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Effect of nitrogen concentration and cotyledons on nitrogen preference in seedlings of *Cedrus atlantica* Manetti

Photo 1.
Cedrus atlantica Manetti, representative aged specimens from the Gouraud and Moudmame forests, Morocco.
Photo B. El Amrani.

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RÉSUMÉ

Effets de la concentration en azote et des cotylédons sur les préférences pour l’azote des plantules de *Cedrus atlantica* Manetti

La matière organique est la source la plus importante de nitrate et d’ammonium, les deux principales formes d’azote utilisées par les plantes, dans le sol des forêts de conifères. La préférence et/ou l’adaptation d’une forme d’azote des génotypes des plantules est un facteur déterminant dans le succès des programmes de reboisement, non seulement pour les espèces de cèdres mais aussi pour toute autre essence forestière. Dans une chambre de croissance contrôlée, des plantules de *Cedrus atlantica* M. de deux origines (Gouraud et Moudmame) ont été cultivées sur un sol naturel ou sur de la matière organique fertilisée avec différentes formes et concentrations d’azote. Nos résultats montrent un besoin élevé en azote et différentes préférences pour les formes d’azote, qui se manifestent principalement au niveau des racines secondaires. D’autre part, les variations au niveau des cotylédons affectent la croissance et la réponse des plantules à la forme d’azote et révèlent ainsi différentes formes d’adaptation à la nutrition azotée, qui sont liées aux cotylédons et à l’âge des plantules. La réponse de la croissance de *C. atlantica* à la forme et à la concentration d’azote est abordée à la lumière de l’effet de l’origine et des cotylédons. L’ensemble de ces résultats suggère que la connaissance des besoins en azote des différents génotypes de *C. atlantica* au stade juvénile devrait s’avérer utile pour la réussite des reboisements de cette essence.

Mots-clés : cotylédons, forme d’azote, racines secondaires, provenance des semis, *Cedrus atlantica*.

ABSTRACT

Effect of nitrogen concentration and cotyledons on nitrogen preference in seedlings of *Cedrus atlantica* Manetti

The organic matter constitutes the most important source of nitrate and ammonium in coniferous forest soil, which are the two major nitrogen forms used by plants. The preference and/or adaptation of one N-form over another by seedlings genotypes is a determinant factor in the success of reforestation programs, not only for cedar species but any other forest essence. In a controlled growth chamber, the seedlings of two provenances of *Cedrus atlantica* M. (Gouraud and Moudmame) are grown on natural soil or on organic matter fertilized with different N-form and concentration. Our results show a high nitrogen requirement and a different preference towards nitrogen forms which is mainly manifested at secondary roots. On the other hand, variations in the cotyledons affect the growth and the response of the seedlings to the nitrogen form and thus reveal different forms of adaptation to nitrogen nutrition which are linked to the cotyledons and to the age of seedlings. The growth response of *C. atlantica* to nitrogen form and concentration is discussed in light of the effect of the provenance and cotyledons. Taken together, these results suggest that knowledge of the nitrogen requirements of the different genotypes of *C. atlantica* at the juvenile stage could be a good tool for successful reforestation of this forest species.

Keywords: cotyledons, N-form, secondary roots, seed provenance, *Cedrus atlantica*.

RESUMEN

Efectos de la concentración de nitrógeno y de los cotiledones en las preferencias de nitrógeno en plántulas de *Cedrus atlantica* Manetti

La materia orgánica es la fuente más importante de nitrato y amonio, las dos principales formas de nitrógeno utilizadas por las plantas, en el suelo de los bosques de coníferas. La preferencia y la adaptación a una forma de nitrógeno sobre otra en los genotipos de las plántulas es un factor determinante para el éxito de los programas de reforestación, no solo para los cedros sino para cualquier otra especie arbórea forestal. En una cámara de crecimiento controlada, se cultivaron plántulas de *Cedrus atlantica* M. de dos procedencias (Gouraud y Moudmame) en suelo natural o en materia orgánica fertilizada con diferentes formas y concentraciones de nitrógeno. Nuestros resultados muestran una elevada necesidad de nitrógeno y diferentes preferencias por las formas de nitrógeno, que se manifiestan principalmente en las raíces secundarias. Por otro lado, las variaciones en los cotiledones afectan al crecimiento y a la respuesta de las plántulas a la forma de nitrógeno y, por tanto, revelan diferentes formas de adaptación a la nutrición nitrogenada, que están relacionadas con los cotiledones y con la edad de las plántulas. La respuesta del crecimiento de *C. atlantica* ante la forma y concentración de nitrógeno se discute a la luz de los efectos de la procedencia y de los cotiledones. En conjunto, estos resultados sugieren que el conocimiento de las necesidades de nitrógeno de los diferentes genotipos de *C. atlantica* en la etapa juvenil podría ser una herramienta útil para el éxito de la reforestación de esta especie forestal.

Palabras clave: cotiledones, forma de nitrógeno, raíces secundarias, procedencia de las semillas, *Cedrus atlantica*.

Introduction

Atlas cedar (*Cedrus atlantica* Manetti) occupies 2.8% of the total forested area of Morocco with a total forest area of 160 km^2 in Rif mountains and 1,160 km^2 in Middle and Eastern High Atlas, and contributes to meeting the needs of the local population (Terrab *et al.*, 2008). This species of international interest is endangered (Thomas, 2013) due to a multitude of problems, mainly climatic and edaphic constraints (Linares *et al.*, 2011; Moukrim *et al.*, 2020; El Amrani, 2022; Tafer *et al.*, 2022; El Mderssa *et al.*, 2022).

Cedrus atlantica has a long-life span of over 600 years. Its migration or genetic adaptation may not follow the dynamics of rapidly changing climatic conditions like other trees. Therefore, it needs to produce seeds adapted to the new environmental conditions that arise (Sniderhan *et al.*, 2018). Consequently, it must produce seeds adapted to the new environmental conditions. This assumption agrees with data of Terrab *et al.* (2006) who found that *C. atlantica* maintains a high genetic diversity in the Middle and High Atlas of Morocco. A similar observation has been shown for *Pinus pinea* L. in which the environmental conditions control the adaptive variability and induce variations in seed characteristics between and within populations (Court-Picon *et al.*, 2004; Terrab *et al.*, 2006).

The cotyledons are the most important seed characteristics because the conifer embryos have a variable number of cotyledons even within the same species (Holloway *et al.*, 2018). Mughal and Thapliyal (2012) consider that the number of cotyledons can constitute a basis for the selection and delimitation of the provenances of *Cedrus deodara*. On the other hand, for conifers, the cotyledons contain the food reserves with which the growth of the seedlings starts and it is themselves which begin photosynthesis (Johnson *et al.*, 2011; Marshall and Kozlowski, 1976). For this reason, during seed growth and development there is an integrated continuum from cotyledons to primary needles to secondary needles. Therefore, the development of primary needles requires metabolites from the cotyledons (Kozlowski and Pallardy, 1997). In this regard, the cotyledons which are physiologically efficient, play a major role in seedling growth, development and establishment (Mughal and Thapliyal, 2012). On the other hand, the physiological state of cotyledons is determined by the mother-plant conditions at the seed formation period, especially how much water and N-form in soil are still available and taken up (Masclaux-Daubresse *et al.*, 2010; Zhang *et al.*, 2018).

The soil organic matter plays a paramount role in the soil properties by affecting soil aggregation and biochemical characteristics (Mangalassery *et al.*, 2019; El Amrani and Bendriss Amraoui, 2020); favouring nutrient retention and cycling (Molina-Herrera and Romanyà, 2015); affecting soil microbial activity and constitutes a nitrogen tank for the coniferous forest soil (Li *et al.*, 2018; Zhang *et al.*, 2018; El Amrani and Bendriss Amraoui, 2022). The nitrogen is one of the most important nutrients for plants it affects their functions from cell metabolism to growth (Boukcim *et al.*, 2006; Saiz-Fernández *et al.*, 2015). However, in most forest soils, the availability of nitrogen is low and unable to meet the requirement of tree growth (Song *et al.*, 2018) due to unreasoned tree felling and low organic matter mineralization. In addition, despite the abundance of nitrogen in the organic state, just 1 to 3% can be in an inorganic form in the soil (Baldock and Nelson, 2000). Under the influence of soil types, vegetation and season this low rate can vary from 0.31 to 5.55 mmol of N/g in forest cedar soil (El Amrani and Bendriss Amraoui, 2018). To our knowledge, no study has been conducted on the preference in nitrogen forms and concentration between different seed provenances of *C. atlantica* in relation to cotyledons. Though, such a study can determine the ability of populations to adapt to environmental changes, the plant productivity, competition, coexistence and the ecological succession (Houlton *et al.*, 2007; Zhang *et al.*, 2018).

The aim of the present paper is to investigate the differences, inter-population, in the preference of the nitrogen form in the progeny of two populations of *Cedrus atlantica* M.

Material and methods

Seeds sampling

Cedar seeds from two localities of the Moroccan cedar forest, Gouraud (Grd) and Moudmame (Mdm), were collected in May from 10 nearly isolated trees of each population. These trees are exposed from northeast to northwest through the south of a 270°, they are approximately the same age (respectively between 3.51 and 3.24 m in diameter, and 26.5 and 27 m height). The selected plants are classed as seed-carrier since they produce a large quantity of seeds with a germination rate greater than 80% (table I).

Soil characterization

After the mineralization of 100 mg of soil, according to the Kjeldahl method (1883), the total nitrogen content [N] was estimated using the colorimetric method of Berthelot (1859) and the total phosphorus [P] content using the colorimetric method of Murphy and Riley (1962). The total organic carbon of the soil (C_{org}) is determined according to the titrimetric method of Walkley and Black (1934). The granulometric characteristics of soils were determined according to Bouyoucos (1935), after drying and sieving 100 g of soil.

Seedling growth conditions

The seeds were soaked for 48 hours, then transferred to damp paper until a radicle of about 1.5 cm appeared and then transplanted into culture-pots of 07 × 07 × 25 cm^3 of dimensions.

In the first experiment, a batch of seeds from Gouraud

Table I.
Characteristics of the rhizosphere and geographic coordinates of the mother plant of the two provenances.

		Gouraud	Moudmame
Latitude		33° 25' 11.05" N	33° 22' 1.84" N
Longitude		5° 10' 11.10" O	5° 13' 23.15" O
Altitude (m asl)		1749	1835
Texture (%)	**Coarse sand**	17.195	16.782
	Fine sand	43.747	39.161
	Coarse silt	20.894	18.998
	Silt fine-clay	18.164	25.059
Physico-chemistry	**% C_{org}**	4.80 ± 0.72^a	8.16 ± 0.24^b
	[N] mmol/g	3.301 ± 0.056^a	3.632 ± 0.255^a
	[P] mg/g	50.094 ± 3.308^a	50.124 ± 1.762^a
Dominant litter		Pure cedar	Cedar, holm oak and oxyhedron
Soil		Develop	Develop, rich in gravel
		Clayey, brown	Clay silt, dark black
		Few undecomposed OM	Rich in OM in the form of humus

% C_{org}: organic carbon content; [N]: nitrogen content; [P]: phosphorus content; OM: organic matter.
Mean ± SD, letters indicate significant differences (P < 0,05 test LSD).

and Moudemame was characterized by physiological and morpho-anatomical features by dissection under a binocular magnifying glass (table V). A second batch of 200 germinated seeds from the two provenances was transferred separately into the culture-pots, at the rate of 20 seeds per treatment, containing an organic substrate (red sawdust). The cultures were fertilized weekly with a nutrient solution based on nitrate [$Ca(NO_3)_2$] or ammonium (NH_4Cl), at low (0.1 mM) or high (5.0 mM) concentration. The mineral composition of the solutions provided was the same except the nitrogen [1 mM KCl; 0.2 mM KH_2PO_4; 0.2 mM $CaCl_2$; 0.1 mM $MgSO_4$; 0.5% $FeCl_3$; 0.2 mL/L of a trace element solution (Morizet and Mingeau, 1976), pH 5.5]. Seedling growth was characterized on 4 seedlings at the 15, 30, 120, 210 and 420-day growth stages.

In the second experiment, the seed content of 10 cones

Table II.
Dry weight average (mg) of two cedar provenances in response to the nitrogen form and concentration during 210 days

Age (days)	Gouraud				Moudemame			
	0.1 NH_4^+	5 NH_4^+	0.1 NO_3^-	5 NO_3^-	0.1 NH_4^+	5 NH_4^+	0.1 NO_3^-	5 NO_3^-
15	33.8 ± 1.34*	33.8 ± 1.34*	33.8 ± 1.34*	33.8 ± 1.34*	30.2 ± 1.76	30.2 ± 1.76	30.2 ± 1.76	30.2 ± 1.76
30	40.0 ± 1.44^a	43.3 ± 4.38^{ab}	54.2 ± 2.11^b	48.3 ± 3.35^{ab}	43.3 ± 2.89^a	54.5 ± 4.46^a	50.0 ± 3.25^a	53.2 ± 2.56^a
120	98.9 ± 5.26^{ab}	88.4 ± 4.23^a*	79.2 ± 1.23^a	127.8 ± 10.74^b	97.4 ± 10.60^{ab}	122.6 ± 13.94^a	86.7 ± 6.15^b	105.3 ± 1.39^{ab}
210	101.9 ± 6.51^a	117.1 ± 21.20^{ab}*	104.4 ± 5.94^a	160.2 ± 6.94^b	125.3 ± 15.93^a	167.5 ± 10.30^a	127.3 ± 17.20^a	124.1 ± 11.23^a

Mean ± SD followed by letters and asterisks indicating significant differences (P < 0.05 LSD test) between the nitrogen treatment and between the provenances, respectively.

from the Moudemame provenance were put to germinate. After the appearance of the cotyledons, the seeds were sorted according to the number of cotyledons and transplanted separately into the culture pots containing soil from the same provenance. The cultures were watered weekly with running water. At the 210-day growth stage, 4 seedlings from each group were harvested for growth analysis.

All cultures are maintained under a temperature ranging from 24 to 32 °C, a photoperiod of 16/8 h and a relative humidity of the order of 50 to 70% in the growth chamber. All seedlings were daily moistened with water during dry periods.

Statistical analysis

The average dry weight of seeds, cotyledons, embryos, seedlings, shoots, roots, needles, stems, main roots, secondary roots and tertiary roots was determined by weighing after drying of the fresh material in an oven for 48 hours at 70 °C. The water content (Wt%) of seeds and embryos was calculated according to the formula: Wt% = [(FW – DW) / DW] × 100. The water content of seeds at saturation (Wt_{sat}%) was calculated according to the formula: Wt_{sat}% = [(W_{sat} – DW) / DW] × 100. With FW: mass of fresh material, DW: dry weight and W_{sat}: seed mass after soaking in water for 48 hours. The length (Lg), number (Nb) and diameter (Ø) of embryos, cotyledons and radicles were estimated by taking images under a binocular magnifier glass and using ImageJ software (Version 1.50i, 2016). The data obtained have been subject to a variance analysis (ANOVA) and the means were compared by Fisher's Least Significant Difference (LSD) Post Hoc test at $P < 0.05$. The Pearson bilateral correlation coefficients at 5% risk of error were calculated to estimate the relationship between cotyledon dry weight and the growth of cedar seedlings organs. A second Pearson bilateral correlation was calculated to estimate the relationship between the difference of cotyledons dry weight and the difference of organs dry weight (DW) on ammonium and on nitrate, as shown by the formula: correlation between ΔC and ΔO with:

- ΔC = [cotyledons dry weight on (NH_4^+) – cotyledons dry weight on (NO_3^-)],
- ΔO = [organs dry weight on (NH_4^+) – organs dry weight on (NO_3^-)].

All these statistical analyses have been done by IBM SPSS Statistics (Version 20.0, 2011). The mean, the maximum, the minimum, the coefficient of variation, and the standard deviation are calculated to describe the physiological and morpho-anatomical characteristics of the seeds of the two provenances, and that of the dry weight for the cotyledons, the seedlings and the root system at the 120-day stage.

Results

Experiment 1: inter-provenance variability

Table II describes the response of two different provenances of *Cedrus atlantica* seedlings to the nitrogen form and concentration over 210 days. In this experiment, control seedlings that do not receive nitrogen (non-physiological control) eventually die during the early stages of growth (data not shown). At the 15-day stage, the seeds of each provenance were transplanted onto the fertilized organic substrate at an average DW of 33.8 and 30.8 mg for Grd and Mdm, respectively. At the age of 30 days the seedlings of provenance Grd show an increased growth when received NO_3^- compared to NH_4^+. At the 120- and 210-day stage the increase of NO_3^- concentration from 0.1 to 5 mM stimulates significantly the cedar seedling growth at the Grd provenance. In contrast to Grd, Mdm is less sensitive to the form and the increase of nitrogen concentration. The comparison between the response of the two provenances shows that Mdm displays a significant preference towards the ammoniacal form at high concentration in 120 and 210 old day stage with 122.6 mg and 167.5mg, respectively. The other forms and concentration show no significant difference (table II).

Given the significant results found, only at the level of the 5 mM concentration, we are looking for the effect of this concentration on the different parts of the seedling. The general shape of the growth curves of the roots and shoots, shows that the average dry weight of the Gouraud provenance is distinguished, from the 30-day stage, by a significant preference towards the nitric form at the level of the roots. This preference also appears at the level of the

Table III.
Change in the dry weight of roots and shoot of the two *Cedrus atlantica* provenances in response to the nitrogen form.

	Age (Days)		15	30	120	210
Shoot	Grd	5 NH_4	29.025 ± 1.38^a	38.325 ± 3.79^a	70.6 ± 2.75^{ab}	89.275 ± 15.54^a
		5 NO_3	29.025 ± 1.38^a	42.625 ± 2.91^a	90.75 ± 6.23^b	105.375 ± 5.47^a
	Mdm	5 NH_5	25.6 ± 1.45^a	47.05 ± 3.98^a	84.675 ± 11.96^a	114.7 ± 7.18^a
		5 NO_4	25.6 ± 1.45^a	47.4 ± 2.30^a	76.675 ± 2.33^a	88.85 ± 5.48^a
Roots	Grd	5 NH_4	4.75 ± 0.33^a	4.975 ± 1.06^a	17.775 ± 1.81^a	27.825 ± 5.66^a
		5 NO_3	4.75 ± 0.33^a	5.675 ± 0.44^a	37.025 ± 4.51^c	54.8 ± 4.55^b
	Mdm	5 NH_4	4.625 ± 0.36^a	7.425 ± 0.94^a	37.95 ± 4.04^c	52.75 ± 3.13^b
		5 NO_3	4.625 ± 0.36^a	5.825 ± 0.34^a	28.575 ± 2.01^b	35.25 ± 6.94^a

Grd: Gouraud; Mdm: Moudemame. Letters indicate significant differences ($P < 0.05$ LSD test) at shoot and roots.

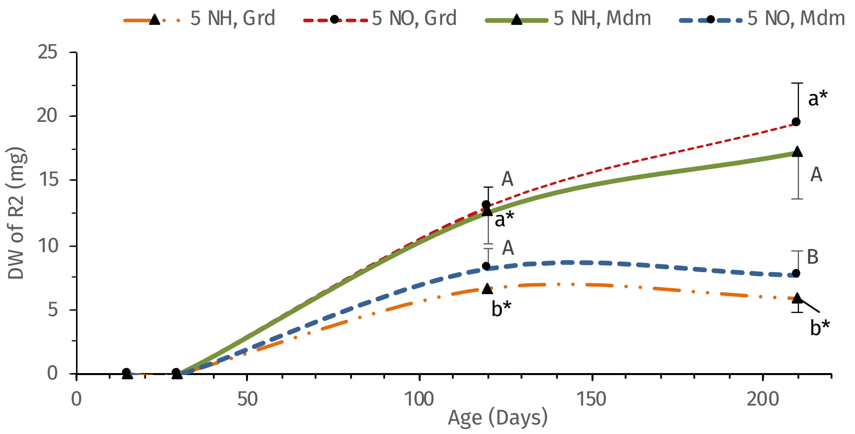

Figure 1.
Averages of the dry weight (DW) of the secondary roots (R2) of *Cedrus atlantica* M. from the two provenances Gouraud (Grd) and Moudemame (Mdm), in response to the variation of the nitrogen form. Letters and asterisks indicate significant differences ($P < 0.05$ LSD test) between nitrogenous forms and provenances, respectively.

shoot but without significant difference. While at the level of Moudemame provenance, the average dry weight of the roots and shoots does not show any significant difference with respect to the nitrogen form. Seedlings from Gouraud provenance differed positively and significantly from Moudemame in response to NO_3^- from the 120-day stage with 37.025 vs 28.575 mg and continued to the 210-day stage with 54.8 vs 35.25 mg, respectively. On the other hand, at the level of NH_4^+, the Gouraud provenance differs negatively and significantly from that of Moudemame on the 210th day of growth with 27.83 against 52.75 mg, successively (table III).

Since the first days of their appearance the gap between DW of secondary roots (R_2) on NO_3^- and NH_4^+ increases with time and characterized by a high preference of that of Grd toward the nitric form. In contrast to Grd, the secondary roots of Mdm show a significant preference toward the ammoniacal form, but until the 210 days of age. The comparison of the two provenances Grd and Mdm shows a highly significant difference of secondary roots, beyond the 120th days, in response to nitrogen form and reveals that R_2 of Grd and R_2 of Mdm prefers NO_3^- and NH_4^+ forms, successively (figure 1).

Whether at the level of Grd or Mdm, NH_4^+ or NO_3^- the variability of the growth response depends on the variability in the cotyledons DW; so that the seeds of Mdm on NH_4^+ show a cotyledon DW that varies between a maximum of 51.9 mg and a minimum of 10 mg which gives rise to a large coefficient of variation (CV) of 45.30, these seeds also show a large variability in the DW of seedlings, total roots and secondary roots with a CV of 22.74, 21.28 and 38.10, respectively. In return, the seeds of Grd on NH_4^+ show a low cotyledon DW variability (CV of 11.37) and a low growth variability at the level of root system and total seedling growth (table IV).

The seeds of the Moudmame provenance are characterized by a clear superiority and a low variability (coefficient of variation), compared to those of Gouraud, at the level of the dry weight and the water content of the seeds, and at the level of the dry weight and the length of the embryo. However, seeds from the Gouraud provenance show a high and variable nitrogen content compared to those of Moudmame which shows a low content of N and a low coefficient of variation. Despite the fact that the water content at saturation, the number of cotyledons and the length of the radicle are greater in Moudmame seeds, they show a high coefficient of variation compared to Gouraud. The water content of the embryo and the length of the cotyledons are characterized by a clear superiority and a low variability at the level of provenance of Gouraud compared to that of Moudmame (table V, figure 2).

Table IV.
Statistic description of analyzed dry weight (mg) for the cotyledons, the seedlings and the root system at the 120 day-stage.

		Gouraud				**Moudmame**			
		Cotd	**Sdl**	**TR**	**R_2**	**Cotd**	**Sdl**	**TR**	**R_2**
5 NH_4^+	x	26.93	88.38	17.78	6.70	29.53	122.63	37.95	12.60
	M	30.20	105.30	23.70	8.40	51.90	178.40	45.90	16.30
	M	20.80	80.40	11.20	4.60	10.00	95.80	21.80	3.00
	CV	11.37	9.58	20.39	15.67	45.30	22.74	21.28	38.10
	SD	3.06	8.46	3.63	1.05	13.38	27.89	8.08	4.80
	N	4	4	4	4	4	4	4	4
5 NO_3^-	x	28.73	127.78	37.03	13.00	27.50	105.25	28.58	8.23
	M	42.30	151.20	47.90	18.80	32.30	107.70	32.70	12.90
	M	18.50	104.00	21.10	9.40	22.30	99.70	21.70	4.80
	CV	35.25	16.81	24.38	22.31	14.18	2.64	14.09	36.78
	SD	10.13	21.48	9.03	2.90	3.90	2.78	4.03	3.03
	N	4	4	4	4	4	4	4	4

x: average; M: maximum; m: minimum; CV: coefficient of variation; SD: standard deviation; Cotd: cotyledon; Sdl: seedlings; TR: total roots; R_2: secondary roots; N: number of population.

Table V.
Statistical description of the physiological and morpho-anatomical characteristics of seeds from the two provenances.

		Seed				Embryo			Cotyledon		Radicle	
		DW	Wt%	$[N]_{mmol/g}$	W_{tsat}%	DW	Wt%	Lg	Nb	Lg	Ø	Lg
Gouraud	$\bar{X}$	63.50	12.28	1.01	74.72	6.50	154.96	0.89	8.70	0.59	0.12	0.24
	M	73.30	16.72	1.17	103.28	8.40	173.08	1.00	10.00	0.67	0.13	0.27
	M	30.50	9.21	0.85	63.77	5.20	128.57	0.74	7.00	0.48	0.10	0.23
	CV	10.61	19.08	7.66	7.98	11.69	7.08	7.01	7.13	7.30	5.86	4.18
	SD	6.74	2.34	0.08	5.97	0.76	10.97	0.06	0.62	0.04	0.01	0.01
	N	10	10	10	10	10	10	10	10	10	10	10
Moudmame	$\bar{X}$	75.02	13.41	0.83	82.12	8.42	129.13	1.02	8.80	0.67	0.12	0.26
	M	79.50	20.35	0.90	120.35	10.60	160.00	1.15	10.00	0.75	0.14	0.30
	M	69.40	10.58	0.77	67.42	5.90	106.52	0.91	8.00	0.62	0.11	0.22
	CV	2.15	16.70	4.40	10.52	11.16	9.38	4.33	9.09	5.17	5.59	8.90
	SD	1.61	2.24	0.04	8.64	0.94	12.11	0.04	0.80	0.03	0.01	0.02
	N	10	10	10	10	10	10	10	10	10	10	10

$\bar{X}$: mean; M: maximum; m: minimum; CV: coefficient of variation; SD: standard deviation; DW: dry weight (mg); Wt%: water content; Wt_{sat}%: water content at saturation; Lg: length in mm; Nb: number and Ø: diameter at the limit between the hypocotyl and the radicle in mm; N: number of population; [N]: nitrogen content.

Experiment 2: intra-provenance variability

At the Mdm provenance, the number of cotyledons in *Cedrus atlantica* is variable between 7 and 11. The study of their effects on the seedling growth shows that the growth of the seedlings follows that of needles which shows a dry weight (DW) decrease in response to the cotyledon number decreasing. The seedlings with 10 and 7 cotyledons (C) have the largest (466.4 mg) and the smallest (229.5 mg) needles DW, respectively, whereas the seedlings with 11 C are significantly similar to those with 10 C. The stem DW shows the same results like needles and seedlings except that the stem of seedlings with 11 C becomes significantly lower than those with 10 C. The main and secondary roots of the 10 C seedlings show the largest DW compared to those with 8 and 7 C, while, the seedlings with 11 and 9 C have an intermediate dry mass average on these root categories. The tertiary roots of seedlings with 11 C show the highest DW value while, the smallest value is shown at the level of the 7 C seedlings (figure 3).

The study of the correlation between the growth of seedling organs and the dry mass of cotyledons throughout 210 days, shows that all correlations are positive, except for the main root and needles at the 120-day stage. Furthermore, the seedling growth is strongly and positively correlated with the cotyledons dry mass at $p < 0.001$ level of signification over all growth stages studied. Secondary and tertiary roots are also positively correlated with the dry weight of cotyledons in late growth stages (table VI).

Table VI.
Pearson correlation coefficients between cotyledon dry weight and seedlings growth during 210 days at the level of Moudemame provenance.

	Days	Seedlings	Needles	Stem	R_1	R_2	R_3
Cotyledon DW	15	0.859**	na	0.693**	0.676**	na	na
	30	0.712**	0.357*	0.614**	0.447*	na	na
	120	0.615**	0.240	0.642**	0.306	0.582**	na
	210	0.615**	0.426*	0.522**	0.564**	0.390*	0.474**

DW: dry weight; R_1: main root; R_2: secondary roots; R_3: tertiary roots; na: not applicable. Bilateral correlation is significant at the 0.01 (**) or 0.05 (*) level.

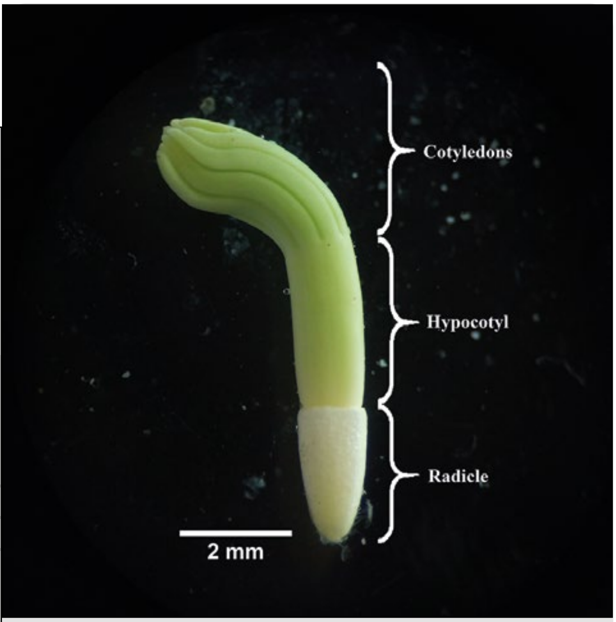

Figure 2.
Photo taken under a binocular magnifying glass (SZX10) showing an embryo of *Cedrus atlantica* M.

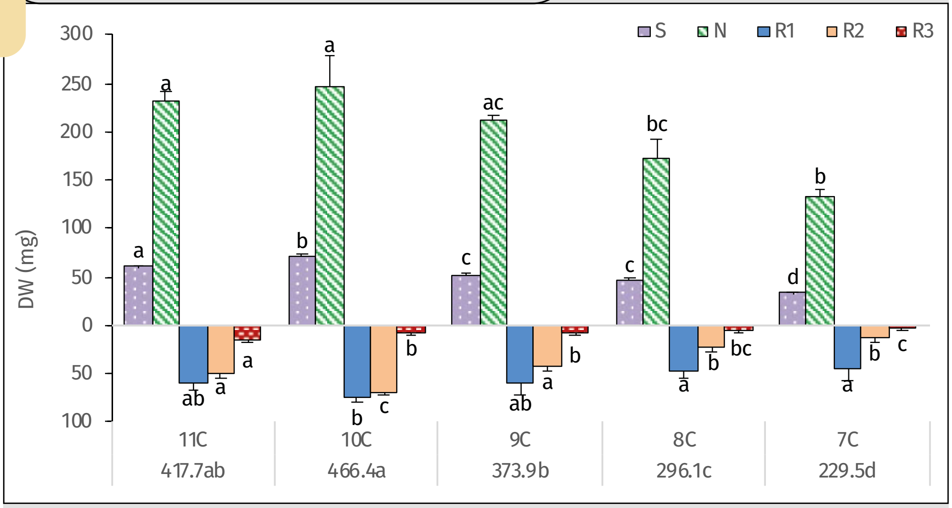

Figure 3.
Dry weight (DW) average of seedlings and organs of Moudemame provenance in response to the number of cotyledons (C). N: needles; S: stem; R1: main root; R2: secondary roots and R3: tertiary roots. The values at the base of the histograms indicate the average DW of the seedlings. Letters indicate significant differences ($P < 0.05$ LSD test).

The study of the Pearson correlation between the cotyledons DW and seedlings DW in ammoniacal and nitric nutrition, shows that the variation of cotyledons DW is significantly and positively correlated with the variation of seedling growth in response to the N-form. The positive correlation also manifested between the cotyledons DW, and the shoots and the roots on all the stages of growth studied, especially, at the stage 120 days where it is significant at a rate of $p < 0.001$. The correlation at the organ level shows that the needles DW is significantly correlated with cotyledons DW only at the 120-day stage, whereas, the main root is significantly correlated to the cotyledon at all stages of growth. The stem, secondary roots and tertiary roots are weakly and positively correlated with cotyledons DW with a coefficient ranging from 0.291 to 0.450 (table VII).

Table VII.
Pearson correlation coefficients during 210 days between (i) the dry weight difference of the cotyledons on NH_4^+ and NO_3^- (ii) the growth difference of the seedlings on NH_4^+ and NO_3^-.

	Age (days)	**$DW_{(NH)} - DW_{(NO)}$**							
		Seedlings	**Shoot**	**Root**	**Needles**	**Stem**	**R_1**	**R_2**	**R_3**
$C_{DW(NH)} - C_{DW(NO)}$	15	na	na	na	na	na	na	na	na
	30	0.584*	0.506*	0.527*	0.291	0.413	0.527*	na	na
	120	0.692**	0.615**	0.632**	0.529*	0.435	0.521*	0.353	na
	210	0.588*	0.517*	0.533*	0.45	0.437	0.510*	0.415	0.36

C_{DW}: cotyledon dry weight; R_1: main root; R_2: secondary roots; R_3: tertiary roots.
Bilateral correlation is significant at the 0.01 (**) or 0.05 (*) level.

Discussion

The cotyledons are a characterizing parameter of the Pinaceae seeds because it can be highly variable, especially in number, between and within the same species (Holloway *et al*., 2018; von Aderkas, 2002), what makes of them a basis for the selection of different genotypes in Pinaceae family. In this study, the analysis of seedling growth in response to the number of cotyledons shows that the dry weight of almost all organs is proportional to the number of cotyledons. The variations in growth in response to nitrogen form, between the two provenances, depended on variations in the dry mass of the cotyledons; so that they show a strong correlation between the growth of the organs and the cotyledons. These results suggest that the cotyledons control the growth of cedar seedlings ($\mathbf{C}_i$). This conclusion is therefore in agreement with previous studies which have shown, in seedlings of *Cedrus deodara*, that physiologically efficient cotyledons are a key component of seedling growth and establishment (Mughal and Thapliyal, 2012).

On the other hand, this study also shows that the form and the concentration of nitrogen affect the growth and development of cedar seedlings differently according to the provenances – with a significant preference of that of Moudemame towards the ammoniacal form and that of Gouraud towards the nitric form. This difference is more apparent at the level of the roots and in particular the secondary roots which show a significant difference between the two provenances in response to the nitrogenous forms. These results agree with previous results which show a very significant difference between the provenances for the growth of the seedlings of *Abies guatemalensis* Rehder, *Pinus nigra* Arnold and *Cedrus atlantica* Manetti in response to substrates (Aoujdad *et al*., 2015; Mataruga *et al*., 2010; Strandby Andersen *et al*., 2008). Taken together, these results suggest the existence of intrapopulation differences within *C. atlantica* for nitrogen nutrition; so that different provenances show a preference for one form of nitrogen or another ($\mathbf{C}_{ii}$).

From the conclusions ($\mathbf{C}_i$) and ($\mathbf{C}_{ii}$), it is questionable whether this preference of the nitrogen form depends solely on the origin of the seeds. To answer this hypothesis, we used the cotyledons as a characteristic separating the different genotypes and we studied Pearson's bilateral correlation between (i) the difference in cotyledon mass on NH_4^+ and NO_3^-, and (ii) the difference in the mass of the organs on NH_4^+ and NO_3^- (table VII). This statistical test shows that the variation in the dry mass of the cotyledons is significantly and positively correlated with the growth response to the nitrogenous form. This means that the gap between growth on NH_4^+ and NO_3^- depends on the cotyledons. The present results are consistent with those of Stöcklin and Armbruster (2016) which show that seed-producing individuals strongly differ molecularly from each other despite their proximity. Overall, it can be suggested that the preference of the nitrogen form by seedlings depends on the cotyledons which themselves depend on the availability and form of nitrogen in the rhizosphere of the mother plant during seed formation. Because the seeds are the sinks of the nitrogen assimilated during the reproductive stage of the mother plant (Strandby Andersen *et al.*, 2008; Tegeder and Masclaux-Daubresse, 2018). This conclusion suggests the need to compare these provenances of soils from the point of view of nitrate and ammonium content as well as the enzymatic activities involved in the two forms of nitrogen nutrition. It would also be appropriate to characterize the mineralization of organic matter by microorganisms in our soils.

Up to the 210-day growth stage, the secondary roots of seedlings from Gouraud and those from Moudemame show a significant preference towards NO_3^- and NH_4^+, respectively (figure 1). At the 420-day stage, when the seedlings have lost their cotyledons by senescence, the Gouraud seedlings and their secondary roots continue to prefer the NO_3^-, while those of Moudemame have lost their preferences for the NH_4^+ form (figure 4). These results suggest that the preference of the NO_3^- by the Gouraud provenance is linked to factors other than the cotyledons. These results agree with previous results of Mughal and Thapliyal (2012) which showed that the heritability values of the species depend on 31.29% of the number of cotyledons suggesting that these characters were under strong genetic control. This means that the cotyledons only separate between the different genotypes and have little effect on the preference of NO_3^-. Contrary, the preference of the NH_4^+ by Moudemame is linked to the presence of cotyledons which agrees with the fact that the photosynthesis and the nutrient storage capacity of the cotyledons control the growth of Pinaceae seedlings (Johnson *et al.*, 2011; Johnson and Smith, 2005; Kozlowski and Pallardy, 1997; Mughal and Thapliyal, 2012). This second suggestion is confirmed by the fact that the seeds of the two provenances are significantly different in dry mass and nitrogen content with 63.50 mg, 1.01 mmol/g for Gouraud and 75.02 mg, 0.83 mmol/g for Moudemame, respectively (table V); and also, at the start-up stage, 15 days, with 33.8 mg for Gouraud and 30.2 mg for Moudemame (table II). This effect of cotyledons on the preference for the ammoniacal form agrees with the results of Zhang *et al.* (2018) who found that preference of the nitrogen form is also related to age. This preference can be obtained by increasing the level of transcription of the SbGln1.3 genes and by the accumulation of glutamine synthetase in the roots and / or in the cotyledons as found by El Omari *et al.* (2010) for ammonium tolerance.

In other terms, the results discussed here show that *C. atlantica* present two forms of adaptation to nitrogen feed; The first is bound to age (presence of cotyledons) and the second is genetically determined (post-cotyledonary stage). From the fact that the differences between seed provenances are attributed to natural selection of different characters in their natural habitats (Rawat and Uniyal, 2011); we can suggest that these responses presented here represent different *C. atlantica* genotype / provenance strategies for adapting to nitrogen fluctuations in soil. This aspect therefore requires further investigations at the level of the enzymatic reserves and activities of the cotyledons between the different genotypes and provenances of *C. atlantica*.

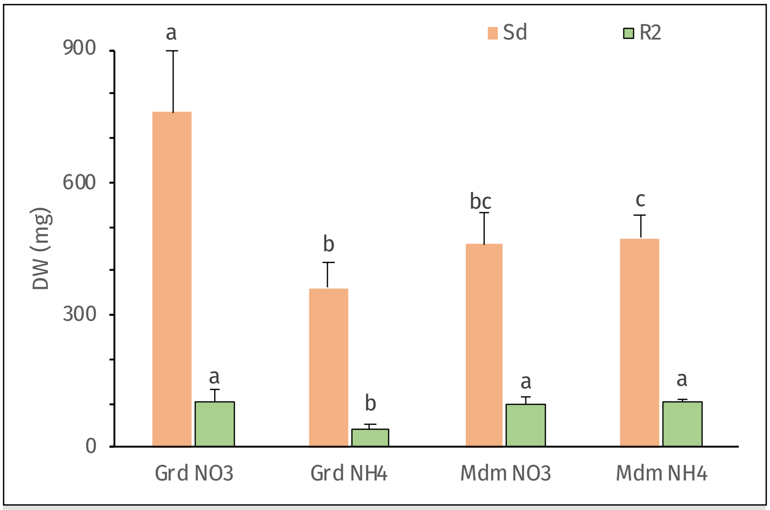

Figure 4.
Dry weight average of two cedar provenances in response to the nitrogen form at the 420-day stage. Sd: seedlings; R2: secondary roots; Grd: Gouraud provenance; Mdm: Moudemame provenance. Letters indicate significant differences ($P < 0.05$ LSD test).

The fact that both provenances modulate the growth of secondary roots in response to the form of nitrogen as shown in this study and to the physico-chemical properties of the soil as shown by El Amrani and Bendriss Amraoui (2018); suggests that secondary roots are the first organ that adapts to fluctuations in the soil. This observation is in agreement with the results of Courbet *et al.* (2012) who underline the important role of *C. atlantica* roots in adaptation to environmental constraints. Based on these findings on secondary roots and others that report the contribution of these roots in the competitiveness of *Pinus radiata* genotypes D. Don (Gautam *et al.*, 2003); we can assume that the competitiveness of different trees within the same provenance is related to the level of preference of each genotype to adopt and use the different forms of nitrogen, and the flexibility of secondary root growth in response to fluctuation nitrogen resources in the soil.

Conclusion

The results of this paper reveal an intraspecific difference within provenances of *C. atlantica* for nitrogen nutrition. They also show that the nitrogen preference is related to the age of the seedlings and the cotyledons as a criterion that separates the different genotypes. *C. atlantica* seedlings have a high nitrogen requirement and their adaptation / preference is especially visible at high concentrations and at the level of the secondary roots as the organ most responsive to the fluctuation of nitrogen in the soil. This study provides preliminary information on the form and availability of nitrogen requirements of the different genotypes at least at the juvenile stage to stimulate cedar regeneration in reforestation programs. However, future studies will be needed to elucidate how cotyledons are linked to genetic factors in the preference of nitrogen forms and to explain the existence of cedar genotypes that preferring NO_3^- despite the fact that coniferous forest soils are generally acidic.

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Data
Data used to produce this study can be accessible with an agreement of the corresponding author.

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