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# Seed germination and viability in two African *Acacia* species growing under different water stress levels

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*Acacia* species are important in forestation programs and for producing non-timber forest products in arid and semiarid zones, but few studies have been carried out concerning the effects of drought in the germination in order to understand the regeneration process of the species. In this paper, we studied the morphology and the germination pattern under different water stress of *Acacia senegal* and *Acacia seyal.* Seeds were subjected to a water stress test for 45 days with four levels of water potential achieved by different concentrations of polyethylene glycol 6000. The germination process was studied by adjusting a Gompertz function, and obtaining related parameters of the curve (total germination, maximum germination rate and the t value corresponding to the inflection point of the curve, and time in reaching the, 50 and 90% of the total germination). The germination process in these species was rapid; there were no significant differences in any of the parameters of the curves depending on the stress treatment except for the total germination. Total germination was higher in *A. senegal*, and this species was more sensitive to the water availability than *A. seyal*, as deduced from the reaction norms in the two environments. The probability of germination was also modeled by a logistic regression, indicating the higher values for non stressed seeds. A consistent pattern is detected among the treatments. The results presented in this paper could be applied in forestation programs to improve germination in nurseries, and by incorporating the logistic models in more complete models describing the dynamic of regeneration under natural conditions.

**Key words:** *Acacia*, water stress, Logistic regression, germination, regeneration.

## INTRODUCTION

Different factors are affecting recruitment in forest species and the establishment of new forest areas, among which dispersal, predation and germination are essential to many species (Blate et al., 1998). One of the main processes is germination, because we can infer some information on the strategy of the species to cope with drought (avoidance, tolerance), and also, knowledge has important implications in the management of the seed during nursery (Kozlowski and Pallardy, 2002; Kozlowski, 2002; Choinski and Tuohy, 1991; Boydak et al., 2003; Sy et al 2001)

Africa is one of the most vulnerable continents to climate change and climate variability, this vulnerability aggravated by the interaction of multiple factors, including the growing deforestation and water stress occurring at various levels, and low adaptive capacity. The deforestation and his consequences are serious problems in developing countries, given that their economies are based on agriculture, and climate changes impose additional pressure on water demand in Africa (Boko et al., 2007). The lack of systematic efforts to conserve and manage resources is a major concern, and in few cases, efforts have been made to cultivate species that yield non timber forest product (Michael and Tadesse, 2004). For many African species with high importance in aforestation or management program, we lack information in regard to the drought stress impact, specially, on *Acacia senegal and Acacia seyal* species.

Non-timber forest products (NTFP) play an important

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role both in the rural economy and population of the African country, in Ethiopia, it play a vital role in the livelihoods of communities contributing to food security and household income (Michael and Tadesse, 2004). Among the species that produce these products; *A. senegal* and *A. seyal* produces gum Arabic and gum talha, respectively. The Arabic gum obtained from species *A. senegal* is used in the food industries and gum talha obtained from *A. seyal* is used in non-food industries, besides the importance of their products, these species adapt to very erratic weather conditions and is considered as species protective environmental conditions (Eisa et al., 2008) and its wood are highly preferred by rural populations as source of fuel and to construct agricultural implements etc.

We have selected two *Acacia* species to address this question, differing in size, dormancy and other life-history traits. *A. senegal* (Linne) and *A. seyal* (Del) produces valuable NTFP. Given the importance that have both national and international level, these species in Ethiopia, due to overexploitation and the problems associated with the process of installing and growing plantations on the extreme dry conditions, is believed to be in danger of extinction in the future (Personal observation). Our hypothesis is that species under arid conditions will show a clear strategy for avoidance (by a very fast germination) or tolerance (by a low germination process).

The objective of the study is to analyze the germination curves and the probability of germination under different water stress conditions of *A. senegal* and *A. seyal*. The analysis of this process will allow us to infer the strategy of the species in relation to drought stress, and also to provide models of germination under different water stress conditions to be included in general models for the regeneration process of the two species.

## MATERIALS AND METHODS

### Description of species, their importance and precedence

Two *Acacia* species has been used in this study. *A. senegal* is a small tree (10-15 m height) distributed from Senegal to red sea, east and South Africa b/n 11 and 16° North from 500 - 1700 m.a.s.l., poor natural regeneration. It is resistant to drought (500 - 1000 mm of rainfall). The species produces Arabic gum (used as a stabilizer in food and pharmaceutical industries and in printing and textile industries) and the wood is highly valued by rural populations as fuel for firewood and charcoal. *A. seyal* is a smaller tree (up to 9 m height), distributed from Senegal to the entire Sahal, Sudan and Egypt, East Africa from Somalia to Mozambique from 0 - 2100 m a.s.l. It is also resistant to drought, with a broader niche (250 - 1000 mm of rainfall) than *A. senegal*. *A. seyal* produces Talha gum (used as a stabilizer in non-food industries) of lower quality than Arabic gum, and the wood is also used as fuel for firewood and charcoal (Argaw et al., 1999)

One population group from each of the species was sampled in southwest Ethiopia (Langano and Shala populations) in the East Shewa region which is classified as a semi-arid area. Langano population (7° 26' N-38° 47' E), is located at 1749 m a.s.l., with a rainfall of 500 - 600 mm, and minimum and maximum monthly

mean temperature of 13.8 and 38°C. Shala population (7° 32' N-38° 40' E) is located under similar conditions: 1620 m a.s.l., 600 mm of rainfall, and minimum and maximum monthly mean temperature of 13.8 and 38°C. Seeds were provided by the National Forest Research Institute of Ethiopia from commercial seed-lots, and stored in a cold chamber at 6°C. The *A. senegal* seeds are bigger (8.62 gr/100 seeds, or 11.600 seeds/kg, length: 8.76 ± 0.95 mm, width: 7.67 ± 0.82 mm) than the *A. seyal* seeds (5.18 gr/100 seeds or 19.305 seeds/kg, length: 6.77 ± 0.77 mm, width: 4.01 ± 0.49 mm). Seeds of the two populations are bigger in comparison to the mean values of the species (18.000 seeds/kg for *A. senegal*, and 22.000 seeds/kg for *A. seyal*), indicating the arid conditions of the two populations.

### Germination process under different drought stress treatments

Germination was tested in a factorial design with Species (2 levels) and drought stress (4 levels) as factorial treatments. The four levels of water potential ( =0 or control, - 4, -8 and -12 bars respectively) were simulated by adding different amount of Polyethylene Glycol 6000 (PEG), to obtain different water stress level (Michel and Kaufmann, 1973; modified by Michel, 1983). These treatments mimic from no stress to very severe water stress (-12 bars).

For each species and drought stress, 100 seeds were used, for a total of 400 seeds per species, and 800 seeds in total. The experimental unit consisted of 25 seeds in Petri dishes (10 cm-diameter on a filter paper) and randomly arranged in four replicates in a germination chamber IBERCEX V-900-D. The conditions for the germination test were: 30°C temperature, a constant relative humidity of 80% and a photoperiod of 12 h. Germination was checked daily, and a seed was considered as germinated when the radicle emerged at least 1 mm from the integument. The test lasted for 45 days from 21/01/2009 until 03/03/2009 (but no germination was recorded after day 20). A Tetrazolium viability test was performed to all the non-germinated seeds in order to quantify the no germinating seeds under drought stress.

The *A. seyal* seeds were submitted to a pre- germination treatment with boiled water (at 100°C) and then left at room temperature for 24 h (Forest Research Directorate, 2000, internal document) to break the dormancy, which is the regular method used for this species. Otherwise dormancy could hide drought stress impact. Seeds were disinfected by applying the methodology proposed by Villamediana et al. (2007) before starting the germination analysis. The solution and the filter paper were changed every four days, to maintain the water potential constant throughout the entire duration of the experiment and prevent hyper-concentration processes, in agreement with Falleri (1994) and Bravo et al. (2010).

### Data analysis

Germination at a given day t was adjusted for each experimental unit (Petri dish) by non-lineal regression to a Gompertz function (Draper and Smith, 1981) with three parameters: c, the predicted germination (asymptote of the curve); b, closely related (proportional) to the maximum germination rate standardized by the total germination (b germination$_{max}$/c); m, the t value corresponding to the inflection point of the curve (date at which maximum growth rate is reached).
The initial parameters (c, m and b) were estimated based on the optimization of the sum of squares of the residues minimized by the Levenberg-Marquardt algorithm (Wolfram, 1999). To test the precision of the model, the value of pseudo-R$^2$ was calculated for each fit. Three other variables were derived for the germination data: b, t50 and t90: being, respectively, the date (in days) corresponding to germination of 50 and 90% of the total. An

**Table 1.** Descriptive values for the different variables derived from the germination of each experimental unit curve.

| Variable | A. senegal | | | | A. seyal | | | |
|---|---|---|---|---|---|---|---|---|
| | mean | std | max. | min. | mean | std | max. | min. |
| c | 16.84 | 6.56 | 25.15 | 6.00 | 8.81 | 4.00 | 15.28 | 2.00 |
| b | 2.79 | 5.79 | 17.75 | 0.15 | 3.32 | 4.51 | 14.64 | 6.31 |
| m | 0.11 | 3.53 | 3.19 | 0,36 | 2.42 | 0.50 | 3.19 | 1.16 |
| t50 | 0.83 | 3.01 | 3.58 | 0,45 | 2.73 | 0.52 | 3.63 | 2.00 |
| t90 | 4.55 | 2.15 | 8.73 | 0.96 | 4.33 | 1.79 | 9.55 | 2.32 |
| $R^2$ | 93.8 | 8.4 | 100 | 69.6 | 94.8 | 7.1 | 100 | 74.36 |

c: total germination, b: maximum germination rate, m: date at which maximum growth rate is reached t50 and t90: date at which germination reaches the 50 and 90% of the total germination. $R^2$: regression coefficient of the adjusted curves.


**Table 2.** Analysis of variance of the variables describing the germination process in *A. senegal* and *A. seyal* under different watering regimes. Mean squares and significance values of the F-test.

| Variable | Species | | Drought stress | | Interacción | |
|---|---|---|---|---|---|---|
| | Squ.of mean | p value | Squ. of mean | p value | Squ. of mean | p value |
| germination | 457.53 | < 0.0001 | 162.78 | < 0.0001 | 44.78 | 0.0403 |
| c | 558.18 | < 0.0001 | 131.54 | 0.0006 | 32.64 | 0.1357 |
| b | 2.78 | 0.776 | 75.17 | 0.1104 | 21.7 | 0.598 |
| m | 35.56 | 0.025 | 16.34 | 0.06 | 12.68 | 0.115 |
| t50 | 0.45 | 0.9017 | 52.62 | 0.1768 | 43.09 | 0.2498 |
| t90 | 701.82 | 0.3311 | 719.93 | 0.4057 | 610.68 | 0.4771 |

c: total germination, b: maximum germination rate, m: date at which maximum growth rate is reached t50 and t90: date at which germination reaches the 50 and 90% of the total germination. $R^2$: regression coefficient of the adjusted curves. *** P < 0.001; ** 0.01 > P >0.001; * 0.05 > P > 0.01; n.s. 0.05 > P

analysis of variance was performed to the different variables (c, b, m, t50 and t90) according to the following factorial model (Equation 1):

$$Y_{ijk} = \mu + \alpha_j + \alpha_i + \alpha_{ji} + \varepsilon_{ijk} \qquad (1)$$

where: $Y_{ijk}$: Value of the variables for the $i^{th}$ species under the $j^{th}$ water stress treatment and the $k^{th}$, and Petri dish; $\mu$: grand mean; $\alpha_j$: drought stress (j = 1 to 4); $\alpha_i$: species (l = 1 to 2) and the error term; $\varepsilon_{jk}$ N (0, $\sigma^2_j$). A Tukey-Kramer multiple comparison test was applied to the main factors if they were statistically significant and the interaction was graphically analyzed.

In the second step, the probability of germination (P) was determined by independent logistics models for each species. Two explicative variables were used: Drought stress (expressed as water potential in MPa), the time and the interaction (Equation 2):

$$P = \cfrac{1}{1 + e^{-(\beta_0 + \sum\limits_{i=1}^{n} \beta_i X_i)}} \qquad (2)$$

The models were tested using the change in the value of -2 log of the likelihood between the model with and without explicative variables (Hosmer and Lemeshow, 1989). Starting from the results of the logistics regression, the probability of germination was analyzed graphically for each species in a specified time, for each treatment. The value of the area below the receiver operating characteristic curve (ROC curve) was used to estimate the precision of the models adjusted. All the analysis was conducted by using the SAS software (SAS Institute Inc, 2004).

## RESULTS

Seed germination occurred after 4.5 days in *A. senegal* and 4.3 days in *A. seyal*. Ninety percent of the total germination was reached and no new germination was observed after 20 days of the experiment (Table 1).

The Gompertz model was very precise in describing the germination process ($R^2$ > 90% in both species). The analysis of variance of the variables showed significant differences in total germination and c Gompertz model parameter. P-values for total germination were under 0.0001 for species and drought stress and equal to 0.0403 for the interaction. The p-values for the c para-meter were under 0.001 for species and drought stress and not significant for the interaction, p-value equal to 0.01357 (Table 2). However, there were no significant differences in b, t50 and t90 for any factors.

The germination of *A. senegal* was greater (mean value 99.0 ± 2.00 in the control treatment) than that of *A. seyal*
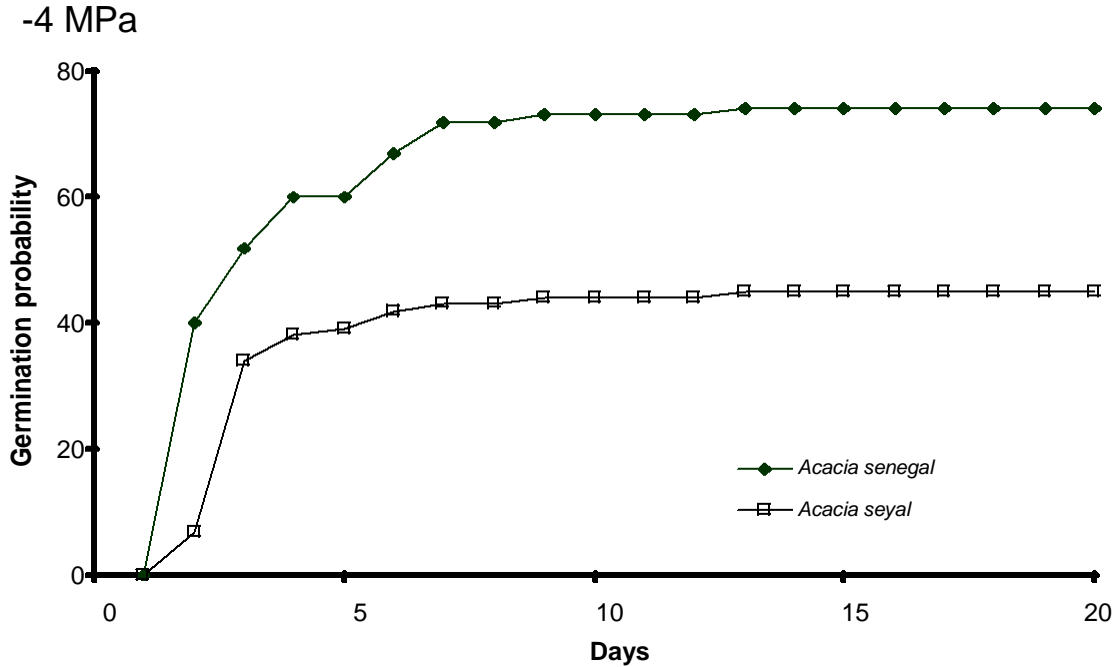
-4 MPa



**Figure 1.** Germination curve at *A. senegal* and *A. seyal*.

**Table 3.** Logistic regression results to the species of *A. senegal* and *A. seyal* under water stress conditions.

| Parameter | DF | Estimate | Standard error | Wald chi-square | Pr > chi-square |
|---|---|---|---|---|---|
| | | | *A. senegal* | | |
| Independent term | 1 | -0.4891 | 0.1550 | 9.9559 | 0.0016 |
| Drought | 1 | 0.1007 | 0.0254 | 15.6682 | <.0001 |
| Day | 1 | -0.1418 | 0.0385 | 13.5816 | 0.0002 |
| Drought*Day | 1 | 0.0195 | 0.00660 | 8.7125 | 0.0032 |
| | | | *A. seyal* | | |
| Independent term | 1 | -1.6820 | 0.2011 | 69.9480 | <.0001 |
| Drought | 1 | 0.0627 | 0.0301 | 4.3302 | 0.0374 |
| Day | 1 | -0.2551 | 0.0391 | 42.5996 | <.0001 |
| Drought*Day | 1 | 0.000478 | 0.00577 | 0.0069 | 0.9339 |

(44.0 ± 17.59). The norm of reaction of the germination according to water stress can be seen in Figure 1. The comparison of these values in respect to the value obtained in the control treatment showed that *A. seyal* did not decrease its percentage until a value of -8 MPa, more quickly than in *A. senegal,* with a decrease in germination for the most severe treatment were very different: for *A. senegal* the germination is only 35% of the control (35.0 ± 12.38) in comparison to a higher value (59%) of the control (26.0 ± 17.74) in *A. seyal*. The intermediate treatments (-4 and -8 MPa) showed a response in the middle of the two severe treatments (mean value of 73.0 ± 16.45% and 57.0 ± 17.09, respectively in *A. senegal*, and 46.0± 13.27% and 29.0 ± 13.61). The viability test applied

to the non-germinated seeds showed that for *A. senegal*, the seeds that did not germinate were not viable at the end of the test, but for *A. seyal*, 50% of the non-germinated seeds were viable.

The logistic regression analysis showed that seed germination in function of a specific time period was lower as time increased, yielding a decreasing tendencies in each treatment. In *A. senegal* drought stress and time, and their interaction, had a significant effect on germination. However, in the case of *A. seyal*, the interaction had no significantly influence on germination (Table 3 and Figure 2), while the results for the rest of the independent variables were the same as in the previous case. The value of the area under the ROC curve were c = 0.85 and
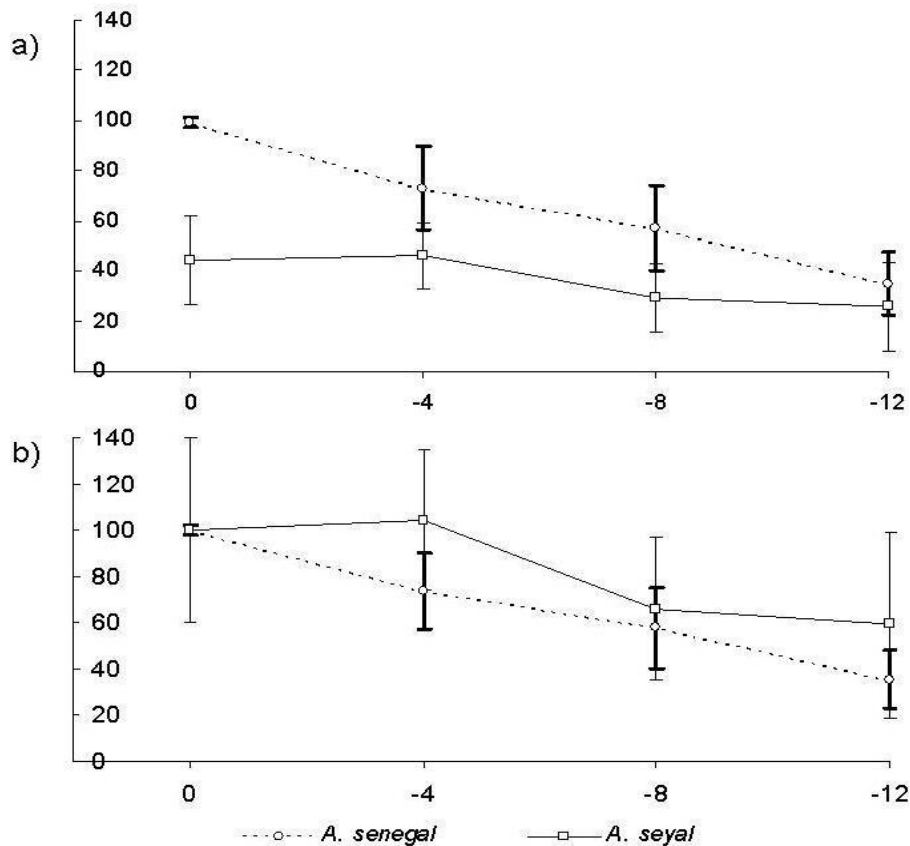
**Figure 2.** Germination for each species and treatment (a) and for each species in relation to the control b.

and c = 0.81 for the species *A. senegal* and *A. seyal* respectively.

## DISCUSSION

Different studies focused on the effect of bush burning on the germination of various species of the genus *Acacia* (Danthu et al., 2003). However, they do not provide information on the effect of water stress (an essential environmental factor in the distribution range of the species) on the germination of *A. sengal* and *A. seyal* species. This paper, analyses four potential effects depending on the intensity of water stress conditions obtained with Polyethylene glycol, including control, on the germination process of this two *Acacia* species.

Germination was different depending on the species, and also a different pattern of viability of non-germinated seeds was found at the end of the experiment. The higher percentage of germination of the species *A. senegal* in comparison to *A.* seyal has been previously reported (Argaw et al., 1999; Teketay, 1996; Zida, 2007), but in our study, the difference in viable seeds among the two species (no viable seeds detected at the end of the experiment in *A. senegal*, and a value of 50% for non-

germinated seeds for *A. seyal*) indicate that this pattern is caused by a heavy induced dormancy in the latter. Most *Acacia* species are characterized by a very hard and impermeable seed coat, which result in temporary dormancy and influences the germination process (Aref, 2000; Argaw et al*.,* 1999; Owens et al*.,* 1995). In *A. seyal*, the application of severe treatments (stronger than the applied in our study) is needed to break the dormancy and speedup the process of germination. The very hard and impermeable seed coat could act as a protection against water stress, with little germination under water stress conditions to prevent problems during the embryo development.

The size of the seeds (measured as the number of seeds/kg) of the two *Acacia* species are larger than those indicated in previous studies (Argaw et al., 1999), suggesting the more arid conditions of the analyzed populations to those previously reported, as shown in *Acacia nilotica (*Miller et al., 2002, Mahamood et al., 2005); *Cordia africana* (Loha et al., 2008), or among populations of the same *Acacia* species (Mahamood et al*.*, 2005). Therefore, we can interpret the result as derived from populations adapted to drought conditions, as a view to explore strategies of the species under stressful conditions.

The analysis of variance showed a decrease in germination as the degree of water stress increases for the two species. This is much more pronounced in the extreme water stress in the case of *A. senegal* (reduction of germination of 64.6% in the level at -12 MPa with respect to the control) than in *A. seyal* (reduction of 40.9% with respect to the control) . This could be related to greater tolerance to drought in *A. seyal*, as shown in leguminous species from sub- Saharan areas where a clear reduction of germination is found depending on the water stress (Sy et al., 2001, in *Cassia abtasifolia, C. occidentalis, Indigofera senegalensis, I. astragalina, I. tinctoria, Sesbania pachycarpa* and *Tephrosia pururea*).

No significant differences have been found in most of the parameter estimated from the Gompertz model (both the inflexion point and the rapidity of growth, as well as the time to reach different germination rates). The analyzed *Acacia* species reached a total germination over 90% in only 5 days. No differences between species and treatment were found. This might be an avoidance strategy to water stress, related to the environment in which the plant will develop.

Under arid condition, these species take advantage of favorable conditions (humidity and temperature) to germinate rapidly. However, we can distinguish two different water -stress avoiding strategies: *A. senegal* produces a rapid germination of all the viable seeds, but for *A. seyal*, the germination under stressful conditions is more heterogeneous (higher std) and is more higher in comparison to the control, and it is limited by an induced dormancy; these non-germinated seeds would germinate under more favorable conditions. Rapid seed germination in arid and semi-arid areas seems to be frequent. This was demonstrated in the study performed with some species (*Spartium junceum* L.) in which germination began at 4 days with 5% and reached a total of 67% over the 18 days of the experiment (Travlos et al., 2007). In *Pines* and Mediterranean *Oaks* under arid conditions, the process is not so rapid, but we can find the strategy of a more irregular and extended germination over time (Boydak et al., 2003).

The probability of germination indicates a rapid decay when the stress increases, and also the large differences among the two species, with implications in the regeneration process. In *A. senegal* the probability of germination under non-stress conditions is quite high and therefore, this species could regenerate easily in such conditions. However, for *A. seyal*, the germination is highly depending on the conditions: The covers protect the embryo from the drought, but it leads to a low probability of germination even under no stress conditions (similar to the values reached by *A. senegal* under the -12.0 Pa treatments.

It can be concluded that water stress has a negative effect on *Acacia* germination in arid and semiarid environments, but the reduction is not enough to impede germination of the seeds if the drought conditions are not prolonged over time. We also detect two different avoiding water stress strategies. All viable seeds of *A. senegal* geminates quickly, in order to be installed as soon as possible based on the seed reserves. However, *A. seyal* with smaller seeds and a heavy and impermeable coat, reacts by inducing dormancy under the heaviest water stress conditions. The analysis of the Gompertz functions as well as the logistic model, describe the process of germination under water stress conditions, and these statistical tools could be included in restoration and management programs in order to favour the conservation and sustainable use under drought conditions.

## ACKNOWLEDGEMENTS

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