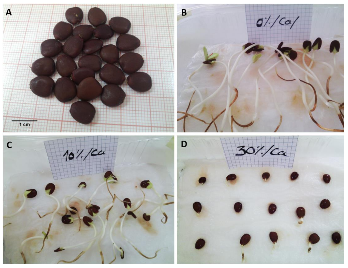

Effect of seawater irrigation on germination and seedling growth of Carob tree (*Ceratonia siliqua* L.) from Gouraya National park (Béjaïa, Algeria)

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Abstract

The carob tree (*Ceratonia siliqua* L.) is an important component of the Mediterranean vegetation and its cultivation is important environmentally and economically. It is also an interesting leguminous species for afforestation-reforestation. In this study, carob seeds collected in a representative area of the Mediterranean basin at the national park of Gouraya (Béjaïa, Algeria), were subjected to germination tests under Mediterranean seawater (SW) irrigation of different concentrations (0, 10, 30, 50 and 100% SW) for 15-day period. Before germination tests, a 20 min pre-treatment with 96% sulphuric acid was necessary to overcome seed coat dormancy which does not permit germination. Results showed that the seeds of *C. siliqua* were able to germinate at different seawater concentration, except for 50% SW and 100% SW which resulted in total inhibition of germination. The maximum number of *C. siliqua* seed germination of 100% FGP (final germination percentage) appeared at 0% SW and 10% SW. Only 35.5% of the seeds have germinated in 30% SW. Ungerminated seeds of *C. siliqua* from different SW treatments showed medium germination recovery (FGP_{Recov}) of 39.9% at 50% SW and low recovery of 18.2% at 100% SW when transferred to distilled water after 15 day-period. Seedlings length and seedling fresh and dry weight were significantly ($P<0.001$) decreased with increasing SW concentrations. Seedling water content remained constant in 10% SW in comparison with the control, while it decreased very slightly in 30% SW. These findings may serve as useful information for *C. siliqua* habitat establishment and afforestation-reforestation programs in coastal sites and for exploiting seawater in the area.

Keywords

Agroforestry; Carob; Fabaceae; Irrigation; Salinity; Seed germination

Contents

1 Introduction

Water is one of the most restricting factors for plant growth in arid and semi-arid areas, and rainfed agriculture has poor and unpredictable yields (Wani et al. 2011). In the Mediterranean region, it is mainly where saline water is used for irrigation, apart from water shortages or high coastal soil salinity, that adverse effects on crops are observed, delaying or preventing germination and seedling establishment (Nicolaou et al. 2020). Seawater can be used for irrigation of plants, particularly in arid and semi-arid areas, which are affected by biosaline agriculture (Ayyam et al. 2019; Khorsandi et al. 2020). Diluted sea water could be used as a source of minerals for the production of a variety of fruit and vegetable crops (Dawood et al. 2014; Mostafa et al. 2015; Amer et al. 2017).

Carob tree (*Ceratonia siliqua* L. of the Fabaceae family) is a long living tree, domesticated and grown since ancient times in most countries of the Mediterranean basin for its large fruits with high sugar content (Sulieman and Mariod 2019). The Carob tree plays an important socio-economic and ecological role in the agrosilvopastoral and animal feed system (Moreno et al. 2014). Fruit pulp and seed gum are widely used in the food industry (El Batal et al. 2013; Karababa and Coşkuner 2013). Some studies have investigated carob pods as a substrate for the synthesis of citric acid (Roukas 1999) and as a readily accessible and inexpensive material for bioethanol production (Makris and Kefalas 2004). It is a drought-resistant fruit species that grows well on poor, calcareous, sandy and calcareous soils (Janick and Paull 2008).

For these characteristics it is particularly suitable to cultivation in arid and semiarid regions of northern Mediterranean basin (Boublenza et al. 2019). Carob tree is recommended for forestation of arid and degraded areas threatened by soil erosion and desertification (Riva et al. 2017) and, due to its ability to preserve and enrich the fertility of the soil. It is particularly useful in the rehabilitation of marginal areas of the Mediterranean basin not adapted to other agricultural uses (Malfa et al. 2010; Correia and Pestana 2018).

The aims of this study are to evaluate and to observe the effect of the diluted natural Mediterranean seawater on germination and seedling growth of *C. siliqua*. The performance of some germination and growth characteristics including germination kinetics, mean germination time, germination recovery, shoot and root length, seedling fresh and dry weight and also seedling water content of the species were investigated.

2 Materials and methods

2.1 Sampling site

Pods of carob tree (*Ceratonia siliqua* L.) were collected on October 2019 from Gouraya National Park (Béjaïa, northeast Algeria) (Latitude 36°45' N; Longitude 5°5' E; 404 m a.s.l.). The mountain of Gouraya is listed as one of the oldest National Parks in Algeria. It covers 2080 hectares and features stunning landscapes and coastal hanging cliffs dipping into the Mediterranean Sea. Gouraya biosphere reserve hosts seven vegetation groups with around 525 plant species. The biosphere reserve occupied by forest, maquis, and scrubland. Carob trees cover the southern slopes of the Biosphere reserve (UNESCO 2019).

2.2 Collection and characteristic of seeds

The mature pods of carob tree were harvested from 20 trees selected randomly and were crushed manually to release the seeds. After harvest, the seeds were mixed to minimize inter-genetic variation. The seed sample intended for our experiment was obtained by mixing the seeds and removing impurities such as vegetable matter (remains of seed coat, stems, and broken cotyledons) (Kheloufi et al. 2019). The seeds of (length: 8.57 ± 1.12 mm, width: 7.23 ± 0.91 mm, thickness: 3.25 ± 0.43 mm, mean ± SD, n = 50) were then stored in a bottle glass at 4 °C, for one month (simulation of the vernalization period). The average 1000-seed weight was 162.3 g. The experiment was conducted at the Laboratory of the Department of Ecology and Environment at the University of Batna 2 (Batna, Algeria).

2.3 Experimental design and treatment

Seawater (SW) was provided from the coastal site of Gouraya (Béjaïa, Algeria) (Latitude 36°46'28.45" N; Longitude 5°5'46.57" E). Different seawater concentrations of 0, 10, 30, 50 and 100% were created using distilled water.

Before germination tests, a 20 minutes pre-treatment with 96% sulphuric acid was necessary to overcome seed coat dormancy which does not permit germination (Cavalo et al., 2016; Kheloufi, pers. observ.). Then, a number of six replicates of 15 seeds each were used for each saline treatment. Germination experiments were carried out in plastic containers (5 cm height, 15 cm length and 8 cm width) with two-layer filter paper (Whatman No. 1) moistened with 25 ml of the appropriate solution of seawater or distilled water for the control (0% seawater).

The germination rate was recorded every day for 15 day-period. Seeds were incubated under continuous dark at 25 ˚C (± 2 ˚C). The papers were changed with the same treatment each two days to prevent salt accumulation (Mansouri and Kheloufi 2017). Seeds were considered as germinated when the radicle had protruded 2 mm through the seed coat (Côme 1970).

The experiment was made as a completely randomized design with 6 replicates of 15 seeds (n=6) for the germination parameters and with 15 replicates (n=15) for the seedling's growth parameters.

2.4 Germination parameters

In order to characterize salinity tolerance, several parameters were calculated:

Germination kinetics: for better apprehending the physiological significance of germination behavior, the number of germinated seeds was counted every day until the 15^{th} day of the experiment.

Final germination percentage (FGP): this parameter constitutes the best identification means of salt concentration which presents the physiological limit of germination. It is expressed at the 15^{th} day of the experiment.

Mean Germination Time (MGT): It represents the meantime, a seed lot requires to initiate and end germination (Orchard 1977).

$$MGT(days)=\frac{\sum(ti.ni)}{\sum ni}$$

Where: *MGT* is the mean germination time, *ti* is the number of days since the start of the test, *ni* is the number of germinated seeds recorded at time *ti*, and *Σni* is the total number of germinated seeds (Orchard 1977).

Germination recovery (FGP_{Recov}): After 15 days, all ungerminated seeds from 50% SW and 100% SW treatments were thoroughly rinsed with distilled water and transferred to distilled water for another 15 days to study recovery of germination from salinity stress. Recovery of germination was calculated as percent of the germinated seeds from salinity treatment germinated after transfer to distilled water.

2.5 Growth characteristics and water content

After 15-day period, seedling shoot and root length were measured and seedling fresh weight (SFW) was determined. For seedling dry weight (SDW) determination, seedlings were dried in an oven at 70 °C for 48 h and weighed. Seedling water content (SWC, $ml.g^{-1}$ DW) was estimated using the following formula as described by Wu et al. (2013):

$$SWC\ (ml/g\ DW) = \frac{SFW - SDW}{SDW}$$

2.6 Statistical analysis

Collected data was statistically performed by Fisher’s Fisher’s analysis of variance (ANOVA) at a significance level of 5% using SAS software version 9.0 (SAS 2002). The treatments were grouped by Duncan’s multiple range tests for the characteristics.

3 Results

3.1 Germination

According to Figure 1 and Table 1, the process of *C. siliqua* seed germination at different seawater concentrations could be divided into three categories: i) seeds germinated immediately, and reached the maximum number of 100% germinated seeds within 5 days and 7 days under 0% SW and 10% SW, respectively; ii) the germination of seeds was delayed, germination occurred after 10 days and reached the maximum of 35.5% in 30% SW; iii) the seeds didn't germinate, such as the seeds at 50% SW and 100% SW did not germinate over a period of 15 days.

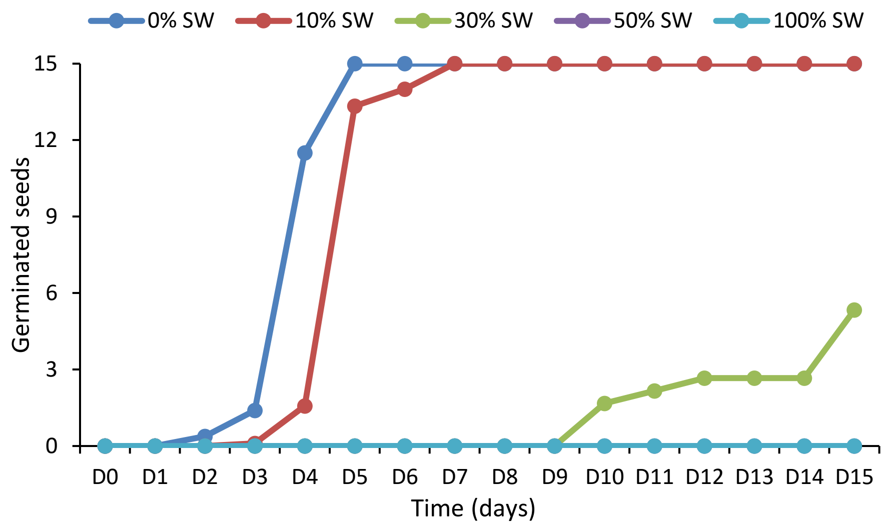

Figure 1. Mean cumulative numbers of germinated *Ceratonia siliqua* seeds as a function of induction salinity by various seawater (SW) concentration.

At the end of germination-experiment (15 days), the data analysis shows that the effect of salinity on the cumulative numbers of seeds germinated were highly significant (Table 1). The maximum number of *C. siliqua* seed germination of 100% FGP appeared at salinity 0% SW and 10% SW, which indicated that these levels of salinity are the suitable germination salinity environment for *C. siliqua* seeds. In the medium salinity environment (30% SW), seed germination was inhibited significantly and only 35.5% of the seeds have germinated (Figure 1, Table 1).

Table 1. Effect of different concentrations of seawater on seed germination and growth characteristics of *Ceratonia siliqua* for 15 day-period.

Seawater Concentration	Seed Germination (n=6)			Seedling Growth (n=15)					
	FGP (%)	MGT (day)	FGP_{Recov} (%)	RL (cm)	SL (cm)	TL (cm)	SFW (g)	SDW (g)	SWC ($ml.g^{-1}$)
0% (Control)	100^a	4.11^c	--	9.87^a	10.40^a	20.27^a	0.983^a	0.263^a	2.73^{ab}
10%	100^a	5.06^b	--	6.66^b	6.94^b	13.61^b	0.840^b	0.217^b	2.87^a
30%	35.5^b	12.81^a	--	1.12^c	1.11^c	2.23^c	0.566^c	0.166^c	2.44^b
50%	0^c	--	39.9^a	0^d	0^d	0^d	0d	0^d	--
100%	0^c	--	18.2^b	0^d	0^d	0^d	0d	0^d	--
F value	756.9	1137.5	32.38	981.5	446.1	1144.9	664.2	974.5	3.17
P (> F)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0329

Means followed by different letters are significantly different at $P \leq 0.05$ as determined by Duncan's multiple range test. FGP-final germination percentage, MGT-mean germination time, FGP_{Recov}-germination recovery, RL-root length, SL-shoot length, TL-seedling total length, SFW-seedling fresh water, SDW-seedling dry weight, SWC-seedling water content.

The data presented on Figure 1 and Table 1 also showed marked differences in the timing of initiation and completion of germination. The MGT increased significantly (P<0.001) by increasing the seawater concentration. Indeed, highest germinations was recorded at (4.11 days) in controls, following by MGT of (5.06 days) and (12.81 days) for 10% SW and 30% SW, respectively (Table 1).

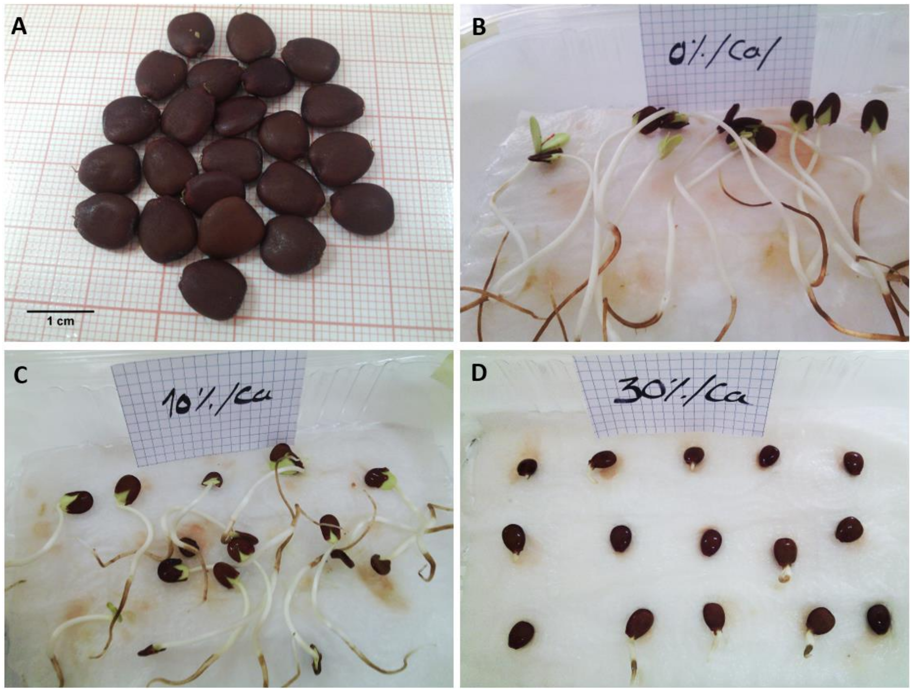

Figure 2. Overview on the emergence of *Ceratonia siliqua* seedlings during a 15-day period in plastic containers under various seawater concentration.

The inhibition of germination could be due either to salt-induced seed mortality, or to unfavourable external osmotic conditions. In order to distinguish between these two factors, the seeds which did not germinate in the presence 50% SW and 100% SW were transferred on distilled water. Ungerminated seeds of *C. siliqua* from different SW treatments showed medium germination recovery (FGP_{Recov}) of 39.9% at 50% SW and low recovery of 18.2% at 100% SW when transferred to distilled water after 15 day-period (Table 1). Germination recovery of *C. siliqua* was significantly (P<0.001) affected by salinity level.

3.2 Seedling growth and water content

Seedlings length was significantly (P<0.001) decreased with increasing SW levels. Stem and root length were strongly affected by all salinity levels. According to Table 1, the results showed that seawater concentrations had a significant effect on seedling growth (P<0.001). Seedling total lengths did not exceed 2.5 cm in 30% SW treatment during the seedling established experiment (Table 1, Figure 2). However, the high values of seedling length were recorded under 0% SW and 10% with 20.27 cm TL and 13.61 cm TL, respectively.

Seedlings fresh and dry weight in seawater concentration of 0% was more than that of other seawater concentrations (P<0.001). However, SFW and SDW were slightly reduced in 10% SW. Indeed, SFW was reduced by 14.5% in 10% SW and by 57.6% in 30% SW, compared to control. Moreover, SDW was reduced by 17.5% in 10% SW and by 36.9% in 30% SW compared to control (Table 1).

The SWC remained constant in the plants treated with 10% SW in comparison with the control, while it decreased very slightly in those treated with 30% SW (Table 1).

4 Discussion

The use of salt-tolerant species as crops would help in salinized zones, where only poor-quality water, unsuitable for most agriculture, is available (Dagar and Minhas 2016; Nachshon 2018). In this context, the exceptional quality of the carob trees could be useful for land reforestation and for improving the low agricultural economy of the Mediterranean semi-arid regions. Our study focused on germination and seedling development, as the establishment of crops depends on successful germination and emergence of seedlings. Our data clearly showed that seed germination and seedling emergence of carob seeds were affected by SW.

Few information is available on the comparative effect of SW and other salts on seed germination of other species of plants. The predominant salt in SW is NaCl (Reig et al. 2014). The greatest negative effects of SW may be due to ion toxicity on germination, as a consequence of a parallel increase in cations and anions (Panuccio et al. 2014). However, the positive effect is its richness of some mineral elements that are important for plant development and growth (Werner et al. 2013; Kheloufi et al. 2016a; Mansouri and Kheloufi 2017).

Salinization of irrigation water and subsequent impacts on agricultural soils are common problems in the Mediterranean region (Paranychianakis and Chartzoulakis 2005; Besser et al. 2017). Under such conditions, carob tree seems to be a salt as well as a drought tolerant species (Correia et al. 2010; Ozturk et al. 2010). In this study, we reported for the first time the effects of SW on *C. siliqua* seed germination and seedling establishment; and our results indicated that SW can significantly influence *C. siliqua* seed germination and seedling emergence.

Germination percentage was found generally higher for *C. siliqua* seeds in the control treatment than for those in the seawater treatment (Table 1). Similar results were obtained in the studies of (El Kahkahi et al. 2015; Cavallaro et al. (2016). They also found that the salt stress declined the germination and also delayed the emergence of seedlings. According to Cavallaro et al. (2016), the effects of NaCl on germination of *C. siliqua* were significantly different in relation to the genotypes.

Our study shows that salinity limits *C. siliqua* growth and seed germination. Seedlings of the control culture had greater shoot and root length and also higher fresh and dry weight than those of the salinity treatments (Table 1). It was detected that the seedling development is reduced when SW concentration increases. As shown on Table 1 and Figure 1, the seeds of *C. siliqua* were too sensitive to salinity level of 50% SW and 100% SW, since no seeds were germinated at this salinity levels. At a water potential of (−1.5 MPa) generated by the NaCl and which is closely equivalent to 100% SW, germination of all the tested genotypes of *C. siliqua* was almost completely inhibited (Cavallaro et al. 2016).

Most seeds germinated at 0% SW (control) and 10% SW because their FGP was 100%. However, the MGT in 10% SW increased by one day compared to control (Table 1). These results are in agreement with the finding of other studies (Kheloufi et al. 2016a; Mansouri and Kheloufi 2017) and reported that the rate and percentage of seed germination were significantly reduced by increasing salinity levels in different leguminous species. Salt induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity (Liang et al. 2018).

Stress can be temporary under natural field conditions and the ability of the plant to complete its life cycle is closely attributed to its ability to recover after exposure to stressful periods (Chinnusamy et al. 2005). Seed germination recovery is an adaptive response to water availability when the soil reaches reduced levels of NaCl content for short periods during the rainy season (Gul et al. 2013). Recovery of germination responses was dependent on SW concentration, ranging from 18.2% to 39.9% after 15-day of exposure in 100% SW and 50% SW, respectively (Table 1). Seed survival rather than germination may be an important criterion for success under highest saline conditions (Zhang et al. 2015). The germination recovery occurs other leguminous species when hypersaline conditions are alleviated: *Caesalpinia crista* (Patel et al. 2011), *Melilotus officinalis* (Vu et al. 2015), *Acacia saligna* (Kheloufi et al. 2016b), *Retama raetam* (Mechergui et al. 2017), *Phaseolus vulgaris* (Mansouri and Kheloufi 2017).

In addition, with increasing salinity, the length of seedlings has been significantly reduced, especially in 30% SW. This may suggest that the seeds of *C. silqua* were more salt tolerant in seawater concentrations of 10% SW. Shoot and root length was also strongly affected by all SW concentrations.

The good hydric state indicator is the water content in seedlings, which decreases slightly in plants under salinity stress (Parida and Das 2005). When the plant is exposed to 10% SW and 30% SW, it is especially noticeable what appears to be a tolerance behavior in salt stress. Indeed, the SWC (seedling water content) remained constant in the plants treated with 10% SW in comparison with the control, while it decreased very slightly in those treated with 30% SW (Table 1). Water absorption is maintained at a degree that is adequate to prevent tissue dehydration and to dilute the salts incorporated into cells (Flowers et al. 2015). The analysis of the relative content of water allows the hydric status of the plant to be defined globally and the ability to achieve the best osmoregulation and maintain cell turgescence (Negrão et al. 2017). Kheloufi (2019) conclude that the salt stress is the result of a hydric deficit in the plant in the form of physiological drought and this osmotic stress is essentially translated by the toxic accumulation of the ions in cells.

5 Conclusion

The use of the seawater in the agroforestry offers an alternative of irrigation in zones suffering from a deficiency of pluviometry or from the shortage of the water of irrigation especially if *C. siliqua* is projected for the revegetation of the coast, a zone very close to the vast source of the seawater. In this context, we showed that irrigation with seawater did not affect germination and also the seedling dry weight and water content under 10% SW. It is further recommended that field trials be conducted for evaluating the performance of this species *in situ*. Effect of seawater on texture and structure of soil should be investigated in future studies.

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