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EFFECT OF COLD STRATIFICATION ON SEED GERMINATION OF THE MULTIPURPOSE FRUIT SHRUB, *ZIZIPHUS LOTUS* (L.) LAM. (*RHAMNACEAE*)

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ABSTRACT. Shrubs and trees of the genus *Ziziphus* are a good example of naturally occurring multipurpose plant species with great potential in arid regions. This study was conducted to investigate the effect of cold stratification on seed germination and seedling growth of *Ziziphus lotus* (L.) Lam. Seeds were subjected to 0, 45, 90 and 120 days of cold stratification at 5°C. We also examined fruit, kernel and seed morphology. For each treatment period, four replicates of 50 seeds were incubated in plastic containers between two layers of moist sand at 15% and under greenhouse conditions for 15-day period. At the end of the experiment, the final germination percentage (FGP), shoot length and root length were assessed. The results clearly indicated that increasing duration of cold stratification improved seed germination. The most effective stratification period was 120 days where *Z. lotus* recorded 83% of FGP and 16.5 cm of total seedling length. Cold stratification treatments significantly increased shoot height, root length, as well as seedling total length. 120 days stratification treatment resulted in the highest shoot and root length (6.80 cm and 9.75 cm, respectively). An overview on the emergence of *Z. lotus* seedlings during a 15-day period was also illustrated.

Keywords: agriculture; arid region; buckthorn; Jujube; medicinal plants; seed quality.

INTRODUCTION

The genus *Ziziphus* belongs to the buckthorn family (*Rhamnaceae*). It is a genus of around 100 species of deciduous or evergreen trees and shrubs distributed across the world's tropical and subtropical regions (Kaleem *et al*., 2014). The fleshy drupes of many species are rich in sugars and vitamins, and this fact has for many centuries made *Ziziphus* important fruit trees. In many traditional medicines, extracts from fruits, seeds, leaves, roots and bark of

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Ziziphus trees are used to alleviate the effects of insomnia, skin diseases, inflammatory conditions and fever (Arndt *et al*., 2001). For these reasons, the *Ziziphus* trees have a significant role to play in the arid lands' integrated economy (Wickens *et al*., 2012).

Ziziphus lotus (L.) Lam., also known as jujube, grows generally in arid and semi-arid regions and is widely distributed in China, Iran, Africa, South Korea and Europe like Cyprus, Spain, Greece and Sicily (Adeli and Samavati, 2015). In Africa, *Z. lotus* is widely distributed in Mediterranean region, like Algeria, Morocco, Tunisia and Libya (Le Houérou, 2001). *Z. lotus* is a shrub 2-5 m in height, with gray and spiny stems, deciduous leaves and small yellow flowers (Maraghni *et al*., 2011). The edible fruit is a dark red drupe (1-1.5 cm in diameter) at maturity (Dahlia *et al*., 2019). *Z. lotus* is an example of multipurpose plants with great potential and utilization capacity in arid regions. Indeed, *Z. lotus* shrubs inhabit arid environments because of their flexibility in adapting to the stress of drought (Refka *et al*., 2013). They play an important role in the conservation of soil, with their strong root system which stabilizes the soil and protects it from erosion. The leaves provide fodder for livestock and the hard wood is used for making agricultural implements, fuel and high-quality charcoal. In many regions, *Z. lotus* is grown as a hedge in many regions, with its spines producing successful live-fencing and its highly nutritious fruits providing a valuable source of energy, vitamins and also income when sold on local markets (Arndt *et al*., 2001).

Seed establishment is an important step in the life cycle of plants (Chambers *et al*., 1994). Dormancy is an interruption in the development of seed embryos (Nikolaeva, 2004). Seed dormancy extends the period of germination over time and contributes to the persistence of plants in agroecosystems (Long *et al*., 2015). Several plants exhibit a cold stratification requirement before seed germination (Li *et al*., 1999; Merritt *et al*., 2007). This may be an evolutionary change to ensure seed germination after the cold winter temperatures, rather than before or during winter (Probert, 2000). Seed germination of *Ziziphus* species is affected by the initial percentage viability at the time of seed collection, storage conditions, environmental conditions at sowing time and treatments applied to break dormancy (Azam-Ali *et al*., 2006). The aim of the present study was, firstly, to investigate some morphological characteristics of the seeds, secondly, to assess the seed germination behavior and seedling establishment of *Ziziphus lotus* under different periods of cold stratification.

MATERIALS AND METHODS

Sampling area and morphometric characterization

The seeds of *Z. lotus* used in the present experiments were obtained from freshly mature and ripe fruits harvested on

November 2019, from 10 shrubs (2-3 m height) growing at 942 m of altitude, in the North East of the National Park of Belezma (Batna, Algeria) (Latitude: 35°40' N; Longitude 6°11' E). Batna has two dominant climates, the semi-arid climate and the Mediterranean climate. In this study, we have retained the average annual temperature (15.8°C), average annual maximum temperature (23.2°C), average annual minimum temperature (8.0°C), total annual precipitation and total rainy days of 67 days during the year 2019 (WCD Tutiempo, 2020).

The fruit sample was obtained by mixing the fruits of the 10 shrubs. After harvesting, kernels (stones) were extracted by opening the fruits and removing the pulp using running water. After air dried, kernels were broken by pressing them transversely or longitudinally in a bench vise. A total of 100 fruits, 100 kernels and 100 seeds were used for biometric determinations using a digital caliper (*Fig. 1*). The length is a distance between the base and the apex and the diameter in the median region of both fruits and kernels (Kheloufi *et al*., 2019). Fruit, kernels and seed weights were also evaluated. Fruit, kernel and seed size of *Z. lotus* are shown on *Table 1*.

Figure 1 - *Ziziphus lotus* (L.) Lam. shrub, fruits, kernels and seeds

Table 1 - Fruit, kernel and seed size of *Ziziphus lotus* (L.) Lam. (n=100)

		Mean ± SD	**Minimum**	**Maximum**
Fruit	Length (mm)	11.0 ± 0.57	10.1	12.2
	Diameter (mm)	11.2 ± 0.80	10.2	13.7
	Weight (g)	0.51 ± 0.10	0.35	0.68
Kernel	Length (mm)	8.38 ± 0.76	6.72	9.22
	Diameter (mm)	6.91 ± 0.47	6.08	7.79
	Weight (g)	0.25 ± 0.06	0.16	0.45
Seed	Length (mm)	4.76 ± 0.36	3.98	5.37
	Width (mm)	4.04 ± 0.31	3.46	4.59
	Thickness (mm)	1.61 ± 0.38	0.94	2.74
	Weight (g)	0.028 ± 0.002	0.026	0.031

Each kernel contains 1-2 seeds.

Experimental design and seed germination

Seeds were subjected to 0 (Control), 45, 90 and 120 days of cold stratification at 5°C. For each treatment period, four replicates of 50 seeds were incubated in plastic containers (18 cm length × 8 cm height × 12 cm width) between two layers of moist sand at 15% (2 cm depth) and under greenhouse conditions (temperature of 27 ± 2°C) for 15-day period. At the end of the experiment, the final germination percentage (FGP), shoot length (SL) and root main length were assessed. Seeds were counted as germinated when the radicle growth reached 2 mm.

Statistical analysis

The experiments were conducted with four replicates of 50 seeds (n=4) for the trait seed germination and with 10 replicates (n=10) for the seedling characteristics, and the results were expressed as mean ± standard deviation (SD). All the data were subjected to one-way analysis of variance (ANOVA) and Duncan's multiple range test ($p < 0.05$) using SAS version 9.0 (Statistical Analysis System) (2002) software.

RESULTS AND DISCUSSION

Results presented in *Table 2* show that cold stratification treatments had a significant effect ($p < 0.0001$) on seed germination of *Z. lotus*. The highest FGP of 83% was recorded after 120 days of cold stratification. The seeds that did not endure cold stratification were unable to germinate. A period of 90 and 45 days of cold stratification was insufficient to induce a good percentage of germination in *Z. lotus* seeds by recording a low improvement of 35% and 15% FGP, respectively compared to the control (*Table 2*). Cold stratification is considered the most effective way of breaking the dormancy in summer annual seeds and the most temperate perennials (Leadem, 1997; Kheloufi *et al*., 2019). However, the patterns of germination response can differ depending on the environment, geographic distribution and phylogenetic relationships (Luna *et al*., 2012). Grime *et al*. (1979) reported interspecific variations in the ability of freshly matured seeds to germinate immediately.

In several plants, seed dormancy is an adaptive mechanism to protect seedlings from freezing damage during the winter (Burke *et al*., 1976; Kozlowski and Pallardy, 2002). Cold stratification, usually between 1-10°C, can break the dormancy of seed for a number of species (Bewley and Black, 1994). The optimum stratification period varies between species, and also between different seed lots of the same species (Andersson and Milberg, 1998). A 60-day chilling duration usually helps to prevent embryo dormancy in many plants (Bewley and Black, 1994). Many species with strict primary dormancy however need a long stratification period of six months (Albrecht and McCarthy, 2006). The current study showed that *Z. lotus* seed germination is enhanced by an extended cold duration of four months, which indicates that *Z. lotus* seeds may have similar primary endogenous dormancy. Control of seed dormancy may depend on the endogenous balance of biosynthesis and catabolism of gibberellic acid (GA) and abscisic acid (ABA), and thus on the ratio of ABA: GA rather than the amounts of these two plant growth regulators (Shu *et al*., 2013). Synthesis and signaling of ABA control the dormant state and synthesis of GA is a signal of the transition to germination (Kermode, 2005).

Table 2 - Seed germination and seedling size of *Ziziphus lotus* 15 days after various cold stratification period

Period of cold stratification at 5°C	FGP (n=4)	Seedling size (cm) (n=10)		
		Shoot length	Root length	Total length
0 days	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]
45 days	15.0 ± 3.05[c]	5.12 ± 0.61[c]	3.71 ± 0.58[c]	8.84 ± 1.00[c]
90 days	32.5 ± 3.05[b]	6.10 ± 0.57[b]	4.95 ± 0.94[b]	11.0 ± 0.98[b]
120 days	83.0 ± 6.42[a]	6.80 ± 0.61[a]	9.75 ± 1.34[a]	16.5 ± 1.56[a]
SEM	32.4	2.75	3.63	6.13
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001

SEM = standard error of measurement. The same alphabet along the column indicates no significance difference (Duncan Multiple Range Test)

Growth parameters including shoot length, root length and seedling total length were also significantly affected by cold stratification ($p < 0.0001$) (*Table 2*). Growth attributes, such as shoot length, root length and total seedling length, were higher among seeds that germinated earlier, since they began early growth compared to seeds that germinated later, where maximum shoot length (6.80 cm), root length (9.75 cm) and seedling total length (16.5 cm) were observed after 120-day period of cold stratification, compared to all other treatments (*Table 2*). Chen *et al*. (2008) showed that seed treatment enhances seedling growth because of interactions between promoters and inhibitors. Indeed, ABA as the inhibitor interacts with gibberellic acid, which is increased after removal of the inhibitor, to improve development (Hoffmann-Benning and Kende, 1992). In addition, the nutrient level in the growth medium may also contribute to the improved growth (Hu and Schmidhalter, 2005; Ashraf and Foolad, 2005).

Fig. 2 illustrate an overview of the seedling establishment of *Z. lotus* which seeds were treated with 120-day period of cold stratification. In freshly germinated seeds, two small false leaves were observed. Seedlings of *Z. lotus* emerged well at depths of 1-2 cm and could not emerge when sand burial depth was > 4 cm (Maraghni *et al*., 2010).

Figure 2 - Overview on seed germination and seedling establishment of *Ziziphus lotus* during 15-day period at 27°C (± 2°C)

CONCLUSION

Through this study, we evaluated the seed germination behavior and seedlings size of *Ziziphus lotus* in different periods of cold stratification. Results analysis revealed that seed germination and seedling emergence of *Z. lotus* are related to the period of cold stratification, where the longer the period of stratification, the greater the germination rate, total seedling length, shoot length and root length. The results showed that when storing *Z. lotus* seeds for 120 days at 5°C before planting, the germination rate was high and their seedlings were longer. These growth parameters are attributed to enhancing the rapid development of vigorous seedlings for nursery or plantation establishment.

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