hoc* Tukey test) (Table 5, figure 3, 4).

Table 5: Results of the final general linear models analyzing variation in height of cedar seedlings according to time, nature of soil and provenance.

Predictor variable	d.f.	F	P
Time	8	15226.07	<0.001
Nature of soil	1	219.60	<0.001
Provenance	3	124.28	<0.001
Time*nature of soil	8	201.09	<0.001
Time*provenance	24	47.06	<0.001
Time*nature of soil*provenance	24	15.53	<0.001

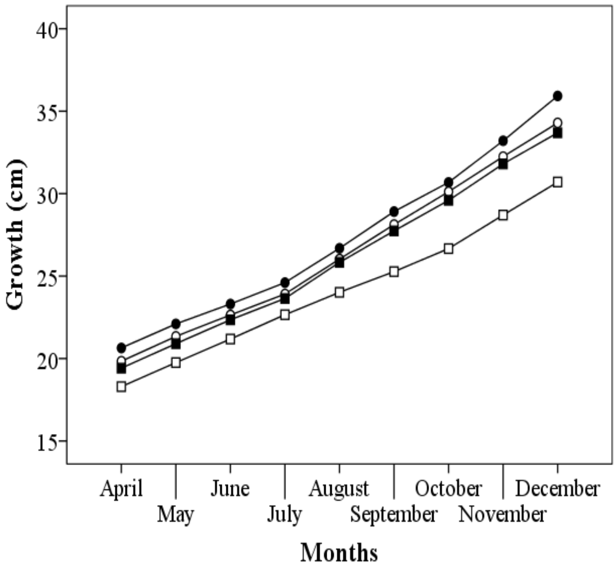

Figure 3. The growth of Cedar seedling depending on the time and the four provenances (● = OMA; ○ = EHA; ■= EMA; □= OR)

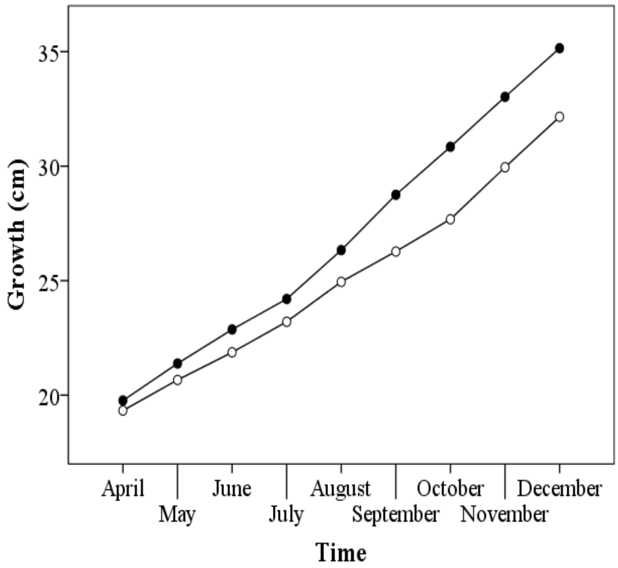

Figure 4. The growth of Cedar seedling depending on the time and the nature of soil (● = basaltic soil and ○ = limestone one).

Discussion

It is acknowledged that Cedrus atlantica played important ecological and socio-economical functions in Morocco [2]. Understanding its morphological behavior in relation with habitats is a key factor to conservation and regeneration of this endemic North African species. Moreover, field provenance trials for perennial species such as *C. altlantica* require longer time and may not answer the immediate needs of the forest managers. In such context, rapid and reliable results can be produced through nurseries experiments.

The results of the first year study have demonstrated that seeds originated from Occidental Rif and Eastern Middle-Atlas were able to germinate earlier than the two other provenances. This finding was significantly maintained throughout 2 weeks of the germination period. Such difference among provenances can be brought by different intensities of natural selection acting upon this trait in their natural habitat [33]. Indeed, seed germination is dependent on the origin and environmental germination factors [34]. The same author stated that germination of cedar varies with their geographical origin, the treatment undergone and the germination conditions. Numerous studies [35-37] conducted in drought situations have overall showed that species seeds and even provenances from dry areas can germinate more easily than others on stressed environments. By considering the ecological characteristics of the four studied provenances, we hypothesized that they may play a role in the germination process. For instance, the Rif area supports higher rainfall values whereas those subjects to the combined influence of the Mediterranean and the Atlantic, are much wetter and warmer than other cedar forests in Morocco [38]. In the Middle-Atlas, climatic conditions are under the influence of the masses of moist Atlantic areas and receive annual rainfall up to 1200 mm. In contrary, High-Atlas Cedar provenances are localized in extremely cold conditions of the Oromediterranean stage with minimum temperatures down to -9°C [2].

On the other hand, the monitoring of height and collar diameter in cedar plants over time showed that the Occidental Middle Atlas provenance is significantly more efficient during the first year of culture. This is consistent with the study that showed that there is a clear effect of origin on growth and productivity of this coniferous [3]. This kind of behavior can be connected to the environmental specificity. The significant correlation between seedlings growth and environmental variables were determined among some conifer tree species both in nurseries and fields [39].

After transplantation of the different provenances in the two types of substrate (basalt and limestone), we noticed that there is, throughout the growth period, a positive correlations between growth traits (height growth and collar diameter). The increasing trends for both shoot height and collar diameter signify the utilization of any of the juvenile characters for interpretation and early selection of provenances for further testing. In this sense the height of cedar seedlings has varied depending on both nature of soil and provenance. The growth of seedlings in basaltic soil and in the OMA provenance was significantly better compared to limestone soil and the other provenances. The height growth of cedar seedlings was significantly affected by the type of substrate used [21], this is consistent with our results. Similarly, a study showed that the nature of the substrate, the texture and the depth are essential parameters in the establishment of the cedar species [40]. The same authors have also reported that cedar natural regeneration is very favorable in deep basaltic soils, while on shallow carbonate substrate; seedlings dry out quickly in summer period. Overall, by their favorable physicochemical properties and their capacity in retaining water, basaltic soils are known to affect positively the growth and the development of the Atlas cedar [41-42-43]. In other way, as the experiment was conducted under similar environment, the variation based on height and collar diameter growth among the seedlings provenance may be due to the genotypes. The growth and collar diamete of seedlings are the consequence of evolved genetic responses to environmental stimuli [44]. Seedling growth is a precondition for conservation and sustainable use of genetic resources which depends upon understanding of breeding system, genetic inconsistency, and evolutionary forces in forest tree improvement [45].

Conclusion

Results show clearly that the use of appropriate seed sources for the specific areas would positively affect cedar reforestation. Although the Occidental Middle Atlas provenance presented a lower germination potential in the first two weeks, it is the best performer in growth traits, and hence can be selected as a source of planting material. Likewise, basaltic substrate is favorable for the development of the cedar species. Further knowledge of field growth performance is highly recommended. Field trials are also advisable when studying growth in relation to factors, such as drought or nutrition. This may provide practical solutions to the cedar reforestation in Morocco, particularly in the context of global climate change, improve the quality of information gained from experiments and direct proper decisions on seed sources.

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