Effect of growing media on morpho-physiological quality attributes of Tetraclinis articulata seedlings

Rachid El Haddadia, Abdelalli El Mekkaouib, Abdemjdid Zouahric, Amina Ouazzani Touhamia and Allal Douiraa

aLaboratory of Vegetal Animal Production and Agro-industry, Team of Botany, Biotechnology and Plant protection. Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco; bLaboratory of Natural Resources Geosciences, Department of geology, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco; cResearch Unit on Environment and Natural Resources conservation, Regional center of Rabat, National institute of agricultural research (INRA), Rabat, Morocco

ABSTRACT
Tetraclinis articulata (Vahl.) Master or thuya is facing several constraints to its successful natural regeneration in Morocco. Containerized seedlings plantation is the only method adopted in thuya forest restoration programs and post-transplant water stress produces high seedling mortality after the first summer following outplanting. There is thus a real need to improve nursery seedlings quality, especially enhancing the growth of the root system by improving the water holding capacity of the root plug. Our aim was to assess the effects of clay on the water holding capacity of the growing media and on various morphological and physiological traits of T. articulata seedlings in the nursery. T. articulata seedlings were raised in nursery using nine composite substrates; seedlings quality was evaluated according to their morpho-physiological and performance attributes. This investigation determined that a clay content of 20–25% constitutes an optimum for obtaining seedlings with a good root growth potential and high root viability. Excessive clay content in the growing media resulted in water-logged root plug decreasing root growth potential. This investigation found that morphological attribute as Root Collar Diameter and Root/Shoot ratio could be considered good predictors of Root Growth Potential.

1. Introduction
As a highly valued and multi-function tree species, Tetraclinis articulata (Vahl.) Master, known as “thuya” is a keystone component of several semi-arid Moroccan forest ecosystems (Boudy 1950; Quézel 1980; Fennane 1988; Charco 1999; Esteve-Selma et al. 2010). However, thuya forests are facing several constraints to its successful natural regeneration due to timber over-exploitation and grazing pressure (Benabid and Fennane 1994; Dallahi et al. 2017). In order to rehabilitate thuya forests, plantation of containerized seedlings still the only method adopted in forest restoration programs in Morocco. However, thuya ecosystems in drylands are characterized by water scarcity, and plants are very often subjected to water stress conditions after the first summer following outplanting. Under such unfavorable conditions, thuya seedlings mortality rates are generally high. This situation is expected to worsen in the future according to Global Climate Change projections for the Mediterranean region, which point to an increase in temperatures and in the frequency and intensity of droughts (Moatti and Thiébault 2016). There is thus a real need to improve forest restoration techniques in these areas, beginning with the application of innovative technologies with the aim of improving seedlings quality, seedling field performance and abiotic conditions on the plantation site (Vilagrosa et al. 1997; Vallejo and Alloza 1998; Cortina et al. 2006; Chirino et al. 2009).

Nursery cultural technique has an impact on seedling quality and, consequently, survival in the field (Oliet et al. 2009; Del Campo et al. 2010). Otherwise, several studies have indicated the importance of the growing media in the quality of the seedlings produced (Mafra et al. 2002; Owen et al. 2008; Chirino et al. 2011; Liu et al. 2012). Seedlings quality can be assessed by measuring several morphological and physiological attributes (material attributes), or by examining plant performance after subjecting them to specific environmental conditions (performance attributes) (Ritchie et al. 2010; Grossnickle and MacDonald 2018). Morphological and physiological traits of seedlings in relation to water stress play an important role in predicting their survival and field performance (Baeza et al. 1991; Waters et al. 1991; Simpson and Ritchie 1997; Royo et al. 2000; Navarro and Palacios 2004). Thus, root growth after outplanting is critical for ensuring seedling establishment (Padilla and Pugnaire 2007).
In this context, seedling quality should focus on enhancing the growth of the root system by improving the water holding capacity of the substrate of the root plug by promoting mechanisms for drought resistance and high water use efficiency (Chirino et al. 2009). A suitable growth medium for container seedlings is critical in order to provide support and nutrients, retain water, and allow oxygen diffusion to the roots. Although there is not an ideal growth medium for all growing seedlings, a growth medium should incorporate physical, chemical, and biological characteristics for good plant growth together with the requirements of practical plant production (Landis et al. 1990; Marianthi 2006). Currently, peat moss still the most common substrate used by forest seedlings growers. However, in recent years, due to the increased cost of peat moss, research is being directed toward the reuse of local materials to meet the rising cost of commercial peat (Marfa et al. 2002; Yu and Zinati 2006; Owen et al. 2008; Manas et al. 2009; Bakry et al. 2012; Ge et al. 2012; Bakry et al. 2013; Heiskanen 2013; Manh and Wang 2014).

The influence of nursery cultivation regimes on container seedlings quality and the outplanting performance of thuya has received little attention (Trubat et al. 2011), and no study on the effect of growing medium on seedlings growth of this species has been reported until now. We hypothesized that increasing of water holding capacity of the growing media used in the nursery, by using local clay material as vertisol, would provide an extra water supply in the critical early post-plantation period. To test this hypothesis, we had two objectives. Our first objective was to evaluate the effect of different composite growing media on seedlings morpho-physiological attributes during nursery culture. Our second objective was to assess the role of clay as a fine texture in improving containerized thuya seedlings performance attribute and consequently to select an optimum growing medium formula for the seedlings production of this species.

2. Materials and methods

2.1. Plant material and experimental layout

*T. articulata* seeds, previously collected (25 September 2018) from a Kourifla forest stand (33.713122°N, 06.707834°W, 220 m elevation), have been stratified at 4°C for 5 months. Seeds pre-germination was performed in early February 2019 as follows: Seeds were surface sterilized in 2% NaOCl for 15 min, soaked in distilled water for 24 h in room temperature (18–20°C) then surface sterilized in 2% NaOCl for 5 min, followed by two rinses with distilled water and then pre-germinated on soaked filter paper and kept in dark at room temperature (18–20°C) for 8 days. The nursery culture was carried out under outdoor conditions with shading nets and auto-spray irrigation systems at the Sidi Amira Forest Nursery, Salé, Morocco (34.050341°N, 06.671741°W; 130 m elevation) with a mean annual rainfall of 534 mm and mean temperatures: $T_{\text{max}} = 27.3\, ^{\circ}\text{C}$ and $T_{\text{min}} = 8.2\, ^{\circ}\text{C}$. For this, 450 rigid plastic containers with 50 cells of 400 mL (anti-spiralling system) were filled up with nine mixture of peat and local materials substrates (peat: red sand, vertisol) by volume %: S1(70:30:0), S2(50:50:0), S3(50:40:10), S4(50:30:20), S5(50:20:30), S6(70:20:10), S7(70:10:20), S8(50:25:25), S9(0:70:30). The substrate formula S1 is the growing medium commonly used in the production of forest seedlings in the region. Red sand and vertisol were the subsoil under 20 cm depth collected from forest near the experimental site which is the most ready available soil in the region with a granulometry (clay; silt; sand) of (5%, 4%; 91%) and (56%;6%;38%) respectively. The peat moss used in all growing media (TS3 Klasmann brand) was fertilized in the factory with a NPK:14-10-18 (1.5 kg fertilizer/m³ of peat). The germinated seeds were sown in the nine growing media formula on 18 February 2019. The watering regime was moderated according to seedlings water demand. Using a digital balance, irrigation was determined by gravimetric water content and maintained equally across all containers at 85% during establishment, 75% during rapid growth and 65% during hardening (Lamhamdi et al. 2006). After one month of growing, the container seedlings were moved up the overhead iron-wire grid in order to induce air root pruning. During the experimental period, normal management practices were carried out, but no fertilization was applied. A randomized complete block design with a monofactorial treatment structure was conducted and carried out with ten replications and every single container with 50 seedlings constituted one experimental unit. A total of 4500 thuya seedlings were grown up for the need of the experiment.

2.2. Growth medium sampling and measurement

Four samples of each growth medium were taken for the estimation of the physical and chemical properties prior to filling the containers. The bulk density (g/cm³) (Arshad et al. 1996), total porosity (%), aeration porosity (%) and water holding porosity (%) were determined as described by Gessert (1976) and Whitcomb (1988). The growing media pH was measured in a soil–water suspension (soil:water = 1:2.5) using a pH-meter (Metfler Toledo Seven Easy-728). Electrical conductivity (EC), in mS/cm, was measured on the saturated paste extracts (Richards 1954) using an ORION conductivity meter, model162. The mineral nitrogen was extracted at KCl 1 N (50 g substrate/100 cm³), NO₃⁻ and NH₄⁺ were dosed by colorimetry (Delphin et al. 1991). Total nitrogen, in (%), was determined using the Kjeldahl method (Bremner 1965). Available phosphorus (P₂O₅), in ppm, was determined using the Olsen method (Olsen and Sommers 1982) where the extraction was made by sodium hydrogen carbonate at pH=8.5. The phosphorus content registered using a JENWAY 6405 Visible model UV spectrophotometer at a wavelength of 825 nm. Exchangeable potassium (K₂O), in ppm, measured by
2.3. Plant sampling and measurement of morphological attributes

At the end of eight months of nursery cultivation (15 October 2019), seedlings morphological characteristics were carried out. A subset of 60 seedlings per treatment were randomly sampled. Edge effect were controlled by avoiding seedlings from buffer block area. Indeed, plants located on the sided-edges of containers are exposed to more light and are more sensitive to variations in ambient temperature. Shoot height (SH) in cm and root collar diameter (RCD) in mm were measured. Sturdiness index (SI) was determined as the relation between shoot height (cm) and root collar diameter (mm): SI = SH/RCD. After carefully washing root systems free of all growing media residue, dry weight of each fraction was determined after oven drying at 65 °C for 48 h. Others variables such as shoot dry weight (S), root dry weight (R), Root/Shoot ratio = R/S, and Dickson Quality Index (Dickson et al. 1960): DI = (S + R)/(SI + (S/R)) were determined using destructive techniques. Seedling mortality was assessed by counting dead seedlings in each container and for each type of substrate (n = 10).

2.4. Root Growth Potential (RGP) test to assess seedlings performance

In order to analyze how the growing media can influence the capacity of new roots to colonize soil, a root growth potential (RGP) test was carried out (Ritchie and Dunlap 1980; Ritchie 1984; Grossnickle 2012). This test was implemented 2 weeks after the end of the nursery culture (29 October 2019). Another subset of 60 seedlings per treatment were randomly selected and transplanted to 2 liter plastic pots (diameter = 10 cm and long = 25 cm) filled with silica sand. The RGP test had a duration of 30 days and was performed in a glasshouse at an average temperature of 20.5 °C and a mean air humidity of 83%. At the end of the RGP test, all new roots growing outside the root plug counted. A total of 540 thuya seedlings were used for the need of the RGP test.

2.5. Root electrolyte leakage (REL) as a seedling physiological quality attribute

A healthy and vigorous root system is essential for seedling establishment, especially in arid areas, where seedlings need to extend rapidly their roots before the onset of summer drought (Padilla and Pugnaire 2007). McKay (1992) described a procedure to assess the vitality of fine roots based on the integrity of cellular membranes: "Root Electrolyte Leakage" (REL). A subset of fifteen seedlings from each growing medium were used for electrolyte leakage test (2 December 2019). The root systems were washed under tap water to remove soil and rinsed in deionized water to clean up surface ions. Small segments (1 cm length, max. 500 mg, fresh weight) of fine roots (≤2mm diameter) were sampled from each root system. Individual samples were put in 30 mL glass test tubes containing 16 mL of deionized water of conductivity < 1 µS/cm. The tubes were capped, shaken and left at room temperature (21–22 °C) for 24 h. The tubes were also shaken after 24 h, and the conductivity of solutions (EClive) was measured using the conductivity meter (Orion M162). Following this measurement, the samples were autoclaved at 110 °C for 10 min and then the samples were allowed to cool to room temperature before taking a second conductivity reading. Later, the second conductivity (ECdead) measurement was made. REL is calculated as the ratio of the EC of live roots divided by the EC of dead roots: REL = (EClive/ECdead)*100. A low REL reading indicates high root viability, allowing water uptake to mitigate transplant shock (McKay 1998).

2.6. Statistical analysis

All of the statistics were performed using the XLSSTAT software (v2016 02.27444), and the results are reported as mean ± standard deviation. Distribution was tested for normality by the Shapiro-Wilk test (p ≤ 0.05), and the homogeneity of variances was tested by Levene’s test. Data on seedling morphological characteristics, mortality, root growth potential and root electrolyte leakage were compared by means of analysis of variance (one-way ANOVA). When normality was not acquired, data were analyzed according to the non-parametric Kruskal-Wallis test at 5% probability and therefore Dunn’s Multiple Comparison Test was used to separate means of variables. Finally, Principal Component Analysis [Pearson (n) correlation matrix], was used to study correlation between different seedlings quality attributes.

3. Results and discussion

3.1. Properties of the growing media

There were significant differences in the physical properties among the nine growth media, except for silt percentage (Table 1). Clay percentage ranged between 4.4% for S2 and 39.98% for S7 while the sand, used as an aerator, showed high percentages varying between 58.77% and 91.48%. The bulk density was largely affected by the amount of peat used in the substrate formula, it was relatively low (0.5 g/cm³) for substrate S1, S6 and S7 and high for S8 and S9 exceeding 1 g/cm³.
Total porosity and aeration porosity (Table 1) showed acceptable values, except for S9 with a total porosity of 48%. Havis and Hamilton (1976) stated that the total porosity of a growing medium should exceed 50%, and that the aeration porosity should be 20–25%. Whitcomb (1988) recommended a higher aeration porosity of approximately 25–35% for container tree seedlings.

Water-holding porosity measures the total micropores space that remains filled with water after the growing medium is saturated with water and allowed to drain freely. Because of the limited volume of small containers, the growing medium must have a high water-holding capacity to supply water to seedlings from one irrigation to the next (Landis et al. 1990). The substrate formula S5 and S7 showed high water-holding porosity levels, 39.02 and 40.62% respectively (Table 1). Water-holding porosity was largely affected by the clay content in the substrate, so that a non-linear regression could be established between these two parameters for substrates with the same peat content (S2, S3, S4 and S5) as follows (Graphic 1): Water-holding porosity (%$=6.43^*\ln(\%\text{Clay})+13.98$ with $R^2=0.883$ and $n=20$).

Chemical properties of the different growing media (Table 2) also showed a wide variation. Organic matter content of the growing media was largely controlled by the percentage of peat used in the substrates mix. This resulted in 3 homogeneous groups: (S1, S6, S7), (S2, S3, S4, S5, S8) and S9. OM(%) exceeded the threshold of 5% for the substrates S1, S5, S6 and S7, while the substrate S9 shows an extremely low value (0.54%). In fact, there is no universal optimum level of organic matter in soil. An optimum level of organic matter ranging between 5% and 12% is commonly adopted for forest and horticultural nurseries (Rose et al. 1995).

The pH of the nine growing media ranged between 4.78 and 7.7 (Table 2). On an operational basis, container nursery managers should strive to maintain the pH of their growing media in the slightly acidic range of pH 5.5–6.5 (Landis et al. 1990). The principal cultural effect of pH in mineral soils is its influence on availability of mineral nutrients, especially micronutrients; several mineral nutrients can become unavailable or even toxic at extreme pH values (Kuhns 1985). The pH can also affect the numbers and types of microorganisms in growing media, including fungal pathogens (Landis 2000). Fusarium spp. are more virulent in neutral to alkaline conditions, and losses to damping-off fungi increase at pH values above 6.5 (Handreck and Black 1984; James 2012). By the way, Fusarium solani (El Haddadi et al., 2019) and Rhizoctonia solani (EL Haddadi et al. 2020) were recently identified as telluric fungi that pose serious problem to containerized thuya seedlings in early stage.

The cation exchange capacity (CEC), is one of the most important factors affecting the fertility of growing media; A high CEC has another desirable property that is of interest to the container nursery manager. Because it is able to selectively adsorb and release cations from the growing medium solution, such a growing medium can buffer the seedling root system against sudden changes in pH or salinity (Whitcomb 1988). For the same level of peat, increasing the amount of vertisol in the substrates formula caused the increase of CEC values (Table 2). The substrate S7 showed the highest CEC value with 39.93 meq/100 cc while S2 and S9 substrates showed the lowest ones. Since Vertisol is very rich in clay, it was found that a linear regression between clay content in the substrate and its cation exchange capacity could be summarized as follows (Graphic 2): $\text{CEC (meq/100 cc)}=2.405+0.783^*\text{(Clay(%) with: n=20 and R}^2=0.949$.

Therefore, clay used mainly to improve the growing media water-holding capacity also showed a positive effect in improving their cation exchange capacity. Electric conductivity, which measures the growing...
Table 2. Chemical proprieties of the nine growing media.

| Growing medium | Organic matter (%) | pH (H₂O) | CEC (meq/100cc) | Conductivity (mS/cm) |
|----------------|--------------------|----------|-----------------|---------------------|
| S1             | 6.02 ± 0.14 b      | 4.78 ± 0.17 a | 13.36 ± 0.67 abc | 0.3 ± 0.04 a |
| S2             | 3.11 ± 0.29 ab     | 5.50 ± 0.08 ab | 7.02 ± 0.9 a     | 0.28 ± 0.04 a |
| S3             | 2.98 ± 0.22 ab     | 6.42 ± 0.15 abc | 14.48 ± 0.64 abc | 0.31 ± 0.03 a |
| S4             | 4.95 ± 0.13 ab     | 7.08 ± 0.12 abc | 23.02 ± 1.2 abc  | 0.19 ± 0.02 a |
| S5             | 5.62 ± 0.05 ab     | 7.07 ± 0.08 abc | 31.91 ± 2.01 bc  | 0.35 ± 0.01 a |
| S6             | 6.01 ± 0.08 b      | 6.25 ± 0.13 abc | 29.91 ± 1.01 bc  | 0.31 ± 0.33 a |
| S7             | 5.46 ± 0.17 b      | 6.12 ± 0.06 abc | 39.93 ± 1.75 c   | 0.24 ± 0.03 a |
| S8             | 2.99 ± 0.28 ab     | 7.14 ± 0.17 bc  | 23.89 ± 1.53 abc | 0.18 ± 0.03 a |
| S9             | 0.54 ± 0.21 a      | 7.70 ± 0.16 c   | 8.64 ± 0.97 ab   | 0.21 ± 0.02 a |

Within the same column, the means followed by different letters are statistically different (p-value < 0.05), using non-parametric Kruskal-Wallis test at 5%.

3.2. Seedlings mortality, growth and biomass

Seedlings mortality: The type of growing medium showed a very highly significant effect on mortality rate of thuya seedlings in early stage (Table 4). Plants from substrate with acidic pH (substrate S1) showed high mortality rates with 21.2%, this means that young thuya seedlings (5–6 weeks) do not tolerate very acidic substrates. On the contrary, seedlings grown in neutral or slightly acidic substrates showed the lowest mortality rates (S3 and S6). Thuya seedlings seem also not withstand alkaline pH substrates, the mortality rate becomes alarming (30% mortality rate for S9 with pH = 7.7). Graphic 3 illustrates the effect of pH level on thuya seedlings during establishment stage: Mortality rate (%) = 406.53 – 132.319 * (pH) + 10.81 * (pH)² with R² = 0.94 and n = 9. Besides, a growing media pH ranging between 5.7 and 6.7 could reduce early thuya seedlings mortalities to less than 5% and a substrate pH = 6.2 seemed to be an optimum.

Shoot height: Growing media formula had a significant effect on seedlings shoot height. However, the richness or the poverty of the substrate with peat is not a real factor, which acts on seedlings shoot height (Table 5). Indeed, even with a mixture of 50% (V/V) of peat (case of substrate S8), the average height of the resulting stem is very close to the mean height of plants raised in a substrate enriched with peat (70% for S1 substrate). Even with a substrate without peat (S9), showing the least amount of organic matter (0.54%), seedlings were able to reach similar heights as those raised in growing media with high level of organic matter (S6 with 6.01% organic matter content). In general, although the substrate showed a significant effect on seedlings shoot growth, the impact of this factor remains less felt on the qualitative level since the majority of substrates resulted in plants with high stem heights. Trubat et al. (2011) recommended a target shoot height (SH = 9cm) for _T. articulata_ seedlings.

Collar Diameter: Substrates S3 and S6 resulted in plants with a collar diameter greater than 3 mm although their organic matter content was 2.98 and 6.01% respectively (Table 5). Same observation can be pointed out for the substrate S9 (with 0.54% organic matter content), allowed the plants to acquire a collar diameter (CD) of 2.72 mm, significantly greater than S5 and S7 for which organic matter content was superior to 5.46% (Table 5).

Sturdiness index: Despite slight variations in this index depending on the substrates, it remains acceptable (4 < SI < 6) for all seedlings. The lowest sturdiness index value was observed in seedlings grown up in substrates S3 and S6 with 4.94 and 4.9 respectively (Table 5). Lamhamdi et al. (2006) recommended a sturdiness index less than 8 for forest seedlings.

Root/Shoot ratio: The S3 and S6 substrates showed the best values for this parameter with a R/S very close to 1(Table 5). This parameter gives an account of the...
The effect of growth medium formula on mortality of *Tetraclinis articulata* container seedlings during the five first weeks (mean ± SE).

| Growing medium | Mortality rate (%) | Mortality rate (%) (*) |
|-----------------|-------------------|------------------------|
| S1              | 10.6 ± 1.3        | 21.2 ± 2.7 g           |
| S2              | 2.3 ± 0.7         | 4.6 ± 1.3 b            |
| S3              | 1.4 ± 1           | 2.8 ± 1.9 a            |
| S4              | 3.8 ± 0.9         | 7.6 ± 1.8 d            |
| S5              | 4.8 ± 1           | 9.6 ± 2.1 e            |
| S6              | 0.9 ± 0.7         | 1.8 ± 1.5 a            |
| S7              | 2.4 ± 0.7         | 4.8 ± 1.4 c            |
| S8              | 7.9 ± 1.6         | 15.8 ± 3.2 f           |
| S9              | 14.9 ± 1.1        | 29.8 ± 2.2 h           |

(*) Within the same column, the means followed by different letters are statistically different at $\alpha = 0.05$ using LSD method ($n = 10$ per treatment).

The analysis of variance of the number of new fine roots emerged showed a very highly significant effect of substrate formula on root growth potential (Table 6), in other words, the substrate formula has an effect on the root development activity after transplanting the seedlings.

Seedlings raised in S4 and S6 substrates with a mixture (Peat: Sand: Vertisol) of (50%: 30%: 20%) (70%: 20%: 10%) successively displayed the maximum root growth potential (43 new fine roots). Thuya seedlings from S3 and S9 substrates were able to reach important RGP values near to 34 new fine roots, while those from S1, S2 and S7 showed the lowest values of RGP (20–21 new fine roots). The application of a non-linear regression on the RGP results in relation to substrates clay content, and thus water-holding porosity (WHP) of each substrate, makes it possible to understand how fine texture in the growing media affects the root growth potential RGP (Graphic 4): 

$$RGP = -6.178 \times (\text{Clay}^{2}) + 2.732 \times (\text{Clay}) + 8.908,$$

with $n = 9$ and $R^2 = 0.88$.

It seems that clay, as a water retainer, plays an important role in supplying the roots with water and nutrients. However, the clay content must not exceed a certain threshold beyond which it begins to negatively affect the performance of thuya seedlings. Hence, a clay content of 20–25% seemed to be an optimum for better root recovery after thuya seedlings outplanting.

### 3.4. Seedlings root electrolyte leakage

Root electrolyte leakage test (REL) performed in early December, corresponding to lifting time, showed a better on sites prone to water stress (Grossnickle and MacDonald 2018). Contrast this with an outplanting site where hot and dry conditions cause high evapotranspirational demand. Here, the advantage lies with plants that have a relatively small transpirational surface area relative to large, absorptive root system. Under these conditions, thuya with a large shoot and small root system (low R/S ratio) are at a disadvantage because they transpire faster than they can absorb water from the soil. Indeed, the S3 and S6 substrates formula optimized the values of the majority of the morphological parameters studied.
significant effect of growing media (p-value < 0.05) on this physiological quality attribute (Table 6). The lowest REL values were observed on seedlings raised in S4 and S6 substrates, with 11.48 and 11.66% respectively; while seedlings from S9 substrate registered the highest value (20.06%). Thuya seedlings grown up in S2 and S3 substrates appeared to belong to a same group with a medium REL values ranging between 12.88% and 14.53%. McKay and White (1997) states that REL usually is proportional to cell membrane damage and it correlates well with seedling out-planting performance. REL test has been used as an indication of root stress such as high and low temperatures (Stattin et al. 2000), desiccation (McKay and Milner 2000), rough handling (McKay and White 1997), and even water logging and disease (Ritchie et al. 2010).

The effect of growth medium formula on Root Growth Potential and Root Electrolyte Leakage of T. articulata container seedlings (mean ± SE) is shown in Table 6. The results indicated that the seedlings raised in S4 and S5 substrates showed the highest Root Growth Potential (RGP) values (14.57 ± 1.67 and 15.12 ± 1.66, respectively), while those raised in S9 substrate presented the lowest RGP value (14.89 ± 1.63). On the other hand, the highest Root Electrolyte Leakage (REL) values were observed in S4 (0.77 ± 0.05), followed by S5 (0.76 ± 0.05) and S3 (0.75 ± 0.06), while the lowest REL values were found in S9 (0.73 ± 0.01) and S2 (0.25 ± 0.06). The REL values were statistically different among the substrates, confirming the significant effect of the growing medium on the seedlings physiological quality.

**Table 5.** Morphological attributes of T. articulata container seedlings (mean ± SE) raised in different growth medium.

| Growing medium | SH (cm) | RCD (mm) | Slenderness index | S (g) | R (g) | R/S ratio | Dickson quality index (%) |
|----------------|--------|----------|-------------------|-------|-------|-----------|----------------------------|
| S1             | 17.39 ± 1.72 e | 2.82 ± 0.52 bc | 6.33 ± 108 d | 0.96 ± 0.18 bc | 0.75 ± 0.11 c | 0.27 ± 0.53 abc | 20.3 ± 0.05 abc |
| S2             | 14.89 ± 1.63 ab | 2.66 ± 0.61 abc | 5.84 ± 0.18 bcd | 0.97 ± 0.12 c | 0.76 ± 0.11 c | 0.30 ± 0.06 d | 20.5 ± 0.06 d |
| S3             | 15.54 ± 1.6 c | 3.22 ± 0.63 d | 4.94 ± 0.64 a | 0.91 ± 0.64 abc | 0.77 ± 0.12 c | 0.38 ± 0.06 c | 28.0 ± 0.06 e |
| S4             | 14.57 ± 1.67 a | 2.85 ± 0.59 c | 5.26 ± 0.83 ab | 0.87 ± 0.54 a | 0.71 ± 0.13 d | 0.31 ± 0.06 f | 21.5 ± 0.04 d |
| S5             | 15.19 ± 1.77 bc | 2.62 ± 0.41 a | 5.89 ± 0.75 cd | 0.87 ± 0.75 a | 0.73 ± 0.12 ab | 0.21 ± 0.04 a | 20.0 ± 0.06 d |
| S6             | 15.12 ± 1.66 bc | 3.14 ± 0.47 d | 4.93 ± 0.56 a | 1.0 ± 0.56 c | 0.84 ± 0.13 d | 0.31 ± 0.06 f | 21.5 ± 0.04 d |
| S7             | 14.59 ± 1.75 a | 2.72 ± 0.53 abc | 5.49 ± 0.83 bc | 0.94 ± 0.83 a | 0.71 ± 0.12 d | 0.25 ± 0.06 cd | 25.0 ± 0.06 c |
| S8             | 16.29 ± 1.77 ab | 2.64 ± 0.47 ab | 6.3 ± 1.03 d | 0.93 ± 1.03 abc | 0.69 ± 1.1ab | 0.22 ± 0.05 ab | 20.5 ± 0.06 c |
| S9             | 14.78 ± 1.49 ab | 2.72 ± 0.55 abc | 5.65 ± 1.24 bc | 0.93 ± 1.24 abc | 0.67 ± 0.1 ab | 0.23 ± 0.06 bcd | 20.5 ± 0.06 c |

**Table 6.** The effect of growth medium formula on Root Growth Potential and Root Electrolyte Leakage of T. articulata container seedlings (mean ± SE).

| Growing medium | Root Electrolyte leakage (%) | Root Growth potential (%) |
|----------------|-------------------------------|---------------------------|
| S1             | 15.27 ± 4.09 abc              | 20.58 ± 2.77 a            |
| S2             | 12.88 ± 4.1 ab                | 21.25 ± 3.261a            |
| S3             | 14.53 ± 3.11 ab               | 33.97 ± 3.48d             |
| S4             | 11.48 ± 1.86 a                | 43.85 ± 3.49f             |
| S5             | 15.8 ± 3.47 abc               | 25.07 ± 3.15b             |
| S6             | 11.66 ± 4.1 a                 | 42.98 ± 3.08f             |
| S7             | 16.1 ± 4.59 abc               | 20.93 ± 3.97a             |
| S8             | 16.89 ± 4.52 bc               | 32.07 ± 3.03c             |
| S9             | 20.06 ± 2.07 c                | 35.58 ± 3.23e             |

**Graphic 4.** Relationship between Root Growth Potential (RGP) of thuya seedlings and growing medium clay content (%).

**Table 7.** Principal components analysis of different thuya seedlings quality attributes – Pearson (r) correlation matrix.

| Vari. | RCD | SH | RGP | REL | R/S | SI  |
|-------|-----|----|-----|-----|-----|-----|
| RCD   | 1   | 0.046 | 1    | 0.530 | 0.308 | 1   |
| SH    | -0.450 | 0.133 | -0.297 | 0.877 | -0.030 | -0.432 | -0.724 | 0.798 | 1   |
| R/S   | 0.297 | 1   | 0.724 | 0.587 | -0.625 | 0.456 | -0.724 | 1   |

Values in bold are different to 0 at level of significance α = 0.05 according to Bartlett sphericity test.

**3.5. Correlations between different assessed seedlings quality attributes**

Morphological quality attributes are easy to measure in operational settings ensuring their use in forest nurseries. In contrast, the study of physiological and performance attributes of the plants remains laborious and time consuming, therefore, it is desirable to look for possible correlations between different seedlings quality attributes. For this purpose, a principal component analysis (PCA) were used to highlight the predictive value of morphological attributes in relation to the physiological and performance attributes of thuya seedlings (Table 7 and Graphic 5). The PCA result demonstrated that the seedlings shoot height (SH) did not show any correlation with either of the two physiological (REL) and performance (RGP) attributes. The root collar diameter (RCD) was strongly correlated with the R/S ratio and negatively correlated with the sturdiness index (SI). Moreover, R/S ratio was negatively correlated with REL and the SI was negatively correlated with RGP.

In the end, seedlings collar diameter and R/S ratio can be considered as good predictors of root vitality.
and integrity (REL) while sturdiness index can be used as a predictor of root system development (RGP). Greater collar diameter and root system size confer a higher chance of survival and growth, because they limit susceptibility to planting stress by improving water uptake and transport to foliage (Chirino et al. 2008).

Our results are consistent with South (1987) who revisited morphological criteria defined by Wakley (1954) and found that collar diameter was still the attribute that best forecast field growth potential. Mañas et al. (2009) argued that the sturdiness index in combination with collar diameter could accurately predict seedling field survival. On another note, Tsakaldimi et al. (2013) pointed out that collar diameter was the best predictor of second-year outplanting survival of five Mediterranean species. On the other hand, a study conducted on five Mediterranean species including T. articulata (Trubat et al. 2011) showed that RGP was a poor predictor of plant performance under field conditions in semi-arid lands when other morpho-physiological attributes were not taken into account.

4. Conclusion

Defining optimum morpho-physiological traits for the establishment of thuya seedlings is a major step toward improving the efficiency of thuya plantations in dry land restoration programs. In this study, morpho-physiological and performance attributes were used to assess the quality of Tetraclinis articulata seedlings raised in nine growing media with different physical and chemical proprieties. Our results show that growing media has a strong potential to modify the morphology of thuya seedlings in the nursery. The growing medium formula (Peat: Sand: Vertisol; v/v) of (70%: 20%: 10%) resulted in seedlings with good values of collar diameter (3.14 mm), low Sturdiness index (4.9), highest R/S ratio (0.84) and the best Dickson quality index (0.31). We have shown that this substrate formula also resulted with plants with low REL (11.66%) and high RGP (42.98 new roots). Growing media formula with only 50% peat mix (50%:30%:20%) ended up to encouraging results from a physiological and performance point of view. Growing media pH largely affects seedlings establishment and increases seedlings dumping-off risk with very acidic or alkaline substrates and a substrate pH = 6.2 seemed to be an optimum. On the other hand, the use of clay as water retainer improves the retention capacity of the substrates and consequently the development of the root system, provided that clay content in the substrate does not exceed the critical threshold of 25%, beyond which the root growth capacity is deteriorated. The findings of these experiments emphasize that no single attribute can assess all thuya seedlings quality issues. Morphological attributes cannot be used in isolation to assess seedlings quality. Thus, a seedling quality program, accomplished through true field trials, combining both morphological, physiological and performance attributes to provide the information necessary for making both sound nursery cultural decision and restoration site conditions.

Authors’ contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript. Growing media samples were analyzed at the laboratory of the National Institute of Agricultural Research (INRA), Regional center of Rabat.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

Arshad MA, Lowery B, Grossman B. 1996. Physical tests for monitoring soil quality. In: Doran JW, Jones AJ, editors. Methods for assessing soil quality. Madison (WI): Soil Science Society of America; p. 123–141.

Baeza J, Pastor A, Martin J, Ibanez M. 1991. Post-planting mortality in reforested sites of Pinus halepensis, Quercus ilex, Ceratonia siliqua, and Tetraclinis articulata in the province of Alicante. Studia Oecol. 8:139–146.

Bakry M, Lamhamedi MS, Caron J, Bernier PY, Zine El Abidine A, Stowe DC, Margolis HA. 2013. Changes in the physical properties of two Acacia compost-based growing media and their effects on growth and physiological variables of containerized carob (Ceratonia siliqua L.) seedlings. New Forests. 44(6):827–847.

Bakry M, Lamhamedi MS, Caron J, Margolis H, Zine El Abidine A, Bellaka M, Stowe DC. 2012. Are composts from shredded leafy branches of fast-growing forest species suitable as nursery growing media in arid regions? New Forests. 43(3): 267–286.

Benabid A, Fennane M. 1994. Connaissances sur la végétation du Maroc: Phytogéographie, phytosociologie et série de végétation. Lazaroa. 14:21–97.

Boudy P. 1950. Economie forestière Nord-Africaine. Tome II, monographie et traitement des essences forestières. Paris, Larousse: Fasc. I. E., p. 525.
Bower CA, Reitemeier RF, Fireman M. 1952. Exchangeable cat-
ion analysis of saline and alkali soils. Soil Sci. 73:251–262.
Bremner JM. 1965. Total nitrogen. In: Methods of soil analysis: part 2, chemical and microbiological properties. Vol. 9. Madison (WI): American Society of Agronomy; p. 1149–1178.
Chacor J. 1999. El bosque mediterráneo en el norte de África: Biodiversidad y lucha contra la desertificación. Madrid: Ed. Mundo Árabe e Islam-AECI; p. 370.
Chirino E, Vilagrosa A, Cortina J, Valdecantos A, Fuentes D, Trubat R, Luis VC, Puértolas J, Bautista S, Baiza J, et al. 2009. Ecological restoration in degraded drylands: the need to improve the seedling quality and site conditions in the field. In: Grossberg SP, editor. Forest management. New York: Nova Publisher; p. 85–158.
Chirino E, Vilagrosa A, Hernández EL, Matos A, Vallejo VR. 2011. Using hydrogel and clay to improve the water status of seedlings for dryland re-
planting. Plant Soil. 344(1-2):99–110.
Cortina J, Peñuelas JL, Puertos J, Savé R, Vilagrosa A. 2006. Calidad de planta forestal para la restauración en ambientes mediterráneos: Estado actual del conocimiento. Madrid: Ministerio de Medio Ambiente.
Dallali Y, Chahhou D, El Aboudi A, Aafi A, Abbas Y, Mounir Del Campo AD, Navarro RM, Ceacero CJ. 2010. Seedling quality.
Delphin J, Chapot J, Schoellen A, Huck C, Schneider C, Schwab Grossnickle SC. 2005. Seedling size and reforestation success: an approach for establishing a quality standard. New For. 39(1): 19–37.
Delphin J, Chapot J, Schoellen A, Huck C, Schneider C, Schwab G. 1991. Relations entre le pouvoir minéralisateur des sols et la minéralisation nette de l’azote au champ. Agronomie. 11(6):439–445.
Dickson A, Leaf AL, Hosner JF. 1960. Quality appraisal of white pine seedlings stocked in nurseries. Forest Chron. 36(1):10–13.
EL Haddad R, Errifi A, Msairi S, Ouazzani Touhami A, Douira Bremner JM. 1965. Total nitrogen. In: Methods of soil analysis: part 2, chemical and microbiological properties. Vol. 9. Madison (WI): American Society of Agronomy; p. 1149–1178.
Dickson A, Leaf AL, Hosner JF. 1960. Quality appraisal of white pine seedlings stocked in nurseries. Forest Chron. 36(1):10–13.
EL Haddad R, Errifi A, Msairi S, Ouazzani Touhami A, Douira Bremner JM. 1965. Total nitrogen. In: Methods of soil analysis: part 2, chemical and microbiological properties. Vol. 9. Madison (WI): American Society of Agronomy; p. 1149–1178.
Dickson A, Leaf AL, Hosner JF. 1960. Quality appraisal of white pine seedlings stocked in nurseries. Forest Chron. 36(1):10–13.
EL Haddad R, Errifi A, Msairi S, Ouazzani Touhami A, Douira Bremner JM. 1965. Total nitrogen. In: Methods of soil analysis: part 2, chemical and microbiological properties. Vol. 9. Madison (WI): American Society of Agronomy; p. 1149–1178.
Dickson A, Leaf AL, Hosner JF. 1960. Quality appraisal of white pine seedlings stocked in nurseries. Forest Chron. 36(1):10–13.
EL Haddad R, Errifi A, Msairi S, Ouazzani Touhami A, Douira Bremner JM. 1965. Total nitrogen. In: Methods of soil analysis: part 2, chemical and microbiological properties. Vol. 9. Madison (WI): American Society of Agronomy; p. 1149–1178.
Dickson A, Leaf AL, Hosner JF. 1960. Quality appraisal of white pine seedlings stocked in nurseries. Forest Chron. 36(1):10–13.
EL Haddad R, Errifi A, Msairi S, Ouazzani Touhami A, Douira Bremner JM. 1965. Total nitrogen. In: Methods of soil analysis: part 2, chemical and microbiological properties. Vol. 9. Madison (WI): American Society of Agronomy; p. 1149–1178.
Dickson A, Leaf AL, Hosner JF. 1960. Quality appraisal of white pine seedlings stocked in nurseries. Forest Chron. 36(1):10–13.
EL Haddad R, Errifi A, Msairi S, Ouazzani Touhami A, Douira Bremner JM. 1965. Total nitrogen. In: Methods of soil analysis: part 2, chemical and microbiological properties. Vol. 9. Madison (WI): American Society of Agronomy; p. 1149–1178.
Dickson A, Leaf AL, Hosner JF. 1960. Quality appraisal of white pine seedlings stocked in nurseries. Forest Chron. 36(1):10–13.
EL Haddad R, Errifi A, Msairi S, Ouazzani Touhami A, Douira Bremner JM. 1965. Total nitrogen. In: Methods of soil analysis: part 2, chemical and microbiological properties. Vol. 9. Madison (WI): American Society of Agronomy; p. 1149–1178.
Dickson A, Leaf AL, Hosner JF. 1960. Quality appraisal of white pine seedlings stocked in nurseries. Forest Chron. 36(1):10–13.
EL Haddad R, Errifi A, Msairi S, Ouazzani Touhami A, Douira Bremner JM. 1965. Total nitrogen. In: Methods of soil analysis: part 2, chemical and microbiological properties. Vol. 9. Madison (WI): American Society of Agronomy; p. 1149–1178.
Dickson A, Leaf AL, Hosner JF. 1960. Quality appraisal of white pine seedlings stocked in nurseries. Forest Chron. 36(1):10–13.
EL Haddad R, Errifi A, Msairi S, Ouazzani Touhami A, Douira Bremner JM. 1965. Total nitrogen. In: Methods of soil analysis: part 2, chemical and microbiological properties. Vol. 9. Madison (WI): American Society of Agronomy; p. 1149–1178.
