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Germination variability of some Tunisian castor bean accessions (*Ricinus communis*.L) in relation to water stress

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Abstract:
The castor plant occupies an important place in the Tunisian heritage. The environmental conditions are difficult (drought and high temperature...) which are the consequence of climate change. To ensure better management, sustainability and profitability, scientists are looking for more efficient species and provenances better adapted to these conditions.
The present research related to a study of the behaviour of seven castor populations (Ennahli Park, Belli, Nefza, Enfidha, Kef, Gabes and Tabarka) at the seed level under the effect of osmotic stress. This stress showed a significant reduction of germination percentage by the -0.2MPa treatment in Belli population (33%) and low in Parc Ennahli, Enfidha (13%) and Nefza (14%) populations while by the -0.4MPa treatment the most important reduction is recorded in Parc Ennahli population (53%) and low in Kef population (7%).
The populations of Belli, Nefza and Enfidha are limited in time while those of Kef and Tabarka are sensitive to water stress; while the population of Parc Ennahli is low.
***Key Word**: Castor, population, intra-specific diversity, germination, Tunisia.*

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I. Introduction

The planet is seriously threatened by the phenomenon of climate change. Tunisia is no exception to this rule and will be confronted with these climatic variations.

In Tunisia, the climate has shown a certain regularity for almost a century until 1975, when a trend towards water stress was observed. The limited quantities of water are unevenly distributed throughout the country and intensively used. This situation has posed serious challenges such as increased degradation and risk of depletion. An analysis of the flora of Tunisia shows that the castor bean occupies not only a considerable area but also an important place in ecological and economic terms. In ecology, it contributes to the fixation and restoration of soils. It is an industrial species known for the use of its oil as fuel for lighting, lubricant, paint, surfactants, and plastics of the polyamide family. Castor is used in pharmacy for its laxative and anesthetic effects, in cosmetics for massages and in horticulture as an organic fertilizer and rodent repellent.

However, considering the undeniable economic role of castor on the one hand and the climatic changes in Tunisia on the other hand, an analysis of the germination of this species seems necessary in order to determine the correlation between the latter and its ecosystem to evaluate the populations and to determine a better production on the quantitative and qualitative level.

In this context, a study of the germination of some castor populations was carried out in a semi-controlled condition (in a nursery) providing investigations at the organ level through the application of a hydric constraint on the young plants by suspension of watering. .

II. Material And Methods

Plant material

The study involved castor seeds from different populations: Ennahli Park (Ariana), Belli (Grombalia), Nefza (Tabarka), Enfidha (Sousse), Kef, Gabes, Tabarka (Jendouba) (Fig.1).

Figure 1 Castor beans (*Ricinus communis.*L)

Characterization of the study sites

Table 1 List of Ricin (Ricinus communis.L) accessions with codes and original location

No	Label	Location					
		City-village	Géographic Répartition	Bioclimate	Latitude	Longitude	Altitude (m)
1	PE	Parc Ennahli	North	medium semi-arid	36°52'52'' N	10°09'30'' E	84
2	B	Belli	North-Est	upper semi-arid	36°34'08'' N	10°33'16'' E	49
3	N	Nefza	North-Ouest	lower wet	36°58'18'' N	09°04'59'' E	32
4	E	Enfidha	North-Est	lower semi-arid	36°07'35'' N	10°22'44'' E	19
5	K	Kef	North-Ouest	semi-arid upper and middle	36°09'15'' N	08°42'51'' E	525
6	G	Gabes	South-Est	lower arid	33°52'33'' N	10°06'52'' E	2
7	T	Tabarka	North-Ouest	lower wetland	36°55'58'' N	08°45'35'' E	50

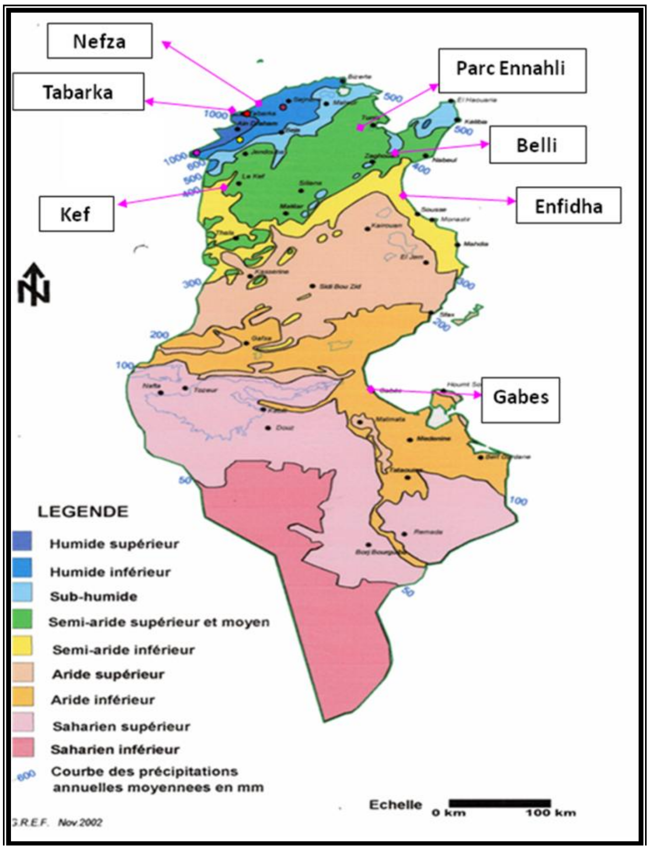


Figure 2 Distribution of the seven castor populations

2. Methodology
2.1. Germination study

The experiment was conducted in the nursery of the I.N.R.G.R.E.F in Ariana under semi-controlled conditions (upper semi-arid bioclimate).

The seeds obtained from the different populations (216 seeds from each population) were used without scarification, disinfected by diluted bleach for 15 minutes and were soaked in tap water in small glass bottles for one day. The seeds were sown in perforated polyethylene bags 16 cm deep and 10 cm in diameter filled with a mixture of sand and cork oak humus with a weight of about 1.7 kg, at a rate of 2 seeds per bag. The sown seeds are covered by turning over and packing the soil to ensure good seed-to-soil contact. After sowing, daily irrigation was provided. In order to avoid a heterogeneity factor due to watering, the same volume of irrigation water was kept for each plant. The soil moisture at the field capacity of the substrate is about 26% and its texture (Tab 1)

Table 2 Texture of the study soil under semi-controlled conditions

Material	Clay %	Silt %	Sand fine %	Sand coarse %	Limestone Coarse %	OM %	pH	P2O5 %	K2O %
	12	12	30	43	3	3.2	7.5	4	0.17

Germination is the resumption of the active life of a plant after a period of rest, of variable duration, it is the environmental conditions (humidity, temperature) which coupled with the characteristics of the seed (thickness of the integument, physiological mechanism, conservation) condition germination.

- Germination rate: It is the percentage of seeds able to germinate under the conditions of the experiment (Abdessamad, 2009). The kinetics of germination were followed from the date of sowing of seeds each day, by noting the number of seeds every day during one month, to determine the maximum rates of germination (Fig.3).
- Germination speed: This is the time taken by the seeds to germinate (Neffati, 1994 in Zouaoui, 2007). It is expressed by the Kotowski velocity coefficient:

$$\mathbf{CV= (\sum ni / \sum (ni+nj))*100}$$

Where: ni= The number of seeds germinated on day ji.

Ji= The number of days after sowing (the day of sowing is not counted).

The average germination time is the inverse of the velocity coefficient multiplied by 100: **MGT= (1/CV) *100**

Figure 3 Castor bean sprouting (height: 1cm)

2.2.2. Effect of osmotic stress on germination behaviour

- Study of water stress at seed level:

The study of the influence of water stress, simulated by the use of osmotic agents, is one of the methods used by researchers to study the drought resistance of plants during the germination phase.

In the Biotechnology Laboratory of INRGREF and under controlled conditions, tests are carried out on the effect of polyethylene glycol (PEG 6000 polyethylene glycol: HOCCH2 (OCH2CH2): a relatively stable, inert, well water-soluble and non-toxic osmotic agent) on the germination rate of different study populations.

For each population, the seeds were washed with tap water and disinfected for a few minutes with benlate. Five seeds were placed in Petri dishes on perlite soaked with either 20 ml of distilled water (control) or PEG 6000 solutions. To create the water stress, we will prepare different solutions of varying osmotic potentials.

Six treatments with different concentrations of PEG6000 were prepared to obtain six levels of water potential: 0,-0,2,-0,4,-0,6,-0,8 and -1,0 MPa. These levels are obtained according to the formula of Michel and Kaufman (1973) (Boubiche, 2009).

$$\boldsymbol{\Psi h=-(1.118*10-2)\ C-(-1.118*10-4)\ C^2+\ (2.67*10-4)\ CT+\ (18.39*10-7)\ CT}$$

Table 3 Treatments of different concentrations of PEG6000 to obtain 6 levels of water potential: 0,-0,2,-0,4,-0,6,-0,8 and -1.0 MPa

T1 :0 Mpa.	0g/l ; T2 : -0,2 Mpa	112,23g /l ; T3 : -0,4 Mpa	169,11g/l T4 : -0,6 Mpa
213,63g/l ; T5 : -0,8 Mpa.	251,02g/l ; T6 : -1,0Mpa	284,02g/l	

Table 4 Experimental conditions in the growth chamber

	Day	Night
Duration	15 hours	9 hours
Temperature	25°C	17°C
Humidity	80%	70%

Figure 4 Device in the growth chamber

Figure 5 Castor seed germination

Each treatment is repeated three times. Germinated seeds are counted every day for 35 days (Fig. 5).
The germination rate and the germination speed or germination energy are calculated, which is explained by the velocity coefficient of Kotowski (1926) in Zouaoui (2007).
Velocity coefficient = $\sum ni / \sum (ni+nj)*100$
ni: the number of seeds germinated on day ji
ji: the number of days after sowing (the day of sowing being taken as time zero).
The velocity coefficient (VC) and the mean germination time (MGT) are calculated.

III. Statistical analysis

The statistical processing carried out aims to compare the variables studied, using SPSS 18 software. All the measurements were subjected to a one-factor or two-factor analysis of variance, depending on the case, using Fisher's F test to verify the hypothesis of equality of the means at the 5% risk threshold. The graphical outputs were produced with Excel software.

IV. Result

4.1 Germination

4.1.1. Germination rates

The maximum germination rates recorded were 99.06, 97.18, 93.29, 97.21, 98.12, 94.37 and 96.27% respectively for the population of Ennahli, Belli, Nefza, Enfidha, Kef, Gabes and Tabarka parks (Fig. 7).

4. 1.2. Germination speed

The latent phase recorded is 5 days for all populations followed by an acceleration phase of 5 days for the populations of Belli, Enfidha and Tabarka; 7 days for the population of Gabes and 8 days for the populations of Park Ennahli, Nefza and Kef. (Fig.6).
The maximum germination rates were reached on day 10 for the populations of Belli, Enfidha and Tabarka, on day 12 for Gabes and on day 13 for Park Ennahli, Nefza and Kef

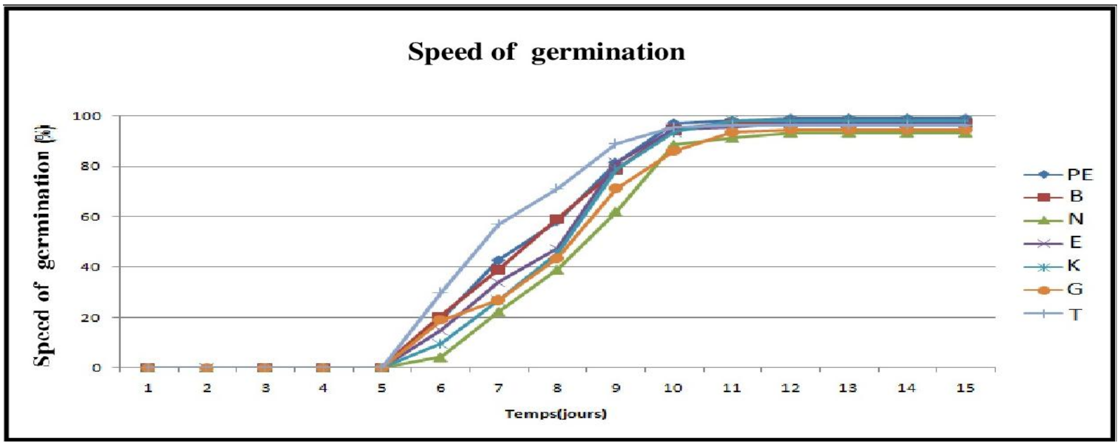


Figure 6 Seed germination rate of the populations (Parc Ennahli, Belli, Nefza, Enfidha, Kef, Gabes, Tabarka) of castor oil (*Ricinus communis*.L)

2 Effect of osmotic stress on germination behaviour

4.2.1. Germination capacity

The germination percentages obtained are of the order of 93; 80; 47; 53; 53; 20; 40% respectively for the Park Ennahli, Belli, Nefza, Enfidha, Kef, Gabes and Tabarka populations for the control (t0) (Fig.7).

While, under the effect of osmotic potential t1 (-0.2MPa), these values are 80; 47, 33, 40, 27, 20 and 20% respectively for the populations Parc Ennahli, Belli, Nefza, Enfidha, Kef, Gabes and Tabarka (Fig.7).

But under t2 (-0.4 MPa), these values are 27, 13, 20, 20, 20, 20 and 20% respectively for the populations Parc Ennahli, Belli, Nefza, Enfidha, Kef, Gabes and Tabarka (Fig.29).

On the other hand, under t3 (-0.6 MPa), t4 (-0.8 MPa) and t5 (-1.0 MPa), there was a total absence of germination (Fig.7).

The statistical analysis shows a non-significant difference between treatments and between populations at the 5% threshold.

The reduction in germination percentage for treatment t1 (-0.2 MPa) compared to the control is in the order of 13; 33; 14; 13; 26; 0 and 20% respectively for the populations of Parc Ennahli, Belli, Nefza, Enfidha, Kef, Gabes and Tabarka. While for t2 (-0.4 MPa) this reduction is of the order of 53; 34; 13; 20; 7; 0 and 0% respectively for the populations of Parc Ennahli, Belli, Nefza, Enfidha, Kef, Gabes and Tabarka.

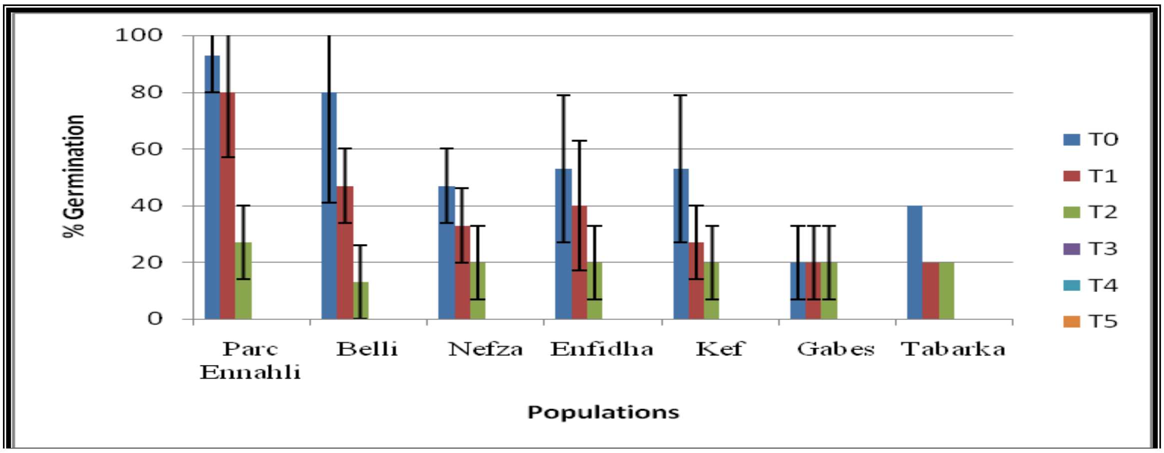
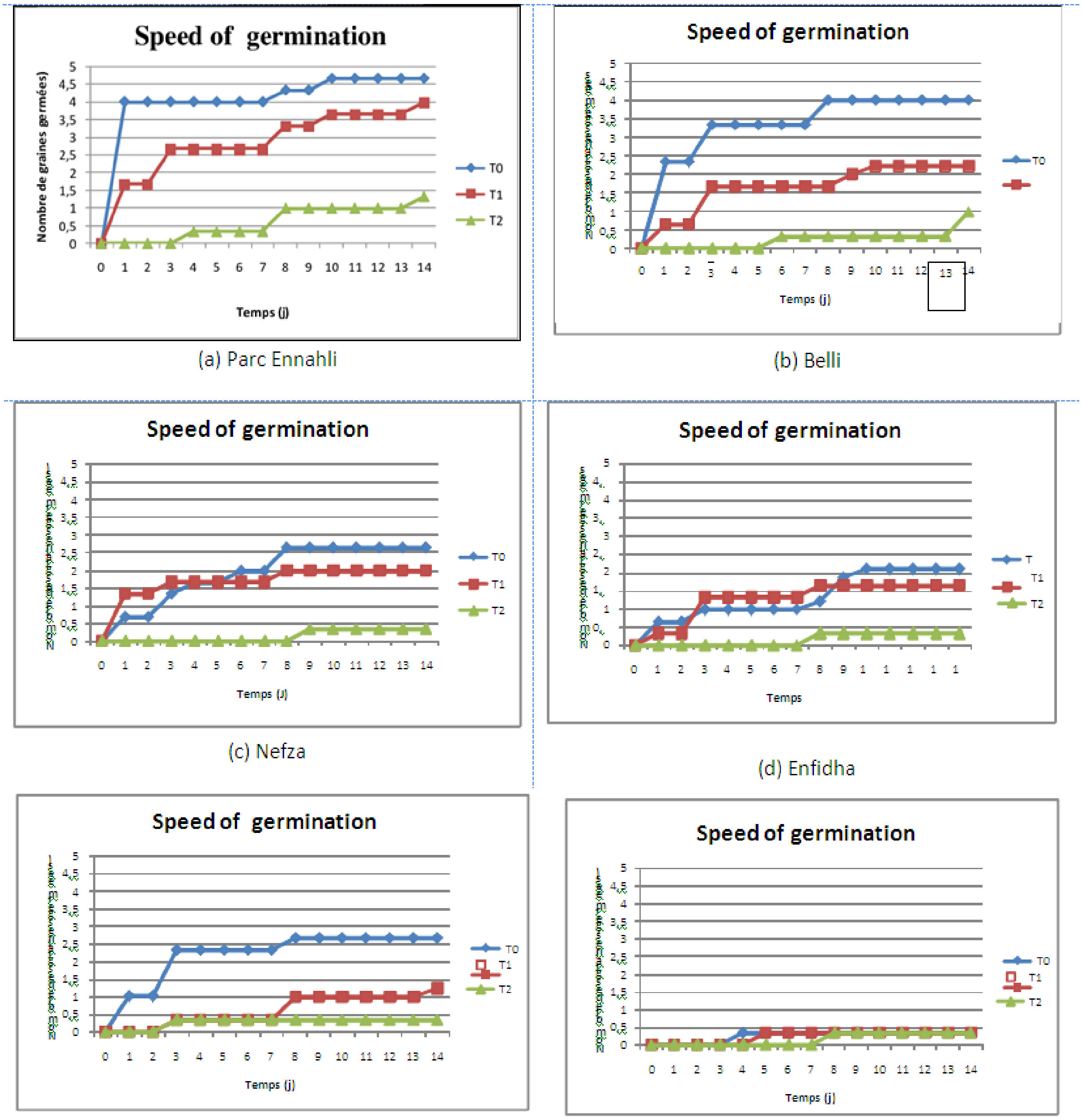


Figure 7 Germination rate of castor bean populations (Ennahli, Belli, Nefza, Enfidha, Kef, Gabes and Tabarka parks) by PEG treatments (0; -0.2; -0.4; -0.6; -0.8; -1.0 MPa)

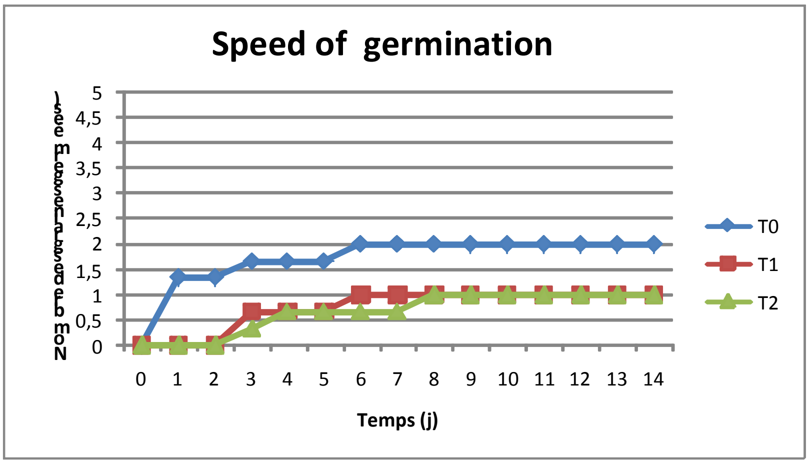
4.2.2. Germination kinetics

Our results show three phases (lag phase, growth phase and a stability phase (plateau). (Fig 30:(a), (b), (c), (d), (e), (f) and Fig 30' (g)).



(e) Kef (f) Gabes

Figure 8 Effect of PEG6000 on the germination dynamics of castor bean populations: (a) Ennahli Park (b) Belli (c) Nefza (d) Enfidha (e) Kef (f) Gabes and (g) Tabarka by different PEG treatments (T0: 0 MPa; T1: -0.2 MPa and T2: -0.4 MPa)



(g) Tabarka

Figure 9 Effect of PEG6000 on the germination dynamics of castor bean populations: (a) Ennahli Park (b) Belli (c) Nefza (d) Enfidha (e) Kef (f) Gabes and (g) Tabarka by different PEG treatments (T0: 0 MPa; T1: -0.2 MPa and T2: -0.4 MPa

4.2.3 Velocity coefficient and mean germination time

The value of the velocity coefficient (VVC) decreases with increasing osmotic stress concentration (t0, t1 and t2) for all castor populations. The mean germination time (MGT) increased with increasing osmotic stress concentration except for the Gabes population.

On the other hand, under treatment t3 (-0.6MPa), t4 (-0.8MPa) and t5 (-1.0MPa), there is a total absence of germination.

V. Discussion

Germination is often considered as a phenomenon that leads from inert seed to a seedling capable of producing its first leaves. This phenomenon has various modes of expression. Being spread over a period of 15 days, germination manifests itself in three different phases, a relatively short latent phase of about 5 days for the seven castor populations. This is explained by the fact that embryonic dormancy is easily lifted by a wet pre-treatment before sowing, as is the case with Ulex parviflorus seeds (Ballini C. 1992).

The germination acceleration phase is relatively short and spreads over a period of 5 days for the populations of Belli, Enfidha and Tabarka, 7 days for the population of Gabes and 8 days for the populations of Parc Ennahli, Nefza and Kef.

However, the maximum germination rate was reached at day 10 for the populations of Belli, Enfidha and Tabarka, at day 12 for the population of Gabes and at day 13 for the populations of Parc Ennahli, Nefza and Kef. Our results are not consistent with those found by Mokrani (2002) on Eucalyptus gomphocephala, Atriplex halimus and Cupressus sempervirens and Dhib (2007) on Calycotum villosa.

The highest germination rates were recorded in the populations of Parc Ennahli (99.06%), Kef (98.12%), Enfidha (97.21%), Belli (97.18%) and Takarka (96.27%) to a lesser degree in the populations of Gabes (94.37%) and Nefza (93.18%). This high germination rate shows a high and important germination capacity.

These results are similar to those found by Ncibi (2005) on oak species and Abdessamad (2009) on zeen, afares and cork oak. On the other hand, they are in contradiction with those found by Ayari A et al (2009) in three species of annual medics and are attributed to embryonic dormancy, impermeability of the seed coat and seed immaturity. The same is true for Argania spinosa which has a low germination rate (Kouardi, 1995).

The germination phase is a critical stage in the plant's development cycle. Indeed, it conditions the installation of the seedling, its adaptation to the environment and probably its subsequent productivity (Tremblin and Binet, 1994 in Baraket M., 2004).

Our results show that the germination of castor seeds (control) is important in the populations of Ennahli Park (93%) and Belli (80%), average in Enfidha (53%), Kef (53%), Nefza (47%) and Tabarka (40%), and low for the Gabes population (20%).

Under the effect of water stress we recorded a decrease in germination rate from the application of the t1 treatment (-0.2MPa) with rates ranging from 20 to 80%. The most important decrease was recorded in the Belli population (53%) compared to the populations of Kef (33%), Tabarka (20%), Nefza (14%), Parc Ennahli and Enfidha (13%), hence the Belli population is more sensitive to osmotic stress and the Kef, Tabarka, Nefza, Parc Ennahli and Enfidha populations are the most tolerant to osmotic stress.

The effect of water stress by the t2 treatment (-0.4MPa) became important with rates between 13 and 27%. The most important decrease was recorded in the population of Parc Ennahli (53%) with a lesser degree in the populations of Belli (34%), Enfidha (20%), Nefza (13%) and Kef (7%) and none in the population of Tabarka. The population of Gabes was very sensitive and the germination rate was low, around 20% for the treatments t0, t1 and t2. On the other hand, from stress -0.6MPa (t3), -0.8MPa (t4) and -1.0MPa (t5), there is a total absence of germination.

The reduction of the germination rate may reflect the sensitivity of the seeds to the osmotic effects induced by PEG6000, which leads to hydration difficulties for the seeds. The seeds are thus unable to absorb the quantities of water necessary to start the germination process (Adbelly, 1997 in Baraket, 2004). Our results were approved by Huseyin (2000) in Abderrazak and Ben Salem (2007) in Cedrus Libani A. Rich.

The latent phase obtained is 1 day for all castor populations for the controls (t0) except for the Gabes population (3 days). This is followed by an acceleration phase of 10 days for the Parc Ennahli and Nefza populations; 8 days (Belli, Enfidha and Kef) and 6 days (Tabarka). Germination stabilised on day 10 for the Parc Ennahli and Nefza populations, day 8 (Belli, Enfidha and Kef) and day 6 (Tabarka).

Under the effect of a water stress of (-0.2 MPa), the latency phase obtained is 1 day for the populations of Parc Ennahli, Belli, Nefza and Enfidha, 4 days (Kef and Tabarka) and 6 days (Gabes) followed by an acceleration phase of 14 days for the populations of (Kef and Parc Ennahli), 10 days (Belli), 8 days (Nefza and Enfidha), 6 days (Tabarka) and 5 days (Gabes) Germination stabilised on day 14 for the populations of Kef and Parc Ennahli, day 10 (Belli), day 8 (Nefza and Enfidha), day 6 (Tabarka) and day 5 (Gabes).

Following a treatment of (-0.4 MPa), the lag phase is 10 days for the Enfidha population, 9 days (Nefza and Gabes), 7 days (Belli), 5 days (Parc Ennahli) and 4 days (Kef and Tabarka) followed by an acceleration phase of 14 days for the Parc Ennahli and Belli populations; 9 days (Enfidha), 8 days (Nefza, Gabes and Tabarka) and 3 days (Kef). Germination stabilised on day 14 for the Parc Ennahli and Belli populations; day 9 (Enfidha), day 8 (Nefza, Gabes and Tabarka) and day 3 (Kef).

Castor is very sensitive to treatments of (-0.6); (-0.8) and (-1.0) MPa, hence the total absence of germination.

The castor seed velocity coefficient is significantly reduced with each increase in osmotic stress concentration for all populations.

It is found that as soon as -0.2MPa is applied, the germination capacity is significantly reduced. It is definitely cancelled at the value of -0.6MPa where water extraction becomes impossible. The average germination time increases with the intensity of osmotic stress and its values become higher and higher with the stress concentration. From this study, we can deduce that there is a difference in resistance to water stress manifested at the seed level between the populations. Thus, it appears that all castor populations are sensitive to osmotic stress in the germination phase.

VI. Conclusion

Castor bean (*Ricinus communis*.L) is a hardy herbaceous species that acclimatizes in tropical and subtropical regions of the world due to its ecological plasticity with respect to temperature, rainfall and soil type. Our work concerns a study of the germination of young castor plants from seven populations: Parc Ennahli (Ariana), Belli (Grombalia), Nefza (Tabarka), Enfidha (Sousse), Kef, Gabes and Tabarka.

The germination rate recorded is important for all the populations of Ennahli, Belli, Nefza, Enfidha, Kef, Gabes and Tabarka respectively. It was reached on the 10th day for the populations of Belli, Enfidha and Tabarka, on the 12th day for Gabes and on the 13th day for Park Ennahli, Nefza and Kef with a relatively short latency phase (5 days) for all castor populations. This significance of germination rates shows a high and important germination capacity for all castor populations

The most important decrease in germination capacity by the -0.2MPa treatment is recorded in the Belli population, average in the Kef, Tabarka, Nefza, Parc Ennahli and Enfidha populations and null in the Gabes population. This decrease became more accentuated with the -0.4MPa treatment. The highest value was recorded for the population of Parc Ennahli, and low for the population of Kef and very low for that of Gabes and Tabarka. This shows that the populations of Belli and Parc Ennahli are very sensitive to osmotic stress at low doses.

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