Effect of co-culture with nurse plant (*Lavandula dentata* L.) on the morphological and physiological parameters in *Cupressus atlantica* G. grown under long term of water stress conditions

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Abstract

The influence of *Lavandula dentata* L. on plant growth and drought tolerance of ten months old *Cupressus atlantica* G. seedlings in potted culture was studies in four different water treatments four month ago. Effect of nurse plant (*L. dentata* L.) in co-culture with *C. atlantica* G. plant growth (including plant shoot and root weight, height and collar diameter of the aerial part of seedlings) were higher for well-watered than for water- stressed plant. The growth of plants in co-culture with lavender plants on non-sterilized soil (CA+Lav) was higher than control plants (CA & CAs+Lav) regardless of water status. P, N, K, Ca++, & Mg++ contents in (CA+Lav) were greater than those in CA and (CAs+Lav) under well watered conditions and severe and moderate level of drought stress (DS).

Keywords: *Cupressus atlantica* G., nurse plant, drought stress, *lavendula dentata* L., reforestation.

1. Introduction

In areas with harsh environmental conditions such as the Mediterranean basin, deforestation has been a common practice for more than 2000 years leading to the loss of most primeval forests (Bauer, 1991; Blondel & Aronson, 1999). Marchand (1990) estimated that only 9- 10% of the Mediterranean area is currently forested.

The most crucial stage in a restoration project is seedling establishment (Grubb, 1977). However, in the Mediterranean ecosystem the prevailing environmental conditions are characterized by abiotic stress and in particular by water stress (Callaway and Walker, 1997; Gomez- Aparicio *et al.*, 2004). Hence, there is a vital need for reforestation in order to reserve this loss of biodiversity but the crucial phase in this project is seedling establishment (Grubb, 1977).

However, recent studies have reported that the development of plant can be facilitated by some pioneer shrubs acting positively on survival and growth of target species, while creating a favorable microhabitat that will protect against environmental constraints and improves fertility soils (Callaway, 1994; Callaway & Walker, 1997), these shrubs are called "nurses plants" (Niering *et al.*, 1963). In recent years, the phenomenon of nurse plant has been investigated in degraded habitats, including Mediterranean ecosystems (Padilla & Pugnaire, 2006; Callaway *et al.*, 2002). The nursing effect may be attributed to the crucial factors, including the increase in the availability of water and nutrients (litter of nurses Plants) crown architecture, low temperature, and soil fertility (Hai *et al.*, 2008).

these shrubs plays an important role in recovering the structures and functions of primary ecosystem, also nurse species facilitate germination, the establishment and growth of forest plants and promote plant community changes in their understory (Bruno *et al.*, 2003; Pugnaire *et al.*, 2011).

Facilitation processes among plants are crucial for maintaining species richness in plant communities, particularly in stressful environments (Hacker and Bertness, 1999; Callaway *et al.*, 2002; Callaway, 2007; Butterfield *et al.*, 2010; Soliveres *et al.*, 2011a), which also promote plant diversity (Butterfield *et al.*, 2013).

Several studies suggest that the effects of positive interactions on species richness at the community level are prevalent at moderate levels of stress (Hacker and Gaines, 1997; Michalet *et al.*, 2006).

Lavandula spp, are representative shrubs species in semiarid Mediterranean ecosystems (Barea *et al.*, 1992). They have constituted “fertility islands” or “resource islands” (Schlesinger *et al.*, 1996) which could play an important role in the regeneration processes of Mediterranean tree species such as *Cupressus* spp (Ouahmane *et al.*, 2007).

In addition, these nurse plants act positively on the mycorrhizal soil potential (Requena *et al.*, 2001; Azcon - Aguilar *et al.*, 2003). The use of native plant species mycotrophic themselves as facilitators species improves mycorrhizal soil load, microbiological properties of the soil and therefore the solubilization of phosphates from the ground.

In Morocco, there is a rich biodiversity with a large number of ecosystems and rich flora (over 4500 species of higher plants with about 200 of them considered as rare or threatened). Overgrazing of pastoral resources, deforestation caused by demographic pressure, irregularity of rainfall distribution and changes in cultural practices limited the natural regeneration of *C. atlantica* G.

We hypothesized that co-culture with *Lavendula dentata* L. (*L.dentata*) can increase *C. atlantica* plant tolerance against long term drought stress (DS). The effect of co-culture with nurse plants on morphological and physiological parameters during drought stress is not well known. In this context, the aim of this study is to compare the effects of lavender plants in growth, nutritional status, potential water, and relative water content (RWC), in *C. atlantica*, in order to develop new technological methods of ensuring adaptation of this specie to harsh environmental conditions like drought stress.

2. Material & methods

2.1. Experimental design and growing conditions

Soil samples were collected in close proximity of *Cupressus atlantica* G. trees at a depth between 10 and 30 cm. Four samples of 1 kg each were collected around the tree trunk a distance of 1 m. This has been done for ten *C. atlantica* totalizing 40 kg of soil. Soil samples were collected in an area located in the N’fis valley (Haut Atlas, Morocco; 8°17’ 02’’W, 31° 54’ 34”, 1700 m above sea level), in November 2012.

The experiment setup consisted of two sets. The first set was designed for water stress using four different water regimes as described bellow, while the second set consisted of co- culture with lavender plants as follow: (1) plants in co- culture with lavender plants growing under non- sterilized soil (CA+Lav), (2) plants in co- culture with lavender plants growing under sterilized soil (CAs+Lav), and (3) plants control growing under non- sterilized soil (CA). The experiment was performed in pot culture of 1500 ml (20 cm diameter, 20 cm height) containing 1 kg of dried soil and it was conducted for ten months. Forty replicates per treatment were set up, resulting in a total of 480 plants (each plant per pot culture).

After ten months of growth and the watering regularly, a water regime (25%, 50%, 75% of field capacity and Control (90% of its field capacity)) was applied to seedlings in controlled conditions for four month.

Pot cultures containing C. atlantica seedlings were watered regularly using distilled water at the following water regimes: 25%, 50% and 75% of field capacity. The watering control consisted of 90% of field capacity. To determine these stress levels (75%, 50% and 25%) of the field capacity, we weighed pots containing 1 kg of dry soil (P1 = 1Kg dry soil) and these pots were then watered until the soil saturation (flow of excess water under gravity). Watered pots were covered with black plastic bag to avoid the evaporation and they were soaked for 48 hours in greenhouse. Pots were weighed again in order to determine P2, which was considered as saturated soils. The difference between P2 and P1 (P2 – P1) provided the amount of water that was required to saturate the soil, which represents 100% of field capacity. In order to determine the watering volumes for four humidity levels of field capacity (90%, 75%, 50% and 25%), we added, to the series of pots containing dry soil, water volumes corresponding to 0.90 x (P2 - P1); 0.75 x (P2 - P1); 0.50 x (P2 - P1) and 0.25 x (P2 - P1) respectively. P3, P4, P5 and P6 weight of the pots at 90%, 75%, 50% and 25% of field capacity, respectively, were then calculated. The experiment was conducted in a greenhouse under natural light. The average day/night temperature was 33/25°C; the relative humidity (RH) was 55/86 %.

2.2. Soil analyses

Soil samples were mixed manually and divided in two subsamples. One subsample was autoclaved at 104°C for 24h and the other was kept in room temperature for further analyses.

Chemical and physical analyses of mixed soil samples, were performed and they provided the following characteristics: pH (H_2O) 6; clay (%) 4.8; fine silt (%) 0.0; coarse silt (%) 1.6; fine sand (%) 28.9; coarse sand (%) 64.7; carbon (%) 3.1; total nitrogen (%) 0.09; Olsen phosphorus 5.2 mg Kg^{-1}; total phosphorus 121 mg Kg^{-1}.

2.3. Seedlings preparation and co-culture with lavender plants

Seeds of *C. atlantica* and seeds of lavender (*Lavandula dentata* L.) were obtained from High Commission of water and forest –Marrakech- Morocco. Seeds of *C. atlantica* were immersed in distilled water for 24h, and kept for germination on wet filter paper in petri dishes at 20°C.

After one week, germinated seeds were individually transplanted into pots that were filled with 1Kg of soil (sterilized or not sterilized).

Germinated seeds of lavender plants were transplanted near of the young plants of *C. atlantica* after two months of their germination.

After ten months of culture under normal conditions of irrigation, plants of *C. atlantica* were subjected to four water regimes for a four months period. T0, T1, T2, T3, and T4 corresponded to timelines of 1, 2, 3 and 4 months respectively where water regimes were applied.

2.4. Measured parameters

After 10 month of the experimental period, samples from ten *C. atlantica* per treatment were used to assess the effect of water stress on growth and water features of co-culture with lavender plants. The remaining plants were used for mineral analysis and the determination of other biochemical parameters.

The response of plants *C. atlantica* in co-culture with lavender plants was estimated by determining the root collar diameter, stem diameter, elongation of the aerial part of the plant and biomass production. Dry mass (DM) was measured after drying in an oven at 105 ° C for 24 hours.

The effect of co culture with lavender plants on the mineral content in the *C. atlantica* was estimated by measurement of P, N, K, $Ca^{++}$and Mg^{++} in the aerial parts of the plant.
The water content of shoots (WC) was determined by the difference between the mass of fresh material (FM) and the dry matter (DM), in grams of water per gram of DM (g/g DM). The relative water content (RWC) was also measured on the shoots for all treatments using the following equation:

$$RWC\ \% = \frac{FM - DM}{FMsat - DM} \times 100$$

Where MFsat corresponds to the saturated fresh material

Needle water potential (ψh) was measured by the method of the pressure chamber developed by Scholander *et al* (1965).

2.5. Statistical analysis

Data were analyzed with two-way ANOVA, with the SPSS statistical package version 18.0 software, to partition the variance into the main effects. Co-culture with a lavender plants, different levels of drought stress, and duration of water stress are the factor, were used as the factors, respectively. Significant differences between factors were calculated at 5%, and significant difference was evaluated with test LSD post hoc ($P \leq 0.05$).

3. Results

After four months of application of DS, non sterilized soil treated with *L. dentata* L. significantly improved the growth of *C. atlantica* G. seedlings (high and collar diameter) compared to the control plants (CA & CAs+Lav) (table 1). Our results the plant of cypress in co-culture with *L. dentata* L. growing under non sterilized soil show significant growth in compared with plants control (CA & CAs+Lav) for all levels of drought stress. At the sever level of drought stress (25% F.C.) growth of control plants remains stable during the four months of stress but the plants CA+Lav show a slight fluctuation of growth. However, the collar diameter of seedling developed under sever and moderate stress show the smaller collar diameter compared with the plants growing under 75% F.C. and T. DS decrease collar diameter for all treatment of plants during the four months of stress applied.
The use of statistical analysis based on the analysis of variance reveals a significant different between the seedling of CA+Lav and both plants control under different level of DS.

Table 1. Effect of the water deficit on height and collar diameter of the aerial part of seedlings *Cupressus atlantica* G. aged ten months and subjected to different treatments (CA: *C. atlantica* G. of non-sterilized soil, CAs+Lav: *C. atlantica* G. in co-culture with lavender plant on sterilized soil and CA + Lav: *C. atlantica* G. in co-culture with lavender plant on non-sterilized soil).

	Traitement plante	**Temps**	**25% FC**	**50%FC**	**75%FC**	**T FC**
Hauteur de la partie aérienne (cm)	**CA**	T0	27.77±0.6	24.87±2.04	29±0.46	32.20±3.05
		T1	27.87±0.66	25.07±2.06	29.50±0.81	32.97±3.04
		T2	28.37±0.85	25.57±1.8	29.87±0.81	33.90±2.94
		T3	28.60±0.81	26.13±1.79	30.97±0.31	35.43±2.02
		T4	28.93±0.05	26.63±1.59	31.20±0.5	36.30±1.39

	CAs+LAV	T0	11.6±0.36	11.10±0.6	13.17±0.35	13.80±0.36
		T1	12.4±0.7	11.20±0.2	12.13±0.23	13.57±0.04
		T2	12.1±0.1	13±0.43	13.20±0.56	14.63±0.25
		T3	13±0.36	13.27±0.6	12.47±0.25	13.2±0.35
		T4	11.53±0.42	11±0.1	13.63±1.19	12.77±0.70
	CA+LAV	T0	11.6±0.36	37.5±0.9	37.5±0.2	36.63±0.61
		T1	12.4±0.7	37.7±0.9	38.67±0.21	37.03±0.49
		T2	12.1±0.1	38.3±0.9	39.77±0.4	37.93±0.55
		T3	13±0.36	38.77±0.85	40.40±0.43	38.47±0.55
		T4	11.53±0.42	39.07±0.8	41.27±0.6	38.93±0.57
Diamètre au collet (mm)	**CA**	T0	3.23±0.2	3.83±0.28	4.27±0.26	4..44±0.07
		T1	3.10±0.32	3.73±0.33	4.33±0.25	4.48±0.07
		T2	2.85±0.10	3.92±0.33	4.36±0.24	4.50±0.06
		T3	2.89±0.03	4.01±0.31	4.41±0.25	4.53±0.08
		T4	3.08±0.10	4.13±0.27	4.42±0.23	4.62±0.12
	CAs+LAV	T0	2.09±0.14	3.83±0.28	2.42±0.21	2.53±0.08
		T1	2.04±0.17	3.73±0.33	2.43±0.2	2.56±0.08
		T2	2.02±0.14	3.92±0.33	2.45±0.2	2.59±0.09
		T3	1.99±0.14	4.01±0.31	2.46±0.19	2.59±0.08
		T4	1.95±0.14	4.13±0.27	2.46±0.19	2.61±0.10
	CA+LAV	T0	4.40±0.5	4.84±0.44	7.3±0.6	7.19±0.77
		T1	4.37±0.44	4.90±0.04	7.48±0.55	7.58±0.59
		T2	4.23±0.62	5.30±0.94	7.66±0.59	7.72±0.57
		T3	4.21±0.68	5.37±0.39	7.78±0.55	7;93±0.59
		T4	4.47±0.6	5.62±1.17	7.99±0.64	8.10±0.59

The dry mass of shoot from the tree treatment of plants was measured. As shown in fig.1. The total dry mass of the plants in co-culture growing under non sterilized soil was significantly higher compared with the CAs+Lav and control under different level of water stress and especially under sever level (25% FC) of drought stress. The result demonstrated that both moderate (50% FC) and severe (25% FC) drought stress significantly decreased the dry mass of shoot for different treatment of plants when compared with plants that had the normal irrigation treatment. The analysis of variance showed this two parameters, drought stress and co-culture with nurse plant (lavender plants), significantly affected the dry mass of shoot seedling of *C. atlantica* G.

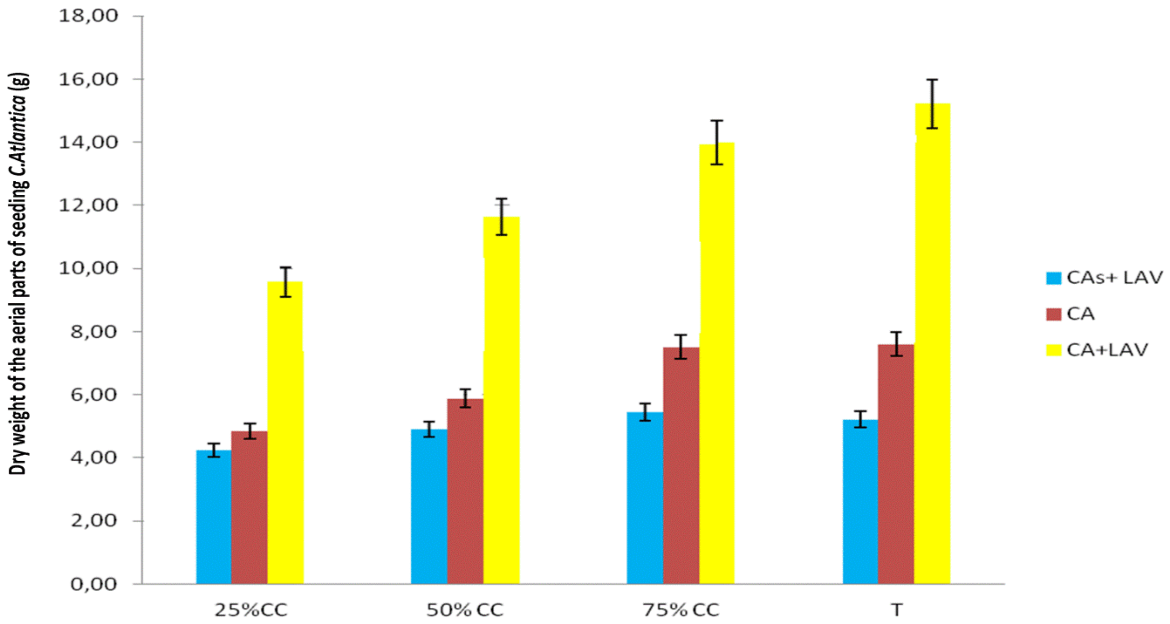

Figure 1. Change in dry weight of the aerial part of seedlings *C. atlantica* aged ten months and subjected to different treatments (CA: *C. atlantica* of non-sterilized soil, CAs+Lav: *C. atlantica* in co-culture with lavender plant on sterilized soil and CA+Lav: *C. atlantica* in co-culture with lavender plant on non-sterilized soil).

The same results are showed in dry mass of root from the tree treatment of plants was measured (fig.2.).

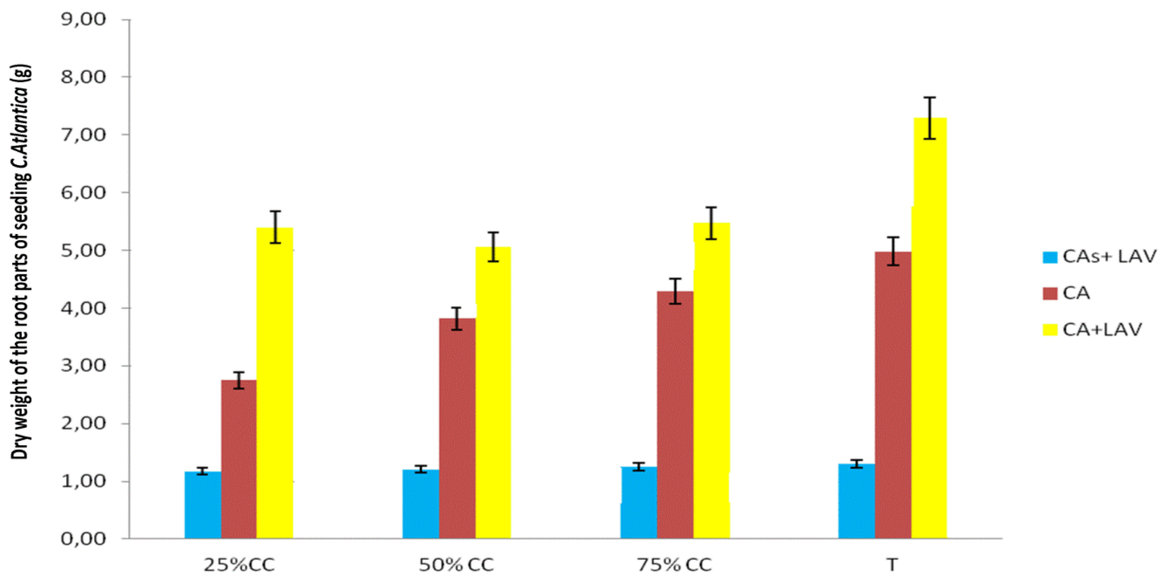

Figure 2. Change in dry weight of the root parts of seedlings *C. atlantica* aged ten months and subjected to different treatments (CA: *C. atlantica* of non-sterilized soil, CAs+Lav: *C. atlantica* in co-culture with lavender plant on sterilized soil and CA+Lav: *C. atlantica* in co-culture with lavender plant on non-sterilized soil).The plants CA+Lav had higher relative water content (RWC) than plants CAs+Lav and control plants (CA). However drought application decreased the RWC ratio in all treatment of plants (figure 3). Moreover, plants CA+ Lav appear the highest RWC ration under sever and moderate level of drought stress (P<0.05).

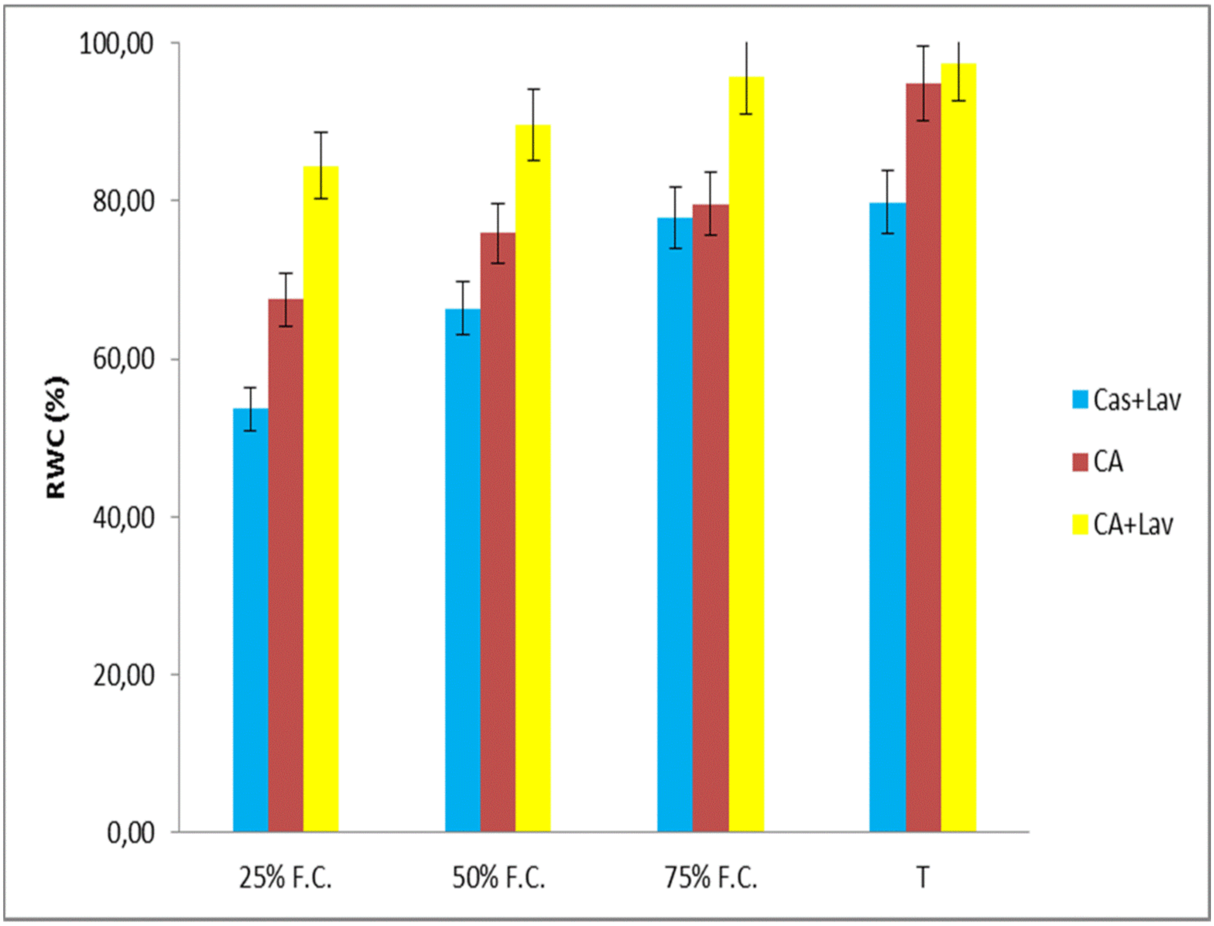

Figure 3. Relative water content (%) of the aerial parts of seedlings C. atlantica aged ten months and subjected to different treatments (CA: *C. atlantica* of non-sterilized soil, CAs+Lav: *C. atlantica* in co-culture with lavender plant on sterilized soil and CA + Lav: *C. atlantica* in co-culture with lavender plant on non-sterilized soil).

It was found that the water potential decreasing as to the water deficit increasing (figure 4). Therefore the most negative values are recorded for 25% F.C. and 50% F.C.

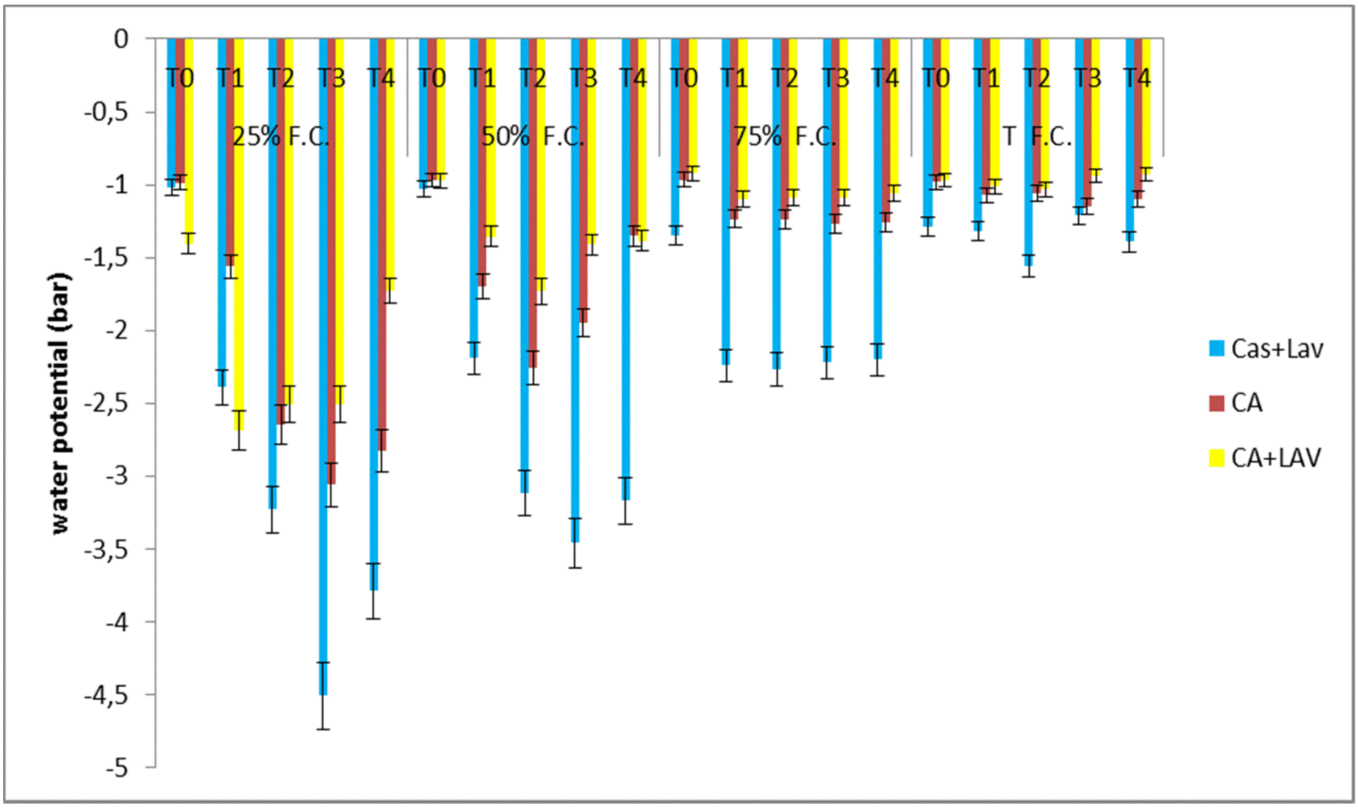

Figure 4. Change in water potential of seedlings *C. atlantica* aged ten months and subjected to different treatments (CA: *C. atlantica* of non-sterilized soil, CAs+Lav: *C. atlantica* in co-culture with lavender plant on sterilized soil and CA + Lav: *C. atlantica* in co-culture with lavender plant on non-sterilized soil).

Drought stress significantly affected N, P, K, Ca^{2+}, and Mg^{2+} in all treatment of plants. In addition, the results showed that the effect of co- culture with lavender plants was significant on all parameters related to nutritional status. The CA+ Lav had a higher P concentration than (CAs+Lav) and CA plants ($P<0.05$) (Table 2). DS reduce the P concentration in the leaves of all plant treatment, under sever and mild drought stress conditions (CA+Lav) had a higher P concentration. The same results of the N, K, $Ca^{2+,}$ & Mg^{2+} concentrations.

Table 2. Effect of the water deficit on the ionic content of aerial part of *Cupressus atlantica* aged ten months and subjected to different treatments (CA: *C. atlantica* of non-sterilized soil, CAs+Lav: *C. atlantica* in co-culture with lavender plant on sterilized soil and CA + Lav: *C. atlantica* in co-culture with lavender plant on non-sterilized soil).

Traitement hydrique	Traitement	P mg/plante	N mg/plante	K mg/plante	Ca^{2+} mg/plante	Mg^{2+} mg/plante
25% CC	CA+ LAV	2.53±0.55	3.81±0.07	4.40±0.16	2.48±0.7	3.34±0.35
	CA	1.02±0.32	1.26±0.4	2.18±0.37	1.28±0.22	2.23±0.52
	CAs+ LAV	0.21±0.6	0.91±0.5	1.07±0.43	0.85±0.61	1.29±0.41
50% CC	CA+LAV	3.13±0.65	5.76±0.11	7.47±0.28	4.28±0.85	3.59±0.74
	CA	1.09±0.8	3.40±0.25	3.74±0.31	2.23±0.52	2.43±0.39
	CAs+ LAV	0.29±0.81	1.04±0.35	1.1±0.44	0.86±0.6	1.18±0.48
75% CC	CA+LAV	4.99±0.33	7.65±0.2	8.43±0.6	7.06±0.81	5.24±0.56
	CA	1.25±0.17	2.68±0.59	5.68±0.55	4.65±0.37	3.45±0.45
	CAs+ LAV	0.34±0.45	1.08±0.23	1.21±0.55	1.05±0.64	1.49±0.31
T CC	CA+LAV	8.22±0.11	8.28±0.19	11.63±0.31	8.86±0.45	5.95±0.64
	CA	1.06±0.09	2.30±0.23	5.28±0.29	5.22±0.31	2.18±0.43
	CAs+ LAV	0.58±0.24	1.1±0.2	2.18±0.45	2.44±0.3	1.41±0.51

4. Discussion

Drought stress is the most serious threat to culture and environment in many part of the world (Parida & Das, 2005). In our study, the co-culture with lavender plant (*Lavandula dentata* L.) positively affected the growth of *C. atlantica* G. plants under well watered and drought conditions. Previous study show the positive effects under stressful environmental conditions, plants use the facilitation process with nurse plant to promote their plants tolerance to extreme abiotic conditions, particularly in arid and semi-arid climates (Flores and Jurado, 2003; Butterfield *et al.*, 2013). In most of previous study drought stress reduced a height growth in *Pinus caribaea* and *Pinus occarpa* (Tesha, 1971), in *Quercus robur* and *Fagus sylvatica* (Van Hees, 1997). And total biomass in *Quercus stellata*, *Acer saccharum* and *Junglans nigra* (Pallardy and Rhoads, 1993). Moderate and severe level of water stress also causes a reduction of the dry matter in the yellow cypress (Arnott *et al.*, 1993). Under drought stress, Harrouni *et al.*, 1995, mentions a reduction in the production of seedlings leaves of the Argan tree, in addition, other study indicate that growth of the aerial part decreases, accompanied by a reduction in the number of leaves (Tazi *et al.*, 2003). Our results indicate that in the absence of lavender plants, the seedlings of cypress growing in non-sterile soil show a cessation of growth in height, though the presence of the lavender plants in co-culture with *C. atlantica* growing in sterilized soil, affects negatively growth, collar diameter and dry biomass (aerial and root) of young cypress seedlings.

In addition, the nurse plants play an important role in the recovery of structures and functions of primary ecosystems. Positive interactions between plants are essential to maintain species richness in plant communities, especially in harsh environments (Hacker and Bertness, 1999; Callaway, 2007; Soliveres *et al.*, 2011b). This positive interactions (facilitation) plant-plant coexists with the negative interactions (competition), while their effect on diversity is highly dependent on environmental conditions (Holmgren and Scheffer, 2010). Common effects of biotic and abiotic stresses on plant-plant interactions are rarely discussed (Suzuki *et al.*, 2008). Several studies have reported the critical role of nurse plants in facilitating the establishment and growth of other plants (Pugnaire *et al.*, 2011). Recent studies show that the positive effect of interactions between plant species, allows them to adapt to different stress (Hacker and Gaines, 1997; Soliveres *et al.*, 2012). Although interactions between nurse plant and soil microorganisms have important implications for ecosystem dynamics (Van Der Putten, 2003).

Water stress can be considered as one of the most common abiotic stress (Anjum *et al.*, 2011, Zhang *et al.*, 2011, 2013). The growth and water status of plants are affected by different types of water stress (Zhang *et al.*, 2009, 2013, Gholipoor *et al.*, 2013, Sun *et al.*, 2014). However, RWC is known as a performance measure of potential growth and water status of plants exposed to water stress (Flower and Ludlow, 1986; Harb *et al.*, 2010; Zhang *et al.*, 2011). Zhang *et al.*, (2014) indicated that the decrease in the RWC signify a loss of cells turgor, which results in limited availability of water to the cell expansion process in plants, but the crucial changes of water status lead to growth inhibition and death of plant tissues and organs (Harb *et al.*, 2010; Anjum *et al.*, 2011). Previously it was reported that RWC ratio is higher in the tolerant cultivars compared with to those sensitive to water stress (Gao *et al.*, 1999; Chandrasekar *et al.*, 2000; Zhang *et al.*, 2009; Ashraf, 2010; Zhang *et al.*, 2014, Zarik *el al.*, 2016). In our study, DS induces a decrease in the RWC in different treatment of plants. Under well watered and DS conditions, plants CA+Lav displayed higher RWC ratio than control plants (CA and CAs+Lav).

In addition, research conducted on nurse plants can validate and enrich the theory that interactions between plant species natural succession leads, which also provides a sense of ecological restoration (Duponnois *et al.*, 2011)

Thus the co-culture of young *C. atlantica* plants with lavender on non-sterile soil induced improvements of water parameters of these plants under severe water stress (25% F.C.) compared to control plants (*C. atlantica* in co -culture with lavender on barren soil and *C. atlantica* on non-sterile soil). Indeed these plants by co-cultivation with the lavender become more efficient in the use of water in a soil drying out as compared to other plants.

The positive effect of nurse plant increased with stressful abiotic conditions which was clearly vigorous in higher mountains and on earlier restoration stage of degraded ecosystem (Callaway *et al.*, 2002).

5. Conclusion

To our best knowledge, the results of the current study suggest that the co-culture of *C. atlantica* G. seedling with nurse plants (*L. dentata*) increased plant drought tolerance by means of drought avoidance and drought tolerance mechanisms. It appears the phenomenon of nursing effects can take place mostly on the premature stages of restoration in degraded ecosystem or succession in plant community.

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