EFFECT OF ABIOTIC STRESSES ON GERMINATION BEHAVIOUR OF *RUTA CHALEPENSIS*

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Abstract

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This work aims to highlight the optimal temperature for the seed germination of *Ruta chalepensis*, an important threatened ethnomedicinal shrub, and assess its germination tolerance against salinity and water stresses as well. For this, the seeds were incubated firstly under different temperatures (5 to 30°C). After that, at 20°C, germination tests were performed under different sodium chloride (NaCl) concentrations (0 to 273.9 mM) and various polyethylene glycol solutions (PEG_{6000}) (0 to -2.20 bar). Final germination percentage, velocity coefficient and latency time were subsequently the germination patterns determined and analysed. The optimum temperature for germination was 15°C, in which the maximum final germination percentage (85%) was noticed; germination fell steadily, however, above and below this temperature and completely ceased at 30°C. Germination behaviour of *R. chalepensis* seeds under osmotic stress conditions showed a significant effect of both NaCl and PEG_{6000}. Germination decreased whether sodium chloride or polyethylene glycol was increased in the medium ($p < 0.05$). Moreover, the salinity tolerance threshold was 205.4 mM, equivalent to 16 g L^{-1} NaCl, while water stress was -1.16 bar, corresponding to 80 g L^{-1} PEG_{6000}. Beyond these thresholds, germination stopped. Dropping in velocity coefficient and postponing in latency time with increasing both stresses were recorded either. The sensitivity of *R. chalepensis* seeds to environmental stresses should be taken into account for successful conservation programmes, which should be implemented by sowing seeds and planting seedlings in fresh habitats with sufficient annual rainfall.

Keywords: conservation, *Ruta chalepensis*, salt stress, seed germination, temperature, water stress.

INTRODUCTION

Algeria is among North African countries that harbour an essential amount of plant diversity; unfortunately, floristic communities are subjected to combined, environmental and anthropogenic, constraining action. The former is linked to the Mediterranean climate, characterised by moderate wet winter with irregular-timing rainfall followed by prolonged hostile dry summer (Quézel, 2002; Sardans et al., 2020), and the latter involved anarchic and thoughtless human activities (e.g. overexploitation, overgrazing, fire, etc.) (Cherifi et al., 2017).

It is crucial, in plant conservation biology, to study seed germination physiology since it is the most critical and vulnerable step in the life cycle of terrestrial Angiosperms (Steckel et al., 2004). Also, selected threatened and valuable native plants, having multiple functions and entirely understanding their germination traits, are a promising option for effective plant conservation (Nedjimi & Zemmiri, 2019). Several environmental factors such as temperature, salinity, light

and soil moisture affect germination (El-keblawy & Al-rawai, 2006). Temperature is the most critical environmental factor that regulates plant growth development and plant community size and distribution (Koger et al., 2004). Seeds have minimum, optimal and ceiling temperatures for germination (Alvarado & Bradford, 2002). Using temperature experiments upon germination allows for knowing the geographical limits of seed establishment and selecting the appropriate sowing period (Ramin, 1997).

High salinity is the most common soil problem that limits plant distribution and productivity (QIN et al., 2010). Affecting almost 932 Mha globally (20% of irrigated lands), this widespread threat, predicted to rise by up to 50% by 2050, is often accentuated by (i) excessive evaporation induced by global warming, (ii) intensive use of groundwater (mainly in a land close to the seashores) or (iii) soil mismanagement (Machado & Serralheiro, 2017). North Africa, Algeria included, shows a high level of soil accumulation not only by sodium chloride (NaCl), but also calcium chloride ($CaCl_2$) and sodium sulfate (Na_2SO_4) salts are excessively sequestrated (Nedjimi et al., 2020). In arid and semiarid regions, generally suffering high salinity inducement, assessing plant tolerance against salinity during germination is critical before establishing conservation programmes (Khan & Gulzar, 2003). The increase of salinity induces a decrease in the percentage of seeds germination due to the osmotic effect, although, for some species, low salt concentrations may stimulate germination (Muhammad & Hussain, 2012). Zhang et al. (2010) have hypothesised that salt absorbed by seeds helps to adjust the osmotic potential, which allows the seeds to imbibe water and, therefore, to germinate in saline conditions. Nevertheless, in various salty-sensitive plants, seeds submitted at high salinity concentrations could display ionic salt toxicity and become unable to recover after re-submitting at proper conditions (Meot-Duros & Magné, 2008). Despite the negative impacts of salts upon germination, salinity's detrimental influence is mitigated at optimum germination temperature (Mehdadi et al., 2017; Dadach et al., 2018; Shah et al., 2020). There is a consensus among researchers that under controlled conditions, it is reliable to use sodium chloride (NaCl) in studying the salinity effect (osmotic or ionic potential) on seed germination of many spe-

cies because, on the one hand, it is the main salinity agent encountered in soils and, on the other hand, it is much easier to handle (Mehdadi et al., 2017; El-Keblawy et al., 2018; Nedjimi et al., 2020).

Drought is one of the most devastating environmental stresses, which disturbs plant establishment. The effects of drought stress on plants depend on the severity and duration of water shortage and the phase of plant growth (Faisal et al., 2019). The aridity of the Mediterranean climate harms plant physiology, including germination, since the conditions of water scarcity and substrate salinization lead to water potential drop limiting then water availability, which eventually is delaying or hampering germination (Maraghni et al., 2010; Mehdadi et al., 2017; Dadach & Mehdadi, 2018). Indeed, tolerance of seeds against drought is usually based on their ability to germinate even in the lowest water potentials, induced by water deficiency. Alternatively, drought-sensitive plants seem to postpone their establishment until the appropriate conditions are available for germination, including sufficient rainfall (Elnaggar et al., 2019). Keeping seed viability and facultative dormancy are other complementary plant adaptive traits involved to cope with drought (Nedjimi, 2013). In the context of plant conservation strategies, knowledge of the tolerance of seed germination to drought will provide more insight into the post-germination behaviour leading to successful plant establishment (Donohue et al., 2010). Amongst processes usually used to simulate moisture stress *in vitro* is treating seeds and seedlings with polyethylene glycol (PEG). PEG molecules are inert, non-ionic, non-toxic and induce constant osmotic stress without penetrating the plant cells. They act as osmotica to reduce water potential, thus creating water stress (Dadach et al., 2015a; Elnaggar et al., 2019; Faisal et al., 2019). In seed sprouting experiments, PEG restrains water percolation through the testa, consequently preventing embryonic axis protrusion.

Regarding global biodiversity drop, mainly two strategies can be implied, in the aim to contain biodiversity loss, *in situ* and *ex situ* conservation processes. The former method permits the maintenance of whole plant communities in their natural habitat and native ecosystems (Spampinato et al., 2018). However, the latter approach is carried out instead of the first one. Whether preserving endangered species in their ecosystem is unfeasible by either heavy loss of

habitats or insufficient population size for regeneration (Leon-Lobos et al., 2010).

This study's main reason was to assess the germinability behaviour of *Ruta chalepensis* L. seeds, a medicinal shrub belonging to the flora covering the Tessala Mount (Sidi Bel Abbes province, Algeria) and dramatically subjected to environmental and anthropozoogenic negative impacts (Cherifi et al., 2017). *R. chalepensis* is an evergreen shrub that erect stems of an average size reach up to 90 cm height, belonging to the Rutaceae family; its common name is rue, called *Fidjel* in Arabic (Quézel & Santa, 1962). This species is a wild dicot native from the Mediterranean basin and cultivated elsewhere in other regions (Martínez-Pérez et al., 2017).

Ruta chalepensis is used in folk medicine in many countries as a laxative, anti-inflammatory, analgesic, antispasmodic, antiepileptic, emmenagogue, and treatment of skin pathologies (Miguel, 2003; Merghache et al., 2009). Further therapeutic virtues of this plant have been confirmed in various scientific works, which have shown the importance of the antimicrobial power of its essential oils and the possibility of its valorization in the treatment of dermatological and respiratory diseases (Merghache et al., 2009; Daoudi et al., 2016) as well as in biological control owing to the inhibitory activity against the larvae of some harmful mosquito vectors such as *Aedes*, *Anopheles* and *Culex* (Conti et al., 2013; Pérez López et al., 2015; Andrade-Ochoa et al., 2017) and post-harvest corn insects (Lakshmi Soujanya et al., 2016).

In the context of *ex-situ* conservation of *Ruta chalepensis*, and considering the lack of data on optimal germination conditions of its seeds, our work aims to highlight the effect of temperatures, salt and water stresses on their germinate behaviour.

MATERIALS AND METHODS

Harvesting site

Mature seeds of *R. chalepensis* were collected from the Tessala Mount (North-Western Algeria) in July 2016 (35°16'0.13" N; 0°46'0.66" W; Alt: 886 m) and then stored in paper bags away from humidity until their use (Fig. 1). The harvesting seed site is typically the Mediterranean, where the climate is semiarid with an average sporadic annual rainfall between 335 and 400 mm, and the average annual temperatures ranging from 8 to 24°C (Cherifi et al., 2017).
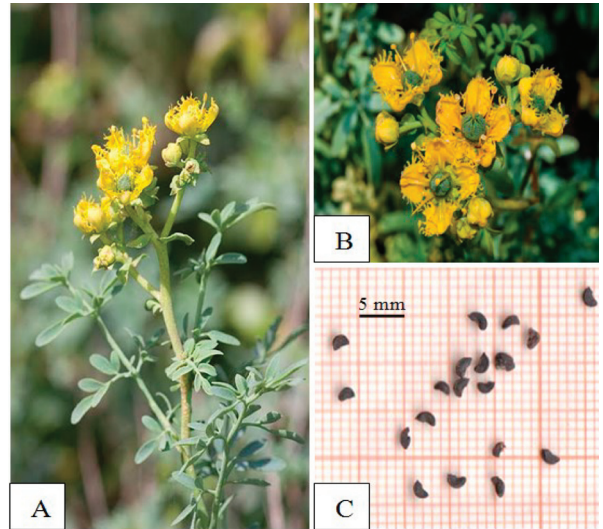

Fig. 1. Shoot part (A), flowers (B) and seeds (C) of *Ruta chalepensis*

Conduct of the germination tests

The germination tests were carried out on healthy seeds, sterilised with sodium hypochlorite solution (5%) for 5 min, then rinsed thoroughly with distilled water and air-dried before being used. The germination tests were performed on five replications of 20 seeds for each. The seeds were placed in Petri dishes lined with two layers of filter paper (Whatman 2), moistened with distilled water for tests concerning to assess the optimal thermal germination conditions, or supplied by different concentrations of sodium chloride (NaCl) or polyethylene glycol (PEG_{6000}) respectively to study the effect of salt and water stress on the seed germination behaviour. During the germination tests, dishes were wrapped hermetically to avoid water loss.

Count of the germinated seeds was carried out every 48 hours for 20 days (till no more germination was noticed). The germination criterion was the breakthrough of seed coat by the radicle (Calone et al., 2020).

Effect of thermal conditions on germination

Since no information is available in the germination performance on *R. chalepensis* seeds, we first submitted the seeds at different continuous temperatures (5, 10, 15, 20, 25, 30°C) in darkness to check for the optimum thermal germination of this species. These temperature regimes were selected after consideration

Table 1. Temperatures and relative rainfall in Tessala, Sidi Bel Abbes province, Algeria

Month	Temperature °C			Rainfall (mm)
	Max	Min	Mean	
January	13.4	2.1	7.8	43.6
February	16.6	2.8	9.7	30.2
March	17.5	4.2	10.8	44.3
April	19.3	5.9	12.6	35.2
May	22.8	9.3	16.0	21.8
June	28.3	12.5	20.4	5.5
July	32.1	15.5	23.8	1.2
August	28.6	13.0	20.8	2.3
September	21.9	13.2	17.6	24.4
October	17.3	8.5	12.9	44
November	14.0	5.8	9.9	47.3
December	13.3	3.1	8.2	50.3

of climatic data (Table 1) collected by the National Meteorological Office in Sidi Bel Abbes province (35°10'26" N; 0°35'43" W; Alt: 482 m). For example, 5 and 10°C simulate temperatures in November and February (before growing seasons), 15 and 20°C reflect temperatures within the growing seasons (March to May) and 25 and 30°C simulate higher temperature by the end of the growing seasons (June–August).

Effect of salt and water stress on germination

The germination tests under salt and water stresses were conducted at 20°C. To demonstrate the effect of salinity on *R. chalepensis* germinability, Petri dishes containing seeds, were irrigated by different saline solutions prepared by sodium chloride (NaCl): 0 (control), 4, 8, 12, 16, 20 g L^{-1} corresponding to the molar concentrations of 0, 68.4, 136.8, 205.3, 273.7 and 342.2 mM, respectively.

Concerning the effect of water stress, the germination tests were conducted in six water potential levels obtained from different concentrations of PEG$_{6000}$: 0 (control), 10, 20, 40, 80, 120 g L^{-1} corresponding to the hydric potentials: 0, -0.08, -0.17, -0.42, -1.16 and -2.20 bar, respectively, determined by the equation of Mɪᴄʜᴇʟ & Kᴀᴜꜰᴍᴀɴ (1973). The used NaCl and PEG levels were selected based on the results of a preliminary experiment assessing salinity and drought tolerance on *R. chalepensis* seeds.

Expression of the results and data processing

The germination tests were expressed in curves representing the cumulative germination percentages as a function of time. Analysis of these curves allowed us to evaluate the final germination percentage (FGP) and the latency time (LT) or the first day of the onset of germination. Furthermore, the velocity coefficient (VC) or germination speed was assessed, too.

The velocity coefficient was calculated according to Jᴏɴᴇs & Sᴀɴᴅᴇʀs (1987) formula as described below:

$$VC = \frac{(N1 + N2 + N3 .... + Nn)}{(N1T1 + N2T2 + N3T3 .... + NnTn)} \times 100$$

where N is the number of germinated seeds every day, and T is the number of days from seeding corresponding to N.

Statistical significance of temperature, salinity, and water stress effect on different germination parameters was verified by analysing variance (ANOVA) using IBM SPSS Statistics 20 software package. Duncan's test was applied ($p < 0.05$) to determine the comparison between means.

To highlight the relationship between the molar concentrations of NaCl and the water potentials with the germination parameters (FGP and CV), we evaluated the coefficient of determination R^2 using simple linear regression analysis.

RESULTS

Effect of temperature

Whatever the tested temperature, the germination curve has three phases: a latency phase, an exponential acceleration phase, and a stationary phase corresponding to a level reflecting the germination accomplishment (Fig. 2).

Germination was possible at the following temperatures: 5, 10, 15, 20 and 25°C, whereas it was completely inhibited at 30°C. Temperature acted significantly on all germination studied parameters (FGP, VC and LT) ($p < 0.05$). Optimum temperature for germination was recorded between 15°C and 20°C (FGP = 85% vs. 76.6% and CV = 11.8% vs. 12.2%, respectively) (Figs 2, 3). FGP tend to fall steadily as soon as the temperature becomes higher or lower than the optimum. While, VC increased slightly when temperature increase, except for the highest temperature.

The shortest LT was noted at 25°C, where germination was onset at the 5th day; however, germination

took more time to trigger at 10°C (LT = 8 days). At 5, 15 and 20°C, LT ranged from six to seven days.
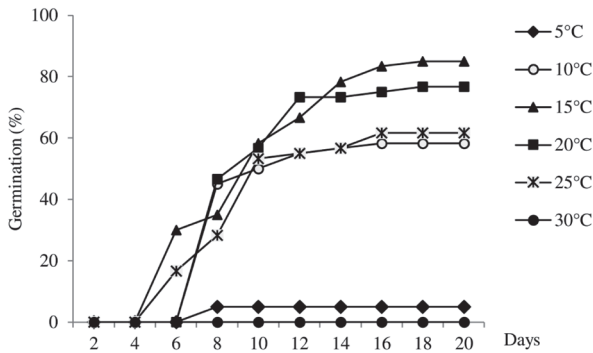

Fig. 2. Cumulative germination percentages as a function of time at different temperatures of *R. chalepensis* seeds
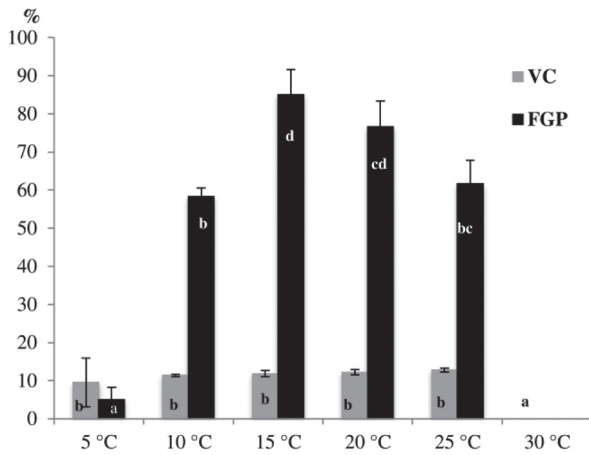

Fig. 3. Effect of temperatures on the final percentage of germination (FGP) and the velocity coefficient (VC) of *R. chalepensis* seeds

Effect of saline stress

Analysis of variance showed a significant effect of salinity on germination in *R. chalepensis* seeds ($p < 0.05$). When salinity increased in the medium, a steady decrease in seed germination was observed (Figs 4, 5). The highest FGP (76.6%) and VC (12.2%) were obtained by the control, which means that seeds germinated abundant and fast in distilled water (0 mM). The lowest values were recorded at 16 g Ll^{-1} (273.7 mM) (FGP = 21.66% and VC = 11.3%) (although not statistically significant differences were recorded for the VC values between 0. 205.3 and 273.7 mM). Germination was inhibited at a salt concentration of 20 g L^{-1} NaCl (342.2 mM). Latency time (LT) was almost the same at the control and for trials treated by 4 g L^{-1} (68.4mM), 8 g L^{-1} (136.8 mM) and 12 g Ll^{-1} NaCl

(205.3 mM) (LT was between six and seven days), however, at 16 g Ll^{-1} (273.7 mM), time of germination onset increased, reaching up to nine days (Fig. 4).

Linear regression analysis showed a high statistical relationship between FGP and the molar concentrations of NaCl with a coefficient of determination $R^2 = 0.98$. This relationship seems to be weakened with VC ($R^2 = 0.49$).
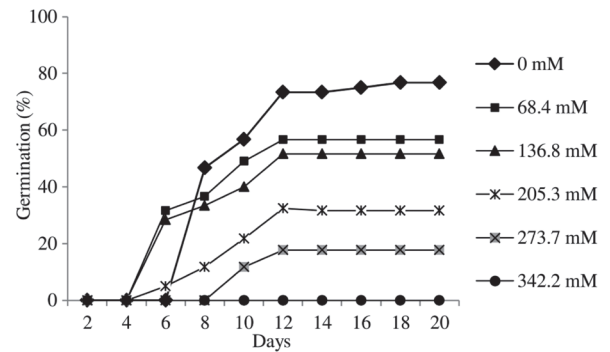

Fig. 4. Cumulative germination percentages under different molar concentrations of sodium chloride as a function of time of *R. chalepensis* seeds
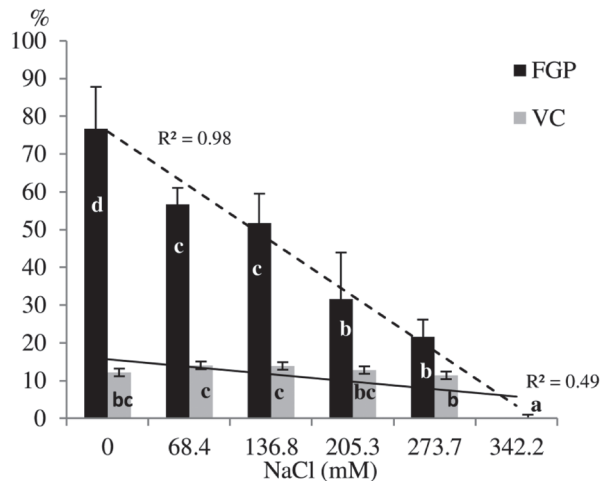

Fig. 5. Effect of different molar concentrations of sodium chloride on the final germination percentage (FGP) and the velocity coefficient (VC) of *R. Chalepensis* seeds

Effect of water stress

Water stress, by using polyethylene glycol (PEG$_{6000}$), significantly inhibited germination of *R. chalepensis* seeds ($p < 0.05$). The highest values of FGP (80%) and VC (15.3%) were noted at 10 g L^{-1} PEG$_{6000}$ (-0.08 bar) (Figs 6, 7). The increase in PEG$_{6000}$ concentration decreased the water potential in the medium, which resulted in a steady decrease in FGP to

reach the minima (30%) at the concentration of 80 g L^{-1} PEG$_{6000}$ (-1.16 bar). Above this concentration, at 120 g L^{-1} PEG$_{6000}$ (-2.20 bar), germination was inhibited entirely (Figs 6, 7). Despite the remarkable extent in LT with increased water stress, the germination took less than seven days to onset (LT ≤ 7 days).

Linear regression analysis also showed a high statistical relationship between FGP and the medium's water potential ($R^2 = 0.86$). In contrast, VC was moderately correlated ($R^2 = 0.50$).
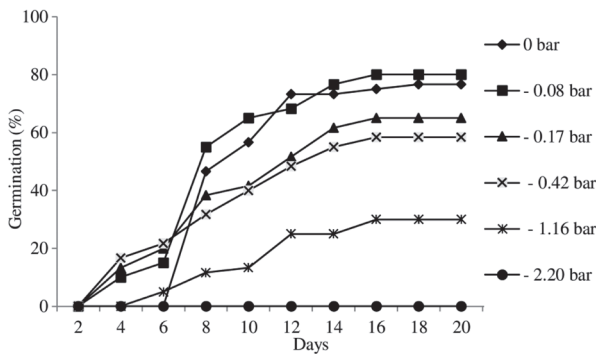

Fig. 6. Cumulative germination percentages under different water potentials induced by different concentrations of PEG$_{6000}$ as a function of time of *R. chalepensis* seeds
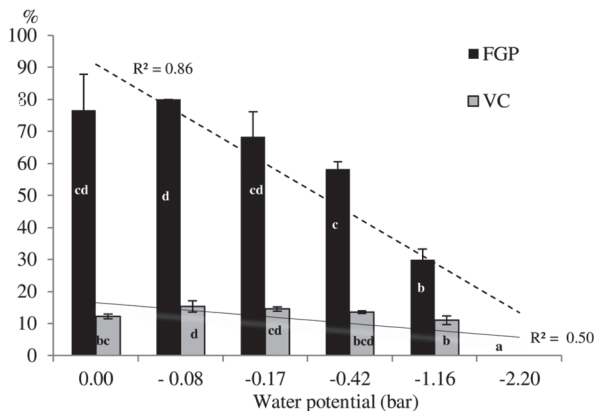

Fig. 7. Effect of different water potentials induced by different concentrations of PEG$_{6000}$ on the final percentage of germination (FGP) and the velocity coefficient (VC) of *R. Chalepensis* seeds

DISCUSSION

The seeds of *R. chalepensis* are non-dormant and showed variations in their germination behaviour. The highest amounts of germinated seeds were reached at 15°C and 20°C. However, germination was negatively affected by low temperatures, whose less percentage was noted at 5°C. At high temperature (30°C), the germination was completely inhibited. This indicates that extreme temperatures, so low (less than 5°C) or so high (30°C and more), can influence the germination physiology of seeds previously able to germinate (Ibanez & Passera, 1997). Indeed, the germinability of *R. chalepensis*, particularly the optimum temperature for germination, is the same as that of many semiarid climate species growing in the Mediterranean mountain habitats such as *Salvia leriifolia* Benth. *(*Dashti et al., 2015), *Thymus fontanesii* Boiss. & Reut. (Dadach & Mehdadi, 2016), *Ballota hirsuta* Benth. (Dadach & Mehdadi, 2018), *Marrubium vulgare* L., *Sideritis incana* L. and *Stachys ocymastrum* (L.) Briq. (Dadach et al., 2018). Otherwise, species occur in rangeland, where climate characteristics hot and dry, the optimum temperatures for seed germination rise slightly and reach up to 25°C as *Stipa tenacissima* L. (Moulessehoul & Mehdadi, 2015), *Lygeum spartum* L. (Lamara et al., 2016), *Retama raetam* (Forssk.) Webb. (Mehdadi et al., 2017) and *Artemisia herba-alba* Asso (Nedjimi & Zemmiri, 2019). *Ruta graveolens* L. is categorised as xerohalophyte, as the optimum temperature for its germination is at 30°C (Mguis et al., 2011). Saeidnejad et al. (2012) have inferred that germination differences could be due not only to the genetic variability of seeds, but also to differences in latitude within seed accessions are collected. El-Keblawy et al. (2020) have emphasised that the geographical origin (the environment under which seeds developed and matured), could have a significant effect on seed germination trait since the optimum temperature for germination varies from one species to another, it corresponds to the ecological adaptation in natural habitats of each species, called as "maternal effect".

Germination of *R. chalepensis* seeds was affected by salinity insofar. We witnessed a significant decrease in FGP and an extension in LT, when the salt content increased in the medium ($p < 0.05$). Germination was inhibited beyond 16 g L^{-1} NaCl (273.7 mM), the concentration seems to be the tolerance threshold for this species' salinity. Such results have been obtained on *R. graveolens* (Yamashita et al., 2009; Mguis et al., 2011). The negative response to saline stress at the germination stage has also been reported in certain studies on glycophytes belonging to other

botanical families (Laghmouchi et al., 2017; Mehdadi et al., 2017; Dadach & Mehdadi, 2018; Mira et al., 2018). On the other hand, being facultative-halophytes, *Arthrocnemum macrostachyum* (Moric.) K. Koch and *A. Indicum* (Willd.) Moq. showed a very high tolerance threshold exceeding 600 mM NaCl (equivalent to the seawater salinity) (Nisar et al., 2019). Interestingly, some euhalophytes such as *Salicornia patula* Duval-Jouve (Gasparri et al., 2016) and *Salsola drummondii* Ulbr. (El-Keblawy et al., 2020), tolerate salinity yet at 1000 mM. Melendo & Giménez (2018) have shown that the harmful effect of salinity is exaggerated at high temperatures (30/20 and 35/25°C) upon *Limonium supinum* (Girard) Pignatti seeds, an Iberian endemic halophyte.

The gradual decrease in germination percentage with increasing NaCl in the environment can be explained by the low water potential generated by saline stress, causing the alteration of enzymes and hormones in the seeds (Botia et al., 1998). Under salinity, the slowdown in germination speed, expressed here by velocity coefficient, and the increase in latency time could be due to seeds' duration to trigger mechanisms allowing them to adjust their osmotic pressure (Medjebeur et al., 2018).

The germination is also regulated by water quantity and quality since hydrolysis stored reserves are embedded in seed endosperm, notably lipids, proteins and starch (Biaecka & Kepczynski, 2010). As is the case with salinity, tolerance to drought during germination is an essential criterion for identifying the species varieties that can withstand water deficit during the earlier stages of plant growth. Our results showed that seeds of *R. chalepensis* were moderately tolerant to water stress. They were able to germinate at concentrations ranging from 10 g L^{-1} to 80 g L^{-1} PEG$_{6000}$, corresponding to water potentials -0.08 to -1.16 bar, even though FGP and VC fell significantly, when water potential decreased in the medium ($p < 0.05$). The gradual decrease of water potential in the medium reduces the intensity of water uptake by seeds, which explains the drop of FGP and the lengthen of LT, when the medium becomes increasingly dry (Zeng et al., 2014). The decrease of water potential (ψ) increases osmotic potential (ψ_π), creating a deficiency in seed hydration and causing mechanisms preventing the emergence of radicle and delaying germination (Gill et al., 2003). Drought-tolerant plants can germinate successfully at water potential exceeding -8 bar as has been the case in *Ziziphus lotus* L. (Lam.) (Maraghni et al., 2010) and *Salsola drummondii* (Elnaggar et al., 2019). In this regard, our results revealed that despite being the Mediterranean native species *R. chalepensis* will have great difficulties germinating in arid and semiarid regions since it was unable to tolerate water potential above -1.16 bar. This value seems to be the germination tolerance threshold to water deficit in this species. Conversely, *Marrubium vulgare* and *Ballota hirsuta*, species sharing the same habitats as *R. chalepensis*, are more tolerant to water stress and could germinate at -0.5 MPa (-5 bar) (Dadach et al., 2015b; Dadach & Mehdadi, 2018). The negative effect of water stress on germination has been formerly observed on *R. graveolens* (Yamashita et al., 2009) and reported by many further authors on various species (Amara et al., 2013; Rios-Rojas et al., 2014; Zhou et al., 2015). Tolerance to water stress varies from one species to another. Species-specific responses may be due to the plant's ability to accumulate metabolites necessary for osmotic adjustment and maintain the cell turgor and physiological activities. Synthesising metabolites confer to seeds an osmotic adjustment that facilitates seed water imbibition and ultimately organises mechanisms responsible for the radicle emergence through seed coverings (Turner, 2018).

Under the Mediterranean climate conditions, seed germination of *R. chalepensis* presumably triggers in spring when temperature and rainfall are promising to successful plant establishment. However, the global change affecting the Mediterranean region is subsequently to provide more frequent and longer harsh periods, rises temperature and changes precipitation patterns (Rey et al., 2011), which consequently threatens the maintenance of *R. chalepensis*. We assume rainfall has the most crucial role in the germination of rue. The rains mitigate soil salinity, increase water potential and normalise temperatures.

These data will certainly support both *ex situ* and *in situ* conservation processes on this species and will open access to new research pathways by selecting resistant genotypes that can be used to reinforce remaining populations evolving in natural conditions.

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ABIOTINIO STRESO POVEIKIS *RUTOS CHALEPENSIS* SĖKLŲ DAIGUMUI

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Santrauka

Šio darbo tikslas – nustatyti optimalią *Ruta chalepensis*, svarbaus nykstančio vaistinio krūmo, sėklų daigumo temperatūrą ir įvertinti sėklų daigumo toleranciją daiginimo terpės druskingumui ir vandens slėgiui. Sėklos buvo daiginamos skirtingose temperatūrose, nuo 5 iki 30 °C. Daigumo bandymai buvo atlikti prie 20 °C, sėklas daiginant skirtingose natrio chlorido (NaCl) koncentracijų (nuo 0 iki 273,9 mM) ir polietilenglikolio (PEG$_{6000}$) tirpalų (slėgis nuo 0 iki -2,20 ba) terpėse. Optimali daigumo temperatūra buvo 15 °C, kurioje nustatytas didžiausias galutinis daigumas (85 %), o prie 30 °C sėklos nedygo. Osmosinio slėgio streso sąlygomis NaCl poveikis turėjo reikšmingą įtaką *R. chalepensis* sėklų daigumui. Daigumas reikšmingai sumažėjo, kai terpėje padidėjo NaCl koncentracija ir PEG$_{6000}$ slėgis. Sėklų tolerancijos riba terpės druskingumui buvo NaCl 205,4 mM, o vandens slėgiui – 1,16 ba, atitinkanti 80 g L^{-1} PEG$_{6000}$. Peržengus šias ribas daigumas sustojo. Sėkmingam *R. chalepensis* išsaugojimo programos įgyvendinimui, įkurdinant augalą naujose buveinėse, reikia atsižvelgti į aplinkos sąlygų įtaką šios rūšies sėklų daigumui.