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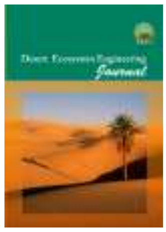

Assessment of Seed Germination of *Moringa peregrina* under Drought and Salinity Stress and its Cardinal Germination Temperatures in Laboratory Environment

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Abstract

The effect of different temperatures on germination was assessed in a laboratory study in the framework of complete randomized design with five repetitions to determine the specific temperature of *Moringa peregrina* seeds. Evaluation of germination response was conducted at constant temperatures of 5, 10, 15, 20, 25, 30, 35, 40 and 45 °C. Cardinal germination temperatures were fitted using three models, including beta, segmented and dent-like. Optimum and maximum temperatures of germination were calculated 17, 25-30 and 47 °C, respectively, based on the dent-like model, which was identified as the best model using statistical indicators. Then, to investigate germination and seedling growth response of *Moringa peregrina* toward different levels of salinity and drought stress at an optimum temperature, another test was conducted. In this experiment, seed germination was assessed in four levels of salinity and drought with the osmotic potential of 0, -4, -8 and -12 bar. The results indicated that seed germination speed and percentage were decreased due to drought and salinity stress. Generally, seed germination of *Moringa peregrina* was more sensitive to drought stress than to salinity stress.

Keywords: Germination Percentage, Germination Speed, Beta Model, Segmented Model, Denta-Like Model.

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1. Introduction

Moringa peregrina (Figure 1) is one of the valuable plants in Iran that has been less considered despite its importance. However, the seed of this plant has been excessively harvested in its local habitats owing to its high economic value; however, it is exposed to severe genetic erosion. This plant is less visible in accessible places due to human interference and generally grows in highland places far from human access. Nevertheless, escape from the salinity has also contributed to this issue.

Furthermore, its habitat is an area with low rainfall (Stadtlander & Becker, 2017). Wide applications are provided for different species of *Moringa,* including *M. peregrine* (Al-Dabbas, 2017). This plant can be used as a stimulant for blood circulation in the heart, and as anti-fever, anti-inflammatory, antihypertensive, antifungal, anti-bacterial, and anti-asthmatic drug for the treatment of various diseases. Another application of this plant that can be considerable in our country is the use of its seed powder for water purification. The local communities in the southeast of Iran have always been a problem for providing fresh water due to the extent of marl sediment in the habitats of this plant. Therefore, it can be used for water purification owing to the protein in the seed of this species that causes coagulation of suspended particles in solutions and their deposit. Concentration of suspended particles, even bacteria in solutions such as turbid water or raw oil, has been studied and demonstrated in Iran and other countries (Mehdinejad *et al*, 2009, Sanchez-Martin *et al*., 2010). Necessary practices should be implemented to conserve this plant owing to its importance and being in the list of the danger of extinction. One of the most sensitive steps in plant survival is germination and seedling establishment that ensures the plant generation survival. Knowledge about seed germination for successful establishment of the plant is necessary to domesticate and cultivate medicinal plants discussion. In this regard, most medicinal plants in nature need more time for germination compared to cultivated and modified species (Laghmouchi *et al*., 2017). It can be due to the low speed of germination or specific ecological needs of the plant for germination and growth (Canter *et al.,* 2005). Various environmental factors influence germination of which temperature and humidity are the most important ones. Climate change is an essential issue on crop production, and it has been predicted that by the year 2100, the average temperature in Iran will increase by 1.5–4.5°C (Amiri & Eslamian 2010; Gohari *et al.*, 2013).

Iran is geographically located in arid and semi-arid regions of the world and approximately 15% of its agricultural lands are influenced by salinity. Therefore, salinity issue should be considered seriously (Raziei *et al*., 2009). Drought and salinity stress are significant abiotic stresses having harmful effects on yield quality and plant performance (Coskun *et al.*, 2016). A higher level of salt stress inhibits the germination of seeds, while the lower level of salinity induces a state of dormancy (Ibrahim, 2016). Indeed, seeds subjected to salinity show variations in germination, some fail to germinate, while others tolerate salinity even at high concentrations (Ashraf & Foolad, 2005). Plants tolerance is different in respect to salinity and drought; therefore, the selection of plants for cultivation in saline or dry lands should be assessed thoroughly (tolerance threshold to stress, resistance development stage and so on) (Khan & Gulzar, 2003). Although these stresses can have adverse effects on all plant development stages, they can strongly influence seedling stage (Rauf *et al.,* 2007). Plants have different temperature and humidity requirements, and understanding these needs can contribute to their survival and reproduction. Plants have three specific temperatures for germination, including basic or minimum, optimum and maximum. The speed of germination is zero at a lower temperature than a primary temperature and a higher temperature than maximum. However, the optimum temperature has the highest rate of germination (Bidgoly *et al.*, 2018). In addition to temperature, awareness of germination and establishment tolerance threshold to salinity and drought can help to detect suitable places for plants cultivation.

Based on the assumption that different temperatures cause a change in the percentage and rate of *Moringa peregrine* germination, the present study aimed to evaluate moisture-temperature response and the possibility of *Moringa peregrina* adaptability prediction under different temperature and humidity conditions that may occur in the near future. Investigation of *Moringa peregrina* resistance to salinity and drought stress and determination of optimum germination temperature can help to develop its cultivation and operation.

Figure 1. *Moringa peregrina* a.*tree and b. seed*

2. Material and Method

An experiment was conducted to investigate seed germination properties of *Moringa peregrina* in 2017 in the seed laboratory of the Agriculture College of the University of Tehran. Evaluation of germination reaction was performed at constant temperatures, including 5, 10, 15, 20, 25, 30, 35, 40, 45°C under a photoperiod of 12-hour light and 12-hour dark in the incubator. The experiment was conducted in the framework of complete randomized design with five repetitions. Twenty seeds were selected for each replication. The seeds were placed in petri dishes (9-cm diameter), including wet Watman filter paper and then transferred to desired constant temperatures. Petri dishes were kept moist enough to preserve moisture and appropriate heat exchange during the test period. Counting of germinated seeds was done daily, 24 hours after starting the experiment, and germinated seeds (root length is 1-2 mm) were recorded (Adam *et al.,* 2007 & Brindle *et al*., 2005). Seed counting continued until germination was finished or reached a constant level. Seed germination speed, and the percentage was counted in per temperatures. Mean germination time was obtained using Equation 1 (Schelin et al., 2003):

$$\mathrm{MGT} = \frac{\sum (f_x x)}{\sum f_x} \qquad (1)$$

fx is the number of germinated seeds in day X, and X is seed counting day. To calculate germination speed and percentage, the program Germin was used (Soltani & Maddah, 2010). Using this program, $D_{10,}$ D_{50} and D_{90} (time that takes for germination to reach its maximum of 10%, 50% and 90%, respectively) were computed. In this program, desired parameters are calculated for each repetition and treatment by interpolating increase of germination curve versus time. Germination speed was obtained using Equation 2 (Saha *et al*., 2008; Soltani *et al*., 2002).

$$\mathrm{R}_{50} = \frac{1}{D_{50}} \qquad (2)$$

D_{50} is the time required to reach 50% germination, and R_{50} is germination speed (Torabi *et al*., 2016). The base, lower optimum, upper optimum and ceiling temperature are called cardinal temperatures (Soltani & Sinclair 2012). Cardinal temperatures were determined using regression models between germination speed and different temperatures. Different temperatures were considered the independent variable, and germination speed was considered a dependent variable. The relationship between temperature and germination speed was determined using regression analysis, and related charts were drawn.

In this study, three functions, including beta, segmented and dent-like, were used to determine the specific temperature of *Moringa peregrina* (Bidgoly *et al.*, 2018; Hardegree, 2006; Jame & Cutforth, 2004., Fang *et al.*, 2012).

Segmented function

$$f(T) = (T - Tb).(To - Tb) \text{ if } Tb < T \leq To$$
$$f(T) = (Tc - T).(Tc - To) \text{ if } to < T < Tc \qquad (3)$$
$$f(T) \text{ if } T \leq Tb \text{ or } T \geq Tc$$

Beta function

$$f(T) = \left\{ \left[\left(\frac{(T - Tb)}{(To - Tb)}\right)\left(\frac{(Tc - T)}{(Tc - To)}\right) \right]^{\left((Tc - To)/(To - Tb)\right)} \right\}^{a} \quad if\ T \leq Tb\ or\ T \geq Tc \qquad (4)$$
$$f(T) = 0 \qquad \text{if } T \leq Tb \text{ or } T \geq Tc$$

Dent-like function

$$f(T) = (T - Tb).(To1 - Tb) \text{ if } Tb < T < To1$$
$$f(T) = (Tc - T).(Tc - To2) \quad \text{if } To2 < T < Tc \qquad (5)$$
$$f(T) = 1 \text{ if } To1 < T < To2$$
$$f(T) = 0 \text{ if } T \leq Tb \text{ or } T \geq Tc$$

In these functions, T is temperature (°C), T_b, T_O, T_C, T_{O1}, T_{O2} and a are basic, optimum, maximum, low optimum, and high optimum temperatures, and the shape parameter for a beta model determining the curvature of the functions, respectively. Specific temperature of germination was calculated using regression analysis, proposed models, and germination speed. Determination of a particular temperature based on the relationship between germination speed and temperature is a standard method in studies concerned with the determination of a specific germination temperature (Bradford, 2002; Colbach *et al.*, 2002). Sigma plot version 12 software was used to fit the model using regression methods.

Another experiment was conducted to investigate germination response and seedling growth of *Moringa peregrina* toward different levels of drought and salinity stress at optimum temperature. In this test, seeds germination was assessed at four levels of salinity and drought with the osmotic potential of 0, -4, -8, and -12. Distilled water was used to create zero stress level (control). Additionally, sodium chloride was used to provide saline solutions with different potential levels. The amount of required sodium chloride was estimated using Van's Hoff equation (Hashemi *et al.*, 2016).

$$\text{Osmotic pressure} = n*(C.M)*RT \qquad (6)$$

n is ionization coefficient, a number of released particles in the solution, which is 2 in sodium chloride, C is concentration (grams per liter), M is the molecular weight of matter, C.M is molar concentration, R is gas constant (0.0833), T is the absolute temperature of the environment based on the Kelvin temperature.

In addition, to create drought stress, desired potential levels were created using Michel and Kaufman's instructions using polyethylene glycol 6000 (1973). The studies have shown that this material is suitable to simulate drought stress conditions.

3. Results and Discussion

According to the results, the effect of temperature on germination speed and the percentage was significant ($p \leq 0.01$) (Table 1). Comparison of the average of germination percentage in different temperature treatments showed that the seeds could not germinate up to 15 °C temperature. As temperature increased to 20 °C, the seeds had germination of 64%. The seeds had proper germination as temperature increased. The seeds had the highest germination equal to 86% at 25 °C. Germination percentage had an adverse reaction to temperatures higher than 25 °C, and we observed a descending trend of germination percentage. The effect of temperature rise was clearly evident at 45 °C, and just 12% of germination was obtained. Furthermore, the highest speed of germination was achieved at 25 °C and 35 °C, and after that, there was a descending trend (Table 2).

Table1: Analysis of variance mean square of germination characteristics under different temperatures

Source	df	Germination percentage	F value	Pr > F	Germination speed	F value	Pr > F
Temperature	8	5543.88**	34.06	< .0001	0.000067**	189.16	< .0001
Error	36	162.77	-	-	0.0000003	-	-
Total	44	-	-	-	-	-	-

** Significant at 1% level

Table 2: Comparison of mean germination speed (number.day) and final germination (percentage)

Temperature	Germination percentage	Germination speed
5	d0	f0
10	d0	f0
15	d0	f0
20	64[ab]	0.005[c]
25	86[a]	0.0085[a]
30	64[ab]	0.0085[a]
35	54[bc]	0.0071[b]
40	40[c]	0.0037[d]
45	12[d]	0.0012[e]

The means of the same alphabet in each column have no significant difference at 5% probability level based on the Duncan test.

Table 3 shows specific germination temperatures of *Moringa peregrina* based on different models. Based on the calculated models, the basic germination temperatures of *Moringa peregrina* in beta, dent-like and segmented models were 15, 16 and 17 °C, respectively. Optimum germination temperatures were equal to 30, 25-30 and 26.6 °C. In addition, maximum germination temperatures of *Moringa peregrina* in different models were 46, 47 and 50 °C. The calculated statistic for the used models includes R^2 and RMSE, which are criteria to measure accuracy of the models. Higher amount of R^2 and lower amount of RMSE show more correlation between model and reality. According to Table 3, RMSE amount of all three models was low. However, among these provided models, the amount of R^2 in the dent-like model was higher than that of other models (it was equal to 93%). Therefore, the dent-like model had more accuracy to determine specific germination temperatures of *Moringa peregrina* (Table 3). The relations obtained from the calculated regression models were also estimated, and their graphs were drawn (Figure 2).

Table 3: Estimated amount of specific germination temperatures based on fitted models

Cardinal temperatures	Beta model	Dent-like model	Segmented model
Basic	15	17	16
Optimum	30	25-30	26.6
Maximum	46	47	50
RMSE1	0.0033%	0.0012%	0.0018%
R^2	0.77	0.93	0.83

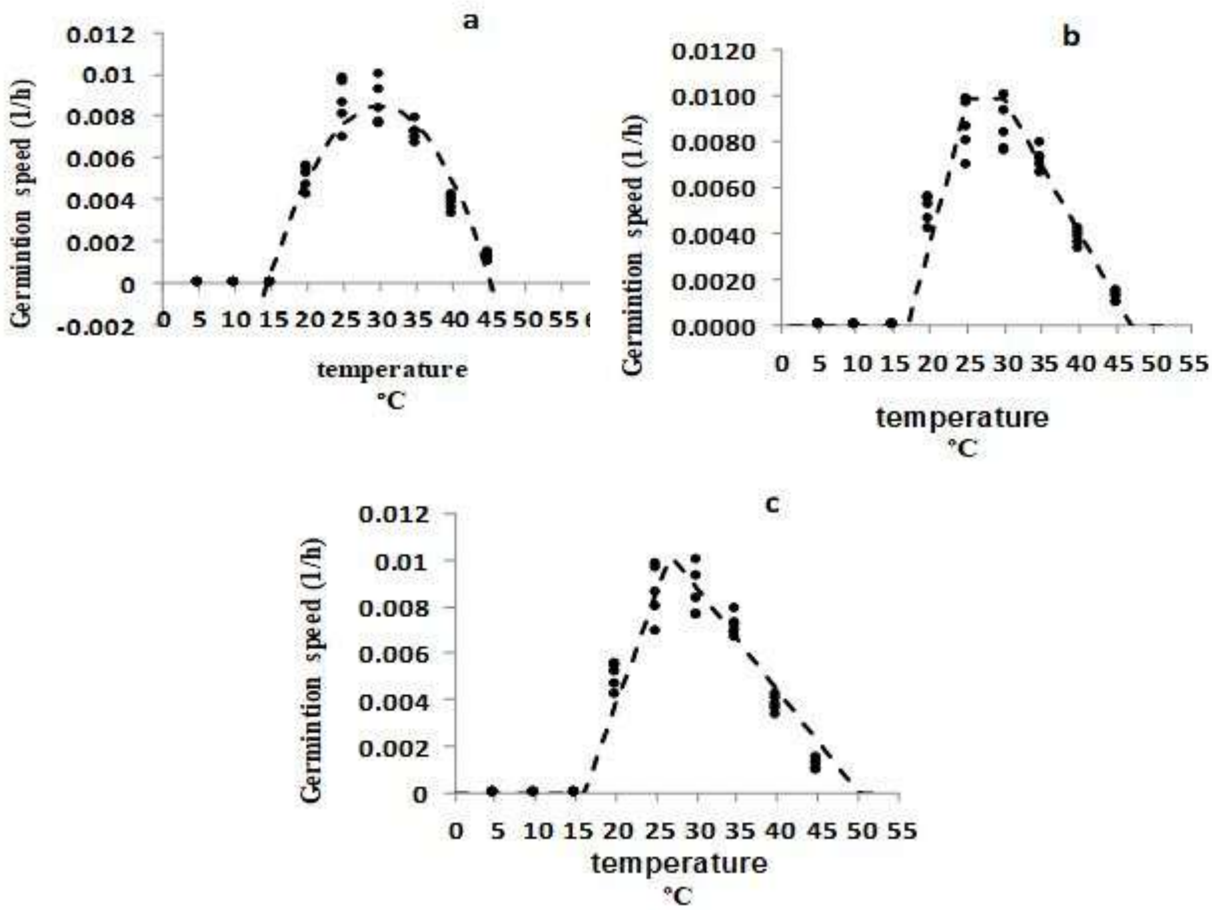

Figure 2: Relationship between germination speed and temperature based on beta (a), dent-like (b) and segmented (c) models.

After determining 25 °C as the optimum temperature of *Moringa peregrina* at different levels, drought and salinity stress were assessed at this temperature. The results of variance analysis of drought and salinity stress showed that the effect of humidity potential on germination speed and the percentage was significant (Table 4).

Table 4: Analysis of variance mean square of germination characteristics under drought and salinity stress

References	df	Germination percentage	F value	Pr > F	Germination speed	F value	Pr > F
Salinity	5	2734**	6.98	.0004	0.000025**	8.37	.0001
Drought	5	6819.33**	34.1	<.0001	0.000072**	7.08	.0003
Error	24	162.77	-	-	0.0000003	-	-
Total	29	-	-	-	-		
The coefficient of the variance of salinity	-	18	-	-	15	-	-
The coefficient of the variance of drought	-	19	-	-	21	-	-

** Significant at 1% level

The germination percentage of *Moringa peregrina* decreased with the increase of drought and salinity stress, but the results showed that the seed germination of this plant was more sensitive to drought stress (Figure 3). The moisture potential of -2 had no significant difference with the control in both drought and salinity stresses, but germination percentage had a significant negative trend with the increase of pressure. In the potential of -4 for salinity and drought, seed germination percentage was 74% and 54%, respectively. With addition of drought stress to -6, germination decreased to 22%, but seed germination percentage was 54% for the same amount of salinity stress. In the potential of -8 for drought, a significant decrease was observed in germination percentage, and only 2% of seeds could germinate. However, the seeds of this plant had excellent resistance to salinity stress, and in the potential of -10, their germination percentage was 22%.

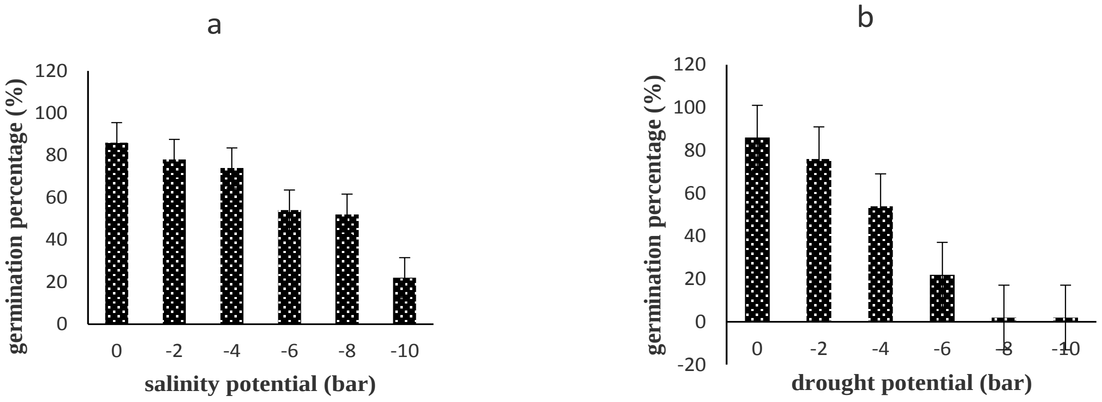

Figure 3: Comparison of germination percentage of Moringa peregrina under different levels of salinity and drought

The same trend was observed regarding germination speed (Figure 4). Germination speed decreased with the increase of both drought and salinity stress. However, this decline was more in drought stress and seeds germinated later in drought stress.

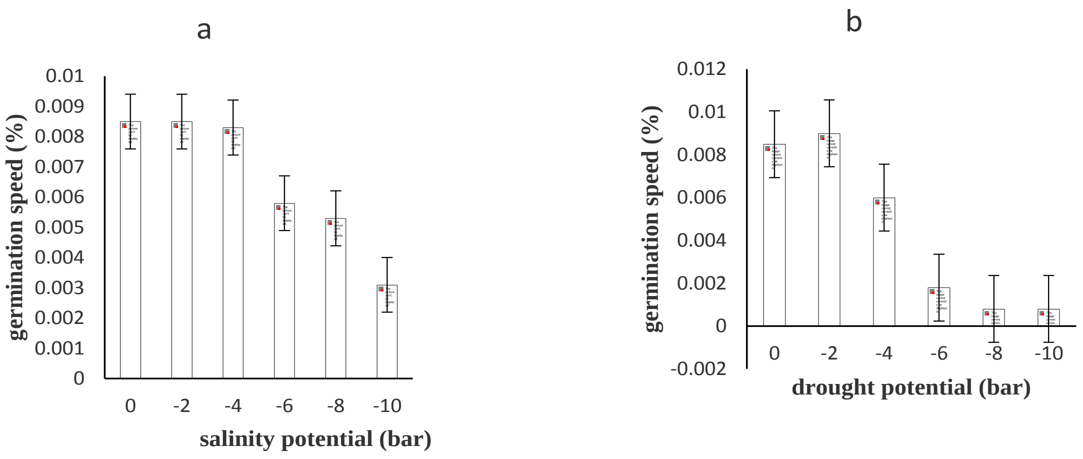

Figure 4: Comparison of germination speed of Moringa peregrina under different levels of salinity and drought

4. Conclusion

In this research, the cardinal germination temperatures of *Moringa peregrina* were calculated using germination speed. Studies have indicated that germination speed is more sensitive to temperature compared to germination percentage so that it increases until temperature reaches the optimum level and then starts to decrease. Therefore, researchers use germination speed, the more critical factor for seedling establishment and proper plant density, to determine specific germination temperatures (Hardegree, 2006).

Several studies have been conducted to evaluate the effect of temperature on the germination properties of different seeds, including the study on *Plantago psyllium*, a medicinal plant. The results of that study showed that the highest germination speed was at the temperature of 25 °C, and germination speed decreased at higher temperatures (Hashemi *et al.*, 2016).

In this experiment, three different models were used to determine cardinal germination temperatures. There are several studies into determination of specific germination temperatures using these models. Jame and Cutforth (2004) used the beta model to assess the particular germination temperature of wheat. Their results showed that basic, optimum and maximum temperatures of seeds were 0, 30 and 42 °C, respectively.

In this research, an increase of drought and salinity stress caused a decrease of germination speed and percentage, however, the effect of drought stress was more than that of salinity stress. The results obtained in this study corresponded to those obtained by other researchers. Karavani *et al.* (2014) reported a decrease of all seed germination properties of *Scrophularia straita* due to salinity and drought stress. They stated that the seeds of this species had more resistance to salinity stress compared to drought stress, and germination indices were less influenced in salinity stress (Karavani *et al.*, 2014). In some studies, germination decline affected by drought stress is attributed to the decrease of cell moisture and its effect on hormones secretion and proteins making. In general, germination speed and percentage were reduced due to cell water potential reduction. In fact, drought stress subsides seeds germination due to water absorption limitation by seeds, impact on seeds reserves movement and transfer during germination or direct effect on the organic structure and protein synthesis (Dodd & Donovan, 1999).

Salinity in the plant bed can hurt germinating seeds in two main ways, namely decrease of water potential in soil due to the soluble salts and the effect of salt poisoning on cells (Garg, 2010). Similar results reported the effect of salinity stress on seeds germination in other plants. In an experiment with four levels of salinity, the results indicated that salinity had a significant impact on germination percentage and uniformity, and the averages of germination percentage in salinity potential of 25 and 50 mm were reduced 17 and 43%, respectively, compared to the control sample (Sharifi, 2007).

Some researchers maintain that salinity stress affects seeds germination by an increase of osmotic pressure, a decrease of water absorption by seeds and the toxic effects of Na and Cl (Zeinali *et al.*, 2002). Reduction of germination speed and percentage is related to water absorption decline by seed during turgor (Bybordi & Tabatabaei, 2009). The increase of salinity levels reduces germination due to the effect on cell division and plant metabolism. It was also determined that the impact of sodium chloride on germination of *Helianthus annuus L*. was dependent on the absorption of chlorine and sodium ions (Turhan & Ayaz, 2004).

The results of this study showed that seeds germination of *Moringa peregrina* was sensitive to low temperatures so that the seeds were not able to germinate up to 15°C. Evaluation of the used models showed that the dent-like model was more appropriate to determine the specific germination temperature of *Moringa peregrina* and based on this model, basic, optimum and maximum temperatures of germination were 17, 25-30 and 47 °C, respectively. Furthermore, the results showed that drought and salinity stress reduced germination speed and percentage, but in general, seeds germination was more sensitive to drought stress compared to salinity stress. However, the plant had excellent resistance to moisture stresses. This feature with the ability of germination at a high temperature can be a valuable factor to cultivate *Moringa peregrina* in warms and dry areas.

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