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Dynamics of Seed Survival and Mortality in *Salvadora persica* L. under Different Temperature Regimes

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ABSTRACT

Germination data can be evaluated using three methods namely: Germination indexes, traditional regression techniques to fit nonlinear asymptotic functions to the temporal sequence of cumulative germination and time-to- event analysis, also called as reliability or survival analysis. All the three methods are currently in use to analyse the germination data. However, nonlinear regression and time-to-event analysis methods are much more popular as they can accommodate temporal dynamics of seed germination. As nature of germination data is often different from other biological data, so classical nonlinear regression techniques may offer some limitations, particularly when ungerminated seeds are present at the end of an experiment. In spite of this, nonlinear regression models are actively applied to seed germination data to obtain survival and mortality curves. Moreover, relatively little literature is available about the application of time-to-event analysis on germination data but this statistical tool works well when some ungerminated seeds are present at the end of an experiment.

This article analyses seed germination data kept under different temperatures by applying Richards (4 parametric) and Weibull (3 parametric) models. This is a temporal analysis and cumulative germination data at any particular point can be evaluated and compared. As seed germination process imitates a sigmoid curve hence the above asymptotic models can be effectively utilized to model germination data. The above two models explained that at 40°C, cumulative germination was severely reduced to less than 50%. But at 27°C and 35°C, cumulative germination percentage was relatively good (above 80%). Moreover, survival analysis demonstrated similar trends. At 40°C, survivor curve displayed a sharp decrease suggesting that faster the survival function decrease over time, higher the hazard.

Keywords Regression, Nonlinear, Time-to-event, Sigmoid curve, Survival analysis.

INTRODUCTION

Seed germination is one of the most crucial physiological phases that allows plant to get establish in a particular environment. Germination experiments are conducted in different fields of biological sciences. Many of these experiments involves determining the

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germination percentage of seeds germinated after specified time intervals by repeated observations and/or the calculations of the germination rates. A variety of methods for analysing the resulting data have been reviewed and proposed which broadly comes under two categories: Germination indexes (Ranal and de Santana 2014) and classical regression techniques to fit nonlinear and asymptotic observed data to the temporal sequence of cumulative germination (Romano *et al*. 2018). There is ample amount of literature available at the moment about all these techniques and many experiments have been performed on several plant species.

Another class of methods to analyse germination data, still not largely used is given by time- to- event analysis, also referred to as reliability or survival analysis (Cox and Oakes 1984). These methods have several utilities, some of them are: To predict reliability in engineering and devices (Wu *et al*. 2011), to evaluate the failure time of machine components in industrial processes (Burhanuddin *et al*. 2014), to analyse time to death or recovery of patients in clinical trials (Austin 2013), in physiological experiments (Keiley and Martin 2005). Survival analysis is also used for forest resource evaluation (Mogensen *et al*. 2014) and forest fire damage assessment (Tremblay *et al*. 2018). In recent years, it has been applied on germination data too viz., *Triticum aestivum* L. (Gunjaca and Sarcevie 2000), *Fallopia japonica* (Houtt.) RD (Mcnair *et al*. 2012) and *Beta vulgaris* L. (Romano and Stevanato 2020). The method describes a binary response variable where data are collected as cumulative counts over time, and consists of modelling the time to response for each individual in the sample. With regard to germination studies, data can be collected as the germination time of each seed, as the number of seeds germinating in each time interval, or the cumulative number of seeds which have germinated by the observation time. Time- to-event analysis can be approached in three different ways: Non-parametric, semi- parametric and fully parametric. Here, non- parametric method is used, for the simple reason that non- parametric methods make no assumptions about an underlying probability distribution. That is how the event of germination changes over time, based on the probability of seed development. Applying non- parametric methods results in estimation and plotting of both survival and hazard curves. Germination is one of the most critical - phase in the life cycle of a plant, particularly in the arid region where seeds have to face several stressful conditions as high temperatures and light intensities, water and salinity stress to name a few. Modelling seed germination data probes into adaptation strategies made by plants in germination phase. A series of experiments was therefore performed on seed germination as influenced by different temperatures, of the habitats where *Salvadora persica* L. grows in nature. The experiment focussed on modelling the germination data over time, applying nonlinear and asymptotic mathematical functions. Moreover, survival analysis was also conducted to evaluate at what rate survival probabilities decrease with time when seeds are kept at high temperatures. The modelling results are being described and interpreted in the present manuscript.

MATERIALS AND METHODS

Fresh seeds of *Salvadora persica* L. were procured from Central Arid Zone Research Institute (CAZRI), Jodhpur, Rajasthan in the month of June, 2022 and stored in glass stopper bottles under laboratory conditions. The experiment started on July 1th 2022 at Ecophysiology Laboratory, Department of Functional Plant Biology, Kumaon University, Almora. Prior to experiment set, technique to increase the germination rate (vigour) was applied which involved de-pulping of seeds by soaking seeds in distilled water for 2-4 hrs and eventually when seeds swelled, the pulp was removed mechanically and seeds were then allowed to germinate. By removing fruit pulp covering, germination rate of seeds improved drastically.

For germination trials fresh, large and uniform seeds were placed in glass petri dishes on two layers of Whatman No. 1 filter paper with 10 ml distilled water. Petri dishes were sealed with parafilm to avoid water losses and distilled water was added as and when required. Each petri dish contained 30 seeds and the treatment was replicated thrice. Seeds with visible radicle were considered to be germinated. Germination count was made daily for a period of two weeks. All the germinated seeds were removed over time in a step-wise manner and removal date was

Table 1. Growth models and their functional forms.

Growth model	Growth function	Parameters	Source
Richards (k- form)	y(t) = α/ [1+νe^-k (t -Ti)] ^1/ν	4	Richards (1959)
Richard's (Tλ- form)	Y(t)= α/[1+νe(1+ν) e{-μi/α*(1+ν) ^(1+1/ν) (t-Tλ) ^1/ν}]	4	Zwietering *et al.* (1990)
Weibull	y(t) = α(1-e^-at^b)	3	Weibull (1951)

registered. All the counts were then converted into percentage germination for data modelling. Similarly, percentage of seeds which were not germinated was also extracted from the cumulative germination data of seeds. For setting different temperature regimes, incubators were used. Seeds were kept at 27°C (control) and 35°C (experimental 1) and were daily observed for two weeks. Light intensity was kept at 16 hrs photoperiod which was provided by cool white fluorescent light (3000 lux).

Accelerated ageing procedure is one of the most frequently used tests to evaluate the physiological potential of seeds. For this, twenty-six (26) fresh seeds (with three replicates) were placed uniformly in petri dishes containing two layer of Whatman No. 1 filter paper with an addition of 10 ml of distilled water. Seeds were then kept at a constant temperature of 40°C for two weeks and germination count was made on daily basis which were later converted to cumulative germination percentage values. A relative humidity of above 80% was maintained throughout the germination trials. After each period of exposure to the accelerated ageing test, germination was determined.

Two nonlinear and asymptotic models viz., Richard's (Richards 1959) and Weibull (Weibull 1951) were applied to the three datasets kept at different temperatures. Re-parameterization (Zwietering *et al.* 1990) of Richard's model was also carried out in order to calculate maximum Absolute Growth Rate (μ_i) and lag time (T_λ) i.e., time required to start the process of germination. With the help of AGR_{max} and upper asymptotic values (α), RGR_{max} was also calculated for Richards model (T_λ- form). Moreover, time at which 50% seeds germination took place (T_{50}: α/2) was also calculated. The functional form applied was: T_{50}= kT_i-log[2^ν-1/ν]. The observed germination data was modelled against time to generate expected values of percentage cumulative germination of seeds that resembled a sigmoid curve. Further, the germination data of percentage of seeds not germinated was also modelled against time which took the shape of a reverse sigmoidal curve. The functional form of the two models and the biological interpretation of model parameters is provided in Tables 1 — 2. Statistical analysis was all implemented in Microsoft Excel, 2021 using Real Statistics Resource Pack.

Nonlinear curve fitting was performed by excel solver. For evaluating model performance, Adjusted R^2 (Chenge 2021), Residual Standard Error (Chenge 2021) and Bayesian Information Criterion (Corral- Rivas *et al*. 2014) values for each model were calculated. Model with least RSE and BIC and maximum adjusted- R^2 was considered to perform the best. Statistical significance of model parameters was evaluated using Jack knife resampling technique (Harris 1998).

Survival analysis on germination data were conducted using Kaplan- Meier survivor function (Kaplan and Meier 1958). This is an add- inn function which is present in Microsoft Excel, 2021 under Real

Table 2. Biological interpretation of the symbols used in functions.

Symbols	Biological interpretation
y(t)	Expected percentage cumulative germination at time "t"
α	Upper asymptote of y
ν	An additional parameter in Richard's equation introduced As a power law so that it can define asymmetric curves, Shape parameter, dimensionless scalar
k	Constant that determines curvature of growth pattern
μi	Maximum absolute growth rate; maximum RGR is μi/α
Tλ	Lag time i.e., time required to start the process of germination
t	A variable representing time
Ti	Inflection point at which growth rate reaches its maximum value
T50	Time at which 50% of seed germination is completed
a and b	Empirical constants defining shape of response
e	Exponential (a universal constant)

Statistics Resource Pack. As KM- survival analysis only accepts germination response in binary form, so all seeds were coded as "1" for those not germinated and "0" for all those that germinated by the end of the experiment. For comparing survival and hazard curves, seed germination at 27°C (control) and at 40°C (experimental 2) was considered. Hazard function [H (t)] was calculated applying the formula H (t) = -LN[S(t)], where S(t) is the survival function.

RESULTS AND DISCUSSION

Seed germination data of *Salvadora persica* L. was analyzed using nonlinear regression models. The dynamics of germination as a function of number of seeds germinated in a given unit of time can be described by a sigmoid function whose absolute growth rate is a bell- shaped curve. Two model functions viz., Richard's and Weibull models were applied to cumulative germination data. Nonlinear and asymptotic models were applied as seed germination data imitated an S- shape curve. Seeds were germinated under three different temperatures viz., 27°C (control), 35°C (experimental 1) and 40°C (experimental 2) and cumulative germination percentage was noted for a period of two weeks. Model fitting and evaluation statistics of cumulative germination is depicted in Table 3.

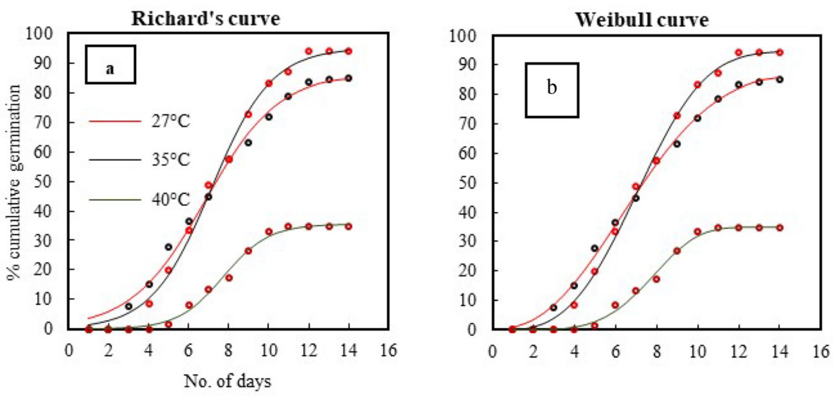
For Richard's model, the upper asymptotic value i.e., maximum or expected cumulative germination at the end of 14th day was 86.31% (27°C), 95.39% (35°C) and 35.65% (40°C). Similarly, for Weibull model the upper asymptotic value came out to be 87.58% (27°C), 94.90% (35°C) and 34.98% (40°C). The fitting and evaluation statistics of Weibull model was slightly better than that of Richard's model perhaps due to the fact that a 3- parametric Weibull function do not need a time constant as it has zero value at time zero which makes the function more flexible with respect to others.

According to Richard's model (Figs. 1a), the cumulative germination percentage in the middle of the germination process was 45.5% (27°C), 45.60% (35°C) and 12.16% (40°C) respectively. Thus, it was observed that at 40°C, the cumulative germination percentage on 7th day was less than 15%.

Reparameterization of Richard's model to T_λ - form demonstrated that lag time was highest (7.415 days) at 40°C. For other two treatments lag time was 2.273 (35°C) and 1.915 (27°C) days respectively which suggested that at constant high temperatures

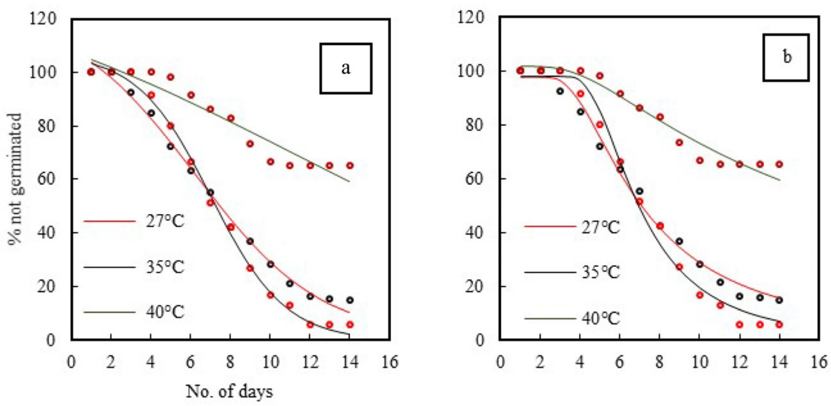
Table 3. Model fitting and evaluation statistics of percentage cumulative germination (* Model evaluation for T_λ – form is similar to that of k- form; model parameter is represented as mean values and ± is the standard error).

Model	Model parameters	27°C	35°C	40°C
Richards (k- form)	α	86.307±0.098	95.389±0.110	35.652±0.062
	ν	2.124±0.006	2.174±0.006	2.275±0.008
	k	1.171±0.003	1.462±0.004	1.993±0.007
	Ti	5.425±0.013	5.976±0.011	6.795±0.010
	Adj. R^2	0.990	0.993	0.990
	RSE	2.60313	2.56132	1.24124
	BIC	42.05487	41.60152	21.31787
Richard's (Tλ- form)	α	86.307±0.098	95.389±0.110	35.547±0.050
	ν	1.372±0.006	1.721±0.005	0.346±0.001
	µi	14.666±0.108	22.708±0.139	3.460±0.016
	Tλ	1.915±0.008	2.273±0.009	7.415±0.018
Weibull	α	87.581±0.120	94.897±0.071	34.975±0.023
	a	0.007±0.0001	0.001±0.000026	0.000036±0.0000025
	b	2.387±0.008	3.171±0.009	4.847±0.038
	Adj. R^2	0.996	0.997	0.994
	RSE	1.55161	1.85349	1.0475
	BIC	23.59361	28.57144	12.59290



Figs. 1. Dynamics of seed germination fitted to (a) 4-parametric Richard's and (b) 3- parametric Weibull curves under different temperatures (* circles are observed values).

the germination was delayed with a rapid seed deterioration and loss of vigour. Moreover, both AGR_{max} (3.460) and RGR_{max} (0.096) values were found to be lowest at 40°C confirming that prolonged high temperature influences both absolute and relative growth rates of seed germination. The T_{50} (α/2) values followed the order: 12.91 > 8.29 > 5.84 which meant that at 40°C, it took about 12.91 days for 50% of seeds to get germinated. This was followed by 8.29 days (35°C) and 5.84 days (27°C) respectively, for the other two treatments. For Weibull model (Figs. 1b), percentage cumulative germination of seeds in the middle of the process was 46.21% (27°C), 45.99% (35°C) and 12.23% (40°C). Towards the end, the cumulative germination was 85.85% (27°C), 94.66% (35°C) and 34.98% (40°C) respectively. Accelerated ageing (AA) test exposes seeds to two environmental variables viz., high temperatures and high humidity which causes rapid seed deterioration. High vigour seed lots with stand these stressful conditions and deteriorates at a slower rate having high germination following ageing compared to low vigor seed lot. Constant high temperatures (40°C and above) and high humidity (80% and above) accelerated the process of seed ageing, which led to rapid loss of seed vigour and significant reduction in germination. As per the models tested, it was quite clear that *Salvadora persica* seeds were capable to tolerate a temperature



Figs. 2. (a) Richards and (b) Weibull regression curves applied to seed germination data of *Salvadora persica* L. under different temperatures (* circles are observed values).

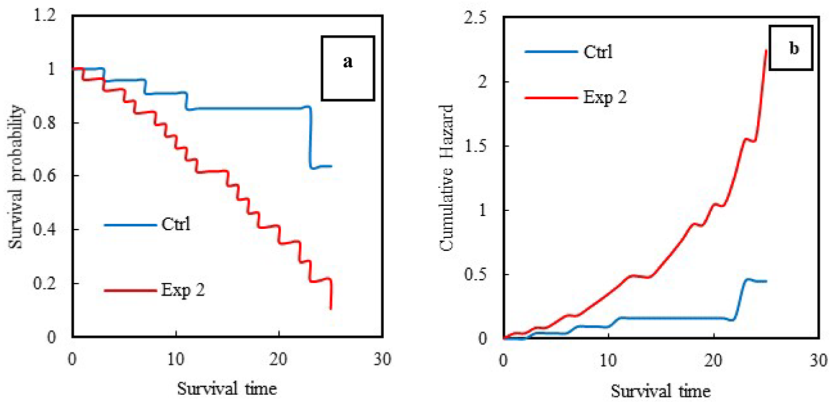
of 35°C with over 90% germination at the end of 14th day while at 40°C (AA) cumulative germination was significantly reduced.

Mortality curve of seeds applying Richard's model suggested that at 40°C, approximately 60% of seeds were unable to germinate at the end of 14 days. At 27°C, the percentage of seeds that remained ungerminated during the same period was 10.17%, whereas at 35°C the value of not germinated seeds were 2.46%. Weibull model also demonstrated similar trends but percentage of ungerminated seeds towards the end was higher than that of Richard's model. At 27°C, about 15.45% seeds remained ungerminated on 14th day and about 7.21% seeds remained ungerminated at 35°C on the same day. However, the percentage of ungerminated seeds towards the ending phase was more or less similar at 40°C in both models and the value was close to 60% (Figs. 2 a - b).

The Kaplan- Meier (KM) survivorship curve is depicted in Figs. 3 (a). The non- continuous nature of KM curves highlighted the fact that they are not smooth functions, but rather stepwise estimates. The value of S(t) is constant between times of germination events, and the estimate probability changes value only at time of each event. The vertical distance between horizontals represented the change in cumulative probability of germinating seeds as the curve advanced.

The steepness of the curve is determined by how long the survival lasts and is represented by length of horizontal lines. For control treatment (27°C), the cumulative incidence or cumulative germination probability at 13th day was 0.85 (85%), conversely, at 40°C, the cumulative incidence at the same time was 0.62 (62%). Similarly, survival probability at 27°C on 25th day was 0.64 (64%) whereas at 40°C, the survival probability at the same time was merely 0.11 (11%). In the survival investigation, germination data is described and modelled in terms of two related probabilities which are survival and hazard functions, both depended on time. The survival function [S(t)], is defined as the probability of surviving at least to time t. The Hazard function [H(t)], is the conditioned probability which stated that the investigated event will happen at the same time "t" having survived to that time (Klein and Moeschberger 2003).

The hazard function is mathematically related to survival function, the faster the survival function decreases over time, the higher the hazard. In other words, higher values of H(t) suggested higher risk of seed germination. Figs. 3 (a) clearly demonstrated that at 40°C, survival probability of seeds decreased at a faster rate (red curve) over time as compared to survival probability of seeds germinated at 27°C (blue curve). Moreover, high values of H(t) at 40°C (Figs. 3b) also revealed that seeds are at a high risk of germination. The present manuscript thus deals with evaluating dynamics of seed germination in *Salvadora persica* L. under different temperatures. Results, demonstrated that at 27°C and 35°C the



Figs. 3. (a) Survivor and (b) Cumulative hazard curves at different temperatures.

germination was over 80% but at 40°C, germination was significantly reduced.

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