



# Quantification of green bean germination response to simultaneous salt and temperature stress: a modeling approach

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## Abstract

Seed germination (SG) is crucial for the survival of plant species; however, it is often subjected to unpredictable environmental conditions such as temperature ( $T$ ) and salinity. Therefore, the objectives of this work were to concurrently investigate salinity and  $T$  effects on SG behavior of green bean using a halothermal time (HaloTT) model, to compute the cardinal  $T$ s, and to explore the effect of salinity on seeds' salt ion assimilation (i.e.,  $\text{Na}^+$  and  $\text{Cl}^-$ ). Our findings revealed that the effect of salt stress on SG was quantified well by the HaloTT model ( $R^2 > 0.82$ ). The estimated cardinal  $T$ s at 0 mM were 8.15, 30.0, and 39 °C for the base ( $T_b$ ), optimum ( $T_o$ ), and ceiling ( $T_c$ ) temperatures, respectively. The median threshold NaCl concentration ( $\text{NaCl}_b(50)$ ) was 305 mM at  $T_o$ , then declined linearly at  $T_s \geq T_o$ , reaching 0 mM at  $T_c$ . Decreasing germination capability at  $T$ s above  $T_o$  was due to the shifts in  $\text{NaCl}_b(50)$ , which appears as a thermoinhibition phenomenon.  $\text{Na}^+$  and  $\text{Cl}^-$  assimilation enhanced significantly with  $T$  and seed  $\psi$  decreased compared to control. In conclusion, green bean SG was initially controlled by salt osmotic effect ( $\leq 150$  mM), when exceeding this concentration, its effect became both osmotic and toxic.

**Keywords** Base NaCl concentration · Cardinal temperatures · Green bean · Halothermal time model · NaCl ion assimilation

## Abbreviations

$\text{Cl}^-$	Chloride ion	$\text{Na}^+$	Sodium ion
GP	Germination percentage	NaCl	The real tested NaCl concentration (mM)
GR	Germination rate, or $1/t_g$	$\text{NaCl}_b(50)$	The base NaCl of the 50th percentile
HaloTT	Halothermal time model	$\text{NaCl}_b$	Median base NaCl concentration
$k_T$	The line slope of the relationship between $\text{NaCl}_b(50)$ and $T$ above $T_d$	SG	Seed germination
mM	Millimolar	SMC	Seed moisture content
MPa	Megapascal	$T$	Temperature
		$T_b$	Minimum temperature (base temperature)
		$T_c$	Maximum temperature (ceiling temperature)
		$T_d$	The temperature at which $\text{NaCl}_b(50)$ trend begins to change
		$t_g$	The time required for fraction or percentage (g) of seeds to germinate
		$T_o$	Optimum temperature
		$\theta_{\text{Halo}}$	Halotime constant
		$\sigma_{\text{NaCl}_b}$	Standard deviation of $\text{NaCl}_b$ within the seed population
		$\psi$	Water potential
		$\psi_b(50)$	The base $\psi$ of the 50th percentile

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## Introduction

Green bean (*Phaseolus vulgaris* L.) is one of the most important fresh vegetable crops cultivated in many countries. The world production of this plant is estimated at 27 million tons in 2019, with an annual rising of 2.8% over the period from 2013 to 2019 (FAO 2019). It is a very widespread crop of most temperate, moist-to-dry tropical and subtropical climates. Green bean is mainly produced in China, India, Indonesia, Turkey, and Thailand (FAO 2019). The global green bean market was \$31.1B in 2019, 4.7% higher than the previous year.

Seed germination (SG) is one of the key phases for the survival of plant species; however, it only occurs if external conditions (e.g., humidity, temperature, light, etc.) and internal factors (e.g., maturity, viability, dormancy, etc.) are favorable (Bewley et al. 2013). Temperature ( $T$ ) is one of the major environmental factors that control SG (Abdellaoui et al. 2017; Bakhshandeh et al. 2021). Each species is characterized by cardinal  $T$ s, varying from one species to another depending on the environment to which it is adapted (Bakhshandeh et al. 2021; Bakhshandeh and Gholamhossieni 2019; Hardegee 2006). An optimum temperature ( $T_o$ , single and/or range of  $T$ s) at which seeds germinate quickly and vigorously, a base temperature ( $T_b$ ), and ceiling temperature ( $T_c$ ) upon which, germination is inhibited (Bewley et al. 2013).

Numerous studies showed that germination rate (GR), that is, the number of days/hours required to reach a specific percentile of germination capacity are seriously affected by  $T$  (Bakhshandeh and Eslami 2013; Bakhshandeh et al. 2013, 2021; Derakhshan et al. 2018; Abdellaoui et al. 2019). It increases linearly at  $T_b \leq T \leq T_o$ , while at  $T \geq T_o$ , it decreases linearly and/or curvilinearly (Alvarado and Bradford 2002; Bakhshandeh and Gholamhossieni 2019; Elahifard et al. 2021).

Several unpredictable environmental factors such as  $T$ , water stress, and salinity, etc. on one hand and seed hydration, storage conditions, seed dormancy, etc. on the other hand often aggravate SG (Bewley et al. 2013; Baskin and Baskin 2014; Bakhshandeh and Gholamhossieni 2019; Bakhshandeh and Jamali 2020; Elahifard et al. 2021).

Changes in climate patterns are significantly influencing SG. Based on climate simulation models, an increase in the average global  $T$ , altered weather patterns with shifts in rainfall frequency and intensity, and an increase in saline land area are expected (Corwin, 2021). Soil salinization is a global problem that has influenced 833 million ha of agricultural land in over the world (FAO 2021). Also, it was estimated that fifty percent of the irrigated land could be salinized by the year 2050 (Talat 2020; Singh 2021). Indeed, soil salinization is increasing at the rate of

1–2 million ha year<sup>-1</sup> globally, influencing a remarkable portion of crop production and making land unsuitable for cultivation (Devkota et al. 2022). To meet global food security, the agricultural land (i.e., irrigated area) needs to be increased from current 202–242 million ha in 2030 (FAO News 2021). In addition, the demand for irrigation is greater in arid- and semi-arid regions, where more than 90% of agriculture depends on irrigation. However, the increase of salt concentration in the medium, delayed SG, and increased latency. This is explained by the time needed for seeds to develop mechanisms to regulate their internal osmotic pressure during saline conditions (Hajlaoui et al. 2007). In general sodium chloride (NaCl) in soil or irrigation water decreases GR and reduces germination capacity (Llanes et al. 2016). This effect depends on the species, the severity, and the duration of the salt stress (Ben Naceur et al. 2001; Tobe et al. 2001). Therefore, monitoring the effects of salinity due to climate change is crucial to study the extent of the problem, to recognize trends, and to formulate some management strategies, particularly during the early stage of plant growth (i.e., SG).

Seed germination decrease is due to either an increase in the external osmotic pressure (i.e., in soil solution), which slows down seed imbibition and limits the absorption of water necessary to trigger the metabolic processes involved in germination and/or excess accumulation of Na<sup>+</sup> and Cl<sup>-</sup> in the embryo, which can lead to impaired metabolic processes of germination and in extreme cases, death of the embryo. However, to some extent, the accumulation of compatible solutes (i.e., soluble carbohydrates, proline, betaine, organic acids, etc.) and the assimilation of inorganic ions (i.e., Na<sup>+</sup> and Cl<sup>-</sup>) are necessary or even mandatory to maintain seed hydration following osmotic adjustment leading to an increase in the negativity of the osmotic potential of seeds (Bakhshandeh et al. 2020; Zhang et al. 2010). Nevertheless, the combined effect of salinity and  $T$  is more pronounced for  $T >$  or  $< T_o$ .

El-Bastawisy et al. (2018) showed that *Vicia faba* SG was markedly affected, especially germination speed, at 200 mM NaCl. Cokkizgin (2012) reported that the coefficient of the velocity of germination, germination index, and seed vigor index, in *Phaseolus vulgaris* L., decreased with increasing NaCl concentration while mean germination time increased. They also revealed that the germination percentage (GP) of this plant decreased up to 60% for seeds germinating in Petri dishes at -1.5 MPa NaCl. Contrarily, Bayuelo-Jiménez et al. (2002) pointed out that increasing salinity (180 mM NaCl) decreased germinability by 50% in species of the genus *Phaseolus*.

Population-based threshold models are frequently used to depict seed response to the environmental conditions during germination and emergence stages (Bello and Bradford 2016; Abdellaoui et al. 2019; Bakhshandeh et al. 2020; Liu

et al. 2020b). One type of these models are halotime (HT) and halothermal time (HTT) models which were successfully used for concurrently describing the effect of NaCl and  $T$  and, their interactions on SG of diverse plants such as *Suaeda maritima* (a model halophyte plant) (Seal et al. 2018), rapeseed (salt-tolerant) (Bakhshandeh and Jamali 2020), chicory (moderately salt-tolerant) (Vahabinia et al. 2019), and cucumber (moderately salt-sensitive) (Bakhshandeh et al. 2021). Indeed, knowing this information at the species level could increase our ability to predict species distribution shifts under climate change. The parameters of these models are strongly flexible, interpreting simply and physiologically, and also describing how environmental factors participate to regulate SG within a seed population (Bradford 2018). Also, a high correlation between the germination results obtained in laboratory tests and the emergence results collected from field conditions was reported by Liu et al. (2020a), who studied the SG of 13 desert species in Arizona over 25 years.

Therefore, our objectives in the present study were (1) to concurrently investigate salinity and  $T$  effects on SG of green bean using the HaloTT model, (2) to compute the cardinal  $T$ s for this plant, (3) to explore the effect of salinity on salt ions accumulation (i.e.,  $\text{Na}^+$  and  $\text{Cl}^-$ ) into the seeds of green bean.

## Materials and methods

### Seed source

Seeds of green bean (*Phaseolus vulgaris* L.; Fabaceae, var. Sunray) were provided in Mazandaran province, Iran, in 2018. It was produced by PROSEED Company (Netherlands). At the beginning of the experiments, the seeds had six months old and their viability was > 90% (assessed by ISTA method). It was also kept at 5 °C before use.

### Treatments

In the present work, the effect of five constant  $T$ s (i.e., 15, 20, 25, 30, and 35 °C) and five salinity levels (0, 75, 150, 225, and 300 mM) were concurrently investigated. This means that, at each  $T$ , all five salinity levels were studied. Distilled water was used at 0 mM treatment (as control) in all  $T$ s. For preparing the above-mentioned salt concentrations, NaCl was used.

### Seed germination test protocol

Twenty-five seeds per Petri dish (9 cm diameter) were placed between germination filter paper (two layers below and one layer on top of the seeds) with three replicates. Then,

13 mL of the test solutions, supplemented with 0.1% Thiram fungicide, was added per Petri dish. The dishes were put into a thin white plastic bag for minimizing water evaporation losses and then randomly placed within an incubator with  $\pm 0.5$  °C precision. The dishes were checked several times daily, depending on the  $T$  and salinity levels and the germinated seeds were immediately counted. In each recording time, the seed at least 0.2 cm radicle length was considered as germinated seed. The experiments were finished when no more germination occurred in each Petri dish for three continuous days.

### Halotime and halothermal time models

To quantify salt stress effect on SG of green bean, a halotime model proposed by Bakhshandeh et al. (2020) was fitted in each  $T$ . This model can be written as:

$$\theta_{\text{Halo}} = (\text{NaCl}_b(\text{g}) - \text{NaCl})t_g \quad (1)$$

A HaloTT model, first developed by Bakhshandeh et al. (2020), was also fitted to the experimental data obtained at all  $T$ s and salinity levels. The model can be expressed as

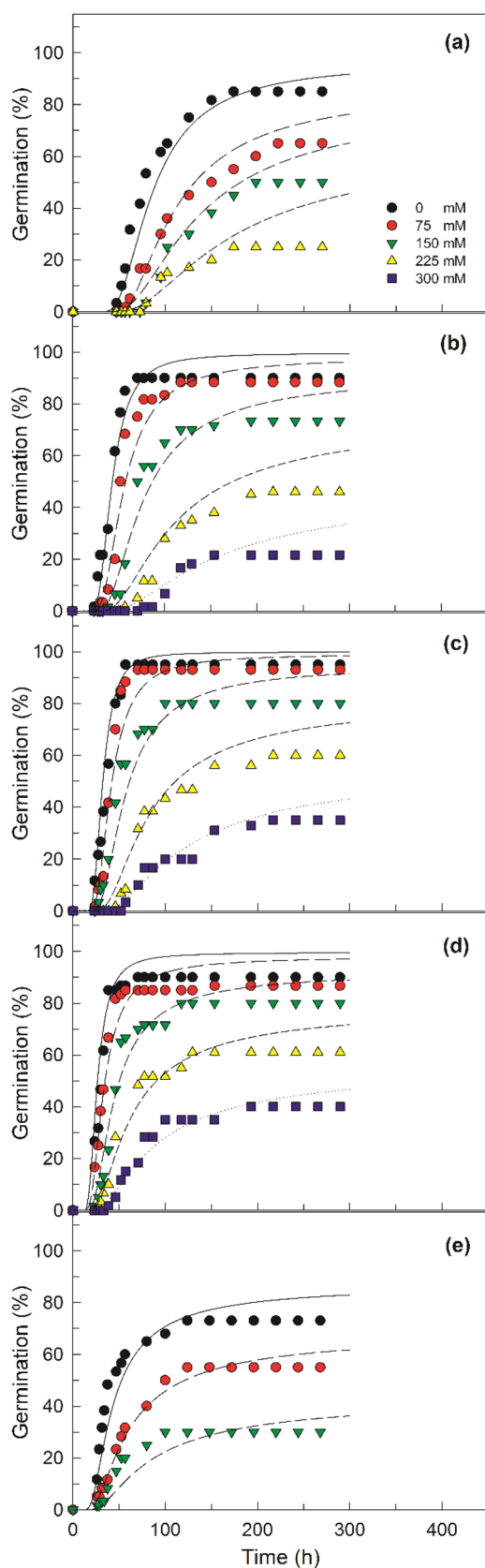
$$\theta_{\text{HaloTT}} = (\text{NaCl}_b(\text{g}) - \text{NaCl})(T - T_b)t_g \quad \text{if } T_b \leq T_s \leq T_o, \quad (2)$$

$$\theta_{\text{HaloTT}} = [\text{NaCl}_b(\text{g}) - (\text{NaCl} + k_T(T - T_d))](T - T_b)t_g \quad \text{if } T_s \geq T_o. \quad (3)$$

According to the results reported by Bakhshandeh and Gholamhossieni (2019), the  $T_d$  value may be equal and/or lower than  $T_o$ , depending on plant species. All the parameters of these models are defined in detail in Bakhshandeh and Jamali (2020).

### Seed moisture content, $\text{Na}^+$ , and $\text{Cl}^-$ concentration

To determine the  $\text{Na}^+$  and  $\text{Cl}^-$  concentration (mmol kg  $\text{DW}^{-1}$ ) into the green bean seeds, at all tested  $T$ s and salinity, the method reported by Bakhshandeh et al. (2020) was used. Briefly, 15 g of the seeds was grown under the same conditions that are described fully in “Seed germination test protocol”. Then, the seeds were harvested from each Petri dish immediately before germination (this time was accurately estimated from Fig. 1) and washed carefully with distilled water for removing any tested solution from the testa. For the seed moisture content (SMC, %) measurement, the seeds were immediately ground and dried in an oven at 130 °C for 1 h (ISTA 2009), then determined as a dry weight basis. For the  $\text{Na}^+$  and  $\text{Cl}^-$  contents measurement, a flame photometric and the Mohr’s titration method were used, respectively (Estefan et al. 2013). To calculate the total osmolality (mmol/kg  $\text{H}_2\text{O}$ ) of the seeds, made by the salt ions (i.e.,  $\text{Na}^+$  and  $\text{Cl}^-$ ), we assumed that all of the water into



**Fig. 1** Germination of green bean at various temperatures under different concentrations of NaCl. **a** 15 °C, **b** 20 °C, **c** 25 °C, **d** 30 °C and **e** 35 °C

**Table 1** Results of analysis of variance (mean squares) for maximum germination percentage ( $GP_{max}$ , previously arcsine transformed) and germination rate ( $GR_{50}$ , for the 50th percentile) of green bean affected by temperature ( $T$ ) and salt stress (NaCl)

Source of variation	df	$GP_{max}$	$GR_{50}$
$T$	4	0.580***	0.000460***
Error 1	10	0.023	0.000011
NaCl	4	1.722***	0.001551***
$T \times \text{NaCl}$	16	0.074***	0.000056***
Error 2	74	0.021	0.000010
Coefficient of variation (%)		14.9	14.1

df is degree of freedom. The number of total data in salt stress is  $n=75$  ( $T=5$ , NaCl (mM) = 5 and replication = 3)

\*\*\*Highly significant at 0.001 probability level

the seed will be symplastic. To convert the total osmolality of the seeds to  $\psi$  (MPa) the method suggested by Van't Hoff (1887) was used:

$$\psi_s = -RTC_i,$$

where  $R$  is the gas constant (i.e. 0.0083 L/atm/mol/K),  $T$  is the absolute temperature in degrees in Kelvin (273.15 °C),  $C$  is the solute concentration in mol  $L^{-1}$ , and  $i$  is the dissociation constant of the salt (i.e. NaCl = 1.8). The unit for  $\psi_s$  is MPa.

## Statistical analysis

To determine the model parameters, a repeated probit regression analysis reported by Bradford (1990) was applied. The statistical analysis system ver. 9.4 (SAS Institute Inc 2015) and Excel software ver. 2013 was used for analyzing the data. Besides, the Sigma Plot ver. 11 software was used to draw the figures. Also, analysis of variance of the data and means comparison using the least significant difference test was carried out using SAS software.

## Results

### Germination characteristics affected by temperature and salt stress

The  $T$ , salinity and the combined effect of them [ $T \times \text{salinity}$ ] had significantly ( $P < 0.001$ ) affected GP of green bean seeds (Table 1; Fig. 1a–e). The seeds in distilled water showed a germination > 90% at 25 °C and then declined remarkably with  $T$  reaching ~ 70% at 35 °C. The control (0 mM NaCl) and treated (75 mM NaCl) seeds showed no significant difference at 20–30 °C relating to the final GP. However, for the same treatment (75 mM NaCl), a significant decrease was

detected at 15 and 35 °C. By increasing salinity (> 75 mM NaCl), GP decreased significantly compared to the control (at all  $T_s$ ) and was inhibited at 300 mM NaCl at both 15 and 35 °C (Fig. 1a–e).

The same results were observed for GR, meaning that the  $T$ , salinity and the combined effect of them [ $T \times$  salinity] had significantly affected GR ( $P < 0.001$ , Table 1). Following the same pattern as GP, the highest  $GR_{50}$  (GR for the 50th percentile) of green bean seeds was found at 25–35 °C (Fig. 1a–e).  $GR_{50}$  also decreased severely with increasing NaCl concentration at all  $T_s$ . It should be noted that the salt stress effect was harsher at  $20 \text{ °C} \leq T_s \leq 30 \text{ °C}$  for both GP and GR parameters (Fig. 1a–e; Table 1).

### Halotime and halothermal time models

The halotime model (Eq. 1), fitted to the green bean SG data showed a coefficient of determination ( $R^2$ ) value of > 0.80 (Table 2). The registered  $\theta_{\text{Halo}}$  value non-linearly decreased from 27,000 mM h for 15 °C to 7000 mM h for 35 °C (Table 2; Fig. 2a). However, at  $T_s < 30 \text{ °C}$ , the  $\text{NaCl}_b(50)$  remained constant (averaged 308.7 mM), then declined linearly ( $k_T = 34.0 \text{ mM °C}^{-1}$ ) with  $T$  at  $T_s > T_o$ , reaching 0 mM at  $T_c$  (i.e., 36.0, 39.0 and 42.2 °C for the 16th, 50th and 84th percentiles, respectively) (Table 2; Fig. 3b). For instance,  $\text{NaCl}_b(50)$  was 132.8 mM at 35 °C (Table 2). The  $\sigma_{\text{NaCl}_b}$  values were similar at all  $T_s$  (averaged 107.1 mM), except for 15 °C (150.3 mM), indicating that SG was homogenous at  $20 \text{ °C} \leq T_s$  (Table 2). A linear model was used to quantify the inverse of  $\theta_{\text{Halo}}$  values ( $1/\theta_{\text{Halo}}$ ) versus  $T$ . The interception value of the linear model broken the  $x$ -axis at 8.2 °C (defined as  $T_b$ ) (Fig. 2b).

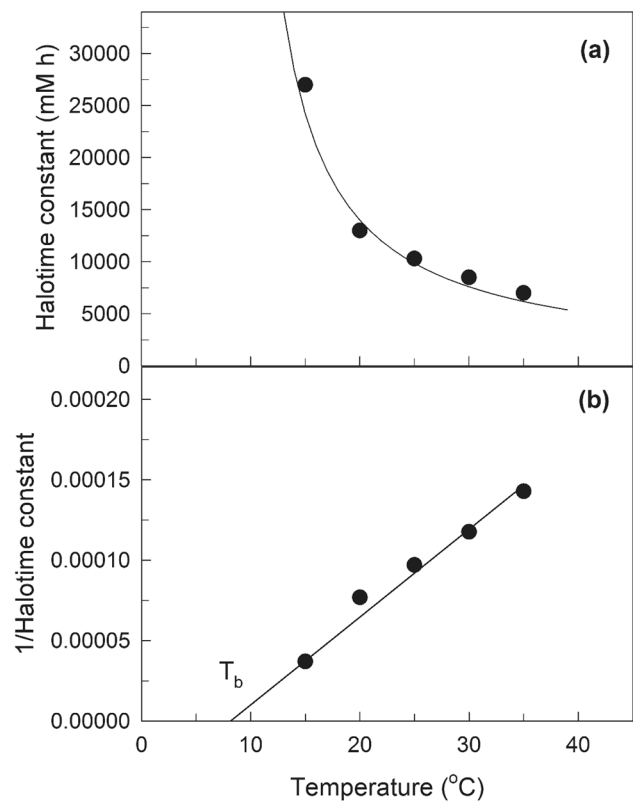
The HaloTT (Eqs. 2 and 3) model was fitted to investigate green bean SG response under different NaCl concentrations at all ranges of  $T_s$ . Results revealed that a high  $R^2$  value ( $R^2 = 0.82$ ), which assigns a high correlation between observed and predicted germination data for each set of  $T$  (Fig. 4; Table 3).

The cardinal  $T_s$  of green bean seeds were calculated based on the HaloTT model (Fig. 4). The  $T_b$  and  $T_o$  values

**Table 2** Estimated parameter values using the halotime (Eq. 1) model to describe green bean seed germination under different  $T$  and NaCl concentrations

$T$ (°C)	$\theta_{\text{Halo}}$ (mM h)	$\text{NaCl}_b(50)$ (mM)	$\sigma_{\text{NaCl}_b}$ (mM)	$R^2$
15	27,000	298.7	150.3	0.80
20	13,000	299.9	102.4	0.81
25	10,306	317.6	96.93	0.86
30	8500	318.7	114.1	0.89
35	7000	132.8	115.0	0.86

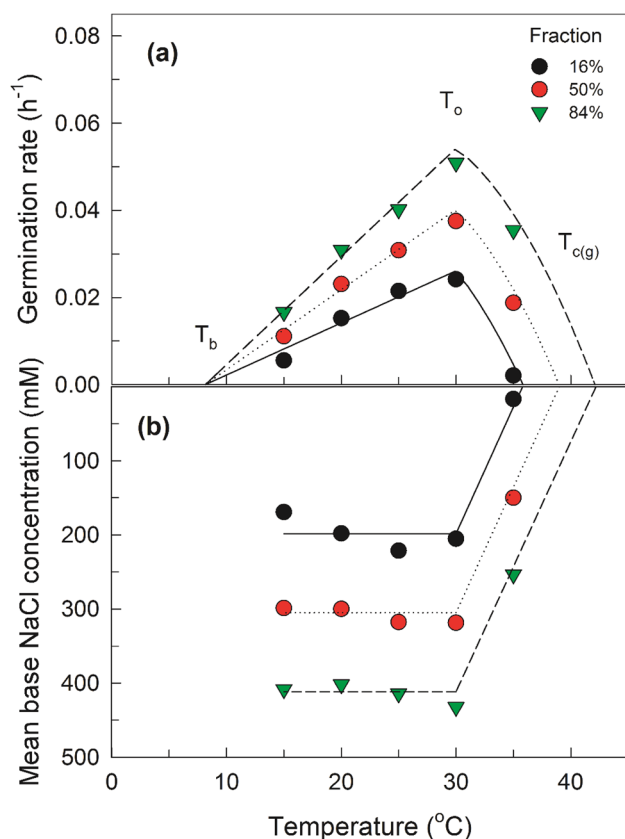
$R^2$  is the coefficient of determination



**Fig. 2** a Changes in halotime constant for green bean as a function of temperature. b The relationship between the inverse of halotime constant values with temperature which is linear and the intercept is equal to 8.2 °C ( $T_b$ ). In each panel, the symbols and the lines are represented the actual and predicted data for the 50th percentile, respectively

remained steady in all seed fractions with 8.2 and 30 °C, respectively (Table 3; Fig. 3a) whereas the  $T_c$  was 36.0, 39.0, and 42.2 °C at 0 mM NaCl (i.e., control) for the 16th, 50th, and 84th percentiles, respectively (Fig. 4a). Besides, the GR variation under the combined effect of salinity and  $T$  indicated a high remarkable relation between GR and NaCl concentrations at all  $T_s$ . At  $T_s \leq 30 \text{ °C}$ , GR showed a linear increase then curvilinearly decreased at  $T_s \geq T_o$  with increasing  $T$  (Fig. 4a).

In Fig. 3b,  $\text{NaCl}_b(g)$  was roughly steady at  $T_s$  below  $T_o$  and then dropped off linearly at  $T_s \geq T_o$ , reaching 0 mM at the intersection of the  $x$ -axis at the  $T_c$  values. At sub-optimal  $T_s$ , the  $\text{NaCl}_b(g)$  values were 198.3 mM for the 16th, 305.0 mM for the 50th, and 411.7 mM for the 84th percentiles (Fig. 4b). After conversing these values to  $\psi$  (MPa) by the Van't Hoff method, they correspond to  $-0.90$ ,  $-1.38$ , and  $-1.86$  MPa, respectively.

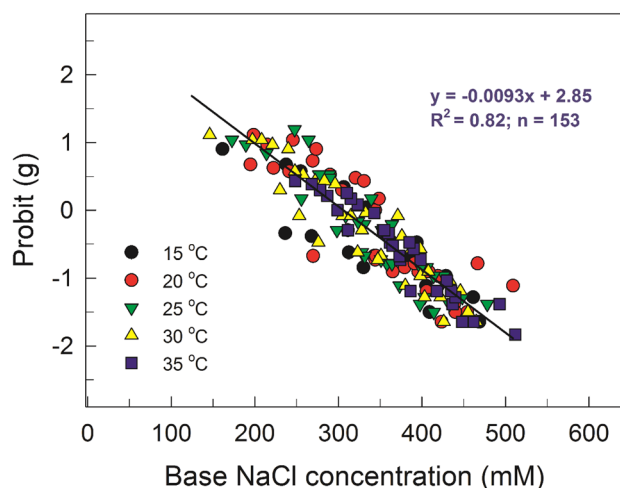


**Fig. 3** **a** The cardinal temperatures for germination of green bean seeds using the halothermal time model. Based on the models' behavior, the base temperature ( $T_b$ ) and optimal temperature ( $T_o$ ) were constant for all seed fractions in this species while the ceiling temperatures ( $T_c$ ) varies with the seed fraction, being 36.0, 39.0, and 42.2 °C in water at the 16th, 50th and 84th percentiles, respectively. **b** The  $NaCl_b(g)$  decreased linearly as  $T$  increased at the supra-optimal  $T$  range for the seed fractions. In panel b, the lines for different seed fractions (16, 50 and 84%) intercept the  $NaCl_b(g)=0$  mM axis at the  $T_c$  values for these fractions. The actual data are shown by the symbols and the lines drawn through these points are based upon the parameters presented in Table 3

### Seed moisture content, salt ions ( $Na^+$ and $Cl^-$ ) content into the seeds

The SMC (determined immediately before germination) showed a significant increase at all studied  $T_s$  under salt stress in comparison with the control (Fig. 5a; small letters) and decreased significantly at  $T < 30$  °C then enhanced notably at  $T > 30$  °C (Fig. 5a; capital letters).

At each studied  $T$ , seed  $Na^+$  concentration enhanced remarkably ( $P < 0.05$ ) under salt stress in comparison with the control (Fig. 5c; small letters). For example, at 30 °C the  $Na^+$  concentration registered was 57.6 mmol kg<sup>-1</sup> DW at 0 mM NaCl (i.e., control seeds) and 181.8 mmol kg<sup>-1</sup> DW for treated seeds (300 mM NaCl). However, the combined effect of both stresses (i.e., NaCl and  $T$ ) gave the most



**Fig. 4** Linear regression of probit (g) plotted against base NaCl concentrations ( $NaCl_b(g)$ , mM) and adjusted base NaCl concentrations ( $-NaCl_b(g) + k_T(T - T_d)$ ). Points are the observed values of probit germination fraction at each set of temperature and the lines represent the predicted values by the models ( $p < 0.05$ )

important  $Na^+$  content (221.5 mmol kg<sup>-1</sup> DW, averaged for all salt stress levels) for treated seeds under 300 mM NaCl at 35 °C. When considering the influence of  $T$  under various concentrations of salinity,  $Na^+$  content decreased with  $T$  at  $T > 15$  °C, remained approximately steady between 20 and 30 °C, then enhanced at the  $T_s$  above 30 °C (Fig. 5c; capital letters).

Similarly, seed  $Cl^-$  content showed the same pattern as  $Na^+$  content; it increased remarkably ( $P < 0.05$ ) with enhancing salt stress levels at all  $T_s$  (Fig. 5b; small letters). The treated seeds (300 mM NaCl) concentrated more  $Cl^-$  content (99.2 mmol kg<sup>-1</sup> DW) compared to control (30.6 mmol kg<sup>-1</sup> DW) at supra-optimal  $T_s$  (i.e., 35 °C). However, the least  $Cl^-$  content was registered for control seeds (15.7 mmol kg<sup>-1</sup> DW) at 30 °C (as  $T_o$ ). The lowest seeds  $Cl^-$  content was observed at  $20 \leq T \leq 30$  °C (averaged for all levels of salt stress), then increased significantly at  $15 > T > 30$  °C (Fig. 5b; capital letters).

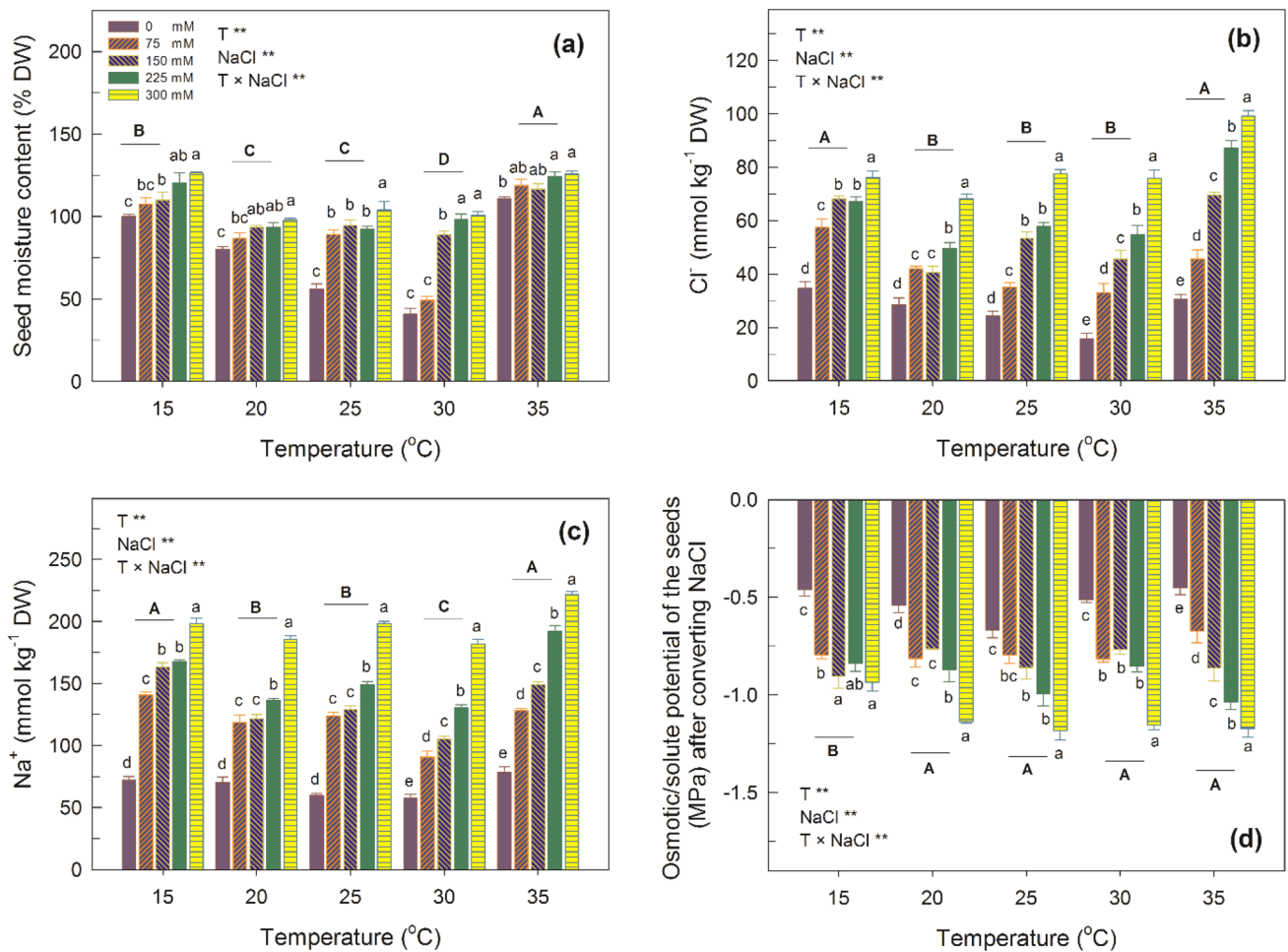
Treated seeds showed a significant decrease ( $P < 0.05$ ) of their osmotic/solute potential (estimated by the Van't Hoff method) across all studied  $T_s$  in comparison with the control (Fig. 5d; small letters). For example, seed  $\psi$  became more negative from -0.45 MPa (control) to -1.17 MPa (300 mM NaCl) at 35 °C. Besides, the  $\psi$  of green bean seeds decreased significantly ( $P < 0.05$ ) at  $T_s$  above 15 °C and remained unchanged for  $T_s \geq 20$  °C (Fig. 5d; capital letters).

Accordingly, at lower osmotic potentials, the salt ions (i.e.,  $Na^+$  and  $Cl^-$ ) could be absorbed by the seeds and so enhancing the turgescence and germination capacity. However, this osmotic adjustment was achieved for treated seeds (75 mM NaCl) at 20 to 30 °C (Figs. 1b–d and 5d). Salt ions

**Table 3** Estimated parameter values using the halothermal time (Eqs. 2 and 3) model for describing seed germination of green bean at five constant  $T_s$  (15, 20, 25, 30, and 35 °C) at each of the following five different NaCl (0, 75, 150, 225 and 300 mM, respectively)

HaloTT parameters	Estimated values
$\text{NaCl}_b(50)$ (mM)	305.0
$\sigma_{\text{NaCl}b}$ (mM)	107.3
$\theta_{\text{HaloTT}}$ (mM °C h)	165,906
$k_T$ (mM °C <sup>-1</sup> )	34.0
$T_d$ (°C)	30.0
Cardinal $T_s$ at 0 mM	
$T_b$ (°C)	8.20
$T_o$ (°C)	30.0
$T_c$ (°C)	39.0
$R^2$	0.82

$T_c$  calculated by extrapolated from the germination rate data at  $T_s$  above  $T_o$ .  $R^2$  is the coefficient of determination. The  $T_d$  was assumed to be equal  $T_o$  in the present study according to Bahkshandeh and Gholamhossieni's (2019) suggestion



**Fig. 5 a–d** Seed moisture content, the concentration of  $\text{Na}^+$ ,  $\text{Cl}^-$  and osmotic/solute potential of the seeds (after converting NaCl to  $\psi$  based on Van't Hoff equation) for green bean following imbibition in different concentrations of NaCl at five constant temperatures. In all panels, the values in the y-axis are different. Lower case letters show

significant differences among NaCl treatments within a temperature and upper case letters show significant differences among temperatures, using the least significant difference test (LSD) at 0.05 probability level. \*\*Significant at 0.01 probability level

uptake is therefore satisfactory at some extent (lower  $\psi_s$ ) to enhance SG rather than being toxic.

## Discussion

Seed germination is strongly affected by different environmental factors (i.e.,  $T$ , NaCl,  $\psi$ , etc.). Therefore, SG modeling under stressful environmental conditions could help us to better understand germination requirements for finding appropriate geographical areas, where a specific species can grow.

The results showed that the GP and GR were significantly influenced by  $T$  (across all studied  $T$ s), which agrees with the findings reported in previous studies (Abdellaoui et al. 2019; Bakhshandeh and Eslami 2013; Bakhshandeh and Gholamhossieni 2019; Derakhshan et al. 2018). In addition, decreasing GR and GP with salt stress was reported, especially when salinity exceeding the threshold above which SG is inhibited (Bakhshandeh et al. 2021; Seal et al. 2018). These results are totally in agreement with those showing that salinity to some extent reduces and/or delays the SG and the loss of seeds before germination (Bakhshandeh et al. 2021; Song et al. 2005). In general, salinity influences SG in several ways: (i) high accumulation of salt ions in the medium causes osmotic and pseudo-drought stress, leading to a decrease in water absorption by the seeds which is necessary for nutrient mobilization during SG (Rajabi Dehnavi et al. 2020), (ii) it can cause changes in enzyme activity by the toxicity effect of ions, leading to a changing in the metabolism of nucleic acid and protein (Gomes-Filho et al. 2008), disturbing the hormonal balance (Ryu and Cho 2015), and reducing the use of seed reserves and (iii) the high levels of salt ions may be toxic to the embryo growth (Kaymakanova 2009). However, different internal factors of a seed, such as coat properties, age, polymorphism, dormancy and, seedling vigor; and external factors, such as  $T$ , light, and water, can influence SG under salt stress (Wahid et al. 2016).

The models applied in this study (i.e., halotime and HaloTT) illustrated well green bean SG under salt stress (Tables 2 and 3). Results showed that the  $\text{NaCl}_b(50)$  of green bean was 305 mM in line with the results of Cokkizgin (2012), who showed that GP of common bean was reduced to 60% at 20 °C when seeds were treated with 17.7 g/L of NaCl (302.8 mM). The  $\text{NaCl}_b(50)$  was unvarying with  $T$ s  $< T_o$  in the studied seed population, similar to the results on chicory (Bakhshandeh et al. 2020) and opposing to the results on *Suaeda maritima* (Seal et al. 2018). At  $T$ s  $> T_o = 30$  °C,  $\text{NaCl}_b(50)$  values decreased linearly confirming results obtained in chicory (Bakhshandeh et al. 2020) and cucumber (Bakhshandeh et al. 2021). Indeed, the decline in SG capability at  $T$ s  $> T_o$  is due to the shifts in the  $\text{NaCl}_b(50)$  value, indicating the seeds needed to absorb much

water for germination, which appears as a thermoinhibition phenomenon (Derakhshan et al. 2018). On the other hand, this phenomenon can be defined as an adaptive strategy by the seeds, meaning that SG will not occur when they are exposed under stressful conditions (i.e., high  $T$  and salinity), keeping their germination capability to reach an appropriate environmental condition (Hosseini Sanehkooori et al. 2021).

In previous researches on common beans, the  $T_b$  was 7–8 °C (White and Montes 1993) and 8.4–9.2 °C (Cardoso and Bianconi 2013) in line with our results (8.2 °C). Raveneau et al. (2011) also reported that  $T_b$  of common bean was 5.1–9.6 °C depending on seed lot. Green bean seeds germination necessitates temperate  $T$ s with moderately high  $T_b$  (8.2 °C) and an extremely high  $T_o$  (30 °C). Contrarily, Balkaya (2004) showed that  $T_o$  ranged from 23.0 to 24.6 °C depending on green bean varieties. In all studied seed fractions, both  $T$ s ( $T_b$  and  $T_o$ ) were steady under salinity levels (Fig. 3a), whereas the  $T_c$  values were changed (i.e., 36.0, 39.0, and 42.2 °C for the 16th, 50th, and 84th percentiles at 0 mM, respectively) confirmedly to Alvarado and Bradford (2002), and Bakhshandeh et al. (2021) results. In common bean, Kurtar (2010) found the  $T_c$  value was 40 and 42 °C, respectively, distinguishable from our results on green bean (39.0 °C). Our findings are also fully agreed with the results obtained from different cultivars of green bean (Carioca, Grauna, and Uirapuru with  $T_c = 38.5, 38.6, \text{ and } 38.9$  °C, respectively) (Cardoso and Bianconi 2013). The  $T$  interval between  $T_b$  and  $T_c$  varies according to the physiological state of the seeds, is often restricted for dormant seeds, and heightens when dormancy is broken (Bradford and Somasco 1994).

The SMC,  $\text{Na}^+$  and  $\text{Cl}^-$  contents of green bean seeds increased with increasing salinity levels (Fig. 5), which is in agreement with the results on *Atriplex canescens* and *Salicornia pacifica* (Khan et al. 1985), *Haloxylon ammodendron* and *Suaeda physophora* (Song et al. 2005), *Suaeda maritima* (Seal et al. 2018), *Cichorium intybus* (Bakhshandeh et al. 2020), and *Cucumis sativus* (Bakhshandeh et al. 2021). In our study, the measured uptake of  $\text{Na}^+$  and  $\text{Cl}^-$

ions during imbibition in salt solutions was sufficient to account for the lower apparent  $\psi_b(50)$  values after converting the NaCl concentrations to osmotic potentials, meaning that the salt ions absorption permitted the seeds grown in a salty medium to preserve lower  $\psi_b(50)$  (i.e.,  $-1.38$  MPa; corresponding to elevated  $\text{NaCl}_b(50) = 305$  mM NaCl at  $T_o$ ) (Bakhshandeh et al. 2021, 2020). This could be explained by the fact that SG was primarily controlled by salt osmotic effect rather than toxic to some extent (150 mM NaCl for green bean), when exceeding this NaCl concentration, its effect became both osmotic and toxic (Bakhshandeh et al. 2021; Seal et al. 2018; Zhang et al. 2010).

Of course, it is not only a physical–chemical effect, but also depends on the physiology and genetics of the species



(Tlahig et al. 2021). However, the water gradient between the seed and the medium is important for the uptake of water in the germinated seed, but also are the cellular strategies that include the detoxification and cell protection mechanisms that allow the seed to germinate until 150 mM NaCl.

Seed germination of green bean, across all studied *T*s and salinity levels, was accurately modeled by the HaloTT model. All the evidence showed that green bean (e.g., Sunray variety) could be considered as a moderately salt-tolerant species in the seed germination stage and also could successfully be cultivated in moderate water-deficient regions (Bourgault 2009; Saleh et al. 2018). Also, we believe that the parameters found in this work can be applied as an extrapolative tool in green bean growth simulation models to predict SG behavior of this plant (especially Sunray variety) under different conditions of *T* and salinity that could exist in the field, although, further study is required under field condition to verify these findings.

**Author contribution statement** EB designed and performed the experiments. EB conducted the modeling. EB, RA, FB and MJ interpreted the data. EB, RA, FB and MJ co-wrote all drafts of the paper and also approved the final draft for submission.

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## Declarations

**Conflict of interest** The authors declare no conflicts of interest.

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