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GERMINATION RESPONSES OF *Ruta montana* (L.) L. SEEDS FROM TESSALA MOUNT (NORTHWEST OF ALGERIA) TO DIFFERENT CONTROLLED ENVIRONMENTAL CONDITIONS

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Abstract

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This work is part of the *exsitu* conservation of *Ruta montana* (L.) L., a spontaneous Rutaceae, known for its many therapeutic properties. The aim is to study, under controlled conditions, the effects of daylight and darkness, temperature, salt, and water stress on the germination of its seeds. Salt stress was induced by different molar concentrations of NaCl (0–200 mM), and water stress was simulated using different concentrations of polyethylene glycol (PEG_{6000}) corresponding to different water potentials (0-1.2 MPa).
The results obtained showed that *R. montana* seeds were viable and could germinate both in daylight and in continuous darkness. Germination was possible at temperatures between 10 and 30 °C, with the maximum final germination percentage (FGP = 81.66%) recorded at 20 °C. At this optimum temperature, FGP decreased significantly when the seeds were placed under salt and water stress ($p< 0.01$).This decrease became more and more significant when the osmotic pressure induced by NaCl increased and when the water potential induced by PEG_{6000} in the medium decreased. The depressive effect of salt and water stress on FGP was verified by a linear regression analysis, which showed high values of the coefficient of determination (R^2):0.934 for water stress and 0.890 for salt stress. This effect on the velocity coefficient and latency time was more moderate or lower. The thresholds of tolerance to salinity and water deficit found were 150 mM and -1 MPa, respectively, for which the lowest FGP values were recorded with 16.25% for water stress and 11.10% for salt stress.

Key words: *Ruta montana*, salt stress, germination, temperature, water stress.

Introduction

The Mediterranean basin is now at the heart of global preoccupations of conservation biology because like other global ecosystems, Mediterranean ecosystems are the most vulnerable (Rambal, 2008).This basin is currently considered one of the 34 hotspots on the planet (Médail, Myers, 2004). These hotspots are characterized by their specific richness and high rate of endemism, but are subject to increasing anthropogenic pressures (Myers et al., 2000; Médail, Diadema, 2006) and the action of prolonged drought (Quézel, Barbero, 1982; Davi, 2015). These conditions contribute to salinization and reduction of the water potential of the soil, which constitutes a threat for a large number of species in their natural habitats. Algeria presents important floristic potentialities (Véla, Benhouhou, 2007), but is subjected to continuous regression in the last decades. This regression is due to the combined action of humans (clearing, overexploitation of resources, fires, etc.) and climatic deterioration, causing water deficits leading to the disturbance of ecological balances and making natural regeneration of certain species difficult in their natural habitat (MATE, 2015).

Tessala Mount, located in the northwest of Algeria, is considered one of the regions with floristic richness. This has been confirmed in certain works such as those of Bouterfas et al. (2013), Ouici et al. (2015), Fertout-Mouri et al. (2016), and Adjoudj, Latreche (2017).

As for most Algerian forests, the potential flora of Tessala Mount is today under threat from several factors such as restricted climatic conditions (extension of the drought period), overgrazing, fires, clearing, and abusive exploitation of species. The combined action of these factors has led to degradation of the vegetation cover (Saidi et al., 2016; Cherifi et al., 2017).

This finding challenges us to undertake actions to safeguard and protect this floristic patrimony. To this effect and in the context of *exsitu* conservation biology, works have been carried out on phytotaxa, whose objectives are to highlight the influence of

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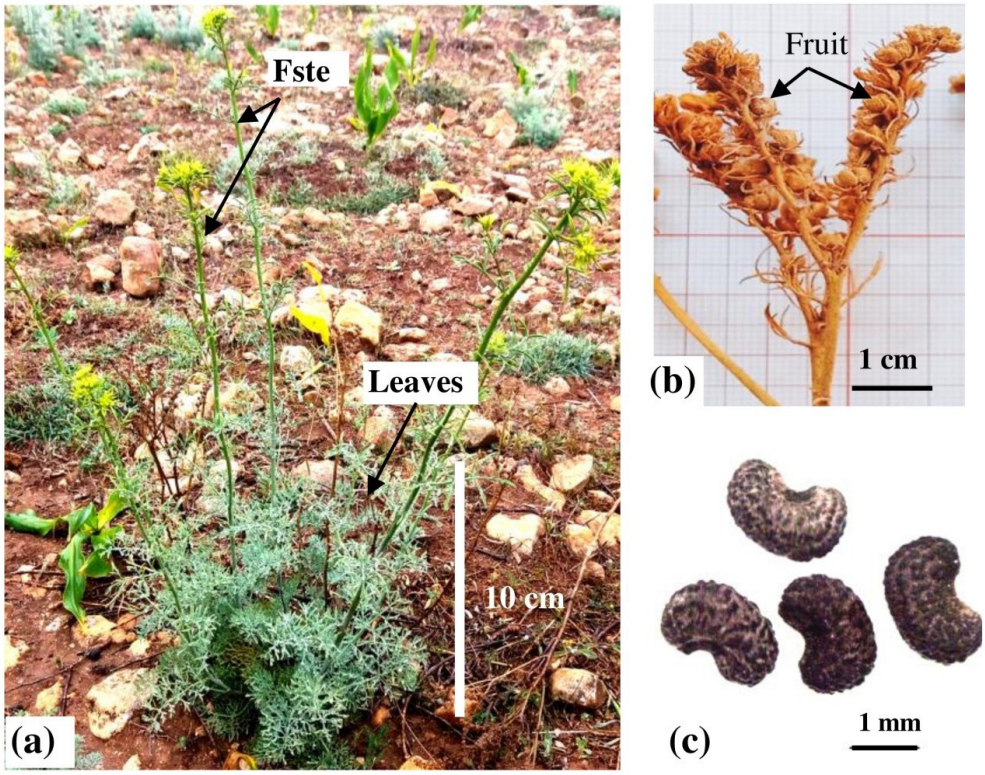


Fig. 1. *Ruta montana* (L.) L.
Notes: a – General view of plant; b – Fruit; c – Seeds (Gr × 30); Fste = floriferous stem.

certain factors such as temperature, water deficit, and salinity on the viability of their seeds.

These factors can also impact their germination, and therefore their establishment in their natural habitat (Nedjimi, Zemmiri, 2019; Dadach et al., 2021; Benadjaoud et al., 2022; Bouker et al., 2022; Hafidou et al., 2021).Within the framework of the creation of seed banks, this will make it possible to know the optimal conditions for the cultivation of plants intended for the reinforcement of natural populations in decline. Indeed, it is reported in some works that temperature, light, salinity, and soil water potential are the most important environmental factors that affect germination, growth, development, and distribution of plants (Kogeret al., 2004; El-Keblawy, Al-Rawai, 2006). The present study is part of this research topic. It concerns *Ruta montana* (L.) L., Rutaceae, which is part of the floristic richness of Tessala Mount. It is widely distributed in the Mediterranean basin. It is a semi-shrubby plant of height from 40 cm to 1m and is very ramified and lignified at the base. Its common name is mountain rue, and it is called fidjela el djebeli in Arabic (Quézel, Santa, 1962; Allouni, 2018). In Algeria, *R. montana* is found in interior mountainous areas up to the Saharan Atlas (Quézel, Santa, 1962). It is an aromatic and medicinal plant, used in traditional medicine in many countries (Hammiche, Azzouz, 2013). The works of Benkhaira et al. (2022) have shown that this plant can be used to treat many diseases like diabetes, hypertension, neurological disorders, infections, and cancer. Its many therapeutic properties are mainly due to its rich composition of secondary metabolites such as polyphenols (Merghem, Dahamna, 2020), essential oils (Benali et al., 2020; Mohammedi et al., 2020; Nahar et al., 2021), alkaloids, and coumarins (Allouni, 2018; Drioiche et al., 2020; Benkhaira et al., 2022), which have antioxidant, antibacterial, antifungal, insecticidal, and larvicidal activities. Currently, no data on the viability and germination capacity of *R. montana* seeds are available. For this purpose and within the framework of its *exsitu* conservation, this work was been undertaken to evaluate, under controlled conditions, the effects of light and darkness, temperature, salt, and water stress on the germinability of its seeds.

Material and methods

Harvest site and description of seeds

The seeds were collected in July 2019 from 40 individuals of a wild population of *R. montana* evolving in the Tessala Mount (northwest of Algeria), whose geographic coordinates are latitude: 35°16.117' N, longitude: 000°46.714' W, altitude: 886 m.

They were stored at the ambient temperature of the laboratory and were protected from humidity until their use. The harvest site is characterized by a semi-arid Mediterranean climate with average annual rainfall ranging between 335 and 400 mm and average annual temperatures varying from 8 to 24 °C (Cherifi et al., 2017). The *R. montana* seeds are reniform, wrinkled, and without ornamentation (Fig. 1).The average length, width, and thickness of 10 seeds are 1.79 ± 0.10, 0.76 ± 0.07, and 0.22 ± 0.05 mm, respectively.

Selection and preparing of seeds

Mature and healthy seeds were selected under a binocular magnifying glass, sterilized with sodium hypochlorite (5%) for 5–10 min, and rinsed with distilled water for use in germination tests.

Germination tests

Each germination test was carried out in 100 seeds distributed on four Petri dishes (diameter: 9 cm; height: 1 cm), at the rate of 25 seeds per box (four repetitions), on two layers of filter paper (PRAT DUMAS, Le Bourg 24150 Couze-Et-Saint-Front, France).

Effect of daily photoperiod and continuous darkness on germination

The seeds were germinated at a constant temperature of 20 °C on a filter paper soaked with distilled water, in two Memmert-type ovens, of which the first one had a glass door exposing the seeds to the light of the day and the second one was closed, providing continuous darkness for the second batch of seeds.

Search for optimal temperature conditions for germination

The results of the effects of light and darkness on the germination capacity of *R. montana* seeds allowed us to carry out germination tests, in continuous darkness, under different temperatures to bring out the optimum thermal germination of seeds. The temperatures tested were: 5, 10, 15, 20, 25, 30, and 35 °C.

Effects of salt and water stress on germination

The effect of salt stress on germination was tested, in the dark, in an oven set at the predetermined optimum germination temperature (20 °C). These tests were carried out by imbibing the different batches of seeds with solutions of different molar concentrations of sodium chloride: 0 (control: distilled water), 50, 100, 150, 200, and 250 mM. The effect of water stress was determined using different concentrations of polyethylene glycol (PEG_{6000}), corresponding to different levels of water potential (Michel, Kaufman, 1973), applied to different batches of seeds placed in optimal temperature conditions and in the dark. The water potentials tested were 0 (control: distilled water), -0.2, -0.4, -0.6, -0.8, -1, and -1.2 MPa. The germination criterion considered is the breakthrough of the envelopes by the radicle (Côme, 1982; Calone et al., 2020).

Expression of the results and data processing

The germination kinetics was expressed by curves representing evolution of the cumulative germination percentages as a function of time (in days).

The final germination percentage (FGP), the latency time (initial germination day [IGD]) which corresponds to the first day of the onset of germination, and the velocity coefficient (VC) or germination speed were evaluated.

VC was calculated according to the formula of Jones, Sanders (1987):

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

where N is the number of seeds germinated each day (T).

The statistical processing of the data was carried out by the IBM Statistical Package for the Social Sciences (SPSS) Statistics 20 software. The analysis of variance was carried out to know the effects of temperature, water, and saline stress on the three parameters of germination (FGP, VC, IGD). Pairwise comparisons of the means of these parameters were carried out using Duncan's test. The relationship of the three germination parameters with the molar NaCl concentrations and water potentials tested was demonstrated by the coefficient of determination R^2 using linear regression analysis.

Results

Effects of daylight and darkness on germination

The results obtained showed that the seeds of *R. montana* germinated better in continuous darkness with a significantly higher FGP (81.66%) than that noted under alternating light of day/darkness of night (60%) ($p< 0.05$). However, IGD and VC were similar in the two experimental conditions: 4 days for IGD and 11.36–12.5% for VC ($p> 0.05$).

Effect of temperature on germination

The germination kinetics curves at the different temperatures are shown in Fig. 2.

For each temperature, the germination curve showed three phases:

1. Latency phase: this is the time required for the radicle to breakthrough the envelopes;
2. Acceleration phase: corresponds to an exponential increase in the number of germinated seeds as a function of time; and
3. Stationary phase: corresponds to the cessation of germination.

Germination occurred at temperatures between 10 and 30°C. The maximum value of FGP was noted at 20°C (81.66%). At temperatures below 10°C and above 30°C, seeds lost their ability to germinate. VC was proportional to the temperature. Inversely, IGD became short when the temperature increased.The analysis of variance showed a highly significant effect of temperature on these three germination parameters ($p< 0.01$).

Effects of salt and water stress on germination

The germination kinetics under salt and water stress conditions are shown in Figs 3 and 4. Whatever the NaCl molarity and the water potential, the germination curve is represented by the three phases described above.

Compared to the control, salt and water stress induced a decrease in FGP. In fact, under saline stress, FGP began to decrease gradually from 50 mM (41.25%) to reach a minimum value (16.25%) at 150 mM. At 200 mM, the germination was impossible (table 1). VC reached its peak at 50 mM (20.29%) and

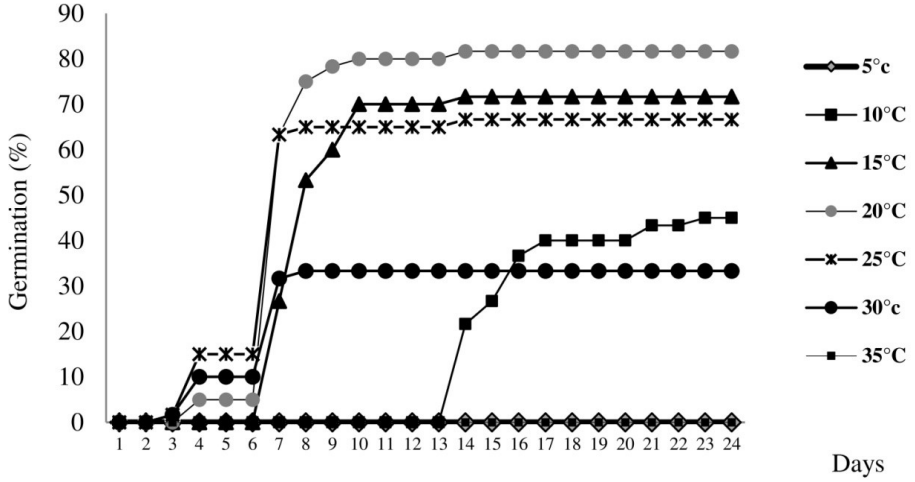


Fig. 2. Cumulative germination percentages of *Ruta montana* seeds as a function of time at different temperatures.

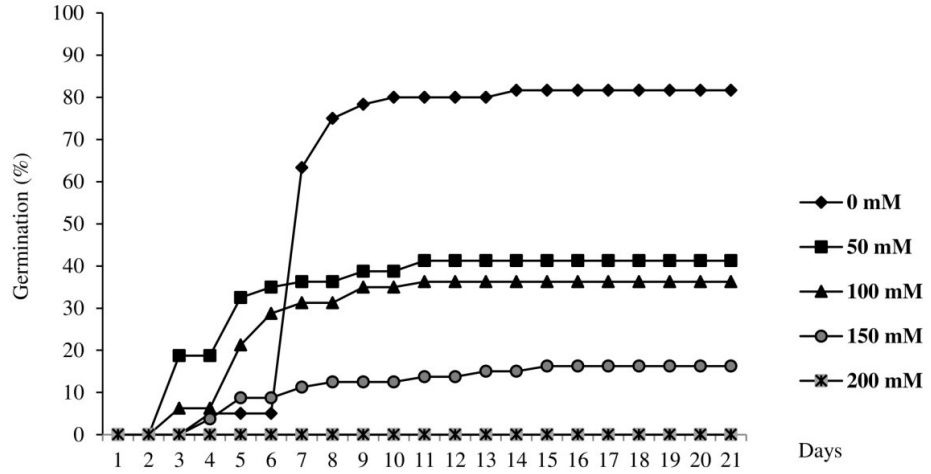


Fig. 3. Cumulative germination percentages of *Ruta montana* seeds under different molar concentrations of sodium chloride as a function of time.

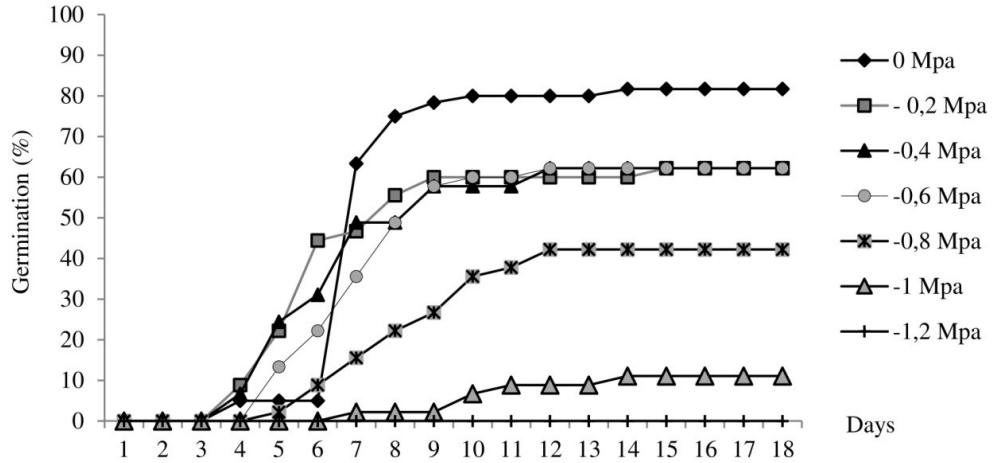


Fig. 4. Cumulative germination percentages of *Ruta montana* seeds under different water potentials induced by different concentrations of PEG_{6000} as a function of time.
Notes: PEG –polyethylene glycol.

Table 1. Variation of IGD, FGP, and VC of *Ruta montana* seeds as a function of different molar concentrations of NaCl (mean ± SE, $n = 4$).

NaCl (mM)	IGD (days)	FGP (%)	VC (%)
0	4.75 ± 1.73^{ab}	81.66 ± 0.95^{a}	13.79 ± 0.64^{b}
50	3^{c}	41.25 ± 1.6^{b}	20.29 ± 3.9^{ab}
100	3.5 ± 1^{bc}	36.25 ± 6.29^{b}	18.06 ± 2.64^{ab}
150	5.25 ± 1.25^{a}	16.25 ± 10.30^{c}	15.2 ± 5.68^{a}
200	0^{c}	0^{d}	0^{c}
F value	17.50^{***}	42.84^{***}	22.93^{***}
R^2	0.310	0.934	0.423

Notes: IGD – initial germination day; FGP – final germination percentage; VC – velocity coefficient; F –Snedecor Fisher variable; R^2– coefficient of determination; SE – standard error. Different lowercase letters (column) show significant differences between the averages. ***Significant differences at $p < 0.01$.

Table 2. Variation of IGD, FGP, and VC of *Ruta montana* seeds as a function of different water potentials (mean ± SE, $n = 4$).

Water potential (MPa)	IGD (days)	FGP (%)	VC (%)
0	4.75 ± 1.73^{b}	81.66 ± 0.95^{a}	13.79 ± 0.64^{ab}
-0.2	4^{b}	62.22 ± 3.84^{b}	15.09 ± 0.86^{a}
-0.4	4^{b}	62.22 ± 3.84^{b}	14.74 ± 1.03^{a}
-0.6	5^{b}	62.22 ± 10.18^{b}	13.80 ± 1.63^{ab}
-0.8	5^{b}	42.21 ± 7.69^{c}	11.85 ± 0.57^{bc}
-1	9.33 ± 2.08^{a}	11.10 ± 7.70^{d}	10.73 ± 3.86^{c}
-1.2	0^{c}	0^{d}	0^{d}
F value	19.48^{***}	62.52^{***}	37.29^{***}
R^2	0.005	0.890	0.600

Notes: IGD – initial germination day; FGP –final germination percentage; VC – velocity coefficient; F –Snedecor Fisher variable; R^2– coefficient of determination; SE – standard error. Different lowercase letters (column) show significant differences between the averages. ***Significant differences at $p < 0.01$.

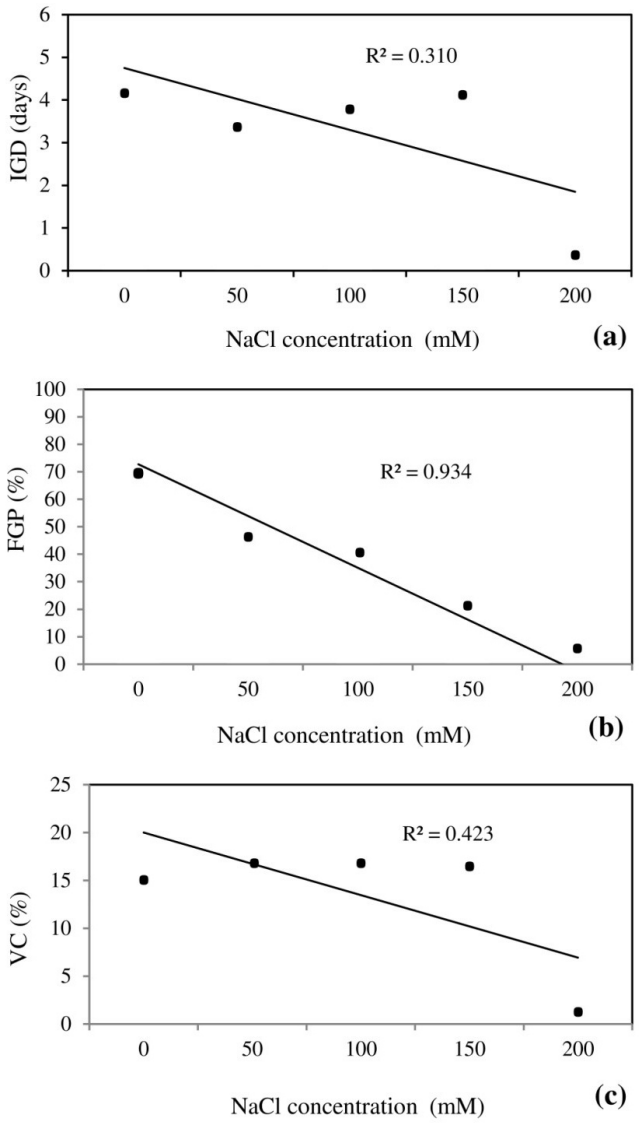


Fig. 5. Linear regression lines between salinity (NaCl) and IGD (a), FGP (b), and VC (c) of *Ruta montana*.
Notes: IGD – initial germination day; FGP – final germination day; VC – velocity coefficient.

then began to decrease gradually when the molar concentration of NaCl increased in the medium, to reach a minimum average value of 15.2% at 150 mM. Compared to the control, the latency time was shortened to approximately 3 days at 50 and 100 mM and then increased to 5.25 days at 150 mM. VC was stimulated under the effect of salinity, whose values ranged from 13.79% for the control to 20.29% and 18.06% at 50 and 100 mM, respectively. It began to decrease from 150 mM (15.2%) to cancel out at 200 mM (Table 1).

The same evolution of FGP was observed under water stress. FGP was stable (62.2%) for water potentials -0.2, -0.4, and -0.6 MPa, then gradually decreased from -0.8 MPa (42.2%) to -1 MPa (11.06%), and then canceled out at -1.2 MPa. VCs and latency times were comparable to those of control for water potentials -0.2, -0.4, and -0.6 MPa. The values ranged between 13 and 15% for VC and from 4 to 5 days for IGD. However, from -0.8 MPa, VC decreased to reach a minimum value of 10.73%, and IGD lengthened to 9.33 days at -1MPa (Table 2).

Analysis of variance showed a highly significant effect of salt and water stress on the three germination parameters ($p< 0.01$). The coefficient of determination showed a strong correlation between FGP and the different molar concentrations of NaCl (R^2= 0.934) as well as the different water potentials (R^2= 0.890). Inversely, this correlation was weak to moderate for IGD and VC (Figs 5, 6).

Discussion

Given the temperate mediterranean origin of *R. montana* and that no information is available on its seeds, the first germination tests were carried out at a continuous temperature of 20 °C (Rao et al., 2006). These tests showed that the seeds of *R. montana* are

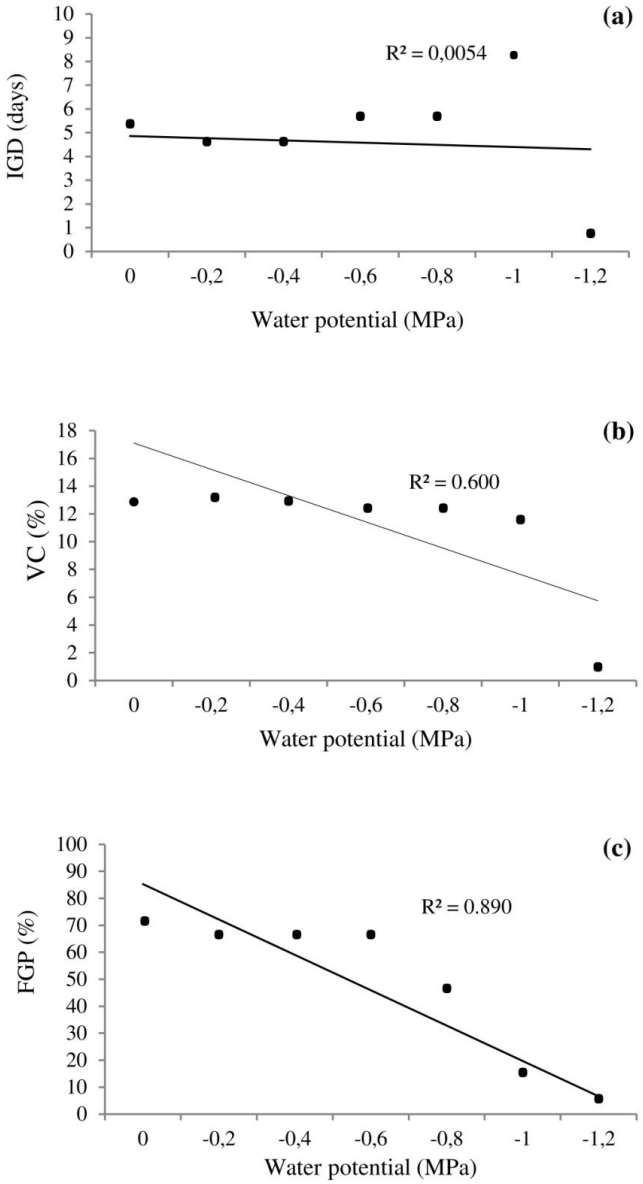


Fig. 6. Linear regression lines between water potential and IGD (a), FGP (b) and VC (c) of *Ruta montana*.
Notes: IGD – initial germination day; FGP – final germination day; VC – velocity coefficient.

viable and able to germinate in both daylight and continuous darkness. The germination rate was better in darkness (81.66%) than in daylight (60%).These data suggest that *R. montana* is one of the species that germinates both in light and darkness. This is the case for many species; while others germinate at a higher rate in the dark than in the light (El-Keblawy, Al-Rawaï, 2005). In continuous darkness, seed germination of *R. montana* is significantly affected by temperature. Germination occurs at temperatures between 10 and 30 °C, and the thermal optimum is between 15 and 20 °C, where the best FGPs are obtained. The extreme temperatures, below 10 °C and above 30 °C, are unfavorable for germination. These results are similar to those obtained by Hamdini et al. (2021) on *Ruta chalepensis* L. evolving in the same natural habitat (Tessala Mount) where the climate is semi-arid. Mguis et al. (2011) indicated that the thermal optimum for germination of seeds of *R. graveolens* L., growing in arid conditions is between 25 and 30 °C, and that temperatures below 10 °C and above 35°C lead to loss of their ability to germinate. The variations in the germinative responses of species to different thermal conditions are related to the genotype and the geographic origin of the seeds, thus reflecting their modes of adaptation to the ecological conditions of their habitats (Nkomo, Kambizi, 2009; El-Keblawy et al., 2020). This has been demonstrated in other works on Mediterranean species belonging to different botanical families such as *Centaurea eriophora* L. (Bouker et al., 2016), *Retama raetam* (Forssk.) Webb. (Mehdadi et al., 2017), *Aristolochia baetica* L. (Hakemi et al., 2020), and *Cedrus atlantica* (Manetti ex Endl.) Carrière (Hafidou et al., 2021).The absence of germination at high temperatures is explained by the fact that these temperatures induce secondary dormancy in seeds able to germinate (Shimono, Washitani, 2004). Corbineau et al. (1993) noted that the caryopsis of *Avena sativa* L., becomes unable to germinate if it is previously soaked and subjected to high temperatures (30 and 35 °C), and that this dormancy induced by these extreme temperatures is called thermodormancy. This phenomenon can be explained by the lack of oxygen necessary for the germination of the embryo and by the synthesis of abscisic acid (ABA), a hormone that inhibits this physiological phenomenon. Ben Dkhil, Denden (2014) showed that the thermodormancy observed in gumbo (*Abelmoschus esculentus* L.) at low (10 °C) and high (40 °C) temperatures was the result of slowing down and then inhibition of protease activity in the cotyledons. These same authors noted that these two temperatures allowed only minimal use of sodium, which becomes toxic for the embryo. In this context, Bewley, Black (1994) explained that the slowing down or stopping of germination at low or high temperatures is a consequence of the dysfunction of certain processes which govern the germination capacity of seeds, such as the activity of membraneand cytosol enzymes. In the face of climate change and its negative impact on Mediterranean flora (Venettier, 2020) and to understand and develop strategies for the sustainable conservation of threatened phytotaxa in their natural environment, it is important to study the effects of other environmental constraints such as salinity and water deficit, which govern the germination of plants and, therefore, their establishment in their natural environment. Germination tests carried out at the optimum temperature (20 °C) showed a highly significant effect of salt and water stress on the germination parameters of *Ruta montana* seeds (FGP, VC, and IGD). We noted a slowing down of germination when the molar concentration of NaCl increased in the medium, resulting in a progressive decrease of FGP, which reached a minimum value of 16.25% at the concentration of 150 mM, which seems to represent the threshold of tolerance to salinity for *R. montana*. Beyond this molarity, germination is completely inhibited.

Salinity is much more correlated to FGP. Indeed, a strong coefficient of determination was noted between FGP and the different salinity levels (R^2 = 0.934). Inversely, this correlation was weak with VC and IGD. Similar results were obtained with *R. graveolens* (Mguis et al., 2011; Barichello et al., 2021) and *R. chalepensis* (Hamdini et al. 2021), which seem more tolerant to NaCl than *R. montana* since their thresholds of NaCl tolerance are 200 and 273.7 mM, respectively. The sensitivity of plants to

salinity at the germination stage is also highlighted in the majority of plants, but with a more pronounced tolerance in halophytes compared to glycophytes (Qadir et al., 2008).This is the case, for example, of some species of the Atriplex genus (Bouda, Haddioui, 2011), *Suaeda aralocaspica* (Bunge) Freitag & Schütze (Wanget al., 2017), *Marrubium vulgare* L. (Nedjimi et al., 2020), and *Lavandula stoechas* L. (Dadach et al., 2021). The variation in seed germination response, essentially FGP, to different salt concentrations is due to the difference in the intensity of osmotic stress caused by these concentrations. The results obtained show that *Ruta montana* can maintain its germination capacity up to 150 mM. This is due to its ability to counteract the osmotic effects induced by NaCl at 50, 100, and 150 mM by the accumulation of ions whose role is to maintain absorption to avoid the harmful effects of salt on the germination of seeds (Dadach et al., 2021). These same authors have shown that the inhibiting effect of salinity on the germination of *Lavandula stoechas* seeds varies according to the nature of the salts and their concentrations in the medium. Furthermore, the work of Shu et al. (2017) on soybeans highlighted the positive effect of NaCl on the biogenesis of ABA, leading to a decrease in the gibberellic acid/ABA ratio, which is the origin of the slowing down or inhibition of seed germination. It is noted in the work of Benadjaoud et al. (2022) on *L. stoechas* that osmopriming seeds with 4% potassium chloride improved their germination capacity. This has also been demonstrated in the seeds of *Satureja montana* L. hydroprimed by potassium nitrate and gibberellic acid (Vidak et al., 2022). In the context of conservation biology, the study of the tolerance of plant species to low water potential at the germination stage is currently a criterion for elucidating their methods of adaptation to water stress conditions. Our results show that *Ruta montana* seeds germination is significantly affected by water stress. The best germination rate corresponds to the control (81.66%). This rate gradually weakens when the water potential decreases in the medium to reach a minimum value of 11.10% at -1MPa, which represents the water deficit tolerance threshold for *R. montana*. As for salt stress, water stress affects essentially FGP, which is strongly correlated with the water potentials tested (R^2 = 0.890). However, these water potentials are weakly to moderately correlated with IGD (R^2 = 0.005) and VC (R^2 = 0.600), respectively. *R. montana* seems to present a better adaptation to potentials -0.2, -0.4, and -0.6 MPa with comparable VC and IGD and an FGP of 62.22%. Sensitivity to water stress is particularly evident at -0.8 MPa and is accentuated at -1 MPa. It results in a significant decrease in FGP accompanied by a slowdown in VC and a lengthening of IGD. This is explained by the fact that the decrease in the water potential leads to an increase in the osmotic potential, which creates a deficit of hydration of the seeds. This water deficit causes changes in the enzymatic activities of seeds, accompanied by a general reduction in hydrolysis and the use of their main natural food reserves, thus preventing their germination (Steiner, Zufo, 2019). Generally, at the germination stage, plants have the same behavior toward water stress, but with tolerance thresholds that differ from one species to another. This has been noted in the work of Boubacar et al. (2018) on the effects of water stress and temperature on the germination of four priority food trees in Niger. This is also the case for some species evolving in the same biotope as that of *R. montana*. These are *R. chalepensis* (Hamdini et al., 2021), *Ballota hirsuta* Benth., *Sideritis incana* L., and *Stachys ocymastrum* (L.) Briq. (Dadach, Mehdadi, 2018, 2021), which are less tolerant than *Ruta montana*. This assumes that the seeds of our species can tolerate drought in the early stages of development, and that they do not present difficulties in germinating in arid and semi-arid areas. Tolerance to water deficit may also be related to genotypic variations that may exist within the same species; this was demonstrated in the work of Dirik (2000) on *Cedrus libani* A. Rich. and of Mahpara et al. (2022) on *Triticum aestivum* L.

Conclusion

From this study, it appears that under controlled conditions, the thermal optimum for *Ruta montana* seed germination corresponds to 20 °C. At this temperature, the seeds are viable and non dormant, and are able to germinate in the presence or absence of daylight. They are sensitive to saline and water stress, but capable of germinating at an osmotic pressure of 150 mM of NaCl and at a water potential of -1 MPa, which seem to constitute their thresholds of resistance and tolerance to these two stresses. The harmful effect of salinity and water deficit manifests significantly on FGP. This was verified by the analysis of variance and by the evaluation of the coefficient of determination R^2.

These data will certainly be used in the conservation of this species and in the production of seedlings for the reinforcement of its populations in their natural habitat. To complete these results, essentially, the modalities of tolerance and adaptation of *R. montana* to saline and water stress, it is important to investigate the other phenological stages. To further understand the conservation biology of *R. montana*, it would be important to also follow the evolution and dynamics of its populations under natural conditions.

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