Journal of Stress Physiology & Biochemistry, Vol. 16, No. 1, 2020, pp. 39-49 ISSN 1997-0838

ORIGINAL ARTICLE

Study of the germinative behaviour of *Aristolochia baetica* L. seeds of Tessala mount (west of Algeria)

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Received December 6, 2019

Our work consists to study the germinative behaviour of the seeds of *Aristolochia baetica* L. of Tessala Mount (western Algeria), endemic species of western Algeria, Morocco and the Iberian Peninsula. The germination tests were carried out under controlled conditions, in Petri dishes lined with filter paper. We studied the effect of some physicochemical pretreatments on the improvement of germination capacity of seeds. The effect of temperature, water and salt stress has also been studied. The results show that the seeds of *Aristolochia baetica* are dormant and that only pretreatment with gibberellic acid (GA_3), soaking in distilled water and stratification have been able to remove their inability to germinate. The optimum thermal of germination is between 15 °C and 20 °C. The seeds of *Aristolochia baetica* are very sensitive to water stress, their tolerance level is -0.19 bar beyond which their germination is inhibited. However, they are intolerant to salinity.

Key words: Aristolochia baetica, germination, salt stress, temperature, water stress

Conservation biology is a multidisciplinary and integrated field of research that has developed for the preservation of species and ecosystems (Robertson 2006). On the subject of conservation, it is important to be able to study germination for different reasons (Campo 1995, Herranz *et al.* 2002). It allows to know the germination capacity of the seeds preserved in bank and to evaluate the method of conservation and its adequacy for each species; to determine if the stored seeds have dormancy, what type and how to remove it. For endemic, rare or endangered plants, these studies are important when sufficient seed is available and harvesting in the field does not constitute a threat to the population. Several environmental factors such as temperature, salinity, light and soil moisture affect germination (El-Keblawy and Al Rawai 2006). Among these different germination factors, temperature is the most important factor in plant regulation and development (Koger *et al.* 2004).

Mount of Tessala (western Algeria) is known for its rich flora (Bouzidi 2009, Dif 2014). As a result, this sector has recently been the subject of several ecological and phytosociological studies (Charif 2001, Ferka Zazou 2006, Bouzidi 2009, Chérifi 2009, Bouidjra *et al.* 2011, Bouterfas 2011, Bouzid 2012, Dif 2014, Saidi *et al.* 2015, Chérifi *et al.* 2017), but its potential for genetic resources has only been partially studied and remains to be evaluated.

Among the species that inhabit this mountain, we are interested in *Aristolochia baetica* L., endemic species of western Algeria, Morocco and the Iberian Peninsula (De Groot *et al.* 2006). According to Maire (1961), this species can be found in clear forests, scrub, in well-watered regions or semi-arid regions. *A. baetica* is a perennial liana, produces 2-7 cm capsular fruit that contains 35-45 oval triangular seeds. At maturity, we observe the dehiscence of the capsules, the seeds become free and under the action of the winds fall on the soil. These seeds are rich in nutrients and are likely to attract ants and other animals (Berjano *et al.* 2003). Flowering occurs between October and May (De Groote *et al.* 2006). In 1962, Quezel and Santa considered this species as fairly common in Algeria. At the level of our site of study and according to our personal observations, this species is represented by some populations of low numbers. This can be explained by the harsh climatic conditions, especially the importance of the dry season resulting in a water deficit, the impact of the anthropozoogenic action resulting in the fragmentation of habitats and the disappearance of species and populations but also for lack of data on seed viability of this species.

In this context, the aim of our work is to study the germinative behaviour of *A. baetica* seeds towards temperature, salinity and water stress.

MATERIALS AND METHODS

Biological material

The seeds used were harvested from *A. baetica* plants growing in Tessala Mountain (western Algeria), between June and July 2016, at the station with the following GPS coordinates: N: 0° 46' 12''; W: 35° 15' 54''; Alt. 726 m. The seeds are stored in paper bags under ambient laboratory conditions and protected from moisture for a period of three months for post-maturation before our germination tests. The climate of the study area is semi-arid Mediterranean with cool winter, with an average annual rainfall of 335.16 mm and average temperatures characterized by a maximum of 26.3 °C in August and a minimum of 9.5 °C in January (ONM, 2014 ; Cherifi *et al.* 2017).

Seed preparation

Healthy and intact seeds were selected for germination tests (Figure 1). They were disinfected with 5% sodium hypochlorite (NaClO) for 5 minutes and rinsed abundantly three times with distilled water to remove all traces of chlorine (Nadjafi *et al.* 2009 ; Dadache *et al.* 2015).

Sequence of germination tests

Given the unavailability of seeds (low fruit production due to flower abortion and lack of polinizers), each germination test involved 30 seeds divided into three replicates of 10 each.

The seeds are germinated in a Memmert incubator in sterile glass Petri dishes containing two layers of Wathman filter paper.

Since very little information on the viability of *A.*

baetica seeds is available, we carried out preliminary germination tests, on the untreated seeds on filter paper soaked with distilled water, in ambient temperature (18-20 °C) in the dark and in the presence of daylight (photoperiod = 9h).

These tests have shown that the seeds used are unable to germinate, which has prompted us to use some physicochemical pretreatments, to check if this inability to germinate is integumentary and / or embryonic origin.

Effects of some physicochemical pretreatments on germination

Germination tests were carried out under the same experimental conditions, on seeds that have undergone some known treatments in the removal of integumentary and embryonic dormancy (Heller *et al.* 1990, Mazliak and Laval-Martin 1995, Nadjafi *et al.* 2009, Dadache *et al.* 2015)

To check if the inability to germinate is integumentary origin, the seeds have undergone two techniques of scarification (mechanical and physical) as well as soaking in distilled water for 24 and 48h. Mechanical scarification consists cracking the seed coat envelopes with a scalpel; and chemical scarification is carried out by immersion in pure sulfuric acid (H2SO4, 96 %) for 45 minutes (Mazliak 1982).

To check if our seeds are affected by embryonic dormancy, we soaked them in a solution of Gibberellic Acid (GA3) at 125 ppm and 200 ppm for 24 and 48 hours; soaking for 24 hours in a solution of potassium nitrate "KNO3" (0.2%); soaking in boiling water (75 °C) for 5 minutes and stratification at -20 °C in a freezer for 3 months (Côme 1970, Mazliak 1982).

For tests of the effect of different temperatures and salt and water stress on germination we used seeds pretreated with gibberellic acid (GA3: 125 ppm). It is the pretreatment that gave the best percentage of germination from the preliminary tests.

Search the optimal thermal conditions

Since no information is available for the optimum thermal germination of *A. baetica* seeds, we carried out germination tests in darkness in Memmert incubator at different temperatures: 5, 10, 15, 20, 25, 30 and 35 ° C. For these tests, the filter paper is soaked each time with distilled water to maintain sufficient and permanent moisture for germination.

Effect of salt and water stress on germination

The effect of salt and water stress on the germination behavior of *A. baetica* seeds was tested at the optimal temperature (15 ° C), defined from the above tests.

The germination tests of salt stress were carried out under different salt concentrations prepared with sodium chloride (NaCl): 0, 4, 8, 12, 16 g/l respectively corresponding to the molar concentrations of: 0 mM (control: water distilled); 68.49 mM; 136.89 mM; 205.33 mM; 273.78 mM.

The germination tests of water stress were carried out under different concentrations of Polyethylene Glycol 6000 (PEG 6000): 0, 10, 20, 40, 80, 120 g/l respectively corresponding to water potentials of 0 (control: distilled water), -0.08, -0.19, -0.47, -1.2, -2.45 bar, evaluated by the formula established by Michel and Kaufman (1973).

For all the germination tests carried out, the slit of the integuments and the appearance of the radical represent the germination criterion (Côme 1970, Zeng *et al.* 2014). The count of germinated seeds was done every 24 hours.

Data analysis

The results of the germination tests were expressed by: the germination capacity (GC); the speed of germination (SG) and the latency time (LT) (Côme 1970, Heller *et al.* 1990, Redondo-Gomez 2007, Dadach *et al.* 2015).

The kinetics of germination was illustrated by curves representing the evolution of cumulative germination percentages as a function of time.

Data were analysed using SPSS for windows, version 20. Analysis of variance (ANOVA) was carried out to test effects of the main factors on the germination percentage. Tuckey test was used to estimate least significant range between means at $p < 0.05$.

RESULTS

Effects of some physicochemical pretreatments on germination

Preliminary tests showed that untreated seeds are unable to germinate in daylight or in darkness. However, it appears the favorable effect of some pretreatments on the lifting of seed dormancy, compared to control seeds which remain unfit to germinate (Table 1). According to the results obtained, the mechanical, chemical scarification and the boiling water soaking have no favorable effect on the dormancy of seeds.

The other pretreatments acted significantly ($p < 0.05$) essentially improving their germination capacities, especially GA3 125 ppm for 48 hours (GC = 36.66%), 200 ppm for 24 hours and cold at -20°C for 1 month (GC = 33.33%). Other pretreatments gave low germination capacity.

The pretreatments used also affected the latency time ($p <0.05$). The longest latency time was observed in seeds treated with KNO3 (60 days); however, the shortest latency time was recorded in seeds treated with GA3:125ppm for 24h (20 days), 200ppm for 48h (25 days) and 27 days for seeds stored at -20 °C for 3 months (Table 1).

Figure 2 describes the evolution of seed germination of *A. baetica* and the first growth stages of the radicle.

Influence of thermal conditions

Seed germination occurred only at temperatures of 15 °C and 20 °C. An absence of germination is noted at the other temperatures tested (0, 5, 10, 25, 30, 35 °C).

The kinetics of seed germination of *A. baetica* at 15 and 20 °C is shown in Figure 3.

For both temperatures, the germination curve is characterized by three phases:

- Latency phase: corresponding to the time necessary for the seeds to begin to germinate.

- Exponential phase: corresponding to a progressive increase in the percentage of germination as a function of time.

- Stationary phase: at this phase, the germination percentage stabilize.

The average germination capacity has a maximum of 56.66 ± 15.55% for the temperature of 15 °C and 53.33 ± 11.54% for the temperature 20 °C. This variability between average germination capacities is confirmed by the Tuckey test ($p <0.05$).

The speed germination is 3.86 ± 0.28% at the temperature of 15 °C, then increases to a maximum of 4.30 ± 0.43% at the temperature of 20 °C, and cancel at 5, 10, 25, 30 and 35 °C.

The latency time is comparable for both temperatures; it varies from 19 days to 20 °C and 20 days at 15 °C.

Effect of water stress on germination

The effect of water stress on the germination, induced by the different concentrations of PEG6000, is represented in Figure 4.

Figure 1. Seeds of *Aristolochia baetica/. SF: superior face; EA: elaiosome on the inferior face*

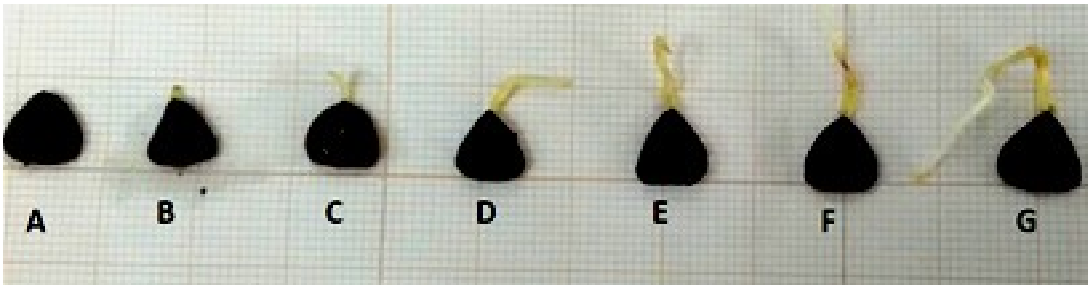

Figure 2. Description of the evolution of seed germination and the first growth stages of *A. baetica* radical. A: imbibition and swelling of the seed; B: appearance of the radicle (germination phase); C, D, E, F, G: elongation of the radicle (growth phase of the radicle).

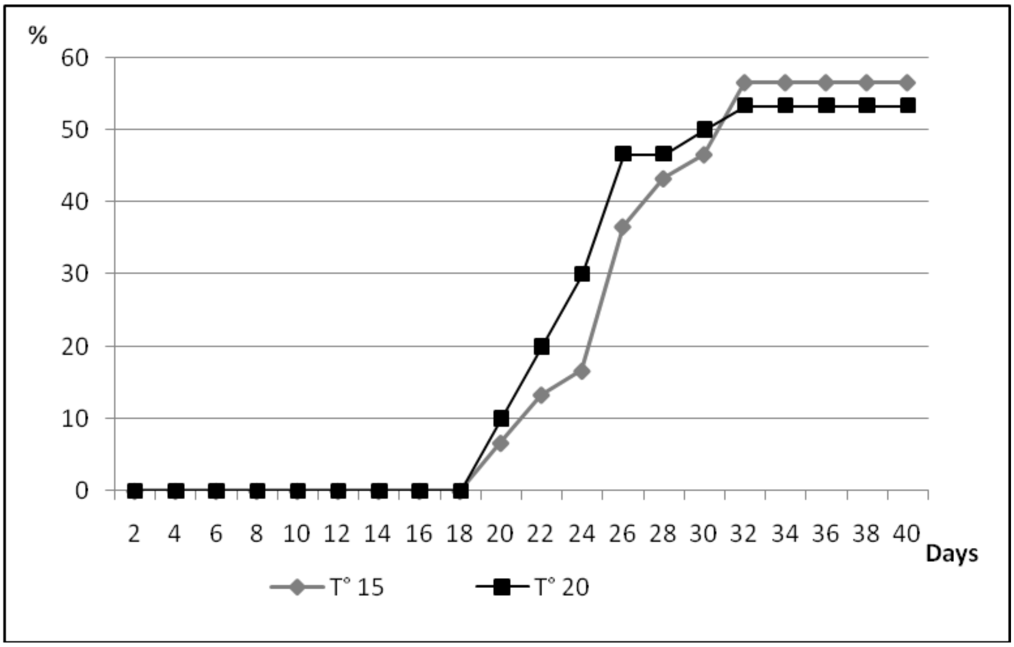

Figure 3. Curves of germination kinetic of *A. baetica* seeds at 15 °C and 20 °C.

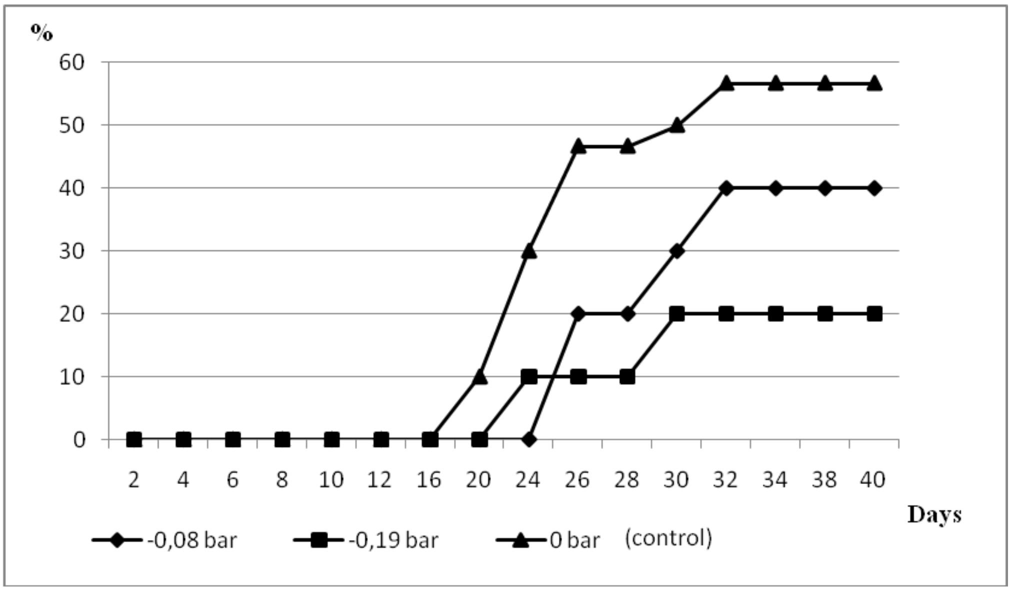

Figure 4. Curves of germination kinetic of *A. baetica* seeds under different water potentials.

Table 1. Effect of different pretreatments on germination parameters of *A. baetica* seeds

Pretreatments employed	**Measured parameters**		
	GC (%)	**GS (%)**	**LT(days)**
Control (without treatment)	0 [a]	0 [a]	0 [a]
Mechanical scarification	0 [a]	0 [a]	0 [a]
Soaking in boiling water (75 °C, 5 minutes)	0 [a]	0 [a]	0 [a]
Chemical scarification by H_2SO_4	0 [a]	0 [a]	0 [a]
Soaking in GA_3: 125 ppm 24h	36.66 ± 11.54 [d]	02.53 ± 00.55 [bc]	20 [b]
Soaking in GA_3: 125 ppm 48h	20.00±0.00 [abcd]	01.71 ± 00.25 [b]	41 [c]
Soaking in GA_3: 200 ppm 24h	23.33±05.77 [abc]	20.02 ± 00.41[b]	25 [b]
Soaking in GA_3: 200 ppm 48h	33.33±11.54 [bcd]	02.46 ± 0.88 [bc]	25 [b]
Soaking in H_2OD 24h	10.00±00.00 [ab]	01.76 ± 00.58 [b]	41 [c]
Soaking in H_2OD 48h	23.33±05.77 [bcd]	01.69 ± 00.46 [b]	35 [c]
Soaking in KNO_3	20.00±00.00 [abc]	01.43 ± 00.15 [b]	60 [d]
Stratification at 5 °C	33.33±04.44 [cd]	03.36 ± 00.02 [C]	27 [b]
Variance analysis test	+	+	+

Values followed by different letters indicate significant differences at the 5% threshold (*p* <0.05).
CG: germination capacity; GS: germination speed; LT: latency time; H_2SO_4: Sulfuric acid; GA_3: Gibberellic acid; H_2OD: distilled water; KNO_3: potassium nitrate; +: significant difference at the 5% threshold (*p* <0.05).

Table 2. Variation of the germination capacity (GC), the germination speed (GS) and the latency time (LT) under the effect of different water potentials.

	GC (%)	**GS (%)**	**LT (days)**
0 bar (control)	56,66 ± 15,27[b]	3,86 ± 0,28[a]	20[a]
- 0,08 bar	40.00 ± 10.00 [ab]	4,06 ± 0,20[a]	26[b]
-0,19 bar	20.00 ± 0.00[a]	4,33 ± 0,25[a]	24[b]
Analysis of the variance	+	-	+

Values followed by different letters indicate significant differences at the 5% threshold (*p* <0.05).
CG: germination capacity; GS: germination speed; LT: latency time ; +: significant difference (p < 0.05); - : no significant difference (p > 0.05).

Variance analysis shows that germination capacity is significantly affected (p <0.05) by water stress (Table 2). The results obtained show that the control seeds as well as the seeds subjected to low concentrations of PEG: 10 and 20 g/l, corresponding to -0.08; -0.19 bar, recorded an average germination capacity respectively 56.66 ± 11.11%, 40 ± 6.66%, 20% ± 0. However, at concentrations above 20 g/l, this capacity was cancel. Compared to the control, we find that more the concentration of PEG increases in the middle, the more the capacity and the speed of germination decrease.

The latency time varied from PEG concentration to another. The shortest latency time of 20 days is noted in the control; while the latency times spread out at 24 days at 10 g/l (- 0.19 bar) and 26 days at 20 g/l (-0.08 bar).

Effect of salinity on germination

No germination was observed under the different salt conditions tested by the use of different concentrations of sodium chloride (NaCl).

DISCUSSION

The results obtained showed that the seeds of *A. baetica* are dormant and therefore unfit to germinate. This inability to germinate is certainly one of the factors that explain the difficulties of the natural regeneration of this species and therefore its restricted distribution at our study site.

Among all pretreatments used on the seeds, only the soaking of the seeds in gibberellic acid (GA_3), in distilled water and stratification (cold treatment) had a favourable effect by stimulating their germination in the dark. The other treatments (mechanical and chemical scarification, boiling water soaking) did not induce any germination, which explains that the seeds used have not physical integumentary inhibition but an embryonic dormancy combined with chemical integumentary dormancy. A maximum rate of germinated seeds (36.33%) is obtained by soaking in gibberellic acid 125 ppm at the end of the 40th day. The favourable effect of gibberellic acid on the dormancy lift of *A. baetica* seeds is also mentioned in the work of Berjano (2006). Also, Gumuscu (2014) in these work of the effect of some treatments on seeds germination of some species of the genus Sideritis, indicates that the highest germination rate was mentioned in seeds treated with GA3. Moreover, Afzalifar *et al.* (2015) have shown that increasing concentration of GA3 decreases the rate of germination in *Satureja khuzistanica* and *S. rechingeri.* Moreover, the application of GA3 contributed in raising seed dormancy in other species, such as *Rosmarinus officinalis* (Madeiras *et al.* 2009) and *Teucrium polium* (Nadjafi *et al.* 2006, Dadache 2015).

The favourable effect of stratification on the lifting of embryonic dormancy and soaking in distilled water on the dissolution of chemical substances inhibiting germination, as polyphenols, existing in the seeds has been referred to by some physiologists as Côme (1970), Mazliak (1982) et Heller *et al.* (2004). This effect is also indicated in the work of Yang *et al.* 2007, Dashti *et al.* 2012, Bouredja (2014) on *Retama monosperma*, Hamdaoui *et al.* (2016) on *Crataegus monogyna,* Lamara *et al.* (2017) on *Lygeum spartum*, Mehdadi *et al.* (2017) on *Retama raetam*.

The results obtained show that the temperature significantly affects the seed germination of *A. baetica* insofar as it is inhibited at 0, 5, 10, 25, 30 and 35 °C while it becomes possible at 15 and 20 °C. The thermal optimum corresponds to 15 °C. This thermal optimum of germination is comparable to some lamiaceae evolving in Tessala mount (western Algeria) (Dadach and Mehdadi, 2018a; 2018b).

The absence of germination at the temperatures already mentioned is explained by the fact that when the temperature is low (0, 5 and 10ºC) or high (25, 30 and 35ºC), the amount of oxygen that arrives at the embryo is not sufficient for germination. Indeed, the embryo requires more oxygen when the temperature of germination decreases or rises. In addition, low or high temperatures can inhibit and / or denature some enzymes essential to the metabolism of germination (Mbaye *et al.* 2002). The effect of temperatures on germination physiology has been mentioned in some studies as Bendimred *et al.* (2007) on the caryopses of Ammophila arenaria and Moulessehoul and Mehdadi (2015), on the caryopses of Stipa tenacissima. Optimum temperatures of seed germination and plant growth differ from one species to another, as indicated by Cochrane *et al.* (2011). These differences can be explained by the genetic variability of seeds used or differences in latitude where seeds are collected (Saeidnejad *et al.* 2012). Milton (1995) indicated that many plants seeds of dryland germinate after the autumn rains and early winter when temperatures are decreasing. However, the seeds do not germinate following the rains in summer, when the temperatures are at their maximum. For annual plants in the Palestinian Negev Desert where rain only falls during the mild winter, the most important survival strategies are that only small batches of seeds germinate in the right place at the right time (Gutterman 2002).

Plants under water stress have frequently an osmotic adjustment that allows the maintenance of water absorption. They also present a synthesis of new proteins which participates in the maintenance of cellular structures, in the synthesis of osmolytes involved in the osmotic adjustment, in the chemical detoxification of species which are frequently produced, and generally to the adjustment of metabolism in the new conditions (Gabriel Cornic 2002).

Regarding germination tests under different water stress induced by different concentrations of PEG6000 used, have shown that the seeds of *A. baetica* germinate best at concentrations of 10 and 20 g/l corresponding to water potentials of -0.08 and - 0.19 bar respectively. At these two concentrations, we noted a germination capacity of 26.66% and 20%, a germination speed of 3.95% and 3.39%, and a latency time of 28 days. However, germination is absent at 40, 80, 120 g/l correspond to water potentials of -0.47, -1.2, -2.45 bar. These results show that the seeds of *A. baetica* are weakly tolerant to water stress. The limit value of water potential for which the seeds do not germinate is 40 g/l equivalent a water potential of -0.47 bar. When the osmotic pressure is high, the germination capacity decreases because the seeds cannot absorb enough water present in their environment. This behaviour was also mentioned in many species of lamiaceae in the work of Bagheri *et al.* (2011) and Dadach *et al.* (2015).

Ndour and Danthu (1998) show that the germination of Acacia tortilis raddiana is possible under higher water stress (-21 bar) than that measured for other Acacia species. Moreover, our results show that the seeds do not germinate in any saline concentration. This shows that our species is intolerant to salt at the germination stage. The decrease in the rate of germination in the presence of salt can be explained either by an increase in the external osmotic pressure, which affects the absorption of water by the seeds and/or an accumulation of Na^+ Cl^- ions in the embryo. This toxic effect can lead to the alteration of the metabolic processes of germination and in the extreme case to the death of the embryo by excess of ions (Hajlaoui *et al.* 2007).

CONCLUSION

In the light of the results obtained, it appears that the seeds of *A. baetica* are dormant. This dormancy could be lifted by soaking seeds in gibberellic acid (GA3), in distilled water and by stratification. Their optimum thermal of germination is between 15 and 20 °C, low (≤10 °C) and high (≥25 °C) temperatures inhibit germination. It is also noted that seed germination is only possible under low water potentials (-0.08 and -0.19 bar). Their germination capacity decreases when the water potential increases in the germination medium; it is absent at 40 g/l of PEG6000 corresponding to a water potential of -0.47 bar. These results show that our species belongs to plants that are sensitive to water deficit at the germination stage. On the other hand, this species is intolerant to salt at the germination stage, which allows to classify it in the category of halophilic glycophytes.

These data allow to know the viability of *A. baetica* seeds and its germination physiology ; they will certainly serve in the ex-situ conservation of this species. It would be desirable to continue this study and to complete our results by addressing the effect of other treatments that can further improve the germination of seeds. The study of the impact of thermal conditions, water stress and salt stress on the other stages of the development of our species is a research path that is interesting to explore.

ACKNOWLEDGEMENTS

We appreciate the support of the research laboratory of plant biodiversity : conservation and valorization of University of Djillali Liabes of Sidi Bel Abbes (Algeria).

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