

## Comparative study on seeds germination of *Stipa tenacissima* L. from two Western Algerian's Habitats

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Received: August 19, 2015

Accepted: October 19, 2015

### ABSTRACT

The aim of our work is to study and compare the germination behavior of seeds of two different ecotypes under various temperatures.

The temperatures tested vary from 5 to 30°C, the study is conducted over a period of 30 days under laboratory-controlled conditions. The studied parameters are: the kinetic and capacity of germination, the speed of germination and latency time.

The seeds from steppe region have a slightly greater germination capacity than those from littoral. This difference was not confirmed by the variance analysis ( $P > 0.05$ ), except at 5°C and 10°C, where this difference is significant. The speed of germination from the two stations are similar at 25°C and 30°C, where the caryopses from the littoral station have higher values than those from the steppe region ( $P < 0.01$ ) and finally between these two stations, any variability in the latency values was detected at different temperatures ( $P > 0.05$ ).

**KEYWORDS:** *Stipa tenacissima* L. - seeds – germination - temperature.

### I. INTRODUCTION

Soil erosion is the main determinant of land degradation in the arid and semi-arid regions, which are widespread in the Mediterranean Basin. One of the foremost causes of soil erosion and desertification is human activity (agricultural practices, overstocking, abandonment of fields, forest fires), which leads the degradation or destruction of the natural plant cover [1, 2].

One way to regenerate degraded soils, reduce soil erosion and prevent the spread of desert-like conditions is by means of revegetation [3]. The first step in any programme of rehabilitation of soils degraded by erosion is to select the most suitable species. This will be based on the capacity of these to germinate and establish themselves in the given environmental conditions, leading to improved vegetation and soil properties [4].

Esparto grass (*Stipa tenacissima* L.) or Alfa is one of the key species of the steppe; it is a Mediterranean endemic perennial xerophytic gramineous. It is considered one of the bulwarks against the encroachment of the desert [5] without forgetting its economic importance because its leaves are used for the fabrication of paper [6].

In Algeria, the area covered by the esparto grass was evaluated at four million hectares. This figure already advanced by Boudy [7] is probably much lower today. Indeed, because of their difficulty to regenerate, including seedlings [8], alfa steppes had been declining and we are seeing a continual loss of vegetation cover and therefore a low plant diversity [9, 10]. This decline is also due to stringent conditions (prolonged dry period), the increasing anthropogenic pressure and excessive overgrazing [11]. Certain characteristics of *S. tenacissima*, such as the protection of soil against erosion, its resistance to long drought periods and its ecological amplitude (soils, climates, slopes) [12].

Germination is a critical stage in the life cycle of plants particularly in arid and semi-arid land, which is subjected to various adverse conditions, and this has serious impact on the subsequent stand of vegetation. However, the environmental conditions of the area of species occurrence are essential to determinate the seed characteristics and its germination responses. Mainly the temperature can promote or inhibit the germination and then influence the seed germination process [13, 14].

The work of Kadi Hanifi conducted on biological and phytogeographical diversity of training at *S. tenacissima* in Algeria reported the installation of this steppe species in northern Algeria (in forests and coastline) that certainly explained by the recesses of bioclimatic floors but also by the probable existence of ecotypes in the esparto grass. A complementary way of work already undertaken and in order to fill the knowledge built on this species, the aim of our study is to determinate and compare the most adequate ranges of temperature for the germination under controlled conditions for two different provenances (steppe and littoral) of *S. tenacissima*. It should be noted that no work has been done on the germination of coastal alfa seeds.

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## II. MATERIEL AND METHODS

### Stations of seed's harvest

*S. tenacissima* mature seeds were collected from two different provenances originating in the western region of Algeria, with the different geographical localizations. The name of the studies accessions, their geographical localization and the climate characteristics of the collect site were summarized in Table 1. After collection, seeds were stored under ambient temperature.

**Table 1:** Characteristics of the stations

Stations (St)	Town	Bioclimatic stage	Latitude (North)	Longitude (West)	Altitude (Meter)	Exposition
St 1 : steppe of Ras El Ma (place called : Kerzouta)	Tlemcen	Arid (ONM, 2014)	34°31'538"	000°54'882"	1113	South-East
St 2 : coastline of Béni-Saf	Ain Témouchent	Semi- aridsuperior (ONM, 2014)	35°19'667"	001°19'617"	533	North-West

### Seeds preparation and unfolding of tests

Previous to the germination test, the seeds were disinfected in 2% sodium hypochloride solution for 1 minute and then washed with distilled water three times (1 minute each).

A total of 450 seeds were used. The seeds were placed on filter paper in 9 cm Petri dishes moistened with different quantities of distilled water selected according to different temperatures. A total of three Petri dishes were arranged per treatment with 25 seeds each. Then, the Petri dishes were placed in a dark incubator, maintained at different temperatures: 5 °C, 10 °C, 15 °C, 20 °C, 25 °C and 30 °C. It is well-known that a seed is proven to germinate at the emergence of the radicle (radicalelength > 1 mm) and the counting of germination was performed daily in each Petri dishes for 30 days.

### Data and statistical analysis

The results were expressed by the following parameters: the germination capacity, the speed of germination, the latency time and the mean time to germination. The comparison between the average values of the above mentioned parameters and the analysis of variance were conducted using Minitab 17 (Version 17.0.1).

## III. RESULTS AND DISCUSSION

### Temperature effect on the germination kinetic

Regardless of the temperature, the germination of the alfa seeds from the two stations runs through three phases: a latency phase; aexponential phase and a stationary phase.

We note that the germination of the *S. tenacissima* samples from the two stations is possible in a range of temperatures from 5 to 30 °C, the optimum temperature is between 20 and 25 °C, and the germination greatly weakens at extreme temperatures: below 5 and beyond 30.

The germination kinetics of seeds from the two stations is comparable; however, some differences are detected in evaluation parameters (Fig. 1).

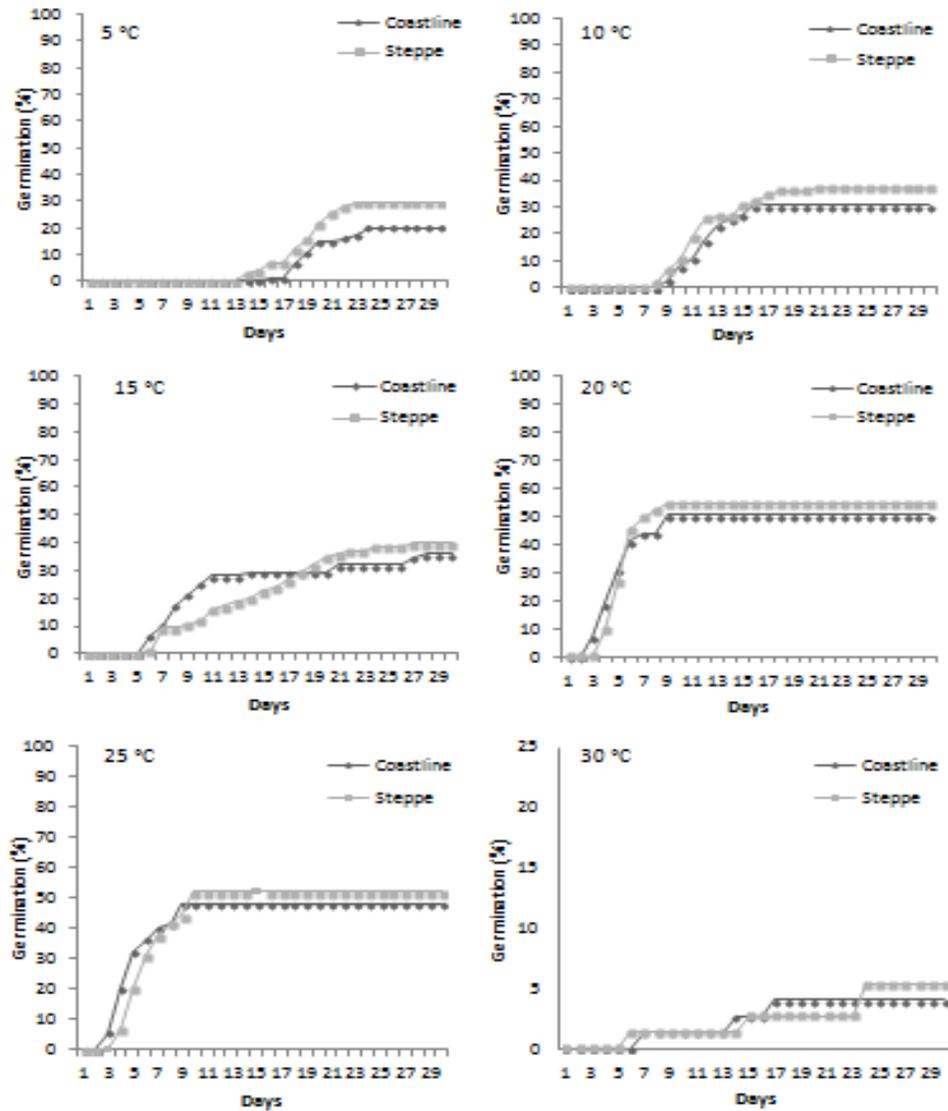


Figure 1: Curves of germination kinetic of seeds from the two stations tested at different temperatures.

**Capacity and speed of germination**

The capacity and speed of germination of seeds are respectively represented by figures 2 and 3.

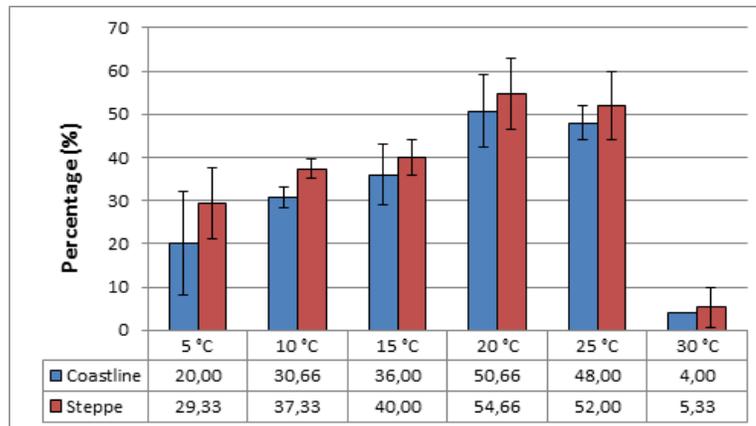


Figure 2: Temperature effect on the germination capacity (final germination percentage).

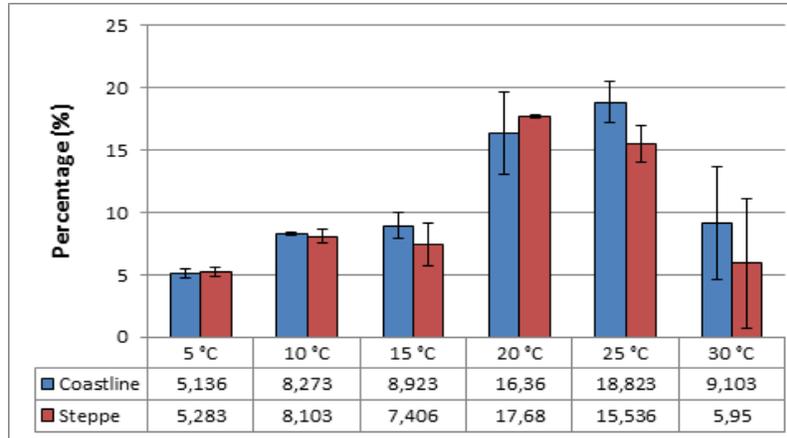
Figure 2 shows that the average capacity of seeds germination from the two stations varies according to the tested temperatures ( $P < 0.05$ ).

At 20 °C and 25 °C, we get optimum values of germination of the seeds from the two stations. At 20 °C, the recorded germination capacity of the seeds from the steppe (Ras El Ma) is  $54.66 \pm 8.326\%$  and it is  $50.66 \pm 8.326\%$  for the ones from the coastline (Beni-Saf). At 25 °C the germination capacity become  $52 \pm 8\%$  for the seeds of the steppe region and  $48 \pm 4\%$  for the seeds from the coastline.

The lowest percentages of germination are recorded at 30 °C: 5.33% for the seeds from steppe region and 4% for the seeds from coastalregion.

The seeds from steppe region have a slightly greater germination capacity than those from coastline. This difference was not confirmed by the variance analysis ( $P > 0.05$ ), except at 5 °C and 10 °C where this one is significant ( $P < 0.05$ ).

Figure 3 indicates for seeds of each station a variability of germination speed at different temperatures ( $P < 0.05$ ).



**Figure 3:** Temperature effect on the germination speed

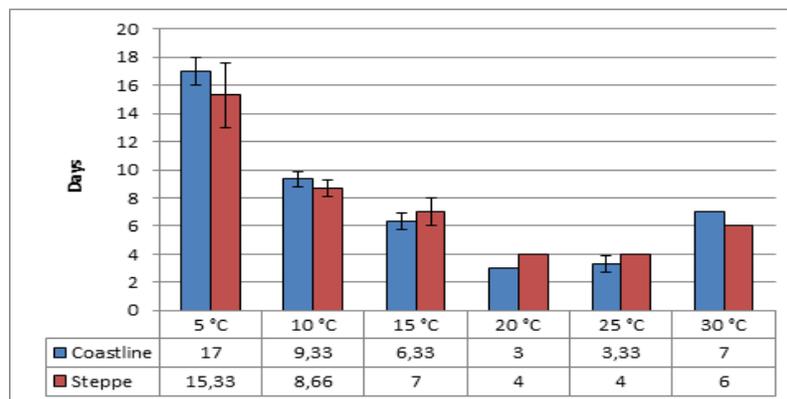
For the seeds from the coastal station, the most important values are achieved at temperature 25 °C ( $18.82 \pm 1.66\%$ ) and 20° C ( $17.68 \pm 0.11\%$ ) for the steppe station. The lowest values are noted at 5 °C for seeds from the coastal station ( $5.136 \pm 4.53\%$ ) and for the steppe station ( $5.283 \pm 5.18\%$ ).

The speed germination from the two stations is similar. Except at 25°C and 30°C, the seeds from the coastal station have higher values than those from the steppe region ( $P < 0.01$ ).

**Latency time**

The analysis of variance revealed a significant variability of the average latency time depending on the temperatures for each station ( $P < 0.05$ ). Between these two stations, any variability in the latency values was detected at the different temperatures ( $P > 0.05$ ).

The optimal germination temperature (20 to 25 °C) show the shortest time of the latency ( $3 \pm 0$  days in the coastal station and  $4 \pm 0$  for the steppe station). The longest times of the latency are recorded at 5 °C ( $17 \pm 1$  day for the coastline and  $15.33 \pm 2.3$  for the steppe). Intermediate values characterize the other temperatures.



**Figure 4:** Temperature effect on the latency time

The results obtained show that the temperature significantly influences the germination of the esparto of the two stations, the optimal temperatures are between 20°C and 25°C.

These results join those found at other species belonging to the kind *Stipa* [15, 16], we observed that the germination is always possible but weak at 30 °C for the seeds of the two regions. According to the classification of Come [17], we can say that the esparto appears among the species which present thermo-dormant seeds, meaning seeds which do not germinate at temperatures higher than 35°C. However, the percentages of germination very reduced at 30°C can be due to a secondary dormancy induced on the seeds which were beforehand ready to germinate [18, 19].

This induced dormancy can be regarded as a strategy developed by the species in order to reduce germination during the period of the year where the chances of survival are low [20]. Indeed, the very high temperatures during the summer deteriorate several éco-physiological processes relating to the ability of the caryopses to germinate and which lies in the activity of proteins and the enzymes of the membrane. In this context, the personnel observations of Gasque and Garcia-Fayos [20] and the results obtained by Mehdadi et al. [8] found that the germination of *S.tenacissima* misses completely before autumn.

At the optimal temperatures of germination (20 and 25 °C), the germination of the seeds of the two stations begins at the end of the 4th day and reaches the maximum of germination at the end of the 9th day. We can conclude that our species develops a strategy to germinate quickly when the conditions are really favorable.

Our results are in agreement with those observed at different perennial poaceae from the high Algerian steppe plains like *Lygeumspartum* L. [21], *Aristidapungens* L. [22], *Stipa tenacissima* L. [23] and from the coastal region like *Ammophilaarenaria* L. The cold (5°C) reduced germination capacity of the seeds for the two regions but does not cancel it. This behavior differs in some species belonging to the same family as it is the case of *A. arenaria* (perennial poaceae of a coastal dunes) whose germination is possible only from 15 °C [24].

Percentages of germination obtained at the optimal temperatures not exceeding 54,66 % for the two studied stations (the age of our seeds was two years). This rate can be explained by the age and the nature of the seeds used (seeds equipped with seminal teguments). Indeed, in other work on the same species having used younger seeds (one year), more significant percentages of germination were noted (86 – 95 %) [23,25]. In this context Gasque and Gacia-Fayos [20] showed in their work at the esparto of Spain that the age influences the percentage of germination, they found that the seeds of *S. tenacissima* were still able to germinate 28 months after harvest when stored under laboratory conditions but their germination percentage decreased substantially and significantly. Also, the work of Bessam et al. [25] revealed that the seeds of esparto are characterized by a tegumentary inhibition blocking and slowing down the process of germination.

For the various temperatures tested, the seeds of the steppe region present a rate of germination slightly higher than the caryopses of the littoral region, particularly to 5 and 10°C where this difference is highly significant ( $P < 0.01$ ). We did not detect differences concerning the coefficient of velocity for temperatures 25 and 30°C where the caryopses of the station of the littoral present a coefficient of velocity more significant than those recorded on the station of the steppe. Concerning the latency time, the seeds of the two stations behave same manner insofar as time necessary for the release of germination is similar for all the temperatures tested ( $P > 0.05$ ).

The variability detected between the seeds of the two stations, particularly the capacity and the speed of germination, could be attributed on the one hand at the geographical origin of the caryopses and on the other hand to a strategy of adaptation to the constraints of the natural environment. Indeed, Besnier [26] and Mehdadi et al. [8] reported that germination depends largely on the geographical origin of the populations of esparto. Also, Lopez et al. [27]; Pico et al. [28] and Bischof et al. [29] explain that the differences between the stations can be attributed to the heterogeneity of the environmental conditions of the sites of taking away. It can be also in relation with a genetic variability insofar as this taxon is characterized by the presence of cytotypes. The work of Boussaid et al. [30] show by molecular analyses (PCR), a genetic diversity concerning 17 populations of esparto (*Stipatenassicima* L.) operating in the semi-arid and arid bioclimatic stage of the Top-Plates of Algeria.

#### IV. Conclusion

In the light of the results obtained, it arises that the thermal optimum of germination for the seeds of the esparto of the littoral and the steppe is comparable insofar as the maximum rates of germination are recorded to 20 and 25°C. The germination of these two categories of seeds strongly weakens to 5 and 30°C to cancel then at 35°C. These extreme temperatures are characterized by slower latency times compared to the optimal temperatures where these times are very short.

On the whole of the temperatures tested, the seeds of the steppe station have better capacities of germination, in particular to 5 and 10°C where this variability is significant.

However, the seeds of the littoral express speeds of germination higher at 25 and 30 °C.

The small percentages of germination recorded in this work can be improved by the recourse to certain traditional methods of lifting of tegumentary dormancy like scarification, the stratification, pre-steeping with distilled water, etc.

To conclude, the results obtained made it possible to elucidate some points of difference between the studied ecotypes. They also identified the optimal thermal conditions for the germination of alfa and therefore its conservation. On the basis of these preliminary data, it would be desirable to supplement this work, by tests of germination under hydrous and saline stress in order to look further into knowledge relating to the germinatif behavior of the caryopses of the two ecotypes and also by advanced techniques as the molecular analyses (PCR), for better determining the origin of the variability recorded between the two stations.

#### ACKNOWLEDGEMENTS

We thank Pr Benhassaini Hachemi for his help in laboratory work.

#### REFERENCES

1. Meyer, S.E., S.B. Debaene-Gill and P.S. Allen, 2000. Using hydrothermal time concept to model seed germination response to temperature, dormancy loss, and priming effect in *Elymuselymoides*. Seed Science Research 10, 213-223.
2. Kadi-Hanifi, A. H., 1998. Syntaxonomie, relation milieu-végétation, dynamique et perspectives d'avenir. Thèse de doctorat, université Houari Boumediene, Alger, 270 p.
3. Francis, C.F. and J.B. Thornes, 1990. Vegetation and erosion. Processes and environments. 1990 pp. 363-384.
4. Aidoud, A. and J. Touffet, 1996. La régression de l'alfa (*Stipa tenacissima* L.): graminée pérenne, indicateur de désertification des steppes Algérienne. Sécheresse, 7, 187-93.
5. Zeriahe, N., 1987. Etude du système racinaire de l'alfa (*Stipa tenacissima* L.) en relation avec l'adaptation xérophytique. Thèse de Magister. Université d'Oran. 113 p.
6. Harche M., 1978 - Contribution à l'étude de l'alfa (*Stipa tenacissima* L.) : germination; croissance des feuilles, différenciation des fibres. Thèse 3ème cycle ; Univ. P. et M. Curie, Paris. 88 p.
7. Boudy, P., 1950. Economie forestière Nord-Africaine Tome II. Paris, 177p.
8. Mehdadi, Z., Z. Benouada, A. Latrech, H. Benhassaini and I. Bouachour, 2004. Contribution à l'étude de la régénération naturelle de *Stipa tenacissima* L. dans les hautes plaines steppiques de Sidi Bel Abbès (Algérie occidentale). Sécheresse, 15 (2), 197-71.
9. Mehdadi, Z., Z. Benaouda, L. Bouachour, S. Moulessehoul, M. Joseph and A. Delcourt, 2000. Etude du comportement du méristème végétatif de l'alfa (*Stipa Tenacissima* L.). Approches cytologiques et histologiques. Journal Soc. Bio., 194 : 195-204 p.
10. Hellal, B., N. Ayad, M. Maatoug and M. Boularas, 2007. Influence du « fatras » sur la biomasse foliaire de l'alfa (*Stipa tenacissima* L.) de la steppe du Sud oranais (Algérie occidentale) Volume 18, numéro 1, 65-71.
11. Aidoud A., 1989. Contribution à l'étude des écosystèmes steppiques pâturés des hautes-plaines Algéro-Oranaise (Algérie) : Fonctionnement, évaluation et évolution des ressources végétales. Doct. ès. Sci, USTHB, Alger, 240p.
12. Le Houérou, H.N., 1969. La végétation de la Tunisie steppique (avec références aux végétations analogues de l'Algérie, de la Libye et du Maroc). Annales de l'INRAT 42, 617.
13. Bewley, J.D., Black, M., 1994. Seeds: Physiology of Development and Germination. Plenum Press, London, p. 445.
14. Fenner, M. and K. Thompson, 2005. The Ecology of seeds. Cambridge University Press, Cambridge.
15. Hamasha, H.R. and I. Hensen, 2009. Seed germination of four Jordanian *Stipa* spp.: difference in temperature regimes and seed provenances. Plant Species Biology 24, 127-132.
16. Hu, X.W., Z.Q. Zhou, T.S. Li, Y.P. Wu and Y.R. Wang, 2013. Environmental factors controlling seed germination and seedling recruitment of *Stipabungeana* on the loess Plateau of northwestern China. Ecological Research 28, 801-809.
17. Come, D., 1970. Les obstacles à la germination. Masson and Cie, Paris, 135 p.

18. Baskin, C.C. and J.M. Baskin, 1998. Seeds Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego, CA, p. 666.
19. Dadache, M., Z. Mehdadi and A. Latreche, 2015. Germination Responses of *Marrubium Vulgare* L. under Various Water stress Conditions. Journal of Applied Environmental and Biological Sciences, 5 (9). 28-33.
20. Gasque, M. and P. Gacia-Fayos, 2003. Seed dormancy and longevity in *Stipa tenacissima* L. (Poaceae). Plant Ecology 168, 279-190.
21. Chadli, R., 1990. Contribution à l'étude du sparte (*Lygeumspartum* L.): germination, croissance des feuilles, structure pariétale, essai d'obtention de pâte papetière à partir des fibres foliaires. Thèse de Magister, Université d'Oran. 96 p. + planches.
22. Mekhaldi, A., 1994. Contribution à l'étude d'*Aristidapungens* L.: germination, structure et ultra structure du tissu fibreux, obtention de pâte papetière à partir des tissus fibreux foliaires et caulinaires. Thèse de Magister. Université d'Oran. 169 p.
23. Krichen, K., H. Ben Mariem and M. Chaieb, 2014. Ecophysiological requirements on seed germination of a Mediterranean perennial grass (*Stipa tenacissima* L.) under controlled temperatures and water stress. South African Journal of Botany 94, 210-217.
24. Bendimred, F.Z., 1997. Contribution à l'étude de L'oyat (*Ammophilaarenaria* L.). Approche écologique. Germination des caryopses, croissance, anatomie et histochimie des feuilles. Mémoire de Magister. Université de Tlemcen, 110 p.
25. Bessam, F.Z., Z.Mehdadi, H.M.Bessam and A. Marouf, 2010. Effets de quelques prétraitements physiques sur l'amélioration des performances germinatives de *Stipa tenacissima* L. et caractérisation des substances inhibitrices. In Acta Bot. Gallica, 157 (2), 349-360, 2010.
26. Besnier, F., 1989. Semillas: Biologia Technologia. In: Mundiprensa (Ed.), (Madrid. 524 pp.).
27. Lopez, G.A., B.M. Potts, R.E. Vaillancourt and A. Apiolaza, 2003. Material and carryover effects on early growth of *Eucalyptus globules*. Canadian Journal of Forest Research 33, 2108-2115.
28. Pico, F.X., N.J. Ouborg and J.M. Van Groenendael, 2003. Fitness traits and dispersal ability in prairie and maternal effects. Journal of Plant Biology 5, 522-530.
29. Bischoff, A., B. Vonlanthen and T. Steinger, 2006. Seed provenance matters-effects on germination of four plant species used for ecological restoration. Basic Applied Ecology Journal 7, 347-359.
30. Boussaid, M., C. Benito, M.Harche, T.Naranjo and M. Zedek, 2012. Genetic Variation in Natural Populations of *Stipa tenacissima* from Algeria. Génétique et biochimie. Volume 1/1967 - Volume 50/2012. (2) 10: 75-96.