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ORIGINAL ARTICLE

The relationship between seeds coat color of *Retama sphaerocarpa* (L.) Boiss and their germinability

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Abstract

The objective of this study was to evaluate the morphological characteristics of *Retama sphaerocarpa* seeds, to assess the relationship between seed coat color and germination quality in order to select seeds with the best germination qualities. Chemical scarification for different durations, mechanical scarification, soaking of seeds in water, soaking of seeds in gibberellic acid (10^{-3} M) were used as presowing treatments to improve germination. For all tests, we evaluated the germination rate and the mean germination time. The results showed that there was significant heterogeneity in seed morphology and color in the study population, with yellowish and greenish seeds behaving differently from the blackish ones. The best germination percentages were recorded by yellowish seeds scarified by pure sulfuric acid (96%) for 1 h, 1h30, 2 h, 2h30 and 3 h, the later leading to a maximum germination rate of 100% ($P < 0.05$), as well as by greenish seeds treated for 2h30, 3 h and 4 h, with 2h30 also leading to a maximum rate of 100% $P < 0.05$. Similarly, hot water significantly increased the germination percentage of yellowish and greenish seeds. On the contrary, mechanical scarification by sandpaper and application of gibberellic acid solution did not significantly increase the germination percentages. The germination percentage of blackish seeds remained almost zero regardless of the treatment type. The results of this work provided new information for the reproductive biology of *Retama sphaerocarpa* especially the germinative behavior of seeds according to their color.

Keywords Germination · *Retama sphaerocarpa* · Scarification · Seed color

Introduction

Arid and semi-arid ecosystems are characterized by specific climatic conditions that are manifested by a long dry season, intense evaporation, low precipitation with a high variability of their spatio-temporal distribution (Le Houérou 1959). All these factors would contribute to the increase of the edaphic aridity of the environment, and consequently the development of the phenomenon of desertification. According to Le Houérou (1996), 47% of the planet is affected by desertification and according to Thirgood (1981) and Vallejo (2009), the natural regeneration of Mediterranean woodlands after a disturbance and the return of the ecosystem to its initial state are often absent or very slow. In view of this situation, it will be necessary to deepen the studies related to the distribution, characterization, evolution and strategies for the perpetuation and development of indigenous species that are generally better adapted to the environment and widely known and used by local populations**;** among these are the Retams.

The Retams are leguminous shrubs, with both pharmacological and ecological interests. They are qualified as dune-fixing plants and their name derives from the biblical name *Rotem* which was changed by the Arabs into *R'tem* or *Retam* (Quézel and Santa 1962). Retams represent a natural means to fight against desertification and play an important role in the balance of the natural environment. There are three species in Algeria: *Retama monosperma* (L.) Boiss., *Retama raetam (*Forssk.) Webb, *Retama sphaerocarpa* (L.) Boiss. This latter is common between 0–1400 m altitude and in the various climates (humid and arid) and open, dry and sunny environments of Mediterranean ecosystems,

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including coastal dunes, scrub, and also deserts, forming the main forest cover in many semi-arid regions (Quézel and Santa 1962; Allen and Allen 1981). It is well adapted to the seasonally stressed conditions and shallow soils typical of Mediterranean semi-arid ecosystems. The species *R. sphaerocarpa* has a thick root system, which can reach a depth of 25 m (Haase et al. 1996) and a stem system that can be 1 to 2 m high, with more or less erect pubescent branches. It bears a large number of green branches of various lengths giving the species an open structure, and representing the majority of its aerial biomass. The deciduous leaves are very small and the flowers are yellow and also very small (5–6 mm), in lateral clusters of 8 to 15 florets arranged on the old branches. The fruits are covered by a hard layer (Domingo et al. 1997). Like many other woody species typical of the Mediterranean region and other semi-arid areas, *R. sphaerocarpa* grows for a short period in spring, with favorable temperature and soil moisture conditions (Haase et al. 2000). The seeds are 3–6 mm long and have hard seed coats (Pugnaire et al. 2006). *R. sphaerocarpa* has edaphic and physical effects on understory vegetation (Moro et al. 1997). It has the capacity to fix nitrogen due to its symbiosis at the root level, to restore degraded areas and stabilize desert areas due to its ability to resist environmental stresses (Boulila et al. 2009). It also contribute to the reduction of the use of nitrogenous fertilizers and the reduction of greenhouse gas emissions (Rispail et al. 2010). *R. sphaerocarpa* is a key element to prevent erosion processes and desertification in semi-arid and arid areas (Caravaca et al. 2003). Many leguminous woody species are pioneer species in arid conditions and have been successfully used for revegetation of arid and nutrient deficient ecosystems (Forti et al. 2006). Nowadays *Retama sphaerocarpa* is spreading due to its high capacity for colonizing abandoned agricultural fields (Espigares et al. 2004).

This species has been listed as a medicinal plant of arid regions. In traditional medicine, the stems and leaves are used as an emetic. The decoction of the leaves is administered as a purgative and their powder is used as healing and antiseptic (Bellakhdar 1997).

Retama sphaerocarpa has been the subject of several works regarding anatomy and histology, but little work has been devoted to the regeneration and conservation of this natural wealth that can represent an opportunity to rehabilitate degraded communities (Rivest et al. 2011). This is what prompted us to begin the morphometric study of the seeds and their germinative behavior.

This study is situated in the general context of the conservation and development of *R. sphaerocarpa* which is an endemic species of the Mediterranean basin. It focuses on the search for adequate solutions for a sustainable conservation of this species and to overcome the various obstacles to germination by developing germination methods and practices of this natural wealth's seeds. A better knowledge of the problems and factors of decline of this species would contribute to a better regeneration and thus to the protection of the biodiversity of arid and semi-arid ecosystems weakened by constant anthropic pressures.

Material and methods

Study area

The study has been carried out in El-Bayadh (south-west Algeria). The sampling site is located at 33°24′36" latitude North and 0°44′19" longitude East, at an altitude of 1284 m (Fig. 1). El Bayadh has a steppe climate, with a very irregular annual rainfall average ranging from 200 to 300 mm, but with long or very long time periods of drought. The average annual temperature is 14.2 °C, with an average minimum of 6 °C in winter and average maximum of 36 °C in summer

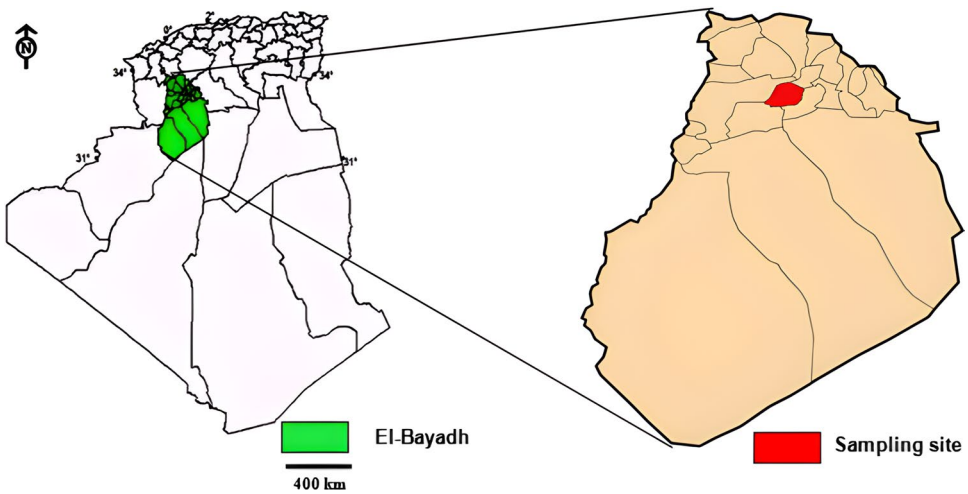

Fig. 1 Location of sampling site of *Retama sphaerocarpa* seeds

(ONM: Office National de la Météorologie 2011; ANDI: Agence National de Dévelopment de l'investissement 2013).

Pods collection and seeds storage

Mature pod heads of *Retama sphaerocarpa* were randomly collected from five different shrubs (shrub diameter averaged 1–3 m and height ranged from 1.0–2.5 m) in September 2018 (Fig. 2). The population sample grows under natural conditions. Seeds were removed from their pods and divided into three batches: the first batch, intended for germination experiments and for morphological study was stored in Kraft paper envelopes and kept at laboratory temperature (20–25 °C), until the beginning of the experiment. The second and third were used to study color change. They were stored under two different conditions: the first at room temperature and the second in the freezer (-21 ± 2 °C) for a period of one year for both groups.

Morphological study

One hundred seeds of each color were analyzed: length (cm), width (cm), weight (g). Length and width were measured using a Vernier caliper and weight using a precision scale. Seed coat color was determined using the standard color chart provided by Dee-cal Frenzy (https://www.dee-calfrenzy.com/products/color-chart-samples).

Determination of water content

A batch of 10 seeds per color is used to determine the percentage of water in seeds; each measurement had 3 replications, totaling 30 seeds per color. The seeds were weighed (P1: Fresh weight), then they were put in the oven at 103 ± 2 °C for 24 h; after they were weighed again (P2: Dry weight).The water content is calculated by the following formula (Beugré et al. 2011)

$$\mathbf{Water\ \%} = \mathbf{P1} - \mathbf{P2} \ * \ 100/\mathbf{P1}$$

Germination tests

Before the germination tests, damaged and insect infected seeds were discarded, and the empty ones were eliminated using the method of floating in distilled water (Downie and Bergsten 1991; Audinet 1993)**.** Seeds of *R. sphaerocarpa* were separated into several lots according to the variation of their colors. One thousand five hundred seeds for each color were tested under different treatments. Seeds were disinfected with Sodium hypochlorite 1% for 5 min, and rinsed three times with distilled water. They were placed in Petri dishes on hydrophilic cotton moistened with distilled water and incubated at 25 ± 2 °C.

In addition to the controls, different pretreatments have been tested of the three colors seeds:

Soaking in water: this pretreatment consisted in immersing the seeds in water for two durations, 24 h and 48 h.
Mechanical Scarification: seeds were scarified by sandpaper for 3 min to carefully remove the seed coat, without damaging the embryo.
Soaking in Gibberellic acid GA_3 (10^{-3} M): seeds were immersed in gibberellic acid for 24 h and 48 h then put to germinate in water.
Chemical scarification in Sulfuric acid: Seeds were soaked in pure sulfuric acid H_2SO_4 (96%) for several durations: 10 min, 15 min, 30 min, 1 h, 1h30, 2 h, 2h30, 3 h and 4 h.

Germinated seeds were counted daily, to determine the parameters characterizing the physiological process. Seeds were considered to have germinated when radical elongation began and reached 2 mm (Côme 1970; ISTA 2003).

Germination parameters

The germination percentage was calculated using the equation below (Czabator 1962; Scott et al. 1984)

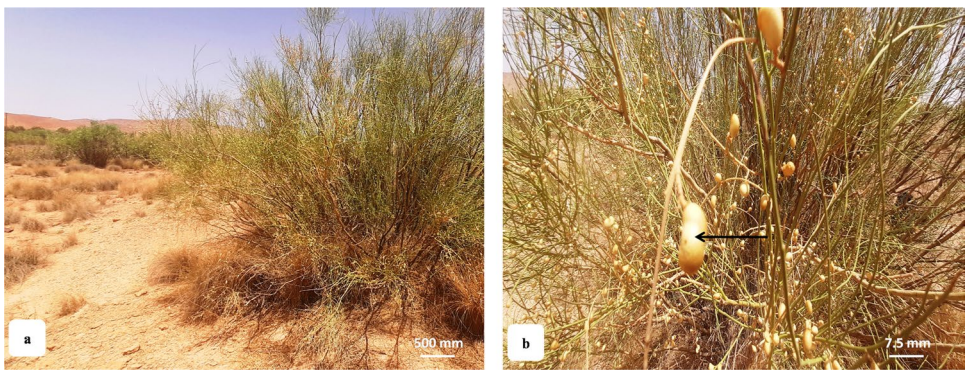

Fig. 2 *Retama sphaerocarpa*. **a**: *Retama sphaerocarpa* shrub; **b**: fruiting shrub. *The arrow* in **Fig. 2. b** indicates: Pod of *Retama sphaerocarpa*

$$Germination\ \% = seeds\ germinated / total\ seed * 100$$

Germination percentage were calculated from the final germination percentages **mean ± SE** of the four replicates (25 seeds for each). The mean germination time (**MGT**) was also determined according to the following formula (Brenchley and Probert 1998)

$$MGT = \sum DN / \sum N$$

where N is the number of germinated seeds per day D and D is the number of days counted from the day of sowing.

Viability test

Blackish seeds that did not germinate were subjected to a tétrazolium viability test. First, the seeds were cut in half to determine if they contained normal embryos capable of germination. Then, half of the seeds meeting this criterion were immersed in a 1% aqueous solution of 2,3,5-triphenyl-2H-tetrazolium monochloride for 24 h at 25 °C in the dark according to the rules prescribed by ISTA (2003). The colorless tétrazolium is reduced by living cells to a red, stable, no diffusing compound (Lakon 1942). Thus, with the living parts stained red, the viability of the seed can be assessed by determining the staining rate of the whole. For the seed to be considered viable, the embryo must be well stained red.

Statistical analysis

Statistical analysis of the results was achieved by SPSS Statistics for Windows, version 25.0. All data were checked for homogeneity of variance using the Levene's equal variance test and normal distribution using Shapiro-Wilk test at first. The values of the final germination percentage were transformed to meet homoscedastic assumptions and subjected to analysis of variance (untransformed data appear in tables) using the SPSS package. The relationship between color and size was determined using one way factorial ANOVA. Moreover, the Pearson correlation between morphological parameters and seed color was analyzed.

One way factorial ANOVA was also used to analyze the effect of the different treatments and duration of treatment on seed germination. In the same way, to determine effect of color seeds on the final percentage of germination. Results are considered not significant if P value > 0.05, significant if P value < 0.05, very significant if $P < 0.01$ and highly significant if $P < 0.001$.

Results

Influence of storage on seed color change

Out of 1000 seeds observed, we found that *Retama sphaerocarpa* seeds exhibited three colors by comparing with color standard chart provided by Dee-cal Frenzy: yellowish seeds, greenish seeds and blackish seeds (Fig. 3).

Storage at room temperature (20–25 °C)

During storage period, the seeds changed the color of their tissue cover. At maturity, the seeds were yellowish, after they became greenish and end upped by becoming blackish. The greenish seeds at harvesting time (September 2018) were at 68.9 ± 1.30%; the yellowish seeds were at 25.9 ± 0.63% and blackish seeds at 5.2 ± 0.47% (Fig. 4). After one year of storage at room temperature (20–25 °C), the modification of seed color was observed in three phenotypes; the greenish seeds decreased from 68.9 ± 1.03% at the harvest time to 66.6 ± 0.80%; the yellowish seeds decreased from 25.9 ± 0.63% to 1.1 ± 0.05%

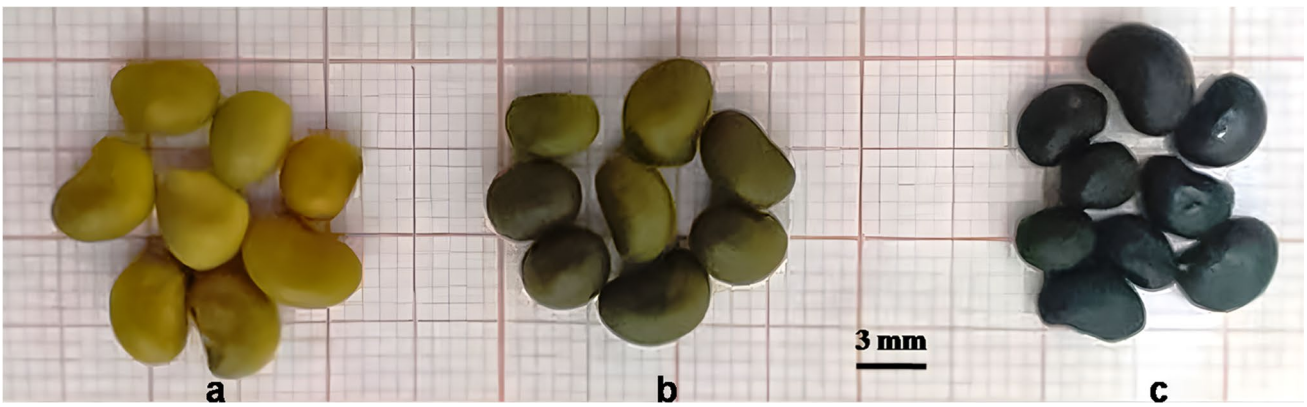

Fig. 3 Variation in the color of *Retama sphaerocarpa* seeds. **a**: yellowish Seeds; **b**: greenish Seeds; **c**: blackish seeds

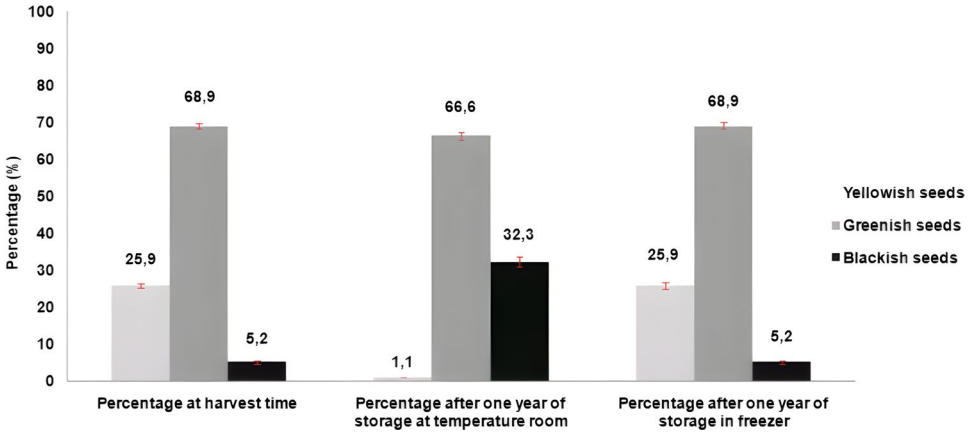

Fig. 4 Variation of the percentage of seeds color according to their storage for one year at room temperature and in freezer; the results are expressed as mean ± SE ($n = 3$)

and the blackish seeds increased from 5.2 ± 0.47% to 32.3 ± 1.33%. At the conclusion, the greenish seeds were the most abundant (Fig. 4).

Storage in freezer (-21 ± 2 °C)

We observed that the number of yellowish, greenish and blackish seeds of *Retama sphaerocarpa* collected and stored directly in the freezer remained the same after one year of storage: greenish seeds had the highest percentage 68.9 ± 1.37%; yellowish seeds 25.9 ± 0.95% and blackish seeds 5.2 ± 0.51% (Fig. 4).

The water content of the seeds

The results obtained showed that the percentage of water content of mature seeds was higher in blackish and in yellowish seeds, respectively 9.55 ± 3.29% and 8.52 ± 0.55%, then in the greenish seeds, containing 7.53 ± 1.56%, but these differences were not significant ($P > 0.05$). Therefore, *Retama sphaerocarpa* seeds are considered as orthodox seeds (Côme and Corbineau 1998).

Relationship between seed color and seed morphometry

Yellowish and greenish seeds had the highest average length, width and weight followed by blackish seeds, which means that blackish seeds were relatively small in size (Table 1). The difference in seed morphometry was highly significant between yellowish and blackish seeds ($P < 0.001$) and between greenish and blackish seeds ($P < 0.001$, Table 1). The differences in length, width and weight between yellowish and greenish seeds were not significant ($P > 0.05$, Table 1).

Positive correlations between the length and the weight of the seeds ($r^2 = 0.33$; $P < 0.01$) and between the width and the weight ($r^2 = 0.38$; $P < 0.01$) were detected.

Germination tests

Influence of seed color on germination

The results of the control seeds showed that they had a strong dormancy with a low percentage of germination; the yellowish seeds showed the highest percentage of

Table 1 Seeds length (cm), width (cm) and weight (g) for each color seeds of *Retama sphaerocarpa*. P: The significance level between the three seed colors. *P* values were considered not significantly ($P > 0.05$ "ns"), significantly ($P < 0.05$*), very significant ($P < 0.01$**) and highly significant ($P < 0.001$***)

Seeds	Yellow seeds(mean value ± SE)	Green seeds(mean value ± SE)	Black seeds (mean value ± SE)	Signification btween three colors (Y: Yellow. G:Green, B: Black)
Lenght	0.61 ± 0.08	0.61 ± 0.09	0.51 ± 0.02	Y/G: *P* value > 0.05 ns Y/B: *P* value < 0.001*** G/B: *P* value < 0.001***
Width	0.42 ± 0.08	0.44 ± 0.01	0.34 ± 0.02	Y/G: *P* value > 0.05 ns Y/B: *P* value < 0.001*** G/B: *P* value < 0.001***
Weight	0.064 ± 0.003	0.070 ± 0.002	0.040 ± 0.003	Y/G: *P* value > 0.05 ns Y/B: *P* value < 0.001*** G/B: *P* value < 0.001***

germination (30.00 ± 3.46%), followed by the greenish seeds (10.00 ± 1.15%), which could be explained by a difference in coat hardness between yellowish seeds and greenish seeds. The percentage of germination of the blackish seeds was null (0%). The analysis of variance showed that the differences in the percentages of germination were highly significant between the three colors of seeds ($P < 0.001$).

Influence of the soaking in water

The results of the germination of yellowish, greenish and blackish seeds of *Retama sphaerrocaarpa* under the effect of soaking in water during 24 h and 48 h are summarized in Table 2.

These results indicated that the soaking in water significantly improved ($P < 0.01$) the germination percentage of both colors (yellowish and greenish seeds), from 30.00 ± 3.46% to 60.00 ± 7.12% in the former, and from 10.00 ± 1.15% to 70 ± 11.49% in the later. Unlike greenish seeds, Soaking yellowish seeds in water had a significant effect on the speed of germination ($P < 0.001$), which remained quite slow in greenish seeds ($P > 0.05$). The duration of soaking in distilled water did not significantly influence seed germination ($P > 0.05$). The germination of the black seeds remained null (0%).

Table 2 Effect of water soaking on germination percentage (mean values ± standard error "SE") and on the mean germination time MGT (mean values ± standard error "SE") of the three seed colors. The significance level "S" between the results of the control and soaked seeds. *P* values were considered insignificant ($P > 0.05$ "ns"), significant ($P < 0.05$*), highly significant ($P < 0.01$**) and highly significant ($P < 0.001$***)

Color of seed	Duration of soaking in water	% of germination (% ± SE)	S	MGT (days ± SE)	S
Yellow	Control	30.00 ± 3.46	/	18.70 ± 1.06	/
	24 h	60.00 ± 9.52	*	10.36 ± 0.53	***
	48 h	60.00 ± 7.12	**	13.00 ± 0.83	**
Green	Control	10.00 ± 1.15	/	13.00 ± 2.48	/
	24 h	70.00 ± 9.30	***	12.89 ± 0.38	ns
	48 h	70.00 ± 11.49	**	12.95 ± 0.50	ns
Black	Control	0	/	0	/
	24 h	0	/	0	/
	48 h	0	/	0	/

Influence of mechanical scarification

The results showed that mechanical scarification by sandpaper for 3 min did not have a significant effect on the percentage of seed germination ($P > 0.05$, Table 3). The germination percentage of greenish seeds increased from 10.00 ± 1.15% to 25.00 ± 7.00%, whereas this treatment gave a negative result in yellowish seeds, causing a decrease in the germination percentage. This means that there was probably a difference in the seed coat hardness between the two colors. However mechanical scarification significantly improved the mean time to germination (MTG, $P < 0.01$, Table 3). So, mechanical scarification with sandpaper for 3 min was not sufficient to eliminate the dormancy of the greenish seeds, while for the yellowish seeds it caused brittleness.

Influence of the soaking in the Gibberellic Acid ($10.^{-3}$ M)

The results regarding the germination behavior of *R. sphaerocarpa* seeds under the effect of gibberellins were summarized in Table 4.

The results showed that the percentage of germination of yellowish seeds decreased compared to the control under gibberellic acid soaking. The difference was significant ($P < 0.05$) compared to the control. In contrast to yellowish seeds, the germination percentage of greenish seeds increased significantly ($P < 0.001$) under the effect of soaking in gibberellic acid for 24 h. Prolonged treatment of greenish seeds with GA3 for 48 h decreased the germination percentage (Table 4).

Influence of soaking in pure sulfuric acid (96%)

The chemical scarification of *R. sphaerocarpa* seeds with the pure H_2SO_4 for several soaking durations showed a very satisfactory effect in greenish and yellowish seeds. The results are summarized in Table 5.

Table 3 Effect of mechanical scarification on germination percentage (mean values ± standard error "SE") and on mean germination time MGT (mean values ± standard error "SE") of the three seed colors. The significance level "S" between the results of control and scarified seeds. *P* values were considered non-significant ($P > 0.05$ "ns"), significant ($P < 0.05$*), highly significant ($P < 0.01$**) and highly significant ($P < 0.001$***)

Color of seed	Control % of germination (% ± SE)	Mechanical scarification (% ± SE)	S	MGT of Control (days ± SE)	MGT of scarified seeds (days ± SE)	S
Yellow	30.00 ± 3.46	20.00 ± 7.48	ns	18,70 ± 1,06	5.43 ± 2.07	**
Green	10.00 ± 1.15	25.00 ± 7.00	ns	13,00 ± 2,48	6.58 ± 0.67	*
Black	0	0	/	0	0	/

Table 4 Effect of soaking in Gibberellic Acid on germination percentage (mean values ± standard error "SE") and on the mean germination time MGT (mean values ± standard error "SE") of the three seed colors. The significance level "S" between the results of control and soaked seeds. *P* values were considered non-significant ($P > 0.05$"ns"), significant ($P < 0.05$*), highly significant ($P < 0.01$**) and highly significant ($P < 0.001$***)

Color of seed	Duration of soaking in GA_3	% of germination (% ± SE)	S	MGT (days ± SE)	S
Yellow	Control	30.00 ± 3.46	/	18.70 ± 1.06	/
	24 h	20.00 ± 1.63	*	9.00 ± 0.71	***
	48 h	1.00 ± 0.41	**	3.00 ± 3.00	**
Green	Control	10.00 ± 1.15	/	13.00 ± 2.48	/
	24 h	40.00 ± 3.26	***	10.13 ± 1.07	ns
	48 h	5.00 ± 2.52	ns	5.92 ± 1.97	ns
Black	Control	0	/	0	/
	24 h	0	/	0	/
	48 h	0	/	0	/

The scarification in sulfuric acid at low duration for 10 min, 15 min and 30 min slightly improved the germination percentage in both types of seeds (yellowish and greenish). The yellowish seeds showed a maximum germination rate of 100% from 1 h soaking time to 3 h. After this soaking time (3 h), the germination percentage decreased significantly to 80.00 ± 8.79% (Table 5). It was also found that pretreatment of yellowish seeds with sulfuric acid for different durations improved the speed of germination. It became significant after a pretreatment time of 1 h to 3 h with a maximum germination percentage of 100%. These results were confirmed by analysis of variance which showed that the duration of chemical scarification had a significant effect on germination rate ($P < 0.001$.)

Greenish seeds reached maximum germination (100%) only after 2h30 of soaking in H_2SO_4. Again, we found that greenish seeds had a more resistant seed coat than yellowish seeds. Analysis of variance showed that scarification of greenish seeds by sulfuric acid for 10 min had no significant

Table 5 Effect of soaking in pure sulfuric acid on the percentage of germination (mean values ± standard error "SE") and on the mean time of germination MGT (mean values ± standard error "SE") of the three seed colors. The significance level "S" between the results of untreated seeds (control) and those of soaked seeds. *P* values were considered as non-significant ($P > 0.05$ "ns"), significant ($P < 0.05$*), highly significant ($P < 0.01$**) and highly significant ($P < 0.001$***)

Colors of seed	Duration of soaking in H_2SO_4	% of germination (% ± SE)	S	MGT (days ± SE)	S
Yellow	Control	30.00 ± 3.46	/	18.70 ± 1.06	/
	10 min	40.00 ± 8.16	ns	15.46 ± 1.59	ns
	15 min	60.00 ± 9.52	*	14.66 ± 2.75	ns
	30 min	60.00 ± 11.43	*	14.19 ± 1.95	ns
	1 h	100.00 ± 0.00	***	10.88 ± 1.41	*
	1 h 30	100.00 ± 0.00	***	8.52 ± 1.03	***
	2 h	100.00 ± 0.00	***	9.39 ± 0.39	***
	2h30	100.00 ± 0.00	***	6.71 ± 0.41	***
	3 h	100.00 ± 0.00	***	6.19 ± 0.89	***
	4 h	80.00 ± 8.79	**	7.48 ± 0.13	***
Green	Control	10.00 ± 1.15	/	13.00 ± 2.48	/
	10 min	20.00 ± 7.12	ns	12.20 ± 4.15	ns
	15 min	60.00 ± 6.78	**	11.94 ± 0.22	ns
	30 min	61.00 ± 5.26	***	11.70 ± 1.88	ns
	1 h	80.00 ± 7.83	***	10.07 ± 0.57	ns
	1 h 30	90.00 ± 2.16	***	9.74 ± 0.77	ns
	2 h	90.00 ± 7. 57	***	7.24 ± 1.27	ns
	2h30	100.00 ± 0.00	***	6.94 ± 0.67	ns
	3 h	100.00 ± 0.00	***	7.70 ± 7.78	ns
	4 h	100.00 ± 0.00	***	7.52 ± 0.72	ns
Black	Control	0	/	0	/
	10 min	0	/	0	/
	15 min	0	/	0	/
	30 min	0	/	0	/
	1 h	0	/	0	/
	1 h 30	0	/	0	/
	2 h	0	/	0	/
	2h30	0	/	0	/
	3 h	0	/	0	/
	4 h	0	/	0	/

effect P > 0.05 on the germination percentage of these seeds. The effect of scarification by sulfuric acid became highly significant from 30 min of soaking ($P < 0.001$, Table 5). Beyond 30 min of soaking, germination became faster with a high percentage.

Statistical tests showed that the mean germination time of yellowish and greenish seeds was strongly correlated with germination percentage ($r^2 = 0.90$, $P = 0.000027$, and $r^2 = 0.71$, $P = 0.002026$) respectively; this correlation was negative.

Regarding the blackish seeds, the results obtained showed that the germination was null whatever the duration of soaking in the sulfuric acid.

Viability test of blackish seeds

Since the blackish seeds did not germinate, this led us to make a test of viability of these seeds by the test of 2, 3, 5-triphenyl-2H-tetrazolium monochloride, which became pink in the presence of CO_2.The observations of the transverse sections of seeds under the binocular loupe showed that more than 80% of the seeds had an embryo touched by the insect which pierced the teguments (Fig. 5a, b, c, d). The few where the embryo was not affected were not also viable because they did not change color in the presence of tétrazolium (Fig. 5e).

Discussion

One of the critical early stages in the life cycle of plants controlling their reproductive success and persistence of their populations is seed germination and seedling establishment (Bu et al. 2008). From an ecological perspective, dormancy can be defined as the prevention of germination even when suitable conditions are prevalent. The dormancy

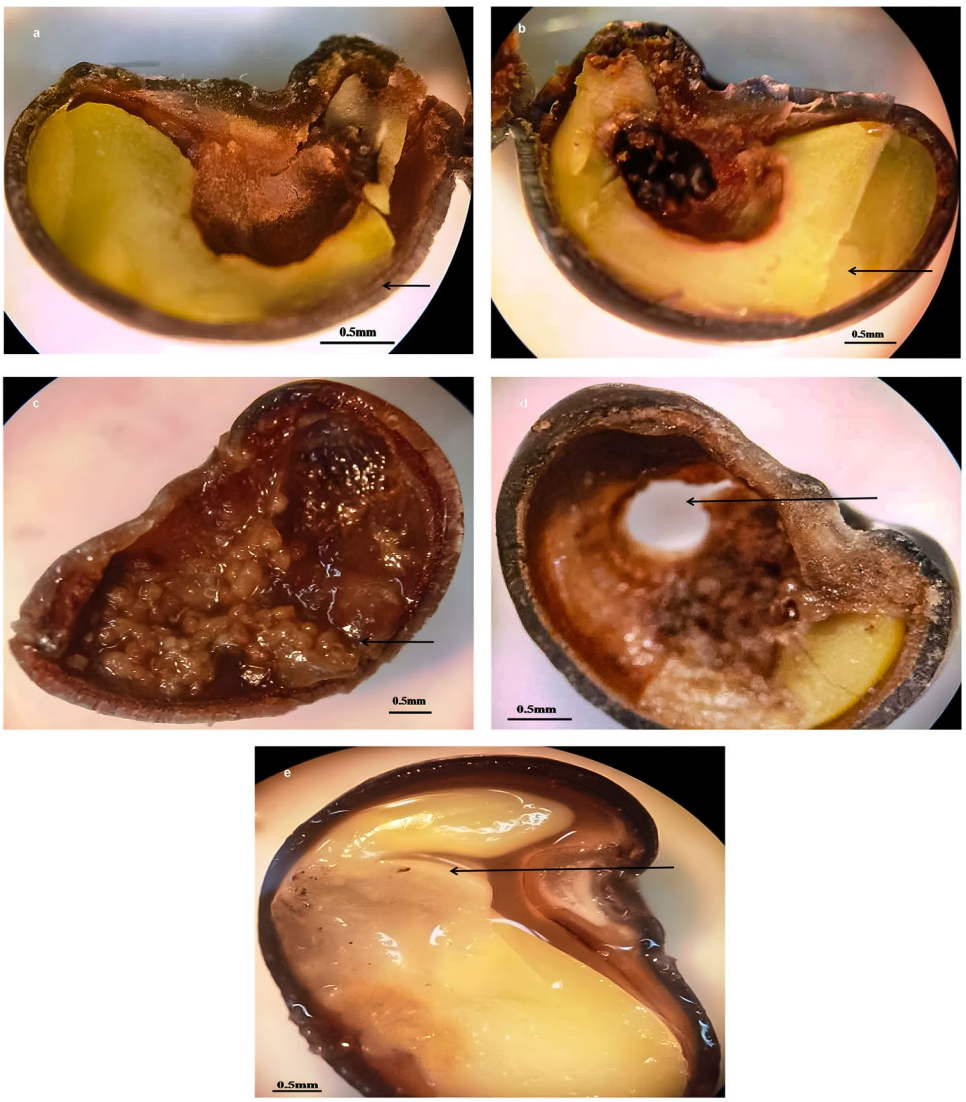

Fig. 5 Observation of a blackish seed embryo with the binocular loupe. **a, b** Embryo partially degraded by the insect; **c, d** absence of the embryo which is completely degraded by the insect which escaped by piercing the mantle; **e** Intact, not viable embryo (negative reaction to tétrazolium). *The arrow* in Fig. 5a indicates: seed coat pierced by the insect. *The arrow* in Fig. 5b indicates: partially degraded embryo. *The arrow* in Fig. 5c indicates: completely degraded embryo. *The arrow* in Fig. 5d indicates: hole caused by the entry or exit of the insect. *The arrow* in Fig. 5e indicates: intact embryo

mechanism allows a species to synchronize its germination with favorable environmental conditions which increases its probability of survival and establishment (Baskin and Baskin 1998). In this context we tried to better understand the morphology and physiology of germination and dormancy of *R. sphaerocarpa* seeds from the region of El Bayadh (North–west Algeria).

This study aims through morphological and physiological examinations to identify the germinative quality and the vigor of the seeds of *R. sphaerocarpa*. The results of the morphometric analyses showed that the difference between yellowish and blackish seeds as well as between greenish and blackish seeds was highly significant ($P < 0.001$). In contrast, morphometric analysis revealed that there was no significant difference between yellowish and greenish seeds ($P > 0.05$). This signified the existence of intra–specific heterogeneity at the seed level of this species. Indeed, morphometric measurements made by Pugnaire et al. (2006) on *R. sphaerocarpa* seeds from northeast Spain showed that there was a remarkable morphological resemblance (length, width and weight) between seeds from northwest Algeria (El Bayadh) and those from northeast Spain.

Benmiloud-Mahieddine et al. (2011) reported by cytogenetic analysis that the morphometric traits of *Retama raetam* seeds were much closer to *Retama monosperma* than those of *Retama sphaerocarpa*. These same authors reported that *Retama sphaerocarpa* had a hybrid origin between *Retama raetam* and *Retama monosperma* (Benmiloud-Mahieddine 2013). This intra-specific difference can be due to the genetic factor (Sajjan et al. 1999), the natural conditions (temperature, soil, rainfall…), the position of the seeds on the plant or in the fruit, the state of maturation of the seed and the age of the mother plant (Gutterman 1980).

The three colors of the seed coats of *R. sphaerocarpa* observed (yellow, green and black) did not show the same germination abilities. However, the yellowish and greenish seeds gave the best rates of germination compared to the blackish seeds which always gave a null rate (0%). As regards the speed of germination, the yellowish seeds always presented a fast and vigorous germination compared to the greenish seeds.

We found that blackish seeds were lighter (0.040 ± 0.003 g) compared to yellowish and greenish seeds that they which had the highest average weights (0.064 ± 0.003 g and 0.070 ± 0.002 g respectively). This means that the blackish seeds were relatively small in size. Observations of the blackish seeds under the binocular loupe showed that they were either completely empty or they had destroyed embryos that had been visibly attacked by insects. Indeed, we observed small holes only in the seeds with black coat (more than 80%) that likely indicate the entry or exit of an insect, which explains the nonviability of the vast majority of these seeds.

We also found that seeds stored in the freezer did not change color unlike seeds stored at room temperature. Preliminary tests showed that seeds stored at -20 °C gave maximum germination (results not shown) unlike seeds stored at room temperature which lost their germination power, meaning that the natural environment is not suitable for storage. Like other orthodox seeds that can be stored for months or years if kept at low temperature and low moisture content (FAO 1985), *Retama sphaerocarpa* seeds also retained their physiological and morphological condition and remained viable at low temperature for 12 months. The low temperatures played a role in reducing of chemical reactions that led to a decrease in respiration, allowing the seeds to retain their life longer (Marcos Filho 2005).

Ferreira (2016) conducted a study on the influence of seed coat color and mother plant on seed germination of *Retama sphaerocarpa* of Portugal. This author reported the existence of three colors of seed coat (black, brown and green) while in our study we observed the following three colors (black, yellow and green). Ferreira (2016) also reported that black seeds of *R. sphaerocarpa* have the lowest germination rate compared to green and brown seeds. This author reported that this did not clarify why black coated seeds exhibited the lowest values of germination rate and vigor index, suggesting that further research must be addressed to seed maturation season, seed coat structure and thickness, and physiological processes involved in seed maturation. *R. shpaerocarpa* shrubs tend to keep some seed pods for more than one year (Haase et al. 2000).The variability can be explained by the differences of the edapho-climatic factors of the various sites (Price et al. 1986; Turpeinen et al. 1999).

Our study showed that the black color of the seeds appears towards the end of the post maturation of the seeds. Even at the time of the conservation of the seeds, this change of color was done progressively of the yellow then the green and the black at the end. We noted that this color change was an indicator of a progressive passage of seeds from a physiological active and viable state to a less active and little or no viable state.

The three colors of seeds require a pretreatment to facilitate and increase the percentage of their germination. Remember, the black seeds did not germinate even with the different pretreatments. Like other legume seeds, *R. sphaerocarpa* showed seed coat impermeability to water and oxygen which means that the seed will not germinate unless the coat is scarified (Cavanagh 1987; Piotto and Piccini 1996). Severals studies showed that the best pursuing treatments for enhancing *R. sphaerocarpa* seeds germination were chemical scarification with acids or treatment with warm water and mechanical scarification. However, sulfuric acid treatment is found to be superior in terms of germination percentage, average germination time and energy of germination (Baskin and Baskin 1998; Zemouri et al. 2020).

Soaking *Retama sphaerocarpa* seeds in water for 24 h and 48 h slightly improved the germination rate compared to the control. Similar results on *R. sphaerocarpa* seeds from Portugal treated with hot water (80 °C) for 30 s were obtained by Fabião (2011) and Fabião et al. (2014). However, a similar treatment (soaking in water) of *Retama monosperma* seeds by Bouredja et al. (2011) did not improve the germination rate. Hatimi et al. (1997); Teketay and Granstrom (1997) reported that soaking *Acacia* and *Retama* seeds of Ethiopian and Moroccan origin in water did not have a significant positive effect on the germination of these species seeds because they had strong coat dormancy. On the other hand, the treatment of a certain number of Fabaceae by soaking the seeds in hot water gave good results (Côme 1970).

The mechanical scarification by the sandpaper during 3 min improved slightly the germination of the green seeds only. This same treatment decreased the percentage of germination of yellow seeds compared to the control. However, this treatment increased the speed of germination by decreasing the MGT of both types of seeds. Scarification of *Retama sphaerocarpa* seeds from Spain with quartz sand for 90 s increased the percentage of seed germination from 24% (Control) to more than 50% (Pugnaire et al. 2006). Therefore, it is necessary either to increase the duration of mechanical scarification or to change the sandpaper by sand using several durations as a test until obtaining a maximum percentage of germination.

Soaking the seeds of *Retama sphaerocarpa* in Gibberellic Acid (GA_3) at 10^{-3} M for 24 h and 48 h reduced the mean time of germination (MGT). In other words, it increased the speed of the phenomenon, but the surprising result was the decrease in the percentage of germination contrary to what we expected; this was more visible in yellowish seeds whatever the duration of soaking. The drop in percentage after this treatment was significant ($P < 0.05$; $P < 0.001$). In greenish seeds we observed an increase in the percentage of germination after 24 h of soaking in GA_3. However, this percentage became almost zero after 48 h of treatment. This negative response to GA_3 can be explained by the lack of prior scarification of these seeds. Gibberellic Acid eliminates embryonic dormancy much more than coat dormancy (Côme and Corbineau 1998). Thus the beneficial effect of GA_3 cannot reach the embryo because the integuments will prevent its passage. These same authors reported that it is always necessary to eliminate the coat dormancy (by chemical or mechanical scarification) before any treatment with GA_3 especially if the teguments are impermeable to water.

Scarification of yellowish and greenish seeds of *Retama sphaerocarpa* for 2 h up to 4 h soaking in pure sulfuric acid (96%) significantly improved the rate and speed of germination. In contrast, blackish seeds still did not germinate after scarification confirming that their inability to germinate was not related to coat hardness but it is related to the embryo itself (male formations, loss of viability). Fabião (2011), Fabião et al. (2014) and Kheloufi et al. (2020) found the same beneficial results of chemical scarification by sulfuric acid on the germination of *Retama sphaerocarpa* seeds from Portugal. Similarly, chemical scarification by sulfuric acid also gave a very satisfactory effect on the rate and speed of germination of *Retama raetam* (Chalabi 2008; Mehdadi et al. 2017) and *Retama monosperma* seeds (Bouredja et al. 2011).

We found that the most beneficial soaking time in sulfuric acid for germination was different according to the color of *Retama sphaerocarpa* seeds. Clearly, greenish seeds were significantly more resistant to longer soaking times in sulfuric acid (up to 4 h of soaking with 100% germination) compared to the yellowish seeds which decreased considerably their percentage of germination beyond 3 h of soaking.

By comparing the three species of the genus Retama, we noticed a difference in their tegumentary hardness. *Retama raetam* and *Retama monosperma* have harder seed coats. They require between 6 and 8 h of soaking in sulfuric acid to reach 100% germination (Chalabi 2008; Bouredja et al. 2011 and Mehdadi et al. 2017) whereas we found that *Retama sphaerocarpa* seeds only require between 2 and 4 h of soaking in sulfuric acid to reach 100% germination.

Conclusions

The results of this work provided new information on the biology of the reproduction of *Retama sphaerocarpa* especially germinative behavior of the seeds in relation to their color. It would be interesting to search for the cause of the tegument color change from yellowish to greenish and blackish at the end. The phytochemical analyses of the teguments can give additional information. It would also be important to better understand why black seeds attract insects and why they lose their viability. The answer to these questions will be the subject of our future research.

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Data availability All data generated or analyzed during this study are included in this published article [and its supplementary information files].

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Authorship Responsibilities I confirm that the manuscript is not currently under consideration elsewhere and the research reported will not be submitted for publication elsewhere until a final decision has been made by Biologia; I also attest that it is not in press at another journal nor will it be submitted elsewhere if accepted by *Biologia Journal*.

I confirm that the manuscript is truthful original work.
I confirm that the paper now submitted is not copied or plagiarized version of some other published work.
I confirm that all authors listed on the title page have contributed significantly to the work, have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission.

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.
All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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