

## Effects of natural long storage duration on seeds germinative response of *Argyrobium uniflorum* (Decne.) Jaub. & Spach

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### Article info

### Abstract

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Little is known about the effect of seed natural long storage on the germination capacity of vascular plant species particularly those used in restoration programs. In the current paper, we aimed to study the effect of different seed storage durations (from 3 months to 22 years) on the germination of *Argyrobium uniflorum* (Decne.) Jaub. & Spach, herbaceous legume of high pastoral value indigenous to the Mediterranean Basin. In this paper, we found that the seeds scarification, hasten germination capacity of scarified seeds (SS) to almost 80%. The seed germination is maintained for 14 years at the level of 78 to 60% and then decreases.

Also, other measured parameters of water content, electric conductivity, seedling lengths and MGT were also modified along seeds age. The duration of storage affects the germination performance of *Argyrobium uniflorum* seeds which decreases after approximately one decade and half which give us an idea about the longevity of soil seeds bank of this species.

## 1. INTRODUCTION

Drylands cover approximately 41% of the earth's land surface and support over 38% of the global human population (Reynolds et al., 2007). In these ecosystems, climate change, human-induced environmental degradation and population growth, caused considerable fatal changes in vegetation units (Hanafi and Jauffret, 2008; Guli et al., 2015). The loss of vegetation structural attributes such as species richness and total vegetation cover is reported to dramatically affect the functioning of the concerned ecosystems (Vitousek, 2004). In fact, plant patches fulfill several ecological functions as they increase soil nutrient levels (Azcon-Aguilar et al., 2003) and improve soil moisture conditions (Callaway, 1995). One of critical challenges facing restoration practitioners is the identification of the suitable environmental factors that help in the establishment of vegetation in the degraded area. Success of revegetation depends firstly on the success of the germination of the target species. Germination represents a critical stage

in the life cycle of vascular plants that could be directly affected by different abiotic factors such as temperature (Flores et al., 2006), water availability (Fenner and Thompson, 2005), salinity (Panuccio et al., 2014) and seed aging (Rice and Dyer, 2001; Huang et al., 2003; Ouled Belgacem et al., 2006). Investigation of seed behaviour of species in response to a combination of biotic and abiotic factors may help to understand factors related to the distribution of the species (Donohue et al., 2010). One of the restoration measures is the seed banks used for a promising revegetation technique (Nishihiro et al., 2006). Seed bank can buffer against years with reproductive failures and reduce the probability of local extinction (Rice and Dyer, 2001). However, it has been reported that the germinative response depend on the period of time of seed storage (Rice and Dyer, 2001; Zheng and Ma, 2014). During aging, seeds deterioration can be expressed by the loss of viability, quality and vigor. The major causes of seed deterioration are enzyme inactivation or protein degradation, disruption of cellular membranes, and damage to genetic (nucleic

acids) integrity (Walters 1998). Previous studies reported strong relationship between germination capacity and seeds water content which is directly proportional to the loss of seed vigor and viability by storage period length (Doijode, 1985; Lee et al., 1995).

Due to global climate change conditions, deep modifications of species phenological behavior (germination and fructification) are expected (Christensen, 2004). Over Northern Africa, reduction in rainfall (Niang et al., 2014) and increases in temperature (Vizy and Cook, 2012) point to an overall warming trend by the end of the 21st century (Barkhordarian et al., 2012). North Africa has a Mediterranean climate characterized by high temperatures and scattered rainfall highly variable both intra- and interannually, which can have important implications on plant germination physiology. Tunisia is one of the North African countries that are seriously affected by desertification and ecosystems degradation generally presented by poor vegetation cover particularly in arid areas. *Stipa lagascae* L. & Sch., *Plantago albicans* L. and *Argyrolobium uniflorum* J. & S. are the best candidate species used in restoration programs in the Rhanterium *suaveolens* Def. steppe (Chaieb and Zaâfour, 2000, Ouled Belgacem et al., 2006). *A. uniflorum* J. is a widely distributed Fabaceae in North Africa especially in the rangelands of central and southern Tunisia.

In the current study, we evaluated the germinative behaviour of the seeds of *A. uniflorum* after storage for different periods of time (from 0.25 to 22 years)

## 2. MATERIAL AND METHODS

### 2.1. Plant material and seed collection site

Experiments were carried out in 2010. Seven studied seeds accessions of *A. uniflorum*, were collected from Southern Tunisia (10° 55,41'E - 33° 17,28'N) and stored in the seed bank of the Institute of Arid Lands (Medenine, Tunisia) respectively since 2009, 2008, 2004, 1996, 1994, 1990 and 1988. Seeds were cleaned and stored in the seed Bank under controlled conditions of relative humidity (30%) and mean temperature (20 °C).

Located in the Mediterranean arid bioclimate (Emberger, 1954), the collection site is exposed to low irregular rainfall (186 mm) and elevated annual average temperatures (19.4 °C).

### 2.2. Effects of storage duration on electrical conductivity and water content of seeds

The electrical conductivity (EC) of the soak water of stored seeds was measured according to Goel et al. (2003). Three replicates, each one was carried out with 50 seeds which were weighted and then soaked in 5 mL of deionised water at 20°C for 24 h then, the EC of the seed soak water determined using a conductivity meter (INOLAB). The EC was expressed as  $\mu\text{S m}^{-1}$  seed<sup>-1</sup> for each category of seeds age.

Seeds water content (WC %) was calculated according to the following formula:

$$\text{WC \%} = (\text{FW} - \text{DW}) / \text{DW} * 100$$
, with FW is the fresh weight and DW is the dry weight (ASAE, 1994). Drying was performed on 100 mg of seeds for every seeds sample which were placed in steam room (or oven) in a temperature of 105°C for 24 h. A total of three replications of 100 mg were held, and the average was calculated for each category of seeds age.

### 2.3 Seed scarification and germination tests

A chemical scarification was made in the laboratory using the sulfuric acid H<sub>2</sub>SO<sub>4</sub> (70%), for one hour and 30 minutes (Neffati, 1994).

To assess the effect of the chemical scarification on germination performance of different aged seeds, two seeds lots are studied (scarified and non scarified seeds).

Germination tests were conducted by placing seeds in Petri dishes with two Whatman N°1 filter papers. 10 replicates of 20 seeds of *A. uniflorum* were placed in each of them with addition of 5 ml distilled water every two days. During 20 days the germinated seeds were counted. Seeds were considered germinated when the radical was at least 1 mm of length (Côme, 1975). Germination tests were performed at the optimal temperature which is 25 °C (Neffati, 1994) in darkness.

To avoid fungus attack before incubation, seeds were surface sterilized in 0.58% of Sodium Hypochlorite solution for 1 min, subsequently washed with distilled water and air-dried before being used in the germination experiments (Gulzar et al. 2001).

Final percentage of germination (G%), seedling length and mean time to germination (MTG) were calculated. This last parameter of MTG was calculated according to the formula:  $\text{MTG} = \Sigma (n_i \times d_i) / N$ , where "n<sub>i</sub>" is the number of germinated seeds at day "d<sub>i</sub>", and the incubation period in days and N the total number of germinated seeds in the treatment (Maraghni et al. 2010). Seedling length is performed simultaneously with standard germination. At the end of the observations period (20 days), all seedlings,

which had complete morphological parts without lesions or defects, were selected and counted as vigorous seedlings. The average of seedling length was calculated by the modified formula of Abdul Baki and Anderson (1973).  
Seedling length = % de germination\* average of radicals length (cm) /100

### 2.4 Statistical analysis

Germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance. Data were analyzed using SPSS for Windows, version 11.5. A one-way and two way analysis of variance (ANOVA) were carried out to test the difference between parameters. Tukey test (Honestly significant differences, HSD) was used to estimate least significant difference between means.

## 3. RESULTS

### 3.1 Effects of storage duration on electrical conductivity and water content of seeds

Electric conductivity during seed soaking increased significantly with storage time (Fig. 1 & Table 1). For fresh collected seeds (3 months) until 16 years, no significant difference was detected of this parameter. This value decreased significantly with storage period to reach 0,4 mSm<sup>-1</sup> seed<sup>-1</sup> for oldest seeds after 22 years of storage. Fig. 2 confirmed that an average of WC% between 6 to 7%, was measured in seeds aged 1, 6 and 14 years. After that, a significant decrease of WC% (Table 1) was observed as the age increased.

### 3.2 Effect of storage on germination performance of seeds of *A. uniflorum*

The statistical analysis in Table 2 showed a significant difference (p<0.05) in percentage of germination capacity (GC%) of different seeds lots. Seeds aged from 1 to 14 years have more than 60% of germed seeds compared to less than the average of 23% for older seeds. The results, showed in Table 2, confirmed that scarification

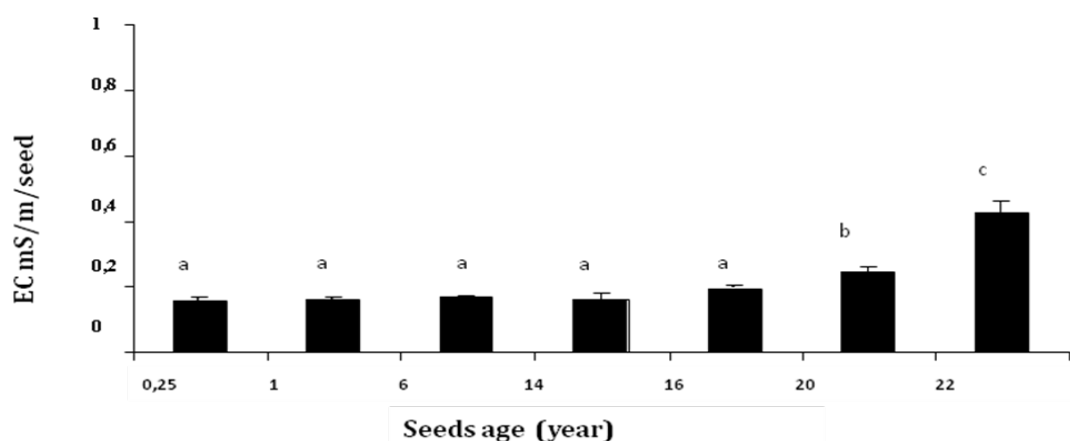


Fig. 1. Changes in electric conductivity (μSm<sup>-1</sup> seed<sup>-1</sup>) of *A. uniflorum* (J.S) seeds over the storage period. (Data represent mean ± SE, (n = 3), test Tukey.).

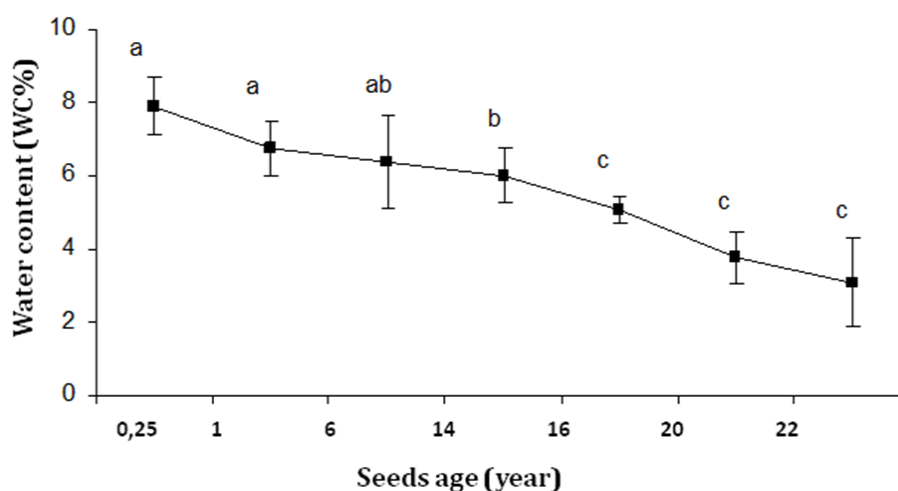


Fig. 2. Changes in water content (%) of *A. uniflorum* (J.S) seeds over the storage period. (Data represent mean ± SE, (n = 3), test Tukey.).

**Table 1.** Analysis of variance for the electric conductivity and the Water content (%)

Source de variation	ddl	Square Means	F	P.
Electric Conductivity	6	0,067	21,374	<0,0001
Water Content (%)	6	6,438	41,598	<0,0001

**Table 2.** Percentage of germination capacity (%GC) of *A. uniflorum* (J.S) seeds stored for different periods.

Seeds age	Germination capacity (%)		Seedling length (cm)		MTG (day)	
	SS	NSS	SS	NSS	SS	NSS
3 months	42.5±3.01 B	15 ± 0.54 ab	2.68±0.25AB	0.73 ± 0.17ab	6.24±0.68A	9.29 ± 2.88ab
1 year	78.5± 3.88A	18 ± 3.8a	4.30±0.62A	0.99 ± 0.15a	3.78±0.28B	7.08 ± 0.99d
6 years	72 ± 3.88A	19.5 ± 3.8a	3.04±0.53A	1.06 ± 0.22a	3.69±0.07B	6.99 ± 1.09c
14 years	61±3.88 B	18 ± 3.8a	3.42±0.36A	0.68 ± 0.20ab	4.49±0.09B	9.77 ± 1.78 ab
16 years	32± 3.88C	7.5 ± 3.8c	1.40±0.15BC	0.47 ± 0.10b	5.1±0.34AB	8.70 ± 1.08c
20 years	23± 2.61C	16.5 ± A A 1 ab	1.35±0.16BC	1.07 ± 0.32a	5.3±0.36AB	9.89 ± 1.48ab
22 years	14.5±5.15 D	9.5 ± 3.08 bc	0.40±0.14C	0.61 ± 0.24b	6.56±5.54A	10.47 ± 2.21a

(Means, 95% confidence limits, (n=10) having the same letter are not significantly different (P>0.05, Tukey test, SS : Scarified seed ; NSS : Non scarified seed)

has a significant effect on germination of *A. uniflorum* seeds ( $p < 0.05$ ). Non-scarified seeds (NSS) showed the lowest germination percentage (less than 20%) compared to scarified seeds (78 %). Scarification effect is maintained along germination test with different aged seeds (only 14.5 versus 9.5%, respectively for scarified and non scarified seeds, after 22 years of storage). Also, Observations of Table 1 confirm that the scarification of seeds hasten the MGT for all stored seeds. The mean time of germination (MTG) decreases reaching less than five days for scarified seeds aged 1, 6 and 14 years, then it exceeded 5 days for older seeds (Table 2).

#### 4. DISCUSSION

In arid and desert ecosystems, successful establishment of plants largely depends on successful germination. Seed bank can buffer against years with reproductive failures and reduce the probability of local extinction (Rice and Dyer, 2001). However, it has been reported that the seeds germination capacity depend on the period of time of seed storage (Rice and dyer, 2001; Zheng and Ma, 2014).

In this purpose, our results reveal that seed germination characteristics were affected by over storage period. Recent collected seed showed a low germination capacity % (only 42%) compared to seeds aged 1, 6 and 14 years

with a germination capacity exceeding 70%. Then beyond this period, germination decreased drastically. Recent collected seeds, aged three months can be considered in physiological dormancy or in “post-harvest dormancy” unfit to germination according to Simpson (1990) and Neffati (1994). In this line Gómez, (1985) have confirmed that the majority of seeds should be aged at minimum between six to 12 months before germination..

For other aged seeds, our results corroborate several other studies (Abdelleoui et al., 2013; McDonald, 1999; Hsu et al., 2003) confirming that deterioration of seeds by time of storage is marked by a decline in their germination capacity. In addition, our data showed that the electric conductivity during seed soaking increased significantly with storage time. This parameter can test the integrity of plasma membrane of seeds (Bewley and Black, 1994; Wang et al., 2003). Indeed, by storage, the deterioration of the seed membrane may enhance the electrolytes flow out of cells, so the vigor of seed reduced by decreased conductivity (Abdellaoui et al., 2013; Wang et al., 1999). The augmentation of conductivity was registered after 16 years of storage in our study case.

Seeds water content showed a significant decrease with the storage period. It is more than 6% for seeds ages 1,6 and 14 years. The older seeds have a very low percentage of water

content, only 4%, causing an excessive desiccation of seed tissue according to Neffati, (1994). When seeds physiological activities reach an irreversible damage state. Discussed parameters below, showed that after almost one decade and half of storage in condition of 20°C and 30% relative moisture, the 1, 6 and 14 years old seeds were still able to germinate with a GC% more than 60%. With stable conductivity and WC% between 7% and 8%.

It is well known that scarification improves germination of hard coat seeds (Simon et al. 2007), reducing the coat inhibition and reaching high capacity of germination which was almost five times more important than no scarified seeds under 25°C in our study case. The germination performance (GC%, seedling length, and MTG) was highly improved significantly ( $P < 0.0001$ ) as it was confirmed in previous studies of Neffati, (1994).

Long storing seeds under conditions of 20°C temperature and of 30% relative moisture leads to a progressive decrease of CG% and seedling length (T), until four times less, for seeds aged 22 year-old.

Our approach provides the optimum WC% of 6 to 7% for *A. uniflorum* seeds stored at 20°C. When MC was  $6 < WC < 7\%$ , seed conductivity was less than 0,2 mS $\cdot$ m<sup>-1</sup> seed<sup>-1</sup> with GC% more than 60%. This optimal percentage of water content of dry orthodox seeds is related to a very high cytoplasmic viscosity and a very low cellular mobility owed to the beginning of the glassy state as Treutter (2006) has indicated.

Important CG % by age showed that *A. uniflorum* seeds improve persistent soil seed banks for more time and have an important role in arid plant communities. Indeed, it enhances the soil stability and can reduce the demographic effects of environmental stochasticity (Thompson, 1992). Consequently, community began more stable (Caballero et al., 2003) and seeds wait to their recruitment years with a good ability of germination. In this context, seeds can be considered as phylogenetic reservoirs (Morris et al. 2002) and can reduce the evolutionary changes by age. (Nunney, 2002).

## 5. CONCLUSION

No modified characteristics and performance of germination of seeds by age, improves the idea of soil seeds bank, helping species to spread more. So in this work we investigate with a seeds stored differently in the same conditions of temperature and moisture to be more close to

real weather because estimations and investigations with accelerated aged seeds by using high temperature or heat can not reflect the natural evolutionary events in seeds by age. Our investigations confirmed that chemical scarification enhances seeds germination by age application of chemical scarification with the same duration to the different seeds ages can be a discussing point because of evolutionary coat characteristics by age what can be experimented later.

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## REFERENCES

- Abdellaoui, R., Souid, A., Zayoud, D. and Neffati, M. (2013). Effects of natural long storage duration on seed germination characteristics of *Periploca angustifolia* Labill. African Journal of Biotechnology 12(15),1760-1768.
- Abdul Baki, A.A., Anderson, J.D. (1973). Vigour determination in soyabean seed by multiple criteria. Crop Science, 13, 630-633.
- ASAE. (1994). Standards S352.3-moisture measurement-ungrounded grains and seeds. In ASAE Standards. ASAE, 469 St Joseph, MI.
- Azcon-Aguilar, C., Palenzuela, J., Roldan, A., Bautista, S., Vallejo, R., Barea, J.M., (2003). Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. Applied Soil Ecology 14, 165-175.
- Barkhordarian, A., Bhend, J., von Storch, H. (2012). Consistency of observed near surface temperature trends with climate change projections over the Mediterranean region. Clim Dyn 38(9-10):1695-1702. <https://doi.org/10.1007/s00382-011-1060-y>
- Bewley, J.D., Black, M. (1994). Seeds Physiology of development and germination. 2nd Ed. New York: Plenum Press.
- Caballero, I., Olano, J.M., Loidi, J. and Escudero, A. (2003). Seed bank structure along a semi-arid gypsum gradient in Central Spain. Journal of Arid Environment, 55, 287-299.
- Callaway, R.M., (1995). Positive interactions among plants. The Botanical Review 61, 306-349.
- Chaieb M. and Zaâfour M.S. (2000). L'élevage extensif, facteur écologique primordial de la transformation phytosociologique du cortège floristique en milieu steppique tunisien. [The

- extensif breeding, ecological primordiale factor of physionomic transformation of floral procession in Tunisian steppic land]. *Options Méditerranéennes*, 39, 217-222.
- Christensen, L., Coughenour, M., Ellis, E.J. and Chen, Z.Z. (2004). Vulnerability of the Asian typical steppe to Grazing and climate change. *Climate change*, 63, 368-368.
- Côme D. (1975). Rôle de l'eau, de l'oxygène et de la température dans la germination. [Role of water, oxygen and tempratur on germination]. pp. 27-44, Gauthier-Villars, Paris.
- Doijode, S.D. (1985). Onion seed quality in relation to seed deterioration under accelerated ageing conditions. *Vegetation Science*, 12, 59-63.
- Donohue, K., et al. (2010). Germination, postgermination adaptation, and species ecological ranges. *Ann. Rev. Ecol. Evol. S.* 2010;41:293-319.
- Emberger, L. (1954). Une classification biogéographique des climats. *Rec. Trav. Lab. Bot. Géol. Zool. Univ. Montpellier. Série Botanique*, 7, 3-43.
- Fenner, M., Thompson, K., (2005). *The Ecology of Seeds*. Cambridge University Press, Cambridge.
- Flores, J., Jurado, E., Arredondo, A., (2006). Effect of light on germination of seeds of Cactaceae from the Chihuahuan Desert, Mexico. *Seed Science Research* 16, 149-155.
- Goel A, Goel AK, Sheoran IS (2003). Changes in oxidative stress enzymes during artificial ageing in cotton (*Gossypium hirsutum* L.) seeds. *J. Plant Physiol.* 160:1093-1100.
- Guli-Jiapaer, Liang, S., Yi, Q., & Liu, J. (2015). Vegetation dynamics and responses to recent climate change in Xinjiang using leaf area index as an indicator. *Ecological Indicators*, 58, 64-76. doi:10.1016/j.ecolind.2015.05.036.
- Gulzar, S., Khan, M.A. and Ungar, I.A. (2001). Effect of salinity and temperature on the germination of *Urochondra setulosa* (Trin.) C.E. Hubbard. *Seed Science and Technology*, 29, 21-29.
- Hanafi, A. and Jauffret, S. (2008). Are long-term vegetation dynamics useful in monitoring and assessing desertification processes in the arid steppe, southern Tunisia. *Journal of Arid Environments*, 72, 557-572.
- Hsu, C.C., Chen, C.L., Chen, J.J., Sung, J.M. (2003). Accelerated agingenhanced lipid peroxidation in bitter gourd seeds and effects of priming and hot water soaking treatments. *Sci Hortic.* 98:201-212
- Huang, Z.Y., Zhang, X.S., Zheng, G.H., Gutterman, Y. (2003). Influence of light, temperature, salinity and storage on seed germination of *Haloxylon ammodendron*. *Journal of Arid Environments*, 55:453-464.
- Maraghni, M., Gorai, M. and Neffati, M. (2010). Seed germination at different temperatures and water stress levels, and seedling emergence from different depths of *Ziziphus lotus*. *South African Journal of Botany*, 76, 453-459.
- McDonald, M.B. (1999). Seed deterioration: physiology, repair and assessment. *Seed Science Technology*, 27:177-237.
- Morris, A.B., Baucom, R.S. and Cruzan, M.B. (2002). Stratified analysis of the soil seed bank in the cedar glade endemic *Astragalus bibullatus*: Evidence for historical changes in genetic structure. *American Journal of Botany*, 89, 29-36.
- Neffati, M. (1994). Caractérisation morphologique de certaines espèces végétales nord africaines: implication pour l'amélioration pastorale. [Morphological characterization of some North African botanical species: implication for the pastoral improvement]. Thèse Doct., Université Gent, 242pp.
- Niang, I., et al., Ruppel, O. C., Abdrabo, M. A., Essel, A., Lennard, C., Padgham, J., & Urquhart, P. (2014). Africa. In V. R. Barros et al., eds. *Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, U.K: Cambridge University Press, pp. 1199-1265. <https://doi.org/10.2134/agronj2010.0303>
- Nishihiro, J., Nishihiro, M.A., Washitani, I. (2006). Restoration of wetland vegetation using soil seed banks: lessons from a project in Lake Kasumigaura, Japan. *Landscape Ecol Eng* 2:171-176.
- Ouled Belgacem, A., Neffati, M., Papanastasis, V., Chaieb, M. (2006). Effects of seed age and seeding depth on growth of *Stipa lagascae* R. & Sch. seedlings. *Journal of Arid Environments*. 65: 682-687.
- Panuccio, M.R., Jacobsen, S.E., Akhtar, S.S., Muscolo, A. (2014). Effect of saline water on seed germination and early seedling growth of the halophyte quinoa. *AoB Plants* 6: plu047.
- Reynolds, J. F., Smith, D. M. S., Lambin, E. F., Turner, B. L., Mortimore, M., Batterbury, S. P. J. Walker, B. (2007). *Global Desertification: Building a Science for Dryland Development*. Science, 316(5826), 847-851. doi:10.1126/science.1131634.

- Rice, K.J., Dyer, A.R. (2001). Seed aging, delayed germination and reduced competitive ability in *Bromus tectorum*. *Plant Ecology* 155: 237–243.
- Treutter D. (2006). Significance of flavonoids in plant resistance: a review, *Environmental Chemistry Letters*, 4: 147–157.
- Vitousek, P.M., (2004). *Nutrient Cycling and Limitation: Hawaii as a Model System*. Princeton University Press, US.
- Vizy, E.. & Cook, K. (2012). Mid-twenty-first-century changes in extreme events over northern and tropical Africa. *Journal of Climate*, 25, pp.5748–5767.
- Walters, C., Kameswara, Rao, N., and Hu, X. (1998). Optimizing seed water content to improve longevity in “ex situ” genebanks. *Seed Science Research*, 8, 15– 22.
- Wang, X.F., Jing, X.M., Lin, J. (2003). Studies on membrane function and sugar components of ultra dried seeds. *Acta Botanica Sinica*. 45:23-31.
- Wang, X.F., Zheng, G.H., Yang, S.J. (1999). Effect of ultradry storage on fluidity of plasma membrane of seeds. *Chinese Science Bulletin*. 44:1008-1013.
- Zheng, Y.L., Ma, H.C. (2014). Effects of Seed Aging on Seed Germination and Seed Reserve Utilization in Mumian. *HortTechnology* 24: 471-474.