GERMINATION BEHAVIOR OF ACACIA TORTILIS (FORSSK.) HAYNE VAR RADDIANA (SAVI) BRENAN AND *A. NILOTICA* VA (L.) WILLD. EX DELILE VAR ADSTRINGENS (SCHUMACH. ET THONN.) ROBERTY SEEDS FROM THE HOGGAR, ALGERIAN SAHARA UNDER OSMOTIC, THERMAL AND SALT STRESS

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Abstract. Seeds of *Acacia tortilis* var *raddiana* and *A. nilotica* var *adstringens* harvested from the Hoggar (Algerian Sahara) were tested for germination under 3 abiotic constraints usually present in their biotope, i.e. thermal, salt and osmotic stress. Results showed a different behavior of the two species. Germination speed is better for *A. tortilis* in all situations. Germination rate under tested temperatures was similar for both species except 15°C which revealed obviously unfavorable on *A. nilotica* germination which dropped from a high of 96.6% at 40°C to a low of 11% at 15°C while that of *A. tortilis* slightly decreased from 92% at 40°C to 80% at 15°C. Regarding germination behavior under salt stress, *A. tortilis* maintained 21% of germination under a salt concentration of 350mM while seeds of *A. nilotica* did not germinate at concentrations above 150 mM suggesting a better tolerance of the former species to salt stress. The same trend applies also in the case osmotic stress with *A. tortilis* maintaining a germination rate of 26.7% at a PEG-6000 concentration of 240g/l while *A. nilotica* showed a germination rate of 21% at a much lower PEG-6000 concentration, i.e. 40g/l. These results indicate a greater adaptive capacity of *A. tortilis* in the face of environmental constraints and may partially explain its wider distribution in the Algerian Sahara. These results contribute to a better understanding of the germination behavior of these two species in a perspective of their *in situ* and/or *ex situ* propagation.

Keywords: *Acacia nilotica; Acacia tortilis;* germination; osmotic stress; salt stress; thermal stress; Hoggar; Algeria.

INTRODUCTION

In the Sahara desert, limiting factors such as temperature (high or low), water stress and sometimes- salt stress are exerted with great intensity. The ability of species to adapt to these constraints will depend on their survival and the most adapted ones are destined to become dominant (Flores et al, 2018; Fengyan et al, 2019) . It is in relation to this problem (i.e. ability of species to cope with extreme factors) that we studied the germination of two Acacia species (*A. nilotica* var *asdtringens* and *A. tortilis* var *radddiana*) from the Hoggar (Algerian sahara). Phylogenetic analyses have shown that they should be included in the *Vachellia* genus (African Plant Database (version 3.4.0),

Conservatory and Botanical Gardens of Geneva City and the South African National Biodiversity Institute, Pretoria; In Medail and Quezel, 2018).

These two species have the particularity of fructifying at the same period (March-April); germination of their seeds takes place in the face of the same constraints. The distribution of these two species in the North African zone differs. *Acacia tortilis* has the widest distribution and is found up to the parallel of Bechar (31°3'N) passing through southern Morocco and Mediterranean Tunisia (Le Floc'h and Grouzis , 2003). It is present in the form of stands up to 1700m of elevation (personal observations) and as isolated individuals at elevations upper than 2000 m. *Acacia nilotica* is found only in the southern Hoggar at an elevation of 1100-1200m and rarely in the form of stands (Sahki and Sahki, 2004) and is absent elsewhere. It is a particularly abundant species in the Sahelian area (Ozenda, 1991). Constraints to germination may explain this distribution. The aggravation of these constraints by human action and climate change would determine a different type of community than the existing one. Similarly, restoration of the ecosystems where such taxa are keystone species and their preservation require a better knowledge of their germination.

Germination of the two Acacia species was relatively poorly and incompletely investigate in North Africa. Some studies have been devoted to *A. tortilis* (Bensaid, 1988 in the region of Bechar, Algeria, Kebbas et al, (2013), Jouadi et al, 2010, 2013 in Tunisia). *Acacia nilotica* has not been studied in North Africa if we except Kheloufi and Mansouri (2017) who tested the effect of chemical scarification on seed germination of this species. Conversely, in Sub-Saharan Africa, germination of these species is more extensively investigated Africa (see Argaw et al, 1995; Danthu et al 1992; Danthu et al, 1996; Danthu et al, 2003; Ndour, 1997; Ndour and Danthu, 1998; Teketay, 1996; Sy et al, 2001). It is therefore interesting for us to evaluate the germinative response of these two species to the constraints usually present in their natural environment (heat stress, salt and water). Such investigation is useful in the perspective of ecosystem restoration and for understanding the dynamics of these two species.

MATERIAL AND METHODS

2.1. Study sites and collection of plant material

Pods of *Acacia nilotica* were collected on the banks of Oued Imezguene (22°34' 695'' N and 05°23' 710'' E), at an elevation of 1150 m. In this stand, *Acacia nilotica* is mixed to *A. erhenbergiana; A. tortilis and to Balanites aegyptiaca.* Density of trees is low. The soil is sandy and the slope shallow (<5%). *Acacia nilotica* trees may reach 15m in height and 1m in diameter and are in good status but young individuals are absent.

Pods of *Acacia tortilis* were collecte on trees located on the banks of Oued Ihenkes (22° 51' 922''N and 5° 35' 634" E) at an elevation of 1469 m. A very low density of trees, a sandy and rocky soil and a very shallow slope characterizes the stand. *Acacia tortilis* was mixed *to A. erhenbergiana* and its individuals have between 4 and 6 m of height.

Climatic data, provided by the National Office of Meteorology, for the period extending from 1969 to 2011 for Tamanrasset, the closest locality to the sampled stands,

indicate a bioclimate characterized by a low annual rainfall (49.3mm) with strong annual irregularities including a low of 0.9 mm in 1973 and a high of 166 mm in 2005.

The average annual temperature at Tamanrasset is 22.2° C, the maximum temperature averaged 35.9°C and the minimum temperature averaged 6.8°C.

Within-stand, pods were harvested randomly on 10 individuals per species at the end of March 2008. *Acacia tortilis* of Oued imezguene was represented by few trees, which did not bear pods. Therefore, pods of this species were collected from another stand, i.e. Oued Ihenkes.

1.2. Laboratory procedures

Collected pods were brought to the laboratory and visually sorted. The sound fraction was conserved at - 4°C until germination tests to prevent development of *Bruschidae,* which completely ravaged a previous collection of *Acacia tortilis* seeds.

2.2.1. Determination of seed weight and moisture content

a. Determination of seed weight

Tree replicates of 100 seed-lots per species were weighed to the nearest gramme. The obtained weight was expressed as 1000-seed weight.

Table 1.

Seed characteristics of *A. tortilis* and *A. nilotica*

species	Water content (%)	1000- seed weight (g)
A.tortilis	6.021	46.59
A.nilotica	6.773	198.59

b. Determination of moisture content

Three replicates of 50 seed-lots were randomly retained for each *Acacia* species. Seeds were weighed for fresh weight and then were put in an oven at 105°C for 24 hours in order to determine their dry weight. Moisture content was determined according to MAZLIAK (1998) as following:

$$Moisture\ content = \frac{fresh\ weight - dry\ weight}{fresh\ weight} \text{ x } 100$$

2.2.2. Seed pretreatments with sulfuric acid (H_2SO_4)

Prior to germination tests, seeds were soaked in 98 % pure sulfuric acid to remove integumentary dormancy (DANTHU et al, 1992, NDOUR, 1997) and then rinsed with distilled water. Preliminary treatments showed that immersing *Acacia nilotica* seeds for 60 minutes and those of *A. tortilis* for 30 minutes in sulfuric acid provided the best germination rates.

1.2.3. Germination tests

After integumentary dormancy breakage, germination tests were performed in different conditions of temperature; salinity and osmotic stress, at darkness, for a period of 15 days.

Each germination test was performed on Petri dishes covered with filter paper and comprised three replicates of 30 seeds. Germinated seeds were recorded daily during the period of the test. Seeds were considered as germinated when the radicle protruded from the micropyle.

Effect of temperature was tested for the following values: 15; 30; 40 and 45°C. Watering seeds with solutions of increasing salt, i.e. Nacl concentrations (0.50; 100; 150; 200; 250; 300, 350 mMol), tested effect of salinity. Water stress, i.e. osmotic stress was simulated by polyethylene glycol (PEG-6000) solutions of increasing concentrations (i.e. 0; 10; 20; 30; and 40 g/l). As the germination rate of *Acacia tortilis* was close to that of the control for the above-mentioned PEG-6000 concentrations, higher concentrations (i.e. 80; 120; 180; 240g and 300 g/l) were tested.

The osmotic potential was calculated in bars according to the equation of Michel and Kaufman (1973). Germination tests performed under salt and water stress were conducted at 30°C.

Two germination parameters were considered: (**1**) final germination (i.e. the maximum germination rate under the conditions of the experiment) and (**2**) mean germination time, in days, according to the formula of Mazliak (1998).

2.2.4. Statistical analysis

Normality of data was tested with Shapiro-Wilk normality test. An analysis of variance was performed on normal data followed by the Newman - Keuls test of multiple mean comparison at P= 0.05, where applicable. Non-normal data were analyzed with the Kruskal-Wallis non-parametric test followed by the Wilcoxon test, where applicable. The R software (version 3.4.1 / 2017) was used for all tests.

RESULTS

3.1. Effect of temperature on germination

Results (table 2) revealed highly significant differences between treatments and species, for the germination parameters under consideration (final germination, mean germination time in days). Seeds of both species did not germinate at 45°C. Final germination of both species were similar for all temperatures (ranging from 92 to 96 %) except 15°C under which *A. tortilis* showed a better value (i.e. 80%) than *A. nilotica* (i.e. 11%). Concordantly, mean germination time at 15°C was better for *A. tortilis* (i.e. 5 days) than for *A. nilotica* (i.e. 8 days). At 30° C also such parameter was better for *A. tortilis* (i.e. 1.88 days) than for *A. nilotica* (i.e. 5.57 days).

Table 2.

Effect of temperature on germination capacity (GC) and germination mean time (GMT) of *A.tortilis* and *A. nilotica*.

Species	Temperature (°C)	GC (%)	Statistical similarity	GMT	Statistical similarity
A.tortilis	30	99.2 ± 0.509	a	1.882± 0.109	d
A.tortilis	15	80 ±0.33	a	5.065 ± 0.11	bc
A. nilotica	30	92.2 ± 0.050	a	5.571± 0.075	b
A. nilotica	15	11 ± 0.019	b	8.055± 0.523	a
A.tortilis	40	92.1 ± 0.069	a	4.704± 0.239	c
A. nilotica	40	96.6 ± 0.033	a	5.475± 0.069	b

3.2. Effect of salt stress on germination

Results (table 3) revealed highly significant differences between species and treatments. Final germination of *A. nilotica* dropped from 80%, for the control, to 25.6 % for NaCl concentration of 100 mMol and to 4.4% at 200 mMol. Conversely, for *A. tortilis* a final germination of 94% was observed at 100 mMol, which decreased to 72.7% at Nacl concentration of 200 mMol and to 65.6% at 250 mMol.

Table 3.

Effect of NaCl on germination capacity (CG) and germination mean time (TMG) of *A. tortilis* and *A. nilotica*.

Species	**Concentration (mMol)**	**GC (%)**	**Statistical similarity**	**GMT**	**Statistical similarity**
A.nilotica	0	80 ± 0.033	a	5.81 ± 0.021	a
	50	55.6 ± 0.019	ab	7.167 ± 0.038	b
	100	25.6 ± 0	bc	7.773 ± 0.076	b
	150	5.6 ± 0.019	cd	9.427 ± 0.05	c
	200	4.4 ± 0.019	d	9 ± 0.028	c
	100	94.4 ± 0.069	a	2.603 ± 0.334	a
	50	93.3 ± 0.033	a	1.25 ± 0.219	b
	0	92.2 ± 0.107	ab	1.773 ± 0.200	c

	150	76.7 ± 0.050	abc	2.614 ± 0.277	d
A. tortilis	200	72.7 ± 0.033	bc	3.679 ± 0.177	d
	250	65.6 ± 0.0773	cd	4.443 ± 0.094	e
	300	24.4 ± 0.019	d	7.077 ± 0.617	f
	350	21.1 ± 0.038	d	6.01 ± 0.171	f

Globally, mean germination time increased with increasing salt concentrations for both species. In the case of *A. tortilis*, it ranged from 1.25 days, for a concentration of 50 mMol, to 7.08 days for a concentration of 300 mMol. In the case of *A. nilotica*, it ranged from 5.81 days, for the control, to 9.43 days for a concentration of 200 mMol.

3.3. Effect of water stress on germination

Results (table 4) revealed highly significant differences between treatments and species for final germination and mean germination time. Final germination decreased with increasing osmotic stress induced by PEG-6000. Seeds of *A. tortilis* tolerated higher concentrations of PEG-6000 than those of *A. nilotica*. Indeed, germination of the former species was ≥ 86.7% for PEG concentrations ranging between 10 and 180g/l; dropped to 26.7% for a PEG concentration of 240g/l and was nil for 300g/l while germination of the latter species dropped from 78.83%, for the control, to 21.07% for a PEG concentrations of 40g/l and was nil for PEG concentrations above 40g /l.

Mean germination time was of 1.73 days for the control and increased to 3.7 days for a PEG-6000 concentration of 240g/l in the case of *A. tortilis*. The same trend was observed in *A. nilotica* with mean germination time of 5.81 days for the control and of 9 days for a PEG-6000 concentration of 40g/l.

Table 4.

Effect of PEG-6000 on germination capacity (CG) and germination mean time (TMG) of *A. tortilis* and *A. nilotica* (B) (c 0: témoin. c1:10g/l. c2 :20g/l. c3: 30g/l. c4 :40g/l. c5: 80g/l. c6: 120g/l. c7: 180g/l. c8: 240g/l. c9: 300g/l).

Species	**PEG6000 Concentration (g/l)**	**GC (%)**	**Statistical similarity**	**GMT**	**Statistical similarity**
	0	92.20 ± 0.051	ab	1.727 ± 0.204	a
	10	94.30 ± 0.038	a	1.579 ± 0.018	a
	20	88.90 ± 0.038	bc	1.609 ± 0.128	a

A. tortilis	30	99.88 ± 0.019	ab	3.106 ± 0.321	b
	40	93.30 ± 0.067	ab	2.562 ± 0.192	c
	80	86.70 ± 0.033	bc	3.5 ± 0.166	c
	120	92.20 ± 0.019	ab	2.418 ± 0.010	c
	180	90.00 ± 0.069	b	2.225 ± 0.288	c
	240	26.70 ± 0.019	c	3.7 ± 0.144	d
	300	00.00			
A. nilotica	0	80.00 ± 0.069	a	5.81 ± 0.661	a
	10	68.90 ± 0.038	ab	7.167 ± 0.425	b
	20	68.90 ± 0.069	bc	7.773 ± 0.516	b
	30	34.40 ± 0.051	cd	9.427 ± 0.827	c
	40	21.10 ± 0.050	d	9 ± 0.748	c

DISCUSSION

4.1.Germination speed:

Germination revealed faster with *A. tortilis* seeds in almost all cases. This could be an important adaptive trait in an environment where annual precipitations can be concentrated on few days and even few hours. *A. tortilis* seeds are smaller and lighter than those of *A. nilotica* (see table 1). The relative slowness of *A. nilotica* germination can be explain by its slower seed imbibition comparatively to *A. tortilis* seeds as reported by Wilson and Witkowski (1998). A thick integument with a large sclerotized palisade parenchyma may be an impediment to water penetration into the embryo as showed by Venier et al (2012) on *Acacia aroma*, *A. caven* and *A. atromentoria*.

4.2. Effect of Temperature

Results showed that 30°C is the optimum temperature for germination of the two *Acacia* species. At 40°C germination took place even slowly while a temperature of 45°C did not lead to germination. Such temperature threshold could be therefore considere as extreme. *Acacia nilotica* revealed more sensitive to decreasing temperatures since its germination dropped to 11% while *A. tortilis* seeds reached 80 % of germination. This may explain the absence of *A. nilotica* above an elevation of 1200 m as stands. Results presently found on *A. tortilis* are concordant with those of previous studies (Abulfatih,

1995; Bensaid, 1988; Dhantu et al, 2003; Jouadi et al, 2010, 2013; Ndour and Danthu, 1998 and Teketay, 1996, 1998).

4.3. Effect of salinity

Salt had an inhibitory effect on germination of both species. However, their tolerance level was different. *A. nilotica* did not tolerate salinity higher than 150 mMol whereas germination of *A. tortilis* reached 21% for salt concentration of 350 mM equivalent to 21 g/l.Ndour and Danthu (1998), In their study on 9 *Acacia* species, showed that germination rates of *A. nilotica* var *tomentosa* and *A. nilotica* var *adansonii* seeds significantly dropped for salt concentrations of 5 to 10 g /l. Jaouadi et al (2010 and 2013) observed a similar trend on *A. tortilis*. Salt stress significantly reduced germination rate from 9g/l of Nacl but seeds maintained a final germination of 21% at 22g /l. In addition to reducing germination, salt slowed down its speed. This is especially obvious in the case of *A. nilotica* (See Table 2).

Effect of salt on germination results from a difficulty of seed hydration due to a high osmotic potential and the negative action of Na^{+} and Cl^{-} ions on structures and cellular enzyme complexes (Manchanda and Garg, 2008; Moraisa et al, 2012). The difference in tolerance between the two species can be explained by a higher concentration of Ca^{++} in seeds of *A. tortilis* comparatively to *A. nilotica* as shown by Rehman et al (2000).

4.4. Effect of water stress

The two species presently studied did not show the same behavior to increasing water stress. *Acacia nilotica* germination decreased to 21.1% for a PEG-6000 concentration of 40g/l while that of *A. tortilis* decreased to 26.63 % for a PEG-6000 concentration of 240 g/l, equivalent to -6.26 bars according to the formula of Michel and Kaufman, and was nil for a PEG-6000 concentration of 300 g/l, equivalent to -9.49 bars.

These results indicate a higher resistance of *A. tortilis* seeds to osmotic stress during germination. Concordantly, Ndour and Danthu (1998) found the same trend for both species. On the other hand, the threshold value of water potential tolerated by *A. tortilis* (i.e. - 8 bars), according to Jaouadi et al (2010), is slightly higher than that of the present study since we observed no germination at -9.49 bars, while that found by Kebbas et al(2013) i.e.- 10 bars is closer to our finding.

CONCLUSION

In the light of the present study, it appears that A. tortilis germinates faster, under a wider range of temperatures and tolerates higher water and salt stress than A. nilotica. This trend may be one of the explanatory factors for its wider distribution in the Algerian Sahara. Furthermore, Zetta et al (2017) found the same trend on two Acacia species (A. ehrenbergiana and A. seyal) of the same region (i.e. Hoggar) with a more extensive distribution and a better adaptive capacity of A. ehrenbergiana. This may imply that the ongoing climate change could lead to changes in vegetation structure because of increased temperature and aridity. Species such as A. tortilis and A. eherenbergiana would replace other species at higher elevation but could also migrate northwards, replacing species less adapted to extreme drought.

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