Effects of pre-sowing treatments and abiotic stress on the germination of *Ceratonia siliqua* seeds of four biomes Moroccan

Abstract

In order to improve the germination rate of carob seeds (*Ceratonia siliqua* L.), we studied the morphological characteristics of the fruits (pod and seeds, integumentary hardness and tolerance to abiotic stress of the seeds of seven ecotypes of carob trees from from four regions of Morocco. The fruits of its seven populations were studied according to seven discriminative characteristics relating to the pods (length, width, thickness, the total number of seeds, the total weight of the pulp, the yield of the seeds in the pod and the yield of pods per tree) and four discriminative characteristics relating to the seeds, namely the length, the width, the thickness and the total fresh weight of the seeds. Integumentary hardness was evaluated by pretreating the seeds with boiling water and 95% sulfuric acid. Similarly, we also followed the evolution of water absorption by the seeds during 4 days and we evaluated on these gra pre-treated ines, the effects of different incubation temperatures (10 °C, 25 °C and 40 °C), their tolerance to different concentrations of NaCl, PEG6000 (0MPa, -0.5MPa, -1MPa and -1.5MPa) and their reversibility. The morphological characterization of the fruits allowed us to group the populations studied into three groups. Soaking the carob seeds in sulfuric acid for 20 minutes improved the germination rate and speed. The evolution of water absorption makes it possible to distinguish two phases. The first phase is obtained during the first 24 hours and is characterized by a rapid penetration of water, and the second phase which lasts over the last 72 hours and which is characterized by a slow entry of water. The optimum temperature for germination of seeds from all provenances is 25 °C. The germination behavior of carob under conditions of osmotic stress demonstrated a highly significant treatment effect (concentration of PEG6000) on the rate and mean time of germination and revealed that this species is very resistant to drought. This study also showed that salt has a depressive effect on the average germination rate and time and the length of radicles. The results of the reversibility test obtained show that the germination of seeds transferred from osmotic stress and salt stress (-0.5, -1 and -1.5 MPa) and from the temperature of 40 °C. is totally inhibited. On the other hand, seeds transferred from a temperature of 15 °C resume germination under optimal conditions. This study allowed us to select a variety with high yield and tolerant to various biotic constraints.

Keywords: morphological characterization, Ceratonia siliqua, seed germination, scarification, reversibility, water stress, salt stress, temperature.

Introduction

Carob tree (*Ceratonia siliqua* L.) is an endemic, aromatic and medicinal plant (Sbay, 2008) which is currently among the most efficient forest, fruit and fodder trees in Morocco. Thanks to its biological and ecological peculiarity, it has been included in the national priority list as a forest resource for conservation. Indeed, it is an agro-sylvo-pastoral species that can be used for the rehabilitation of degraded soils, thanks to its ability to adapt to different edapho-climatic conditions (Ait Chitt et *al.,* 2007; Barwick, 2004) . This evergreen tree has a very high economic profitability compared to other fruit species. The carob tree is a tree-like Caesalpiniaceae, spontaneous or cultivated, and of great economic importance, it tolerates drought well, explaining its wide distribution in arid and semi-arid regions of the Mediterranean climate (Correia et *al.,* 1992; lo Gullo et salleo, 1988; Gharnit et *al.,* 2001; Gharnit et *al.,* 2005). Abiotic stresses cause significant losses in agricultural production across the world (Jakab et *al.,* 2005; Hanumantharao et al., 2016). Salinity and drought in particular significantly affect crop yields in arid and semi-arid regions (Patanè et al., 2013; Gamalero et *al.,* 2020). In Morocco, soil salinization is taking on alarming dimensions by reducing arable land and threatening the food balance of these regions (Kinet et *al.,* 1998). The introduction of indigenous species resistant to aridity, has been adopted since 1920, as one of the means used to recover degraded soils in West Asia and North Africa (Martins-Loução et *al.* , 1996). Seed germination is usually the most critical stage for the establishment of seedlings in domestication andor planting (Almansouri et *al.,* 2011; Hamrouni et *al.,* 2012; Walbott et *al.,* 2018; Bhatt et *al.,* 2019). The study of the germination requirements of the species in combination with the physical parameters of its environment makes it possible to reason the early choice of the plant material best suited to the dry environment (Bell et *al.,* 1992; Tetsuto et *al.,* 2011; Cavallaro et *al.,* 2016; Hadi et *al.,* 2018). Likewise, water stress is also considered among the unfavorable factors that can affect seed germination and therefore seedling quality and yield (Larbi et *al.,* 2000; Hamrouni et *al.,* 2012). Water stress affects several plant functions, such as stomatal conductance (Penuelas et *al.,* 1992; Yagoubi., 1993), photosynthesis (Moran et *al.,* 1994; Yuan et *al.,* 2014) and leaf area (Penuelas et *al.,* 1992; Haffani et *al.,* 2017) A decrease in the water content of the plant immediately results in a reduction in growth in size even before photosynthesis is affected (Turner 1997). According to Amigues et *al* (2006), the consequences of a drought depend on its onset period compared to the crop stage and on its duration of action. With this in mind, and in order to study the germination requirements of the seeds of the carob tree and their tolerance to abiotic constraints, we have studied the scarifying power of sulfuric acid and boiling water in combination with the main environmental constraints affecting the seed germination. In this context, we focused on the study of germination under conditions of salt, water and heat stress.

Materials and Methods

Sample collection

Seven populations of Carob tree (Ceratonia siliqua L) from four regions of Morocco were studied (Table 1). The pods were harvested in August 2017 in four localities: Meknes, Fez, Marrakech and Khémisset. Samples were collected in sterile Stomacher bags and transferred for testing to the Laboratory.

Table 1 : Geographical and climatic data of the *Ceratonia siliqua* L. seed collection stations

Group	Sex	Origin	Latitude N	Longitude W	Altitude (m)	Geographic region
P1	Femelle	Meknes	33° 53′ 42"	5° 33′ 17″	560	Tray saïs
P2	Femelle	Fez	34° 03′ 00″	4° 58′ 59″	579	Tray saïs
P3	Femelle	Marrakech	31° 37′ 48″	8° 00′ 00″	450	High Atlas
P4	Femelle	Marrakech	31° 37′ 48″	8° 00′ 00″	450	High Atlas
P5	Femelle	Marrakech	31° 37′ 48″	8° 00′ 00″	450	High Atlas
P6	Femelle	Khemisset	33° 49′ 0″	6° 4′ 0″	409	Central Board
P7	Femelle	Fez	34° 03′ 00″	4° 58′ 59″	579	Tray saïs

Morphological characterization of carob fruits

The morphological characters of the fruits (pod and seeds) were determined randomly on fifteen pods per carob tree population (*Ceratonia siliqua L.*). Six characteristics were measured at the pod level, namely length, width, thickness, number of seeds, fresh pod weight (g) and yield (weight of seeds/weight of pod *100). Four discriminative characteristics relating to seeds were determined: length, width, thickness and total weight of seeds /pods (g).

Effect of pretreatment on germination

The seeds of the carob tree have a thick, hard seed coat. In order to determine their optimal germination conditions, we investigated the variability in mantle hardness that might exist between seeds from different regions. Two pretreatments were carried out in comparison with the control, the first consists in soaking the seeds in boiling water at 100°C and for 20 min and the second process was carried out by immersing the seeds in concentrated sulfuric acid (H_2SO_4 at 95%) for 20min. Prior to pretreatment, the seeds were washed with 10% bleach for 2 min and then rinsed three times with sterile distilled water. After overnight soaking at 4°C, the seeds were germinated in the dark in plastic petri dishes containing the sterile filter paper soaked in sterile water. To study the influence of the thermal factor (Ben Dkhil and Denden, 2014), three repetitions per treatment for a period of ten days and at a rate of 20 seeds per box were carried out at three different temperatures (10°C, 25°C, and 40°C).

Effect of salinity on germination

The germination tests under salt constraints were established using the optimum germination conditions determined from the previous pretreatments. The seeds were first immersed in sulfuric acid for 20min, then washed in distilled water for 15min. Seed germination was carried out in the dark and at the optimum germination temperature (25°C) in Petri dishes watered daily with distilled water containing different concentrations of NaCl (0MPa, -0.5MPa, -1MPa, and -1.5MPa) (Cavallaro V., et *al.,* 2016). The counting of germinated seeds was carried out daily, for a duration of the test of 15 days.

Effect of osmotic stress on germination

The germination tests were carried out in the dark and at the optimum germination temperature (25°C) under different levels of water potentials using PEG (molar mass = 6000), while maintaining a stable and uniform water potential during the entire experimental period (Michel and Kaufmann, 1973). The values of the water potential tested are 0MPa, -0.5MPa, -1MPa and -1.5MPa (Cavallaro et *al.,* 2016). The duration of the test was fixed at the period of germination which was spread over 12 days and the counting of sprouted seeds was done daily.

Three variables were used to assess the germination of the seven groups of carob:

-The final germination rate (GF) which is expressed by the ratio of the number of germinated seeds to the total number of seeds,

- The mean time to germination (TMG): TMG = ΣDn / Σn, where D the days from the start of the germination test, and n is the number of newly germinated seeds on day D (Gunes et *al.,* 2013b)

- Radicle length: Radicle length measurements were also performed for each treatment during the germination test (Cavallaro et *al.,* 2016).

Germination reversibility test

This test looks at seeds germinated under different abiotic stresses (osmotic, saline and thermal) and which have not germinated. This involves transferring them again for germination under optimal conditions (sterile distilled water and T = 25°C. (Ben Dkhil and Denden, 2014).

Water retention capacity of the seeds

For each treatment, the fresh weight of seeds germinated at 24, 48, 72, 96 h of imbibition was measured. The seeds were placed in petri dishes as described for the germination experiments, removed every 24 hours after the start of imbibition and weighed (Cavallaro V., et *al.,* 2016).

The water retention capacity is calculated as follows: Water retention capacity = (P2-P1) / P1 * 100

StatisticalAnalysis

All data were analyzed by ANOVA using SPSS Statistics 26 software. Means ± standard error were calculated from the morphological parameters studied. The difference in means was compared by Tukey's test at the threshold $p \leq 0.05$ and the measurements were carried out in fifteen copies (n = 15). Hierarchical classification was performed using SPSS 26.0 software according to the group distance aggregation method, which is based on the proximity distance matrix. The resulting dendrogram was normalized against Pearson's correlation. However, to evaluate the effects of water stress, salinity, temperature and their interactions on different parameters of germination and on seedling growth. The difference in means was verified by Dunnett's test at the threshold $p \leq 0.05$. Each test is carried out in three replicates of 30 seeds per group. The presented graphs and tables were produced by Graphpadprism 6 software. The different letters on the tables and the graphs indicate a different meaning at $p \leq 0.05$.

Results

Morphological characterization of the seven groups of carob trees

The morphological characterization of the fruits of seven populations of carob tree was studied. The results obtained are shown in Table 2. The characteristics related to the size of the pods vary from one group to another. The values for length, width, thickness, number of seeds per pod and fresh pod weight (g) varied from 9.947 ± 1.047 cm (P3) to 21.400 ± 1.160 cm (P5), from 1.600 ± respectively 0.130cm (P5) to 1.713 ± 0.119 cm (P1), from 0.373 ± 0.046 (P2) to 0.653 ± 0.062 cm (P5), from 7.510 ± 0.892 (P4) to 17.133 ± 1.187 (P7) and 3.765 ± 0.456g (P2) at 14.650 ± 1.137g. From the above, it emerges that there is a significant difference between the means of length, number of seeds per pod and fresh pod weight ($p \leq 0.05$). In contrast, there were no significant differences in width, thickness. Regarding the seeds, the length of the seed varies between 0.606 ± 0.034cm (P4) and 0.807 ± 0.046 cm (P7), the width varies between 0.391 ± 0.017 cm (P4) and 0.633 ± 0.049 cm (P7), the thickness seed varies between 0.287 ± 0.035 cm (P2) and 2.311 ± 0.244 cm (P5), the total weight of seed per pod varies between 0.787 ± 0.046g (P5) to 3.079 ± 0.280g (P7) and seed yield is between 21.381 ± 1.947% (P4) and 32.595 ± 2.348% (P7). There is a significant difference between the means of the thickness of the seed, the total weight of seed per pod and the seed yield ($p \leq 0.05$). On the other hand, the length of the seed, the width are not significantly different.

The hierarchical analysis based on the results of the study of morphological characters allows us to identify in three groups of populations (Figure 1). The first group includes populations P1, P2, and P3 from Meknes, Fès and Marrakech. They are characterized by their small and thick pods and medium yield. The second group consists of P4 and P5, these populations of Marrakech origin are related. They are characterized by long and heavy pods, with a number of seeds and a reduced yield. The third group consists of P6 (Khémisset) and P7 (Fès) which is characterized by a yield and a fairly large number of seeds with an average size of pods.

Table 2 : Morphological characterization of pods and seeds of seven groups of *Ceratonia siliqua* L

	Pods						**Seeds**			
Group	**Length (cm)**	**Width (cm)**	**Thickness (cm)**	**Fresh pod weight (g)**	**Number of seeds/ pod**	**Yield (%)**	**Total weight of seeds (g)**	**Length (cm)**	**Width (cm)**	**Thickness (cm)**
P1	10,300±0,720[a]	1,713±0,119[a]	0,533±0,062[a]	6,226±0,679[a]	9,867±1,167[a]	29,125±2,849[a]	1,819±0,259[a]	0,800±0,065[a]	0,607±0,026[a]	0,399±0,004[a]
P2	10,900±0,996[a]	1,420±0,041[a]	0,373±0,046[a]	3,765±0,456[c]	11,200±1,042[b]	28,651±2,342[a]	1,108±0,201[a]	0,753±0,052[a]	0,573±0,046[a]	0,287±0,035[a]
P3	9,947±1,047[a]	1,507±0,153[a]	0,513±0,064[a]	6,349±0,946[a]	10,533±1,187[a]	25,647±1,346[b]	1,639±0,210[a]	0,793±0,046[a]	0,613±0,035[a]	0,393±0,026[a]
P4	14,240±1,218[b]	1,712±0,083[a]	0,565±0,061[a]	11,550±1,46[d]	7,510±0,892[c]	21,381±1,947[c]	0,803±0,029[a]	0,606±0,034[a]	0,391±0,017[a]	1,603±0,216[b]
P5	21,400±1,160[c]	1,600±0,130[a]	0,653±0,062[a]	14,650±1,137[b]	8,912±0,786[a]	25,967±2,060[b]	0,787±0,046[a]	0,676±0,045[a]	0,413±0,042[a]	2,311±0,244[c]
P6	13,080±1,022[d]	1,553±0,074[a]	0,520±0,056[a]	7,243±0,570[c]	13,867±1,685[e]	26,498±1,870[b]	1,919±0,188[c]	0,787±0,035[a]	0,560±0,063[a]	0,393±0,059[a]
P7	15,507±1,522[e]	1,653±0,099[a]	0,547±0,052[a]	9,573±1,287[e]	17,133±1,187[d]	32,595±2,348[d]	3,079±0,280[b]	0,807±0,046[a]	0,633±0,049[a]	0,400±0,000[a]

Data represent Mean ± SE of replicates (n = 15). Values in the same columns with different letters are significantly different by Tukey's multiple comparison test at $p \leq 0.05$

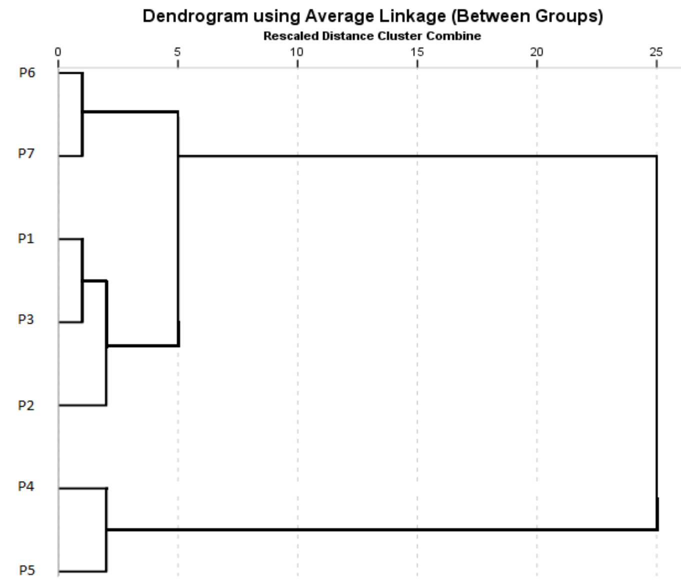

Figure 1. Hierarchical classification according to the morphological characters of the seven groups of carob tree (*Ceratonia siliqua* L.).

Effect of scarification on germination.

The variations in germination rates according to the different scarification pretreatments over 10 days (Figure 2), shows that scarification with sulfuric acid allows for a faster start of germination. Indeed, higher germination rates of 96.67% were obtained with scarification with sulfuric acid compared with the other pretreatments tested namely scarification with boiling water (47.78%) and with sterile distilled water (35.55%). These results are confirmed by analysis of variance ($p < 0.05$) which revealed a very highly significant pretreatment effect on germination rates.

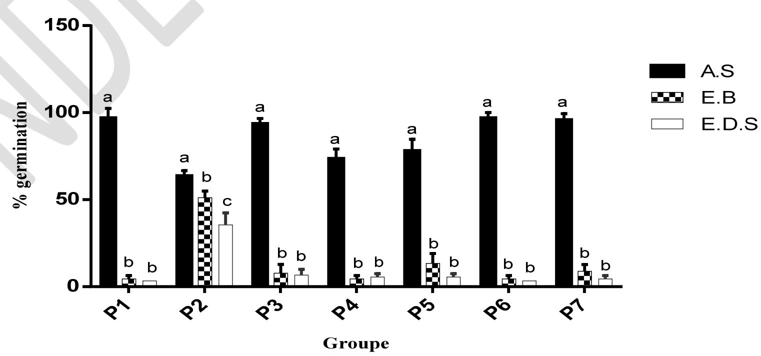

Figure 2 : Germination rate of carob seeds from four regions of Morocco under the effect of different pretreatments. EDS: sterile distilled water (Control), EB: boiling water, AS: Sulfuric acid. Different letters indicate different meaning.

Effect of water, salt and heat stress on the water retention capacity of seeds

A separate statistical analysis was carried out for each population according to the abiotic constraints studied, namely temperature, water stress and salt stress (figures 3, 4 and 5). The moisture content of the seeds, measured every 24 hours, was significantly affected by temperature (figure 3), water potential (figure 4) and salt stress (figure 5). Regardless of the population, there are increasing reductions in water absorption over time at high and low temperatures, at low concentrations of PEG6000, and NaCl. The evolution of water absorption makes it possible to distinguish two phases. The first phase is obtained during the first 24 hours and is characterized by a rapid penetration of water, and the second phase which lasts over the last 72 hours and which is characterized by a slow entry of water. Our results also show on the one hand that the water retention capacity of seeds soaked in PEG6000 is greater than those recorded in seeds soaked with NaCl and on the other hand a high absorption in seeds soaked at 15°C by compared to those soaked at 40° C.

Effect of temperature on germination.

The comparison of the average germination rates and times of seeds from different regions of Morocco as a function of temperature, shows very high seed germination rates and speeds at 25°C. The germination power in all the populations studied varies between 93.33% to 100% for the temperature of 25°C and 3.33% to 17.78% for the temperature of 10°C, while the seeds germinated at 40°C undergo inhibition thermal (figure 6a). The best average germination time of carob seeds under different temperatures is 3.16 days at 25°C (Figure 6b). Thus, following these results obtained by these various pretreatments, the salt and water stress tests are carried out at a germination temperature of 25°C and with a preliminary pretreatment with sulfuric acid.

The variation in the length of the radicles of carob seeds under the effect of the different temperatures tested (figure 6c), also shows that the temperature 25°C has a remarkable effect on the length of the radicle of the seeds of the carob tree coming from different regions. of Morocco compared to other temperatures 10°C and 40°C.

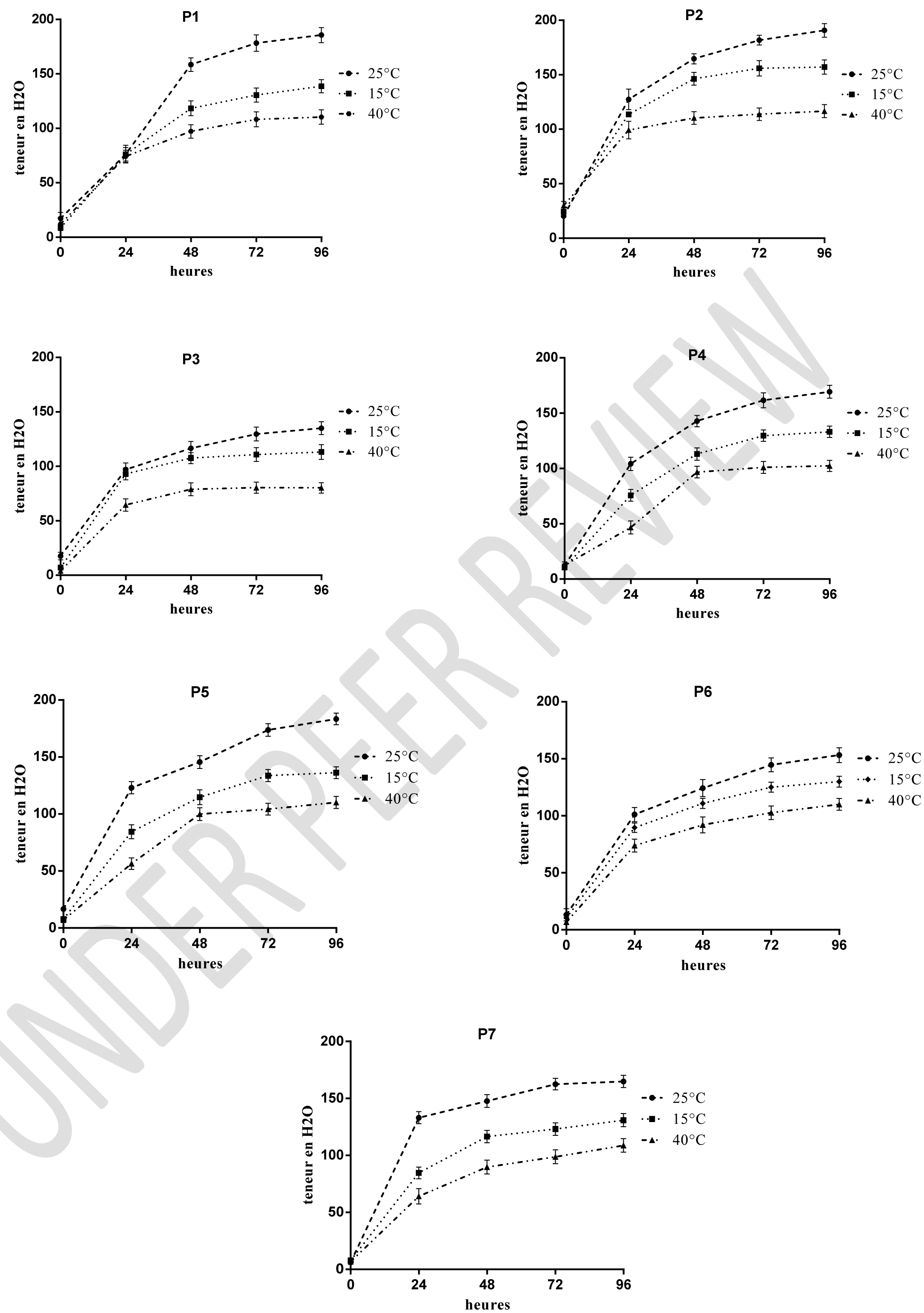

Figure 3 : Determination of the moisture content of carob seeds germinated at 10, 25 and 40 ° C ($p \leq 0.05$)

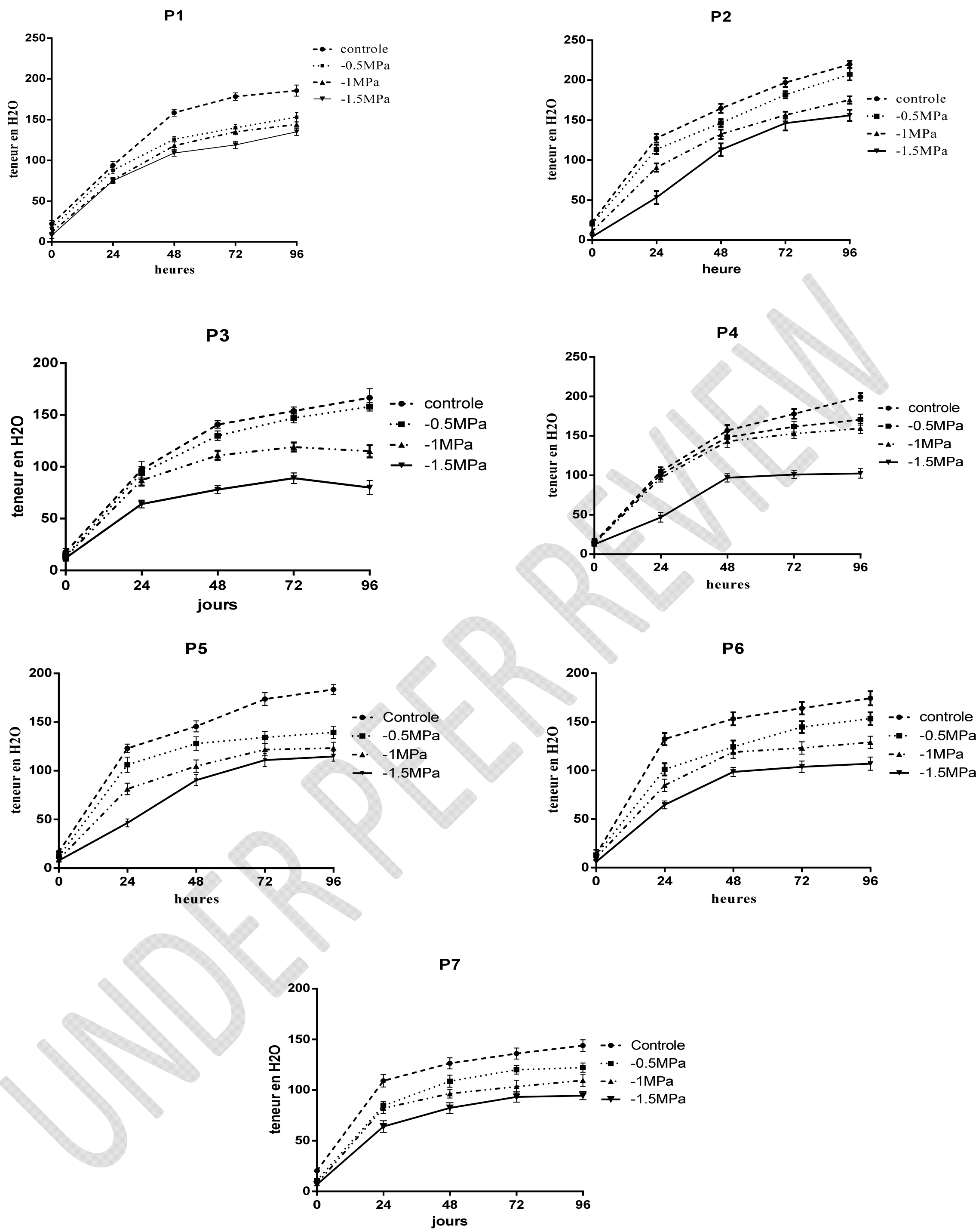

Figure 4 : Determination of the water retention capacity of carob seeds germinated at 0, -0.5, -1 and -1.5 MPa under water stress ($p \leq 0.05$).

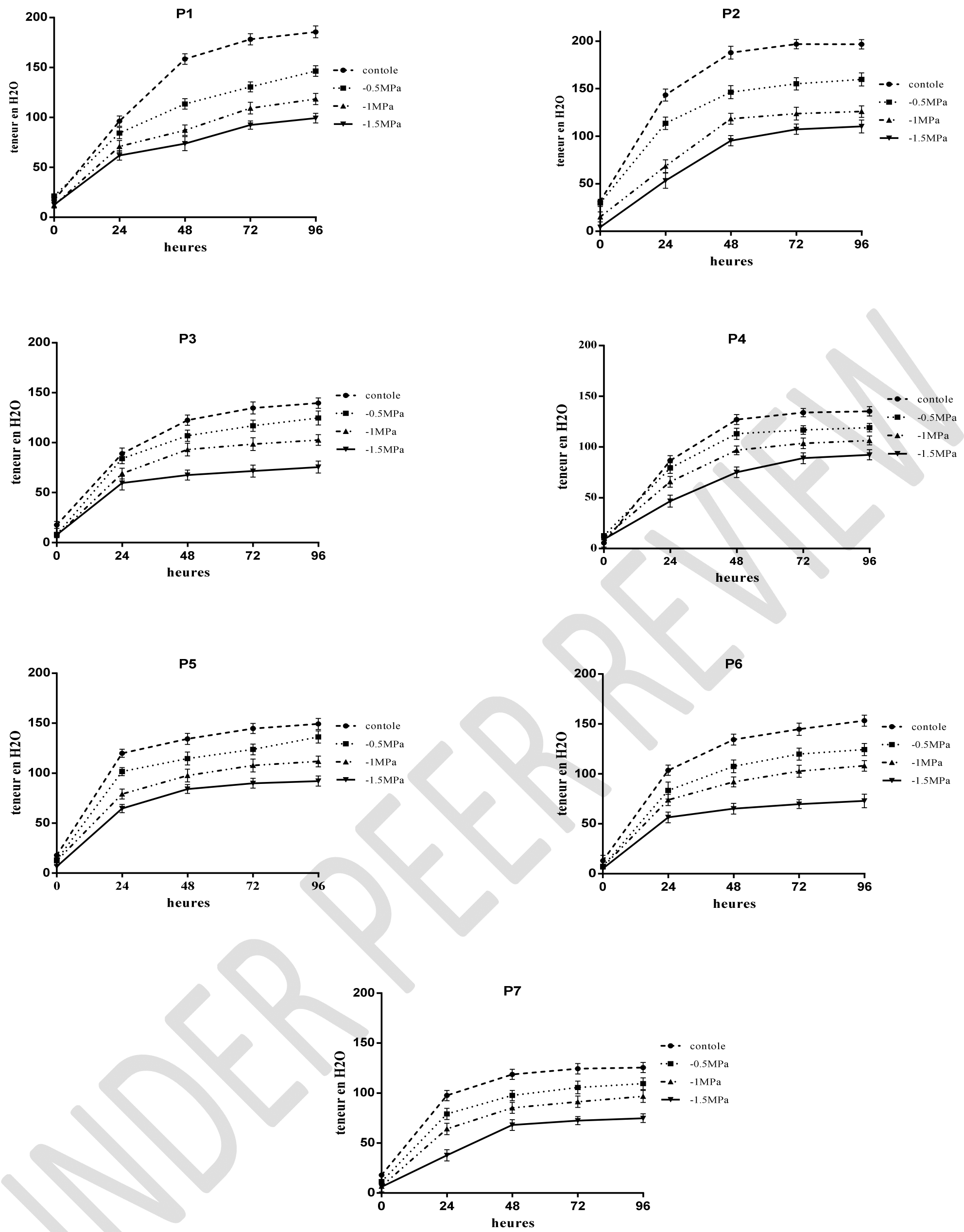

Figure 5 : Determination of the water retention capacity of carob seeds germinated under salt stress at 0, -0.5, -1 and-1.5MPa ($p \leq 0.05$).

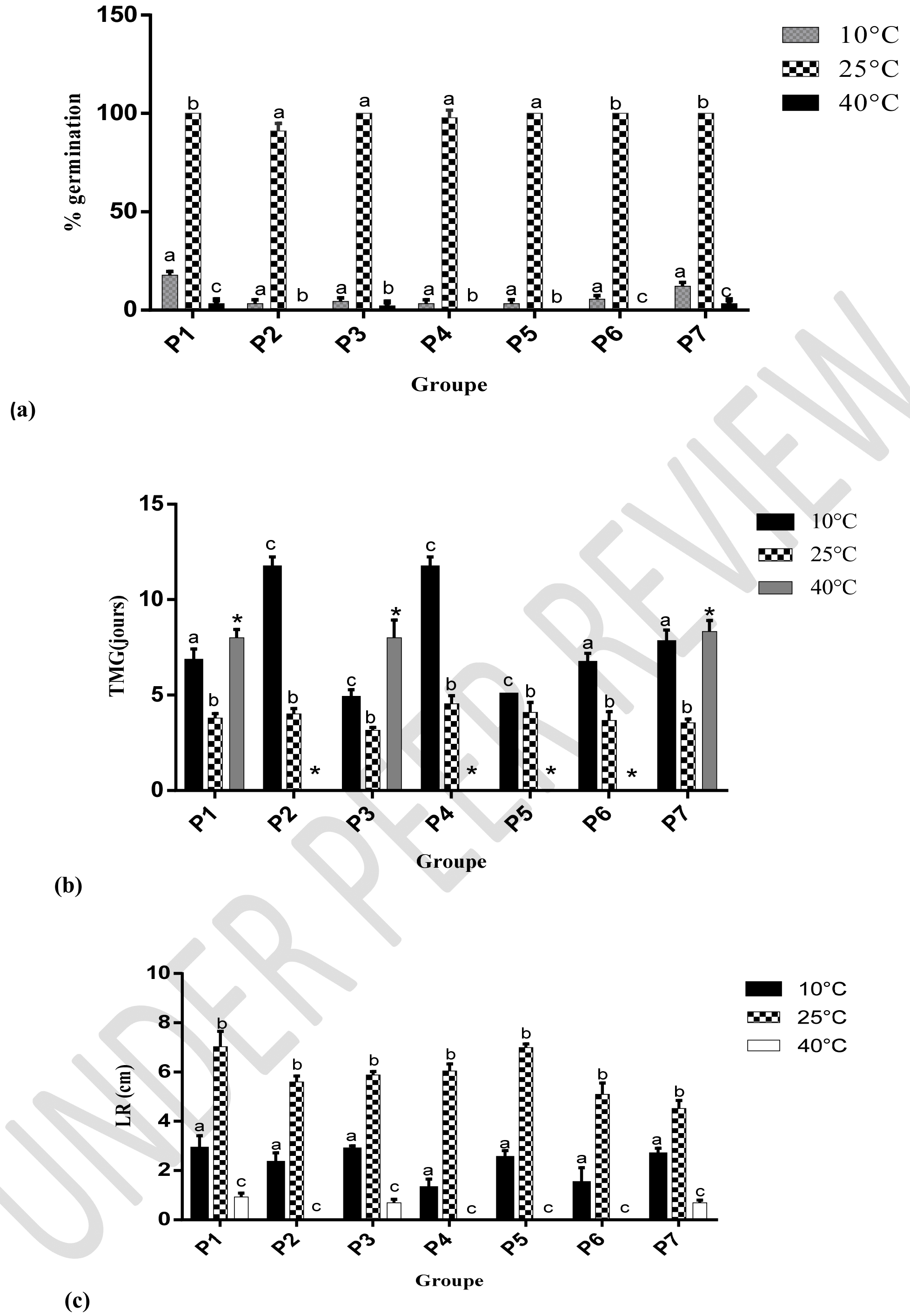

Figure 6: (a) Germination rate, **(b)** Germination time), **(c)** Root length of carob seeds under the effect of heat stress. Different letters indicate different meanings (p≤0.05; Dunnet's multiple comparisons test). (* TMG is not possible to calculate, there is no data for some row-column combinations).

Effect of salinity on germination

The evolution of the rate and average time of germination of the seeds of the carob tree according to the increasing concentrations of NaCl (figure 7a), shows that the increase in salt stress compared to the control, leads to a reduction in the germination rates for all the seeds. carob tree from different regions of Morocco. The germination of Ceratonia siliqua seeds is affected by NaCl only from the -1MPa treatment (0% germination rate). The mean germination time also lengthens depending on the intensity of the salt stress (Figure 7b). It is only significantly affected by NaCl from -1MPa. Overall, there is a significant reduction in the length of the radicles in all the seeds from different regions of Morocco compared to the control (Figure 7c). The -1MPa concentration inhibits total seed growth.

Effect of water stress on germination

The germination of the seeds of the seven ecotypes used in this study according to the water stress, shows that the highest germination rate is recorded in the control seeds (100%) (Figure 8a). Overall, for the different ecotypes, and at With the exception of populations P2 and P7, we have observed that in general the seed germination rate is only slightly affected by the different concentrations of PEG6000. The P2 population shows a germination rate of less than 25% for the -1.5MPa treatment and the P7 population shows successively germination rates of 15% and 8% for the water potential levels of -1MPa and -1.5MPa (Figure 8a). For the mean germination time (Figure 6b), our data show a variation with water potential (Figure 8b). It is about 3.8 days for the seven populations subjected to a 0MPa water potential, but much longer under a water potential of -1MPa and -1.5MPa (6.1days). Comparison of the average germination rates and times with Dunnett's test shows that the application of the water potential of -1.5MPa significantly reduces the germination capacity, while the average germination time remains at 6.1 days. The variation in the length of the radicles of carob seeds under the effect of different water potentials also shows a progressive reduction in the length of the radicles in all carob seeds from different regions of Morocco (Figure 8c).

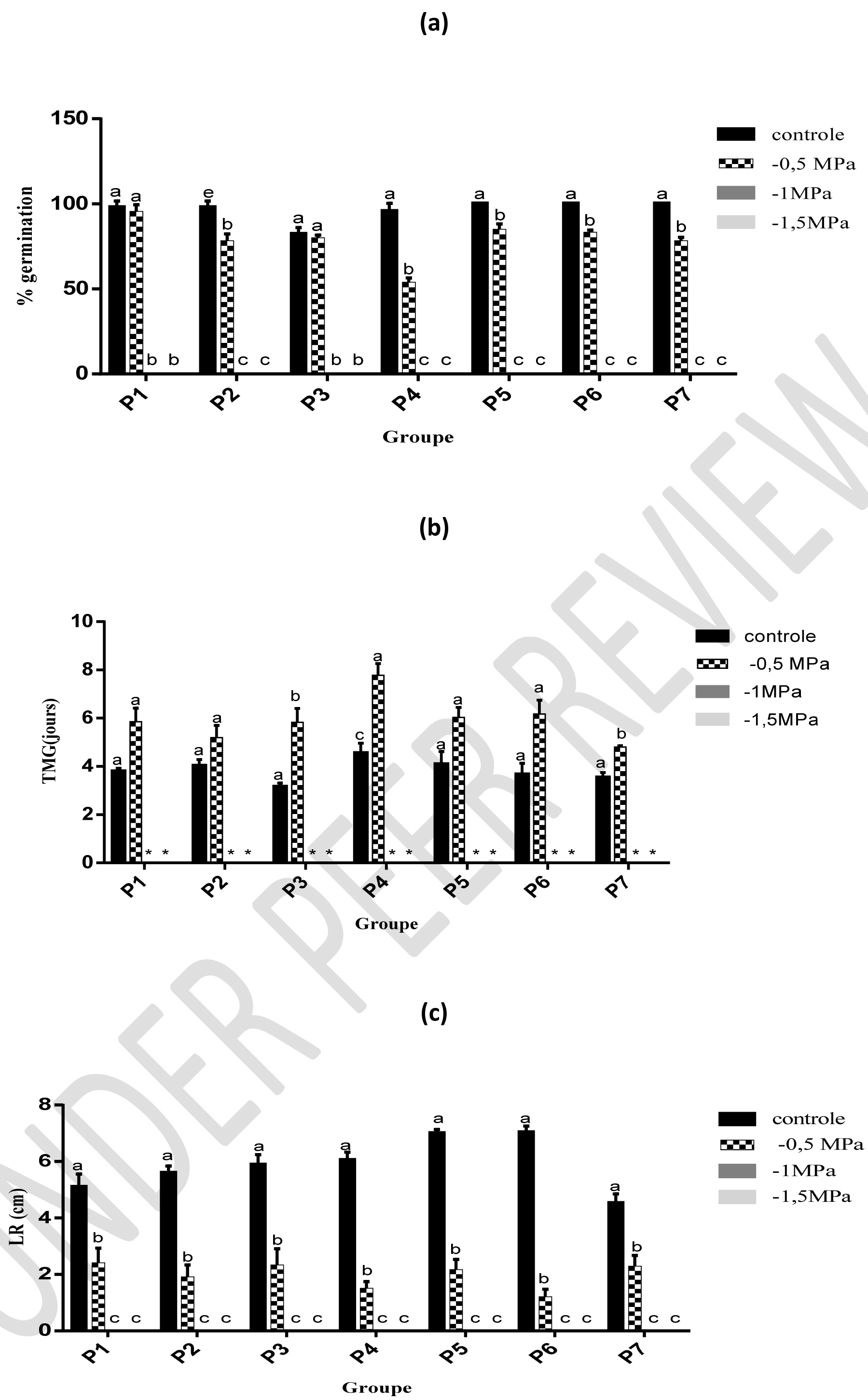

Figure 7: (a) Germination rate, **(b)** Average germination time and **(c)** Root length of carob seeds from different regions of Morocco under the effect of different concentrations of NaCl. Different letters indicate different meaning (p≤0.05; Dunnett's test).

Effect of water stress on germination

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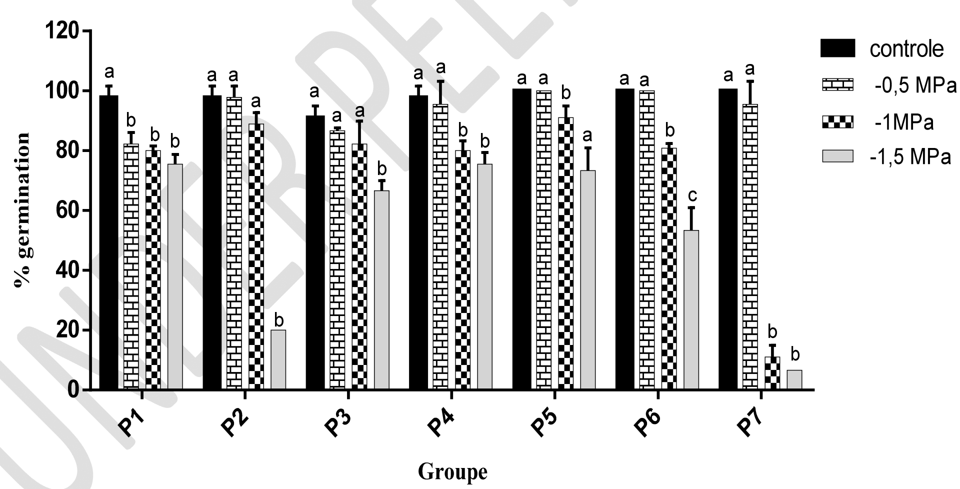
(a)



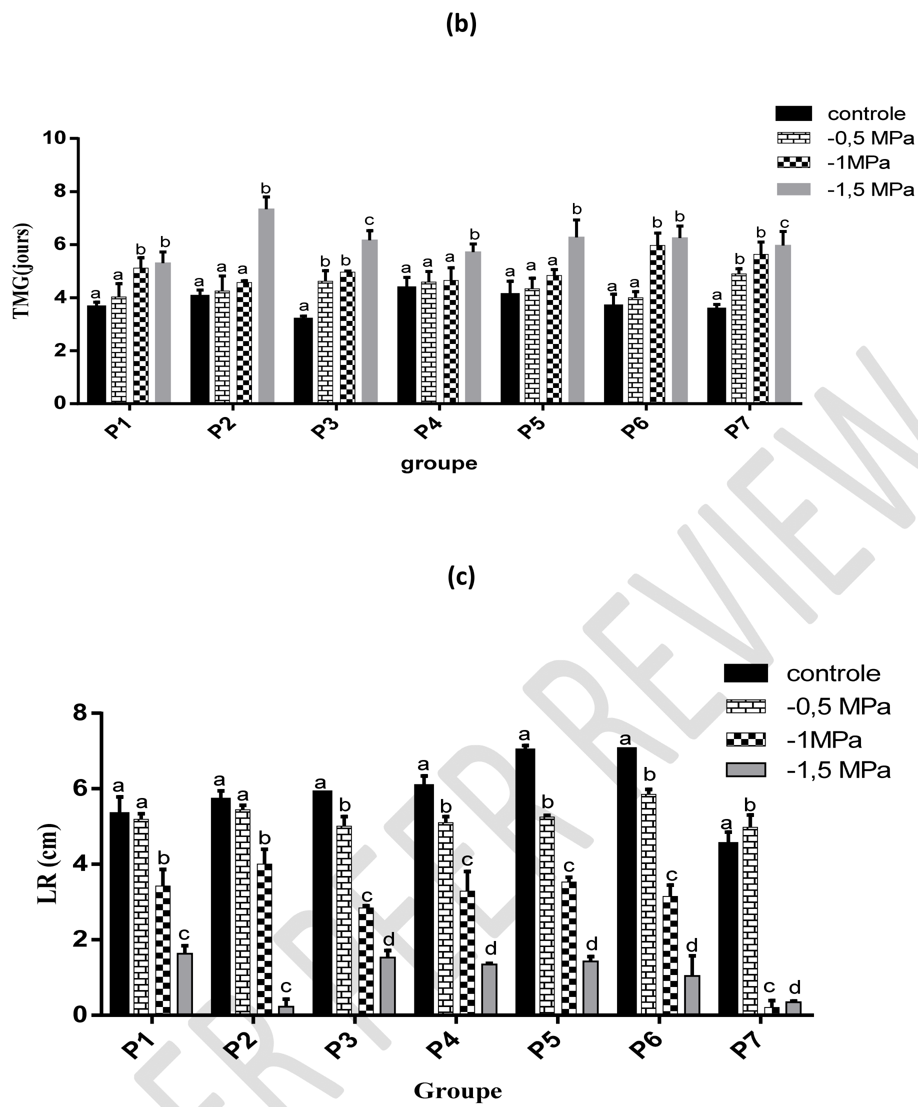


Figure 8: (a) Germination rate, **(b)** Average germination times, **(c)** Root length of carob seeds from different regions of Morocco under the effect of different concentrations of PEG 6000. Different letters indicate a different meanings (p≤0.05; Dunnett's test).

Germination reversibility test.

The germination reversibility test has been studied to determine the effect of abiotic stress on seeds. The results obtained show that the germination of seeds transferred from osmotic stress and salt stress (-0.5, -1 and -1.5MPa) and from the temperature 40°C is totally inhibited. On the other hand, seeds transferred from a temperature of 15 ° C resume germination under optimal conditions (sterile distilled water, T = 25°C) for all of the seven populations studied. The germination power for all the populations studied shows a germination rate which varies between 16.3 and 34.44% and an average germination time which lasts 8.19 days) (Table 3). Likewise, the length of the radicles also shows a decrease in all the seeds of the carob tree coming from different Moroccan regions

Table 3: Effect of reversibility test of carob seed germination on rate, mean germination time and radicle length (p ≤0.05)

Group	Germination rate	Average germination time	Root length
P1	34,444±1,849[a]	6,987±1,124[ns]	1,600±0,20 [ns]
P2	16,300±0,935[b]	6,200±0,739 [ns]	0,467±0,106[ns]
P3	28,889±1,925[c]	5,190±0,656[ns]	1,067±0,153[ns]
P4	17,778±1,925[b]	6,467±1,194[ns]	0,933±0,152[ns]
P5	28,889±1,925[c]	8,093±0,584[ns]	1,467±0,152[ns]
P6	24,444±1,925[d]	8,190±0,873[ns]	0,967±0,115[ns]
P7	31,111±1,925[c]	6,974±0,739[ns]	1,133±0,098[ns]

Discussion

Morphological characterization

In order to determine the variability of the carob tree, the study of morphological characters is commonly used. ((El Kahkahi et *al.,* 2014 ; Elfazazi et *al.,* 2017)). The results obtained in our study are compared with those of some other countries. For 24 accessions of Syrian carob tree, they found great diversity according to the morphological criteria studied. The length, width, pod thickness, number of seeds and pod weight are respectively between 12.83 and 22.32cm, 1.98 and 3.12cm, 0.58 and 1, 26cm, 8.26 and 16.53 and between 8.99 and 16.11g (Mahfoud et al., 2018). The study carried out by Boublenza et al., In 2019 showed significant variability between the 10 varieties of Algerian carob, length, width, pod thickness, number of seeds and pod weight are respectively between 13.13 and 18.75cm, 1.81 and 3.10cm, 0.61 and 0.80cm and between 6.79 and 13.23 and between 7.04 and 30.57g. In Morocco, the studies completed on the agro-morphological characterization of the Moroccan carob tree show significant phenotypic diversity (El Kahkahi et *al.,* 2014), (Elfazazi et al., 2017). In Beni Mellal (middle atlas), the carob tree population is characterized by a length of 12 and 15cm, width 1.5 and 2.5cm, pod thickness 0.4 and 1.37cm, number of seeds 10.67 and 12.5 (Elfazazi et *al.,* 2017). In our study, the seed yield varies between 21.38 and 32.59%. It is higher compared to a few Mediterranean countries (Portugal by 20% (Barracosa et *al,* 2008), Turkey 17% (Pazir et *al.,* 2018) and Algeria 14.58% (Boublenza et *al.,* 2019).

Pretreatment of carob seeds

Our results showed the beneficial effect of certain pretreatments on improving the germination capacity of carob seeds. Among these pretreatments, the chemical agent (Sulfuric acid) has been shown to be necessary for the rapid and homogeneous germination of the seeds of the carob tree (*Ceratonia siliqua* L.). Indeed, the best results recorded were obtained with seeds treated with sulfuric acid. Thus, and for a period of 20 min of soaking the seeds in 95% sulfuric acid, we recorded the highest germination rate (96.67%). According to Cavallaro et *al.,* (2016), germination equivalents of 100% and 95.3% were obtained for seeds of the carob tree soaked in sulfuric acid for 20 min. A germination percentage of 100%, 93.06% and 88.89% were obtained respectively for carob seeds soaked in sulfuric acid for 30min by El kahkahi et *al.,* (2014), Güneş et *al.,* (2010 and 2013) and Bostan and Kiliç, (2014). For the seeds of the carob tree and according to the authors, the duration of the treatment with sulfuric acid was very variable. It is 10 min for Christodoulakis et *al.,* (2002) et al (2002), 20 min for Valeria Cavallaro et *al.,* (2016). 30 min for Lamlom & Abdalrasol, (2016), Bostan and Kilic (2014), El Kahkahi et *al.,* (2014), Güneş et *al.,* (2013), Pérez-García, (2009), (Sbay, 2008) and from 45min to 60min for (Konaté 2007). On the other hand, the use boiling water as a scarifying medium from seven provenances revealed a very low germination rate, the highest rate (47.77%) being recorded only in the case of P2 accession.

Water retention capacity

Water is a necessary element for the start of the germination process. It is a source of oxygen which activates the metabolism of seeds through respiration (Patanè et *al.,* 2006; Cavallaro et *al.,* 2014). Therefore, our results show that the absorption of water was significantly affected by the water potential of PEG6000 and NaCl. The same results were obtained by Caruso et al (2018). According to these authors, the absorption of water during the first hours of imbibition, in solutions of Mannitol and NaCl, was significantly influenced at 0MPa compared to other levels of osmotic pressure (-0.25, - 0.50 and -0.75MPa). This water absorption is reduced according to the increase in the water potential of the NaCl and Mannitol solutions tested in two varieties of durum wheat (Maucieri et *al.,* 2018). The solution tested -0.750MPa induced the lowest water absorption (Maucieri et *al.,* 2018). Note also that the water absorption in PEG solutions is lower than that recorded in NaCl solutions. This adequacy of water absorption was observed for seeds germinating in NaCl solution at -1.5MPa potential and in PEG solution at -0.5MPa (Cavallaro et al., 2016). On the other hand, our results show that the water holding capacity was also significantly affected by temperature variation. In okra seeds, it is dependent on temperature variation (Ben Dkhil & Denden, 2014). Water absorption which is relatively slow at 10°C and greater at 25 and 40°C. According to Ben Dkhil & Denden, (2014), two distinct phases were observed, the first phase which lasts 6 hours is distinguished by a rapid entry of water whatever the temperature and a second phase of 18 hours which is characterized by a slow water absorption.

Effect of heat stress on germination

Carob seeds from different regions of Morocco show a variation in the germination rate with respect to the thermal factor under germination conditions. Our results show a thermal optimum of 25°C for the germination of seeds of the carob tree which is similar to that of Valeria Cavallaro et *al.,* (2016). El kahkahi et *al.,* (2014) showed that a temperature of 30°C allows for a high germination rate. The results of Konaté (2007) also showed that the temperature 28°C ensures maximum germination. Likewise, germination at extreme temperatures (15 and 40 ° C) clearly affected the germination of carob seeds. Ben Dkhil and Denden in 2014 showed that extreme temperatures (10 and 40°C) inhibit the germination processes in okra seeds. This inhibition is explained by an inactivation of the enzymes responsible for the degradation of protein reserves at 10°C and by blocking the assimilation of hydrosols resulting from the degradation of reserves by the embryo at 40°C. Our results show that temperature is one of the factors regulating germination in the carob tree.

Effect of salt stress on germination

Tests relating to the behavior of the carob tree with respect to salinity have shown that the seeds from different provenances studied are particularly sensitive to doses of salt, in particular of -1 MPa of NaCl. Indeed, the reduction of the germination rate is significant from the concentration of -0.5MPa of NaCl. These results are in agreement with those cited in the literature (Medjebeur et *al.,* 2018, Cavallaro et *al.,* 2016). According to Caruso et *al.,* (2018), increasing salt concentrations progressively inhibit seed germination, significantly lower germination values were observed from -0.25MPa. At a concentration of 200mM, a depressive effect of salt on germination was noted for Medicago ciliaris Medicago intertexta and Medicago scutellata. In addition, a reduction in epicotyl and radicle length was observed in all populations for all salt doses tested (Mbarki et al., 2020). Salt stress also has highly significant effects on the germination rate of Salvadora persica seeds subjected to different concentrations of NaCl (Hadi et *al.,* 2018). The effect of NaCl on germination was also observed by Bhatt et *al.* (2019), increasing salt concentrations reduce germination and retard the germination rate of Deverra triradiata seeds in the presence of 200mM NaCl. Gharbi et *al.,* (2011) show that NaCl affects the germination ability of the three species of Eucalyptus (*E. gomphoecephala, E. astringenset E. sargentii)*. These authors report that Eucalyptus astringens is the most sensitive species in a range of concentrations up to 14g/l of NaCl.

Effect of water stress on germination

Water deficit is also one of the environmental factors that most often affects the germination and growth of plants (Bhatt et *al.,* 2019; Lhlou et *al.,* 2013). Our results show that carob populations from different regions of Morocco behave differently with respect to water stress at the time of germination. Water stress, simulated by PEG6000, showed a significant effect on the germination of seeds of different genotypes of the carob tree (Cavallaro et al., 2016) .It has an inhibitory effect on germination and radicle growth at -1MPa and - 1.5MPa. The application of osmotic stress with PEG8000 for 14 days showed a significant inhibitory effect on the germination of seeds of populations of Lotus creticus and Lotus ornithopodioides collected from different regions of Tunisia (Hajri et al., 2018). Compared to the control, the seed germination rate of L. creticus populations was reduced by treatments between -0.6MPa to -1.0MPa. On the other hand, in L. ornithopodioides seeds, germination is reduced to -0.2MPa and it is almost inhibited with treatments of -0.6MPa. Our results confirm those recorded by (Boubacar et *al.,* 2018 ; Samb et *al*., 2015 ; Jaouadi et *al* ., 2010). These authors have shown that the germination rate of seeds of Accaciaraddiana and Tamarindusindica gradually decreases according to the water potential. The same authors show that the water potential of -8 bars deeply affects the germination of seeds. Thus, the seeds of Balanites aegyptiaca and Ziziphus mauritiana manage to germinate, but with a longer waiting period. Similar results were obtained in Ziziphus lotus (Wahbi 2012; Zouaoui et *al.,* 2013).

Reversibility test

The transfer of seeds to an optimal medium makes it possible to determine the osmotic and / or depressive effect of abiotic stress on germination (Hajlaoui et al., 2007). The osmotic effect results in a resumption of germination once the osmotic constraints are removed. However, the high temperature and high concentrations of PEG6000 and NaCl inhibit germination (Hajri et al., 2018). Germination is also irreversible for Medicago polymorpha and Trifolium subterraneum at high concentrations of NaCl (Nichols et al., 2009). Salinity can affect the seed germination process by modifying certain enzymatic and hormonal reactions (Patanè et al., 2013; Maucieri et al., 2018). The depressive effect, on the other hand, results in a weak germination power. Thus, carob seeds transferred from a medium at a temperature of 15 ° C to a medium at a temperature of 25 ° C, resume germination but with a germination capacity which remains quite low compared to the control. Similar results are obtained in Cicer arietinum and Abelmoschus esculentus (Hajlaoui et al., 2007; Ben Dkhil and Denden, 2014). For sorghum and carob (Patanè et al., 2013; Cavallaro et al., 2016), abiotic constraints have an osmotic effect on seed germination. According to Cavallaro et al (2016), the presence of the solute decreases the rate of water penetration which results in a delay or inhibition of germination. This phenomenon is not a general rule since the abiotic constraints studied in our study exert a toxic effect on the germination of seeds of seven groups of carob trees studied.

In order to demonstrate the difference between the seven populations studied, an analysis of the variance at $p \leq 0.05$ was carried out on the germination rate of the seeds under the different treatments carried out (PEG6000, NaCl, temperature 10 ° C and 40 ° C). With the exception of seeds under water stress at -0.5MPa, our results show that there is a fairly significant difference between the germination rate of control seeds and those germinated under stress. In general, we note a high germination rate for the P1, P3, P4, P5 and P6 populations which are germinated at different concentrations of PEG 6000 and for the concentration -0.5MPa of NaCl (Table 4).

The hierarchical analysis (Figure 9) allows us to group our seven populations of the carob tree into three groups. The first group is made up of populations P1, P3, P4, P5 and P6 coming from Meknes, Marrakech and Khemisset. This group is characterized by a high tolerance to water stress and salt stress of -0.5MPa. The P2 population of origin of Fez alone constitutes the second group which is characterized by a low tolerance to water stress of -1.5MPa, salt stress and heat stress (10°C and 40°C). The third group consisting of P7 (Fes) is distinguished by a fairly low tolerance to water stress -1MPa and -1.5MPa, salt stress and thermal stress.

Table 4: Germination rate of carob seeds from seven groups under the different stresses carried out (PEG6000, NaCl, 10°C and 40°C).

Group	Control	-0,5MPa PEG	-1MPa PEG	-1,5MPa PEG	-0,5MPa NaCl	-1MPa NaCl	-1,5MPa NaCl	10°C	40°C
P1	97,78^{a}	82,22^{b}	80,00^{b}	75,56^{b}	95,55^{a}	0,00^{c}	0,00^{c}	17,78^{d}	3,33^{c}
P2	97,78^{a}	97,78^{a}	88,89^{b}	20,00^{c}	78,33^{d}	0,00^{e}	0,00^{e}	3,33^{e}	0,00^{e}
P3	91,11^{a}	86,67^{a}	82,22^{ab}	66,67^{c}	80,00^{ab}	0,00^{d}	0,00^{d}	4,44^{d}	2,22^{d}
P4	97,78^{a}	95,56^{a}	80,00^{b}	75,55^{c}	53,89^{c}	0,00^{d}	0,00^{d}	3,33 ^{d}	0,00^{d}
P5	100,00^{a}	100,00^{a}	91,11^{b}	73,33^{b}	85,00^{c}	0,00^{d}	0,00^{d}	3,33 ^{d}	0,00^{d}
P6	100,00^{a}	100,00^{a}	80,89^{b}	53,33^{b}	83,33^{c}	0,00^{d}	0,00^{d}	5,56^{d}	0,00^{d}
P7	100,00^{a}	95,56^{a}	11,11^{b}	6,67 ^{c}	78,33^{d}	0,00^{c}	0,00^{c}	12,22^{bc}	3,33^{c}

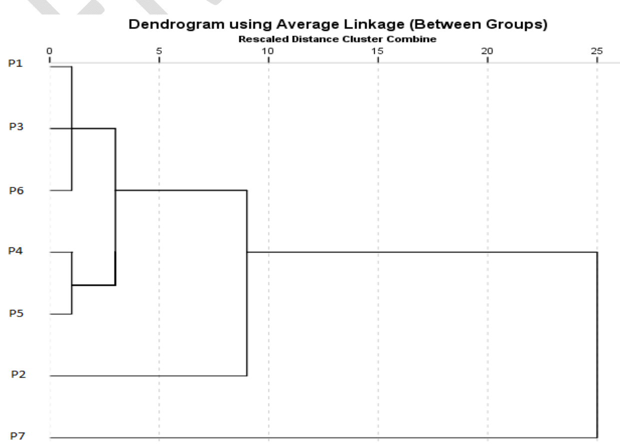


Figure 9. Hierarchical classification of the seven groups of carob tree (*Ceratonia siliqua* L.) according to the germination rates of carob seeds of seven groups under the different stresses achieved (PEG6000, NaCl, 10°C and 40°C).

Conclusion

Our results on the effects of abiotic constraints on the germination of carob seeds from four Moroccan regions show that with the exception of populations P2 and P7, the germination of seeds of carob populations is sensitive to salt stress and tolerant to water stress. . A decrease in the germination rate from -0.5MPa of NaCl, and at osmotic pressure of -1.5MPa was recorded. The germination of carob seeds is practically inhibited at -1 MPa of NaCl. Just like the germination rate and the average germination time is also affected by salt and water stress. The delay in germination increases with the severity of the stress. The evaluation of carob tree by the criterion of tolerant to abiotic constraints cannot be done solely on the basis of the response of this species in the germination phase. An investigation of the later phenological stages of this species, under constraining hydric and saline conditions, would complete these results and help to better define its pedoclimatic requirements.

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