DOI: 10.2478/cerce-2018-0039 **Original Article**
Available online: www.uaiasi.ro/CERCET_AGROMOLD/
Print ISSN 0379-5837; Electronic ISSN 2067-1865

Cercetări Agronomice în Moldova
Vol. LI , No. 4 (176) / 2018: 96-107

MORPHOLOGICAL CHARACTERISTICS AND SEED GERMINATION IMPROVEMENT OF TWO ECOTYPES OF *ASTRAGALUS ARMATUS* WILLD. SUBSP. *ARMATUS* IN ALGERIA

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Received: July 24, 2018. Revised: Oct. 21, 2018. Accepted: Nov. 19, 2018. Published online: Oct. 3, 2019

ABSTRACT. *Astragalus armatus* Willd. subsp. *armatus* is an endemic shrub of the Northern Africa. Its cultivation and domestication are very limited because of difficulty with seed germination and establishment. In this study, we investigated some plant morphological characteristics in real time and in situ (leaves, fruit and seeds) of different ecotypes of *A. armatus*, collected from two sites in Algeria (Arid Steppe of Aïn Naga and Condorcet Mountain), which elevation and climate data are very different. Moreover, the role played by the seed coat in seed dormancy of these two different populations was tested by the effects of the pretreatment and its duration on the performance of seed germination, by considering the final germination percentage (FGP) and the mean germination time (MGT). These parameters are estimated for 10 days in Petri dishes and stored in darkness at (25°C). Pre-sowing treatments included immersion in concentrated sulphuric acid for 30, 60 and 90 min, and immersion in hot water (100°C) for 10 min. Statistical analysis showed that the treatment and the ecotypes effects on both FGP and MGT were highly significant ($p< 0.0001$). Untreated seeds of both ecotypes of *A. armatus* failed to germinate (except for a few of Condorcet Mountain ecotypes). For both populations, the most effective treatment was immersion in sulphuric acid for 60 min for the ecotype of Arid Steppe of Aïn Naga, and only 30 min for Condorcet Mountain. An excellent germinative strength is characterized by a higher FGP and a reduced MGT. The morphological characteristic and seed germination could be attributed to intraspecific variations resulting from the natural selection of the same species.

Keywords: *Astragalus armatus*; desert shrub; ecotype; germination; scarification.

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INTRODUCTION

As annual or perennial herbs, subshrubs, or shrubs, the plants of *Astragalus* (L.) (*Fabaceae*) are widely distributed throughout the temperate and arid regions (Zakhia *et al.*, 2004). So far, the genus has been estimated to contain 2000-3000 species and more than 250 taxonomic sections in the world (Li *et al.*, 2014). Several *Astragalus* species were studied for their antiviral, cardiotonic, antioxidant, cytotoxic, anticancer, immunostimulant, anti-inflammatory and analgesic activities (Labed *et al.*, 2016). *Astragalus armatus* Willd. subsp. *armatus* is a thorny shrub locally known as "ketad". It is an arid and semi-arid flowering plant that constitutes a significant element of the North African vegetation (Labed *et al.*, 2016). This shrub is an endemic species to Algeria and is mainly found in North Sahara (Ozenda, 1991) and is adapted to severe climatic conditions of edaphic poverty (Rodelas and González-López, 2013).

It has been reported that harvesting most of commercial medicinal plants that are obtained by destructive harvest techniques of these species occurs essentially during the flowering period, before seed set, thereby lowering regeneration and causing the gradual degradation of wild populations (Saharkhiz *et al.*, 2015). One of the most appropriate actions for safeguarding overexploited species would be to improve propagation techniques and to encourage cultivation. This strategy has been widely adopted in Europe, China, and India for many medicinal plants (Lubbe and Verpoorte, 2011).

There are two types of frequently used pretreatments, mechanical nature (break) and wet nature (immersion in a corrosive solution). The soaking into hot water or acids presents the advantage to treat an important quantity of seeds at the same time. However, the duration of the immersion must be determined to conclude the best time required to raise the coat dormancy. The present study aimed at identifying easily applied pretreatments (time of soaking into concentrated sulphuric acid and soaking in boiled water) that can be used to treat massive quantities of seeds to assure fast, homogeneous and synchronized seed germination of two ecotypes of *Astragalus armatus* subsp. *armatus* collected in Algeria (Biskra and Batna). A secondary aim of this study is to investigate and measure some morphological characteristics of fruit, seeds and leaves for each ecotype. This comparison provides a test of the hypothesis that species in mountains region should be controlled by altitudinal barriers than lowland species.

MATERIALS AND METHODS

Collection and origin of seeds

Seeds of *A. armatus* were collected from two wild populations at Batna (Mountain of Condorcet), and Biskra (Arid Steppe of Aïn Naga), located in Algeria (*Fig. 1*).

The geographic characteristics of the collection sites of the different ecotypes are presented in *Table 1*. Georeferenced maps were realized by GIS software (ArcGIS 10.3). Geographical positions were recorded using a Magellan eXplorist 200 GPS Receiver.

In this study, we have retained the average annual temperature, average annual maximum temperature, average annual minimum temperature, total annual precipitation and total rainy days during the year, of 38 year-period (1980-2018).

Climatic data was provided by the World Climate Data: Tutiempo available on the website: www.tutiempo.net.

Morphological characteristics

Leaves and mature fruits were collected directly from a total of 10 shrubs per ecotypes of *A. armatus* and stored in paper bags (*Fig. 2*). After collection, they were transported to the Laboratory of Ecology and Environment, University of Batna 2 (Algeria), where they were selected properly, described and photographed. In the laboratory, 50 leaves, 50 fruits and 50 seeds were analyzed.

The fruits and the seeds were analyzed in relation size (length, width) using a caliper. The leaves were observed for number of leaflets. After processing, seeds were reserved for germination test (*Fig. 3*).

Improvement of seed germination

The seed sample was obtained by mixing the seeds. Seeds of every population underwent several pretreatments: a chemical treatment, which consisted of immersion in 98% sulphuric acid for 30, 60 or 90 min, followed by washing in distilled water; or hot water treatment, soaking seeds in hot water (100°C) for 10 min, followed by washing in fresh distilled water. For control, seeds were not treated. It was conducted to be able to compare the effect of no pretreatment on germination.

The sowing (four replicates of 10 seeds × 5 treatments × 2 ecotypes) was realized in Petri dishes of 10 cm diameter, papered with two layers of Whatman filter paper and soaked with 20 ml of distilled water and then placed in a culture chamber in the obscurity at the laboratory temperature 25 (±2°C), during 10 days of incubation.

The Petri dishes were arranged every two days, according to a randomized design to eliminate any effect of the position in the seed culture room (Kheloufi *et al.*, 2017). The counts of germinated seeds were made every day until the 10th day of incubation and were expressed in percentage (The criterion of germination was 2 mm radicle protrusion).

In the germination tests, final germination percentage (FGP) and mean germination time (MGT) for each ecotypes and pretreatment were calculated by using the following procedures and formulas:

$$\text{FGP (\%)} = \frac{\sum ni}{N} \times 100$$

where, FGP is final germination percentage, *ni* is the number of germinated seeds at final day of test, and *N* is the total number of incubated seeds per test (Côme, 1970).

$$\text{MGT (days)} = \frac{\sum (ti.ni)}{\sum ni}$$

where, MGT is mean germination time, *ti* is the number of days from beginning of the test, *ni* is the number of germinated seeds recorded at time t(i), and Σ*ni* is the total number of germinated seeds (Orchard, 1977).

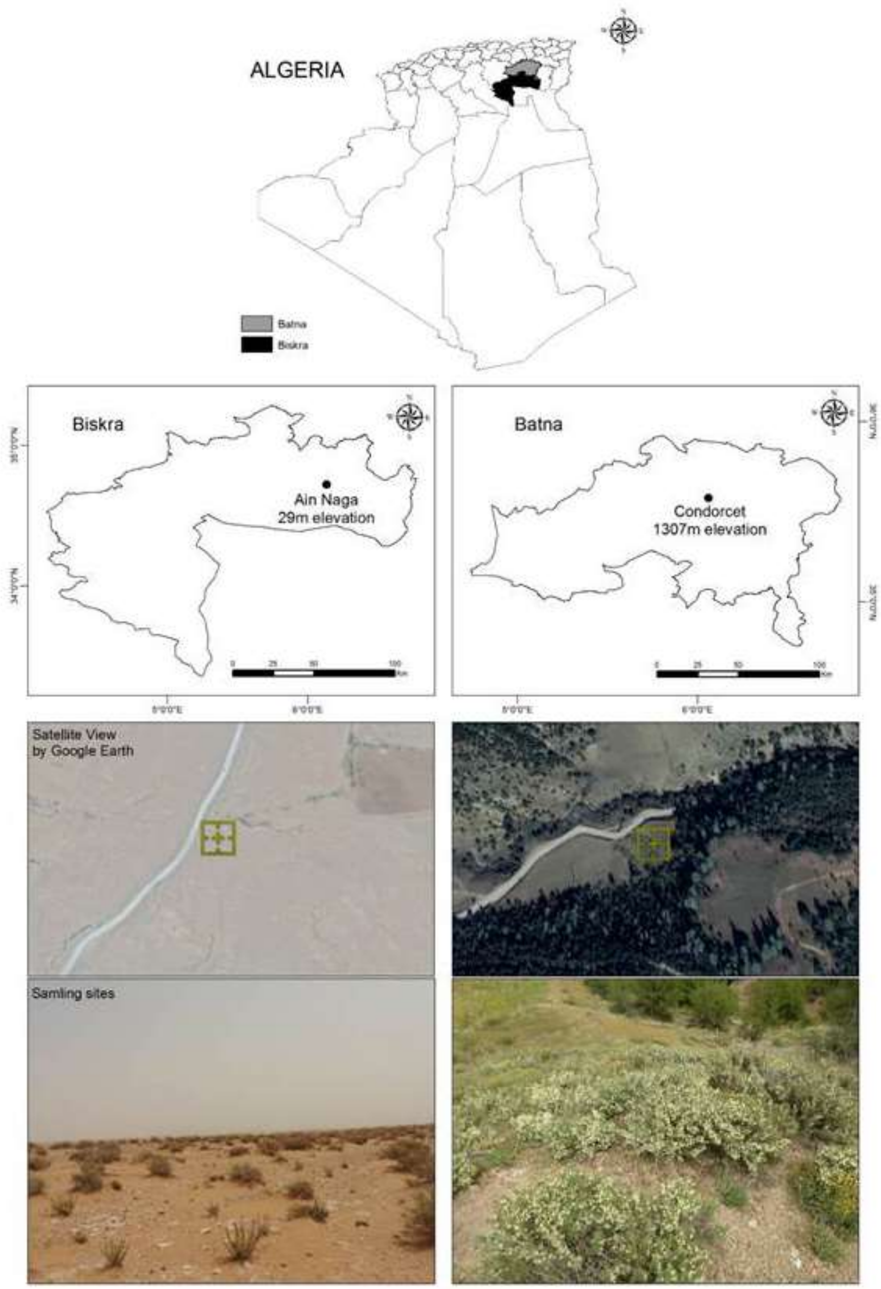

Figure 1 - Study area and location of sampling sites in Algeria

Table 1 - Geographical coordinates and climate of two populations of *A. armatus* in Algeria

Climatic data (1980-2018)	Batna (Mountain of Condorcet)	Biskra (Arid Steppe of Aïn Naga)
Elevation	1307 m	29 m
Latitude	35°34'49.99" N	34°44'20.86" N
Longitude	6° 4'11.31" E	6° 7'5.89" E
Average annual temperature (°C)	15,6	22.7
Average annual maximum temperature (°C)	22.8	28.4
Average annual minimum temperature (°C)	8.3	16.9
Total annual precipitation (mm)	323.8	160.6
Total rainy days during the year	73	30

Figure 2 - Two ecotypes of *Astragalus armatus* Willd. subsp. *armatus* (A) ecotype of Aïn Naga Steppes; (B) ecotype of Condorcet Mountain

Statistical analysis

The morphological characteristics and the effects of pretreatments on both variables were tested by analysis of variance (ANOVA). Multiple comparesons of means were performed with Duncan's test ($\alpha \leq 0.05$).

All statistical methods were performed using SAS Version 9.0 (Statistical Analysis System) (2002) software.

RESULTS AND DISCUSSION

Morphological description

The distribution of plant species among habitats is determined by a wide range of climatic and edaphic factors.

Habitat heterogeneity combined with natural selection often lead to multiple, genetically distinct ecotypes within a single species (Hufford and

Mazer, 2003). Thus, ecotypes are populations of a particular species that are evolutionally adapted to specific environmental conditions. These ecotypes, or populations that occurring in distinct habitats, vary from one to another in morphological traits such as shape, size, or leaf color (Kjemtrup *et al.,* 2003), as well as traits related to seed germination (Hufford and Mazer, 2003).

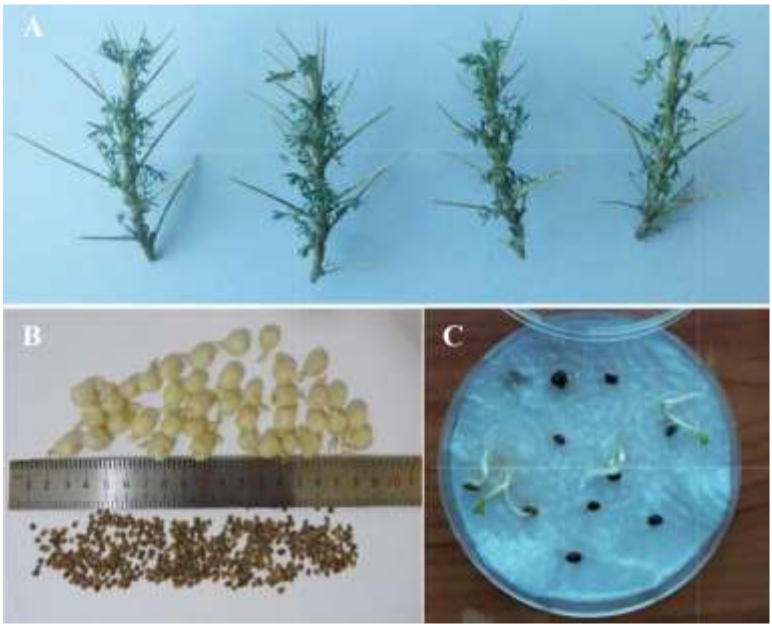

Figure 3 - Stems of 10 cm long of *A. armatus* (A); seeds and fruits morphometrics of *A. armatus* (B); seed germination in Petri dish of *A. armatus* (C)

ANOVA showed that ecotype had a significant effect on the traits: leaflets number per leaf (p = 0,0206), fruit length and width (p< 0,0001), and seed width (p< 0,0001), whereas seed length was not significant at 5% probability level, as illustrated in *Table 2*. Effectively, seed length of both populations of *A. armatus* seems similar.

The greatest difference was observed in the case of 1000-seed weight. The Condorcet Mountain ecotype showed the highest weight (10,81 g), followed by the Arid Steppe of Aïn Naga (8,18 g) (*Table 2*). Relationships between seed mass and germination are less well understood than relationships between seed mass and other life-history stages.

Differences in seed mass among species are due in part to different levels of starch and endosperm nutrients, and may influence germination percentage (Kidson and Westoby, 2000).

Table 2 - Variance analysis and morphological characteristics of leaves, fruit and seeds of *A. astragalus* ecotypes (Aïn Naga Steppe and Condorcet Mountain)

Ecotypes	1000-seed weight (g)	Leaflets per leaf	Fruit size		Seed size	
			Length (mm)	Width (mm)	Length (mm)	Width (mm)
Arid Steppe of Aïn Naga	8,18	7,06 ± 1,44^{B}	14,9 ± 0,92^{A}	6,14 ± 0,60^{B}	3,35 ± 0,38^{B}	2,1 ± 0,24^{B}
Condorcet Mountain	10,81	7,78 ± 1,60^{A}	12,3 ± 1,28^{B}	11,1 ± 1,23^{A}	3,56 ± 0,38^{A}	2,5 ± 0,24^{A}
F of Fisher	--	5,54	136,05	649,28	0,0055	56,26
P	--	0,0206	< 0,0001	< 0,0001	8,07	< 0,0001

For each ecotypes species, the same alphabet along the column indicates no significance difference (Duncan Multiple Range Test) (n=50).

As shown in *Table 2*, ecotype had a significant effect on the fruit length. The highest length of was found on plants from the Arid Steppe of Aïn Naga (14,9 ± 0,92 mm), followed by Condorcet Mountain with (12,3 ± 1,28 mm), respectively. However, fruit width of Condorcet Mountain population was larger with (11,1 ± 1,23 mm).

Ecotype had no significant effect on seed length. However, the high seed width of (2,5 ± 0,24 mm) was observed for Condorcet Mountain population.

Seed germination improvement

According to Kheloufi *et al.* (2017), the intensity of dormancy for the same species may vary according to genotype and environment in which seeds are produced.

The efficiency of scarification with sulphuric acid to overcome seed coat impermeability and increase seed germination has been reported for different species. However, the efficiency of this treatment varies with the acid concentration, plant species and treatment duration (Kheloufi, 2017).

The final phase of seed development involves the loss of water, cessation of reserve synthesis where after the seed enters a metabolically inactive state. Seed dormancy has been defined as a temporary failure of a viable seed to germinate in conditions that favour germination (Bewley, 1997). These conditions are a complex combination of water, light, temperature, gasses, mechanical restrictions, seed coats, and hormone structures. Dormancy in nature serves to protect the seed from conditions, which are temporarily suitable for germination, but which quickly revert to conditions too harsh for survival of the tender young seedling (Koornneef *et al.*, 2002).

Thus, a seed coat relatively impermeable to moisture prevents germination during isolated showers in the middle of a long dry season, while permitting it during a sustained rainy season (Vázquez-Yanes and

Orozco-Segovia, 1993). The seed coat has been shown to be a multifunctional organ which supplies nutrients to the embryo sac throughout development (van Dongen *et al.*, 2003) and is functional during drying of the seed (Howe and Smallwood, 1982). The structural and chemical properties of the seed coat impose impermeability (Rolston, 1978), regulate water entry once dormancy has been broken (Serrato-Valenti *et al.*, 1993), provide a barrier against fungi (Mohamed-Yasseen *et al.*, 1994), and reduce leakage from the embryo during imbibition (Simon and Harun, 1972). So, understanding this type of dormancy and identifying natural ways of breaking it becomes economically important.

Statistical analysis showed that the treatment and the ecotypes effects on both FGP and MGT were highly significant ($p< 0.0001$) (*Table 3*). Untreated seeds of both ecotypes of *A. armatus* failed to germinate (except for a few of Condorcet Mountain ecotypes). Mean germination time varied significantly across different provenances and due to pretreatments. Reduced mean germination time in acid treated seeds implies that the period of dormancy in seeds was reduced due to pretreatment of seeds in sulphuric acid. It is reported that acid treatment is an efficient method of enhancing seed germination of species with hard impermeable seed coat (Sy *et al.*, 2001; Pérez-García and González-Benito, 2006; Hu *et al.*, 2009). It stimulates fast and uniform germination of seeds.

Table 3 - Variance analysis for the traits investigated of two *Astragalus armatus* ecotypes seeds in response to different pre-sowing at different durations for 10 days-period

Parameters	Sources of variation	Df	F of Fisher	*P*
FGP	TRT	4	68,25	< 0,0001
	Sp	1	17,36	0,0002
	TRT × Sp	4	20,57	< 0,0001
MGT	TRT	4	20,85	< 0,0001
	Sp	1	45,78	< 0,0001
	TRT × Sp	2	0,00	1,0000

For both populations, the most effective treatment was immersion in sulphuric acid for 60 min for the ecotype of Arid Steppe of Aïn Naga, and only 30 min for Condorcet mountain. Increasing the duration of soaking in sulphuric acid from 30 to 60 min was favourable for lowland seeds, improving FGP from 90 to 100 (*Table 4*). However, extension of the soaking period to 90 min was unfavourable, reducing the FGP by 50%. Seeds soaked in boiled water had poor germination for Condorcet Mountain ecotypes and no germination for the ecotype of Arid Steppe of

Aïn Naga (*Table 4*). According to Trivedi and Joshi (2014), soaking in water, whatever its duration or temperature, does not enhance germination for species with hard seed coats. According to de Paula *et al.* (2012), the required soaking time in hot water at 100°C also depends on the thickness and hardness of the coat. The highest germination was obtained following a pretreatment of 30 min in sulphuric acid for the mountain seeds with 95% final germination, while 90 min soaking inhibited all germination process.

According to Nonogaki *et al.* (2010), the evaluation of germination capacity depends not only on the final germination percentage, but also the speed of germination. The correlation of these two factors is often used to determine the success of a pretreatment on overcoming dormancy.

The optimal duration of soaking is proportional to the coat rigidity. In this study, the seeds of Condorcet Mountain seem very sensitive to increase in the duration of soaking in the acid and under the boiling water treatment. This is in agreement with the works of Kheloufi (2017), which revealed that prolonged soaking in acid could damage the embryo and reduce germination.

Table 4 - Final germination percentage (FGP) and mean germination time (MGT) for *A. astragalus* ecotypes species (Steppe of Aïn Naga and Condorcet Mountain) exposed to different pre-sowing treatments

Ecotypes	TRT	FGP	MGT
Arid Steppe of Aïn Naga	Untreated	0,00 ± 0,00[D]	--
	BW	0,00 ± 0,00[D]	--
	30 min SA	90.0 ± 11,54[B]	2,58 ± 0,20[A]
	60 min SA	100 ± 0,00[A]	2,90 ± 0,57[A]
	90 min SA	50.0 ± 0,00[C]	1,20 ± 0,28[B]
Condorcet Mountain	Untreated	15.0 ± 10,00[C]	3,00 ± 0,00[B]
	BW	15.0 ± 10,0[C]	4,67 ± 1,15[A]
	30 min SA	95.0 ± 10,0[A]	2,94 ± 0,12[B]
	60 min SA	60.0 ± 23,1[B]	3,69 ± 0,37[AB]
	90 min SA	0.00 ± 0,00[D]	3,00 ± 0,00[B]

For each ecotypes species, the same alphabet along the column indicates no significance difference (Duncan Multiple Range Test) (n=50).

To conclude, for several forest species, a special seed pretreatment is needed to get satisfactory germination. The pretreatments do not allow the seeds to germinate, but improve the germination ability subsequently, when all favourable conditions are united. According to our results, germination of a seed depends on the potential of embryo growth or potentials of growth preventor. These potentials depend particularly on seed structure that surrounded the embryo (endosperm, pericarp, glumes) (Schopfer and Plachy, 1985; Germanà *et al.*, 2014). Other factors, like hormones and environmental factors, also affect embryo growth (Shu *et al.*, 2016).

This kind of dormancy happens when factors like water and gas are not permitted to enter the seed, so imbibition is not occurred and, consequently, resulting in decreasing seed germination (Bewley, 1997).

CONCLUSION

According to the obtained results, scarification treatments with sulphuric acid (98%) were effective, which caused dormancy breaking and seed germination induction of both populations of *A. armatus*.

This suggest that, considering only germination, it could be relatively easy for high altitude populations of the species studied to colonize open sites at lower elevations. However, lowland populations of the same species may either require specific genetically based adaptations to low temperatures, or acclimation to low temperatures during seed development, before their seeds can germinate on high-elevation sites.

This study is significant for land managers and conservation agencies with an interest in optimizing the germination of arid-zone seeds for rehabilitation of this threatened species.

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