

Research article

SEED GERMINATION RESPONSE OF *Nicotiana rustica* L. (SOLANACEAE) TO SALT STRESS

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ABSTRACT

Nicotiana rustica L., an economically important plant species cultivated for medicinal and industrial purposes, remains understudied in terms of seed germination dynamics under salinity stress. This study examines six key germination attributes, including final germination percentage (FGP), mean germination time, time to 50% germination, seedling length, germination tolerance index, and seed vigor index, over a period of seven days. This study investigates the influence of varying concentrations of sodium chloride (NaCl), ranging from 0 mM to 300 mM, on the germination kinetics of *Nicotiana rustica* seeds. The results reveal a significant effect of salinity on *N. rustica* germination, with increasing NaCl concentrations leading to reductions in FGP, delayed germination kinetics, inhibited seedling growth, and compromised seed vigor and tolerance. *N. rustica* seeds demonstrated a moderate sensitivity, with FGP declining from 99.3% at 0 mM NaCl to 7.66% at 300 mM NaCl. Statistical analysis confirmed these trends, showing highly significant differences ($p < 0.001$) among NaCl treatments for all germination traits. These findings improve our understanding of the physiological responses of *N. rustica* to salinity stress and highlight the importance of developing strategies to mitigate the adverse effects of salinity on seed germination and crop productivity.

Key words:

Nicotiana rustica, salt stress, germination traits, salinity tolerance, germination kinetics

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INTRODUCTION

Seed germination is a crucial stage in the plant life cycle, directly impacting subsequent growth and productivity (Carrera-Castaño et al., 2020). In order to improve crop management and cultivation practices, it is essential to understand the factors that affect germination. In addition to biotic stresses, salinity is one of the most significant abiotic factors that affect seed germination and plant growth by causing osmotic stress, ion toxicity, and nutrient imbalances (Tlahig et al., 2021). The increasing use of irrigation and the effects of climate change are accelerating soil salinization in arid and semi-arid areas (Devkota et al., 2022). Currently, more than 100 countries face the problems of soil salinity and groundwater salinization. Irrigation of agricultural crops with saline water increases the concentration of soluble salts in the soil, thereby reducing the plant productivity (Srivastava et al., 2019). Under saline conditions, osmotic pressure disrupts water uptake and toxic ions accumulate, causing ionic imbalances that

hinder metabolic processes necessary for seed germination and seedling development (Gul et al., 2022). In Algeria's arid rangelands, saline soils mainly contain various chloride and sulfate salts, with NaCl accounting for more than 50% (Halitim, 1988; Mansouri et al., 2019). A fundamental aspect of breeding and agricultural practices designed to improve stress resilience is recognizing the varying levels of salinity tolerance among plant species (Singh et al., 2021).

Nicotiana rustica L., commonly known as Aztec tobacco, belongs to the Solanaceae family and is widely used in traditional medicine and rituals due to its high nicotine content. This plant has various applications, including ceremonial uses by indigenous cultures and application as a natural pesticide (Mehmood et al., 2020). Additionally, *N. rustica* has potential therapeutic properties, such as antimicrobial, anti-inflammatory and antioxidant effects (Zou et al., 2021). *N. rustica* is subjected to diverse environmental stresses, including salinity, which significantly affects its growth, development, and yield potential (Hajiboland et al., 2017; Xu et al., 2018; Fatehi and Ehsanpour, 2022). On the other hand, there is a scarcity of information regarding the ecophysiological factors influencing germination in this species. Investigating *N. rustica* germination under saline conditions could provide valuable insights for optimizing its cultivation in saline environments, ensuring its productivity, sustainability and the use of its beneficial properties.

This study aims to evaluate the effects of salinity on the seed germination of *Nicotiana rustica*, focusing on key germination responses and their varying tolerance to different levels of sodium chloride stress. The primary objective is to evaluate the germination response of *N. rustica* seeds under different salt stress levels, specifically by identifying the threshold salinity levels that inhibit germination, and assessing the impact of various NaCl concentrations on germination percentage, rate, and seedling vigor.

MATERIAL AND METHODS

Seed collection

Mature seed capsules from 10 *Nicotiana rustica* (L.) plants (one genotype) were randomly collected from a farm in the municipality of Oum El Bouaghi, Algeria (35°53' N, 6°27' E). These seeds represent a widely cultivated genotype in this region, known for its medicinal and industrial applications. The seeds, which are dark brown and either oval or elongated in shape, were manually extracted from the capsules. To minimize genetic variation, the seeds were mixed after harvesting. The goal of this approach was not to reduce the genetic diversity inherent to the species, but rather to ensure that the observed effects on the germination traits could be attributed to environmental treatments (salinity) rather than to genetic differences among individual plants. By mixing seeds from 10 plants, we aimed to create a homogeneous sample representative of a typical *N. rustica* genotype in order to more accurately assess its general response to salinity stress (Mansouri and Kheloufi, 2024). The seeds were then cleaned and stored in paper bags at room temperature until February 2024. A batch of 1000 seeds weighed approximately 0.19 g. For a sample of 100 seeds, the average dimensions were 1.09 ± 0.08 mm in length and 0.74 ± 0.07 mm in width.

Experimental design and application of salt stress

The germination test was performed using plastic Petri dishes (9 cm Ø), each containing one Whatman filter paper moistened with varying saline concentrations (0, 50, 100, 200, and 300 mM) of NaCl (Table 1). For each salinity level, five replicate Petri dishes, each with 100 seeds, were incubated in total darkness at a temperature of 25 °C (± 2 °C). To maintain appropriate levels of humidity for the seeds, the filter papers were replaced every two days to prevent salt accumulation. A complete randomized design was used to conduct the germination test (Mansouri and Kheloufi, 2024).

Table 1. Saline solutions and their corresponding pH and electrical conductivity

Concentrations (mM NaCl)	pH at 19°C	Electrical conductivity (EC) (mS cm ⁻¹) at 23.3 $\pm$ 0.2 °C
0 (Control)	9.11	0.03
50	8.80	5.08
100	8.09	9.52
200	8.04	18.7
300	7.92	25.4

Germination parameters

Final germination percentage (FGP): FGP represents the total number of germinated seeds out of the total number of seeds sown in each Petri dish. It was calculated using the formula:

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

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

Mean Germination Time (MGT): MGT index shows how fast the seeds emerge in a population. A lower MGT value reflects a high germination rate and vice versa. It was calculated using the following formula:

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

where MGT is the mean germination time, ti is the number of days since the beginning of the test, ni is the number of germinated seeds recorded on day $t(i)$, and $\sum ni$ is the total number of germinated seeds (Orchard, 1977).

Time to 50% germination (T_{50}): T_{50} was developed to determine the time required for 50% of seed germination. It was calculated using the formula:

$$T_{50} \text{ (days)} = \frac{ti + (N/2 - ni)(tj - ti)}{(nj - ni)}$$

where N is the final number of seeds emerged, nj and ni are the cumulative numbers of seeds emerged after adjacent counts during tj and ti , when $ni < N/2 < nj$ (Coolbear et al., 1984).

Germination Tolerance Index (GTI): GTI is a quantitative measure used to assess the ability of seeds to germinate under different salinity levels. It is calculated by comparing the germination rates of seeds subjected to stress conditions with those grown under optimal conditions (control, non-stress). It is calculated using the following formula (Coolbear et al., 1984):

$$\text{GTI (\%)} = \frac{\text{FGP under stress condition}}{\text{FGP under control condition}} \times 100$$

Seed Vigor Index (SVI): The length of ten seedlings (SL) from each Petri dish (replicate/concentration) was measured in cm and then calculated using the following formula (Abdul Baki and Anderson, 1973). The seed lot with higher SVI is considered to be more vigorous.

$$\text{SVI} = \text{SL (cm)} \times \text{FGP (\%)}$$

Statistical analysis

The effects of different NaCl concentrations on the six studied variables were tested using a one-way analysis of variance (ANOVA). Differences between treatments following ANOVA were made by means comparison. Multiple comparisons of means were carried out using Tukey's test ($p \leq 0.05$). A repeated measures analysis of variance was applied to examine the germination kinetics. All statistical analyses were performed using SAS software Version 9.0 (SAS, 2002).

RESULTS

Germination kinetics

Figure 1 illustrates the cumulative germination percentages of *N. rustica* seeds treated with varying concentrations of NaCl over a period of seven days. Each day represents a different stage of germination, allowing for an analysis of germination kinetics and the influence of salinity on the germination process. The figure highlights three distinct phases:

(i) Latency phase: On the first day of observation, all treatments showed a cumulative germination percentage of 0%, indicating that no seeds had initiated germination at this early stage. This initial phase is commonly referred to as the latency phase, during which seeds undergo imbibition and prepare for germination.

(ii) Exponential phase: From the second day onwards, seeds entered the exponential phase of germination, characterized by a rapid increase in germination percentage. At 0 mM NaCl (control), germination progressed gradually, reaching a cumulative percentage of 66.3% by day 3 and plateauing near complete germination (>99%) by day 5. This design indicates optimal germination kinetics under normal conditions. However, increasing NaCl concentrations progressively inhibited the progression during this phase. Seeds treated with 50 mM NaCl exhibited slightly delayed germination compared to the control, with cumulative germination percentages of 58.7% on day 3 and 96.3% on day 6. These findings suggest a moderate effect of salinity on the germination rate, leading to a slower transition through the exponential phase.

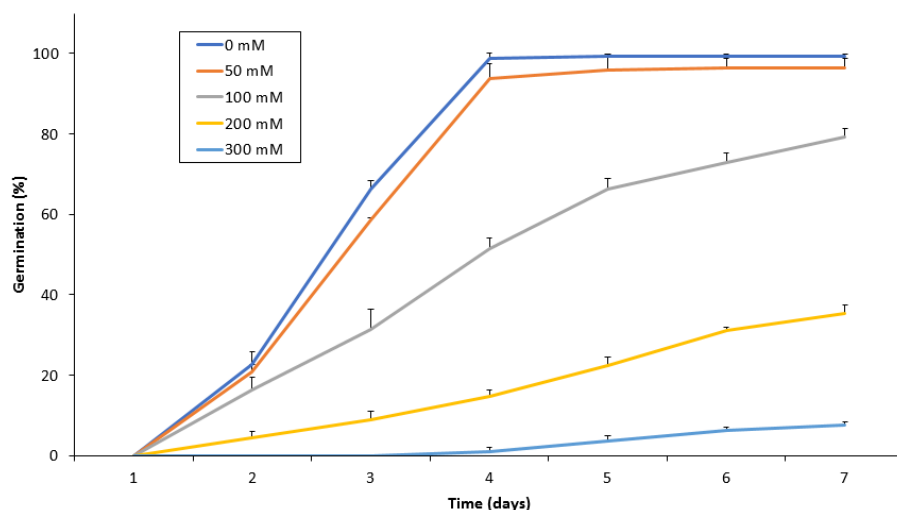


Figure 1. Cumulative germination percentages of *Nicotiana rustica* (L.) seeds treated with NaCl for seven days

(iii) Plateau phase: At higher NaCl concentrations (100 mM, 200 mM, and 300 mM), the inhibitory effect on germination became more pronounced. Seeds treated with 100 mM NaCl showed a noticeable delay in reaching the plateau phase, with cumulative germination percentages of only 79.3% by day 7. Also, seeds treated with 200 mM and 300 mM NaCl exhibited significant reductions in cumulative germination percentages, plateauing at 35.3% and 7.6%, respectively, by day 7.

The latency phase remained consistent across all treatments, indicating that initial seed imbibition was not significantly affected by salinity. However, in the exponential phase, the inhibitory effects of salinity became apparent, leading to slower and less synchronous germination. The plateau phase, when germination rates stabilize, was reached more slowly under salinity stress, with higher NaCl concentrations resulting in lower cumulative germination percentages even after seven days. These findings suggest that the final germination percentage was significantly reduced under elevated NaCl concentrations, highlighting the detrimental impact of salinity on seed germination kinetics.

Germination traits

To assess the response of *N. rustica* to salinity stress during seed germination, the effects of different NaCl concentrations were evaluated on the final germination percentage (FGP), mean germination time (MGT), time to 50% germination (T_{50}), seedling length (SL), germination tolerance index (GTI), and seed vigor index (SVI) (Table 2).

According to Table 2, *N. rustica* exhibited distinct responses to increasing NaCl concentrations. At lower concentrations (0 mM and 50 mM), FGP remained relatively high, reaching 99.3% and 96.3% respectively. However, as salinity levels increased to 100 mM, FGP declined substantially to 79.3%. Further increases in NaCl concentration to 200 mM and 300 mM resulted in a notable reduction in FGP to 35.3% and 7.66% respectively, indicating a significant inhibitory effect on germination.

In terms of germination timing, *N. rustica* seeds exposed to different NaCl concentrations exhibited comparable MGT values, ranging from 3.11 to 5.56 days. However, T_{50} gradually increased across treatments, with seeds exposed to 300 mM NaCl requiring significantly more time (5.09 days) compared to other concentrations.

Table 2. Effects of different NaCl concentrations on the final germination percentage (FGP), mean germination time (MGT), time to 50% germination (T_{50}), seedling length (SL), germination tolerance index (GTI), and seed vigor index (SVI) of *Nicotiana rustica*

Salinity [NaCl]	FGP (%)	MGT (days)	T_{50} (days)	SL (mm)	GTI (%)	SVI
0 mM	99.3 ^a	3.11 ^d	2.63 ^d	22.2 ^a	100 ^a	22080.7 ^a
50 mM	96.3 ^a	3.21 ^d	2.72 ^d	21.3 ^a	96.9 ^a	20576.1 ^a
100 mM	79.3 ^b	3.99 ^c	3.43 ^c	15.9 ^b	79.9 ^b	12625.7 ^b
200 mM	35.3 ^c	4.71 ^b	4.39 ^b	10.4 ^c	35.6 ^c	3683.3 ^c
300 mM	7.66 ^d	5.56 ^a	5.09 ^a	1.83 ^d	7.72 ^d	141.1 ^d
F-value	1561.4	171.7	77.6	182.4	1549.1	215.5
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Legend: Different letters in the same column indicate a significant difference at $p \leq 0.05$, as evaluated by Tukey's test.

Seedling length (SL) was significantly affected by salinity stress. *N. rustica* seedlings exposed to higher NaCl concentrations exhibited reduced growth. At 0 mM NaCl, seedling length was the highest (22.2 mm), but it progressively decreased with increasing salinity, reaching the lowest value of 1.83 mm at 300 mM NaCl. Despite the significant reduction in emergence rates under saline conditions, the seedlings that successfully emerged at 200 mM NaCl maintained vigorous morphological integrity (Figure 2).



Figure 2. Seed germination and seedling emergence of *Nicotiana rustica* (L.) following seven days of 200 mM NaCl treatment

The germination tolerance index (GTI) and seed vigor index (SVI) provided comprehensive measures of seedling performance under salinity stress. *N. rustica* seeds exposed to lower NaCl concentrations exhibited higher GTI and SVI values, indicating better tolerance and vigor under moderate salinity levels. However, as salinity increased, both GTI and SVI progressively declined, reflecting the adverse effects of high salinity on seedling vigor and overall germination performance.

These findings contribute to a deeper understanding of the plant responses to salinity stress and underscore the importance of developing strategies to mitigate the adverse effects of salinity on crop productivity in saline-affected regions. The one-way ANOVA statistical analysis revealed that NaCl concentrations had significant effects on all evaluated seed germination parameters, including final germination percentage, mean germination time, time to 50% germination, seedling length, germination test index, and seedling vigor index, demonstrating the considerable influence of NaCl concentration on seed germination and seedling growth traits (Table 2).

DISCUSSION

The results indicate that *N. rustica* seeds are highly sensitive to salinity, with increasing NaCl concentrations substantially reducing the germination percentage. These findings are in line with previous studies on salinity effects on seed germination in other species of the genus *Nicotiana* (Yan et al., 2014; Florentine et al., 2016; Nejatzadeh-Barandozi, 2018; Abrantes et al., 2019). Our data suggest that *N. rustica* seeds can tolerate up to 50 mM NaCl without substantial inhibition in germination rates. However, concentrations above 100 mM NaCl significantly damage germination, with severe inhibition observed at 200 mM and nearly complete inhibition at 300 mM NaCl.

This threshold is consistent with the general salinity tolerance observed in many crop species (Uçarlı, 2020; de Paiva Gonçalves et al., 2024).

The delay in germination and inhibition of growth induced by salinity is caused by various factors such as reduced external water potential, ion imbalance, and specific ion toxicity (Soni et al., 2023). In such conditions, there is both a reduction in water uptake and excessive absorption of ions. The salinity tolerance of seeds includes both their persistence in the soil, during which they may encounter elevated salinity levels and extreme temperatures, and their capacity to germination under such stress (Haider et al., 2023).

N. rustica exhibited varying degrees of salinity tolerance, with significant reductions in final germination percentage (FGP) and seedling length (SL) observed at higher NaCl concentrations. These findings are consistent with previous studies investigating the effects of salinity stress on seed germination and seedling growth in *Nicotiana* species (Florentine et al., 2016; Nejatzadeh-Barandozi, 2018). However, it is important to note that the responses of *N. rustica* to salinity stress may differ from those of other *Nicotiana* species (Abrantes et al., 2019). Recently, *Nicotiana tabacum*, a closely related species, has been reported to exhibit varying levels of salt tolerance depending on the cultivar (Zhang et al., 2024). These differences in salt tolerance among *Nicotiana* species underscore the importance of species-specific approaches in managing salinity stress in agricultural systems.

Furthermore, the decline in seedling vigor index (SVI) with increasing NaCl concentrations highlights the adverse effects of salinity stress on overall seedling performance. Similar observations have been reported in other crop species, where high salinity levels negatively impact seedling vigor and subsequent crop establishment (Finch-Savage and Bassel, 2016; Truşcă et al., 2023). Therefore, mitigating the detrimental effects of salinity stress on seedling vigor is crucial for ensuring successful crop establishment and optimal yield in saline-affected regions. The contrasting responses of *N. rustica* to salinity stress during seed germination and seedling growth underscore the need for targeted breeding efforts to develop salinity-tolerant cultivars. Identifying the physiological and molecular mechanisms underlying salt tolerance in *N. rustica* will allow scientists to improve crop resilience to saline environments by breeding crops with these traits. Furthermore, soil amendments and cultural practices can be employed in saline-affected regions to mitigate soil salinity and improve soil structure. One effective soil amendment is the application of gypsum (CaSO_4), which helps in displacing sodium ions from the soil and improves its structure, especially in sodic soils. Additionally, incorporating organic matter, such as compost, manure, or biochar, can enhance soil porosity, water retention, and promote beneficial microbial activity, which in turn alleviate salt stress. Mulching is another useful practice, which reduces evaporation and maintains soil moisture, creating a more favorable environment for plant growth. Irrigation management through leaching (applying frequent, light irrigation) can help in flushing excess salts from the root zone, particularly in areas where water quality is not highly saline. Moreover, crop rotation with salt-tolerant species can aid in regulating salt accumulation in the soil over time. In areas with high groundwater salinity, raised beds can be used to prevent salt from reaching plant roots (Kheloufi and Mansouri, 2019; Kheloufi et al., 2019; Rashed, 2020; Shin et al., 2022; Awaad, 2023).

McGaughey et al. (2018) demonstrated that alterations in salinity levels can impact seed aquaporin function, thereby influencing seed germination. In saline environments, the prompt germination of Mediterranean halophyte fodder species plays a crucial role in facilitating plant establishment. As a result, these plants propagate rapidly and develop deep root systems capable of absorbing moisture from deeper soil layers during drought periods. This adaptive mechanism enhances their resilience during dry seasons by enabling access to moisture from deeper areas in the soil (Reed et al., 2022; Kheloufi et al., 2023). A proteomic analysis uncovered that exposure to salt decelerates the extensive conversion of reserve proteins into 20 amino acids, which can be further transformed into other amino acids, as well as the conversion of fatty acids into carbohydrates. However, under favorable recovery conditions, these conversion processes are promptly reinitiated (Debez et al., 2018).

CONCLUSION

This study presents a comprehensive analysis of the effects of sodium chloride (NaCl) concentrations on the germination kinetics of *Nicotiana rustica* seeds. These findings underscore a clear response to salinity stress, with increasing NaCl levels correlating with significant reductions in final germination percentage, delayed germination kinetics, inhibited seedling growth, and compromised seed vigor and tolerance. Statistical analysis confirms the reliability of these results, with highly significant differences observed among NaCl treatments for all germination attributes. The delayed and reduced germination kinetics observed in this study suggest that *N. rustica* seeds are particularly sensitive to saline conditions, which can severely limit their establishment and growth in salt-affected soils. These results have important implications for agricultural practices, particularly in regions subjected to soil salinization. Future research should focus on identifying and developing salt-tolerant *N. rustica* cultivars and exploring mitigation strategies to counteract the negative effects of salinity on seed germination and crop production.

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