Agrociencia

OZONE AND AQUA REGIA SEED TREATMENTS ENHANCE GERMINATION IN TOMATO (*Solanum lycopersicum* L.) AND ROSELLE (*Hibiscus sabdariffa* L.)

Carlos Arturo **Guzmán-Nuñez**[1], Antonia **Macedo-Cruz**[1], Sergio Humberto **Chávez-Franco**[2], Guillermo **Carrillo-Castañeda**[3], Axel Eduardo **Rico-Sánchez**[1*]

[1]Colegio de Postgraduados Campus Montecillo. Posgrado de Hidrociencias. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. C. P. 56264.
[2]Colegio de Postgraduados Campus Montecillo. Posgrado de Fruticultura. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. C. P. 56264.
[3]Colegio de Postgraduados Campus Montecillo. Posgrado de Recursos Genéticos y Productividad-Genética. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. C. P. 56264.
* Author for correspondence: rico.axel@colpos.

Citation: Guzmán-Nuñez CA, Macedo-Cruz A, Chávez-Franco SH, Carrillo-Castañeda G, Rico-Sánchez AE. 2025. Ozone and aqua regia seed treatments enhance germination in tomato (*Solanum lycopersicum* L.) and roselle (*Hibiscus sabdariffa* L.). **Agrociencia.** https://doi.org/ 10.47163/agrociencia.v59i3.3233

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: May 20, 2024.
Approved: April 01, 2025.
Published in Agrociencia: April 28, 2025.

ABSTRACT

Global food demand has surged, necessitating innovative agricultural practices to mitigate environmental impacts and ensure food security. This study investigates the effects of pre-sowing treatments, including ozonated water and aqua regia, on the germination of tomato (*Solanum lycopersicum* L.) and roselle (*Hibiscus sabdariffa* L.) seeds. A randomized complete block design was used, comparing treatments to a deionized water control. Seeds were subjected to one-minute exposures, and germination metrics were assessed over 10 days. Results indicated that the ozone treatment significantly enhanced tomato seed germination rates compared to the control and aqua regia (Kruskal-Wallis, $p \leq 0.01$). While statistical significance was not observed for all treatments in roselle, a trend towards improved germination with ozonated water and aqua regia was noted. Germination indices, including germination percentage, rate, and synchrony, were analyzed using principal component analysis (PCA), revealing distinct treatment effects. PCA highlighted that the ozone treatment promoted rapid and enhanced germination in tomato, correlating with metrics such as emergence rate and peak germination percentage. Aqua regia demonstrated efficacy in roselle, likely due to scarification of the seed coat. The study suggests that optimized ozone treatments can serve as a biostimulant, enhancing germination velocity, while aqua regia effectively improves germination in species with hard seed coats. These findings contribute to understanding the potential applications of pre-sowing treatments for improving crop production and sustainability.

Keywords: Solanceae, Malvaceae, crop yield, germination, priming, hormesis.

INTRODUCTION

Population growth in the past century has significantly increased global food demand, challenging the development of novel agricultural practices. This rapid has

had profound consequences, including environmental pollution, the encroachment of agriculture onto natural lands, and the exacerbation of greenhouse emissions, among other unsustainable practices (Koop and van Leeuwen, 2017). In response to recent food crises, there has been a notable surge in the importation of food products, particularly in Mexico, primarily to address the region's escalating food demand (Valencia-Romero, 2019).

To mitigate the current set of food security challenges, a concerted effort is required to develop and implement new technologies and innovative agricultural methods. A critical aspect of this endeavor lies in a thorough understanding of plant development to optimize crop yields and enhance food production (Tester and Langridge, 2010; Tolhurst and Ker, 2015). Plant domestication has been instrumental in enhancing food crop production through selective breeding for desirable physiological and morphological traits. This process involves improving plant characteristics, such as seed number and size, while addressing issues like seed shattering, dormancy, flowering time, and stress tolerance. By optimizing these traits, crop yields can be significantly increased (Gross and Olsen, 2010; Meyer and Purugganan, 2013).

Seed germination is a critical stage in plant development, influencing subsequent growth and yield (Ashraf and Foolad, 2005). It involves three distinct phases: imbibition, metabolic activity, and radicle emergence (Bradford, 2017). Seed priming is a pre-sowing technique that can enhance germination and improve crop performance under various stress conditions, such as salinity, temperature extremes, and drought (Ashraf and Foolad, 2005; Draganić and Lekić, 2012; Kaya *et al*., 2006; Shahrokhi *et al*., 2011). Priming methods, including hydropriming, osmopriming, and chemical priming, can improve seed vigor and tolerance to environmental stresses. These techniques are cost-effective, low-risk, and readily applicable in various agricultural settings (Ashraf and Foolad, 2005).

Ozone (O_3), a triatomic allotrope of oxygen, while often associated with its detrimental effects in the lower atmosphere as a pollutant, plays a crucial and often overlooked role in plant physiology, particularly in seed germination and early seedling development (Kangasjärvi *et al*., 1994; Roshchina and Roshchina, 2003; Violleau *et al*., 2008; Patwardhan and Gandhare, 2013; Pandiselvam *et al*., 2020). While high concentrations of tropospheric ozone can induce oxidative stress and negatively impact plant growth (Pandiselvam *et al*., 2020), carefully controlled and optimized applications of ozone at lower concentrations have demonstrated significant beneficial effects across a range of plant species (Patwardhan and Gandhare, 2013; Tütüncü *et al*., 2024; Violleau *et al*., 2007).

The positive effects of ozone in plants include enhanced seed germination rates, improved seedling vigor, and increased tolerance to various environmental stresses (Kangasjärvi *et al*., 1994; Sudhakar *et al*., 2011; Pandiselvam and Thirupathi, 2015). The mechanisms underlying these beneficial effects are complex and involve a variety of physiological and biochemical processes, including the modulation of antioxidant systems, hormonal signaling pathways, and the activation of specific gene expression

related to germination and growth (Godínez-Mendoza *et al*., 2023). Understanding these mechanisms is crucial for developing effective strategies for utilizing ozone in agriculture to improve crop production and sustainability.

This study focuses on the impact of ozone treatment on seed germination in two economically and nutritionally important species: tomato (*Solanum lycopersic*um L.) and roselle (*Hibiscus sabdariffa* L.). Tomato is a globally cultivated vegetable crop, valued for its nutritional content and versatility in culinary applications (Kumar *et al*., 2017; Casals *et al*., 2021; Canul-Ku *et al*., 2023). Roselle, on the other hand, is a valuable crop known for its edible calyces, used for making beverages and jams, as well as its potential medicinal properties (Herrera-Arellano *et al*., 2004; Montaño-Arango *et al*., 2024). While previous research has explored the effects of ozone on various plant species, including some crops (Pandiselvam and Thirupathi, 2015; Sudhakar *et al*., 2008; Violleau *et al*., 2007), a detailed investigation of the specific responses of tomato and roselle seeds to ozone treatment is still lacking.

This research aims to address this gap by investigating the effects of different ozone concentrations and exposure durations on the germination parameters of tomato and roselle seeds. It was hypothesized that optimized ozone treatments can significantly improve seed germination, germination rates, and seedling vigor in both species. The findings of this study will contribute to a better understanding of the potential applications of ozone in enhancing seed germination and promoting sustainable agriculture. This study aims to evaluate the impact of ozonated water (ozone) and aqua regia as pre-sowing treatments, compared to a control, on the germination of tomato and roselle seeds.

MATERIALS AND METHODS

Research facility

This research was conducted at the Molecular Genetics Laboratory at Postgraduate College Campus Montecillo, located in Texcoco municipality, State of Mexico, Mexico. The experiments were carried out during the final week of May and the first week of June 2023.

Experimental design

A randomized complete block design was used, with individual seeds serving as the experimental units. To investigate the effects of varying seed coat hardness treatments, tomato (*S. lycopersicum* var. Río Grande) and roselle (*H. sabdariffa*) seeds were selected. The treatments included ozonated water (ozone), aqua regia, and a deionized water control. Each treatment was replicated six times.

For each treatment, 150 seeds were utilized (α = 0.05). This was conducted in two independent experiments, one for each plant species. The seed treatments were as follows: a) Seeds were submerged in deionized water for 1 min as a control treatment;

b) seeds were imbibed in 200 mL of deionized water and then treated with an ozone-oxygen mixture (ozone concentration of 9.3 mg L^{-1}) generated by a Yuomo Aquapure APSO 0.5-D ozone generator for 1 min under constant stirring and bubbling at 21 °C; and c) seeds were imbibed in 200 mL of aqua regia for one minute under constant stirring, then rinsed thoroughly with deionized water.

The germination assay was conducted using primed seeds. Fifteen Petri dishes were prepared for each treatment, each containing 10 treated seeds placed equidistantly on a 90 mm Whatman filter paper. A total of 45 Petri dishes per plant species were randomly placed in a lidded box and incubated at 29 °C for 10 days. Seed germination was recorded daily for 10 days.

Data analysis

Germination rates data for each treatment and plant species were assessed for normality and homoscedasticity. Differences among control, aqua regia, and ozone treatments were then evaluated using a Kruskal-Wallis test, followed by Dunn's *post hoc* test. Numerous germination indices were calculated for each analyzed plant using the *germinationmetrics* R package (Aravind *et al*., 2019) and a germination matrix organized by day, replicate, and treatment (Table 1).

Table 1. Germination indices and their description for tomato (*Solanum lycopersicum* L.) and roselle (*Hibiscus sabdariffa* L.) seeds.

Index	Formula	Description	Reference
GerminationPercent (GP)	$GP = \frac{N_g}{N_t} \times 100$	N_g = Number of germinated seeds, N_t = Total number of seeds	(ISTA, 2023)
PeakGermPercent (PGP)	$PGP = \frac{N_{max}}{N_t} \times 100$	N_{max} = Maximum number of seeds germinated per interval	(Vallance, 1950; Roh *et al*., 2004)
FirstGermTime (t_0) LastGermTime (t_g)	$t_0 = min\{Ti : Ni \neq 0\}$ $t_g = \max\{Ti : Ni \neq 0\}$	Ti = Time from the start of the experiment to the *ith* interval, Ni = Number of seeds germinated in the *ith* time interval (not the accumulated number, but the number corresponding to the *ith* interval)	(Edwards, 1932; Czabator, 1962; Goloff and Bazzaz, 1975; Labouriau, 1983; Ranal, 1999; Quintanilla *et al*., 2000)
TimeSpreadGerm	$(t_g - t_0)$		
t50_Coolbear Median Germination Time (t50)	$t_{50} = T_i + \frac{(\frac{N+1}{2} - N_i)(T_j - T_i)}{N_j - N_i}$	N = Final number of germinated seeds, N_i and N_j = Cumulative germinated seed counts at times T_i and T_j where $N_i < (N+1)/2 < N_j$	(Coolbear *et al*., 1984)
t50_Farooq Germination time (t50)	$t_{50} = T_i + \frac{(\frac{N}{2} - N_i)(T_j - T_i)}{N_j - N_i}$	N = Final number of germinated seeds, N_i and N_j = Cumulative germinated seed counts at times T_i *and* T_j where $N_i < N/2 < N_j$	(Farooq *et al*., 2005)

Table 1. Continue

Index	Formula	Description	Reference
MeanGermTime ($\bar{T}$)	$\bar{T} = \frac{\sum_{i=1}^{k} N_i T_i}{\sum_{i=1}^{k} N_i}$	T_i = Time to *i*th interval, N_i = Seeds germinated in *i*th interval	(Edmond and Drapala, 1958; Czabator, 1962; Smith and Millet, 1964; Ellis and Roberts, 1980; Labouriau, 1983; Ranal, 1999)
VarGermTime (s_T^2)	$s_T^2 = \frac{\sum_{i=1}^{k} N_i (T_i - \bar{T})^2}{\sum_{i=1}^{k} N_i - 1}$	T_i = Time to *i*th interval, N_i = Seeds germinated in *i*th interval	(Labouriau, 1983; Ranal and de Santana, 2006)
SEGermTime ($s_{\bar{T}}$)	$s_{\bar{T}} = \sqrt{\frac{s_T^2}{\sum_{i=1}^{k} N_i}}$	N_i = Seeds germinated in *i*th interval, k = Total number of intervals	
CVGermTime (CV_T)	$CV_T = \frac{\sqrt{s_T^2}}{\bar{T}}$		(Ranal and de Santana, 2006)
MeanGermRate ($\bar{V}$)	$\bar{V} = \frac{\sum_{i=1}^{k} N_i}{\sum_{i=1}^{k} N_i T_i}$	T_i = End time of *i*th interval, N_i = Seeds germinated in *i*th interval, k = Total intervals	(Labouriau and Bicalho-Valadares, 1976; Labouriau, 1983; Ranal and de Santana, 2006)
CVG Coefficient of velocity/Rate of germination	$CVG = \bar{V} \times 100$		(Kotowski, 1962; Nichols and Heydecker, 1968; Labouriau, 1983; Scott *et al*., 1984; Thompson *et al*., 1995)
GermRateRecip_Coolbear (v_{50})	$v_{50} = \frac{1}{t_{50}}$	*t50* = Median Germination Time (Coolbear)	(Coolbear *et al*., 1984)
GermRateRecip_Farooq		*t50* = Median Germination Time (Farooq)	(Farooq *et al*., 2005)
GermSpeed_Count (S) GermSpeed_Percent (S)	$S = \frac{N_1}{T_1} + \frac{N_2}{T_2} + \frac{N_3}{T_3} + \cdots \frac{N_k}{T_k}$	N_i = Number or percent of germinated seeds at time T_i, k = Total intervals	
GermSpeedAccumulated_ Count GermSpeedAccumulated_ Percent	$S_{accumulated} = \frac{N_1}{T_1} + \frac{N_1 + N_2}{T_2} + \frac{N_1 + N_2 + N_3}{T_3} + \cdots \frac{N_1 + N_2 + \cdots N_k}{T_k}$	N_k = Number or percent of seeds germinated at times $T_{k,i,j}$ k = Number of intervals	(Throneberry and Smith, 1955)
GermSpeedAccumulated_ Normal	$S_{accumulated} = \sum_{i=1}^{k} \frac{\sum_{j=1}^{i} N_j}{T_i}$	T_i = Time from the start of the experiment to the *i*th interval, $\sum_{j=1}^{i} N_j$ = Cumulative number of seeds germinated up to the *i*th interval, k = Total number of time intervals	

Table 1. Continue

Index	Formula	Description	Reference
GermSpeedCorrected_ Normal GermSpeedCorrected_ Accumulated	$S_{corrected} = \frac{S}{FGP}$	S = Represents germination speed (calculated using germination percentage, either standard or accumulated, depending on the method argument: "normal" or "accumulated," respectively), FGP = Final germination percentage	(Evetts and Burnside, 1972)
WeightGermPercent	$WGP = \frac{\sum_{i=1}^{k}(k-i+1)N_i}{k \times N} \times 100$	N_i = Seeds germinated in interval i, N = Total seeds, k = Total intervals	
MeanGermPercent ($\overline{GP}$)	$\overline{GP} = \frac{GP}{T_k}$	GP = Final Germination Percentage, T_k = Time at final interval	(Czabator, 1962)
MeanGermNumber ($\overline{N}$)	$\overline{N} = \frac{N_g}{T_k}$	Ng = Final germinated seeds, T_k = Time at final interval	(Khamassi *et al*., 2013)
TimsonsIndex	$\Sigma k = \sum_{i=1}^{k} G_i$	G_i = Cumulative germination percentage at interval i, k = Total intervals	(Baskin and Baskin, 1998; Brown and Mayer, 1988; Grose and Zimmer, 1958)
TimsonsIndex_Labouriau (Σk_{mod})	$\Sigma k_{mod} = \frac{\Sigma k}{\sum_{i=1}^{k} g_i}$	g_i = Partial germination percentages	(Khan and Ungar, 1984; Ranal and de Santana, 2006)
Timsons Index_KhanUngar	$\Sigma k_{mod} = \frac{\Sigma k}{T_k}$	T_k = Total germination time	(Khan and Ungar, 1984)
GermRateGeorge (GR)	$GR = \sum_{i=1}^{k} N_i K_i$	N_i = Seeds germinated in interval i, K_i = Time until end of test from start of interval i, k = Total intervals	(Nichols and Heydecker, 1968; Tucker and Wright, 1965)
GermIndex (GI)	$GI = \sum_{i=1}^{k} \frac{\lvert(T_k - T_i)N_i\rvert}{N_t}$	T_i = Time to end of interval i, N_i = Seeds germinated in interval i, Nt = Total seeds, k = Total intervals	(Melville *et al*., 1980)
GermIndex_mod (GI)	$GI_{mod} = \sum_{i=1}^{k} \frac{\lvert(T_k - T_i)N_i\rvert}{N_g}$	T_i = Time to end of interval i, N_i = Seeds germinated in interval i, N_g = Total germinated seeds, k = Total intervals	(Ranal and de Santana, 2006)
EmergenceRateIndex_ SG (ERI)	$ERI = \sum_{i=1}^{k-1} N_i(k-i)$	N_i = Number of seeds germinated in the ith time interval (new germinations only), i = Index of the time interval, i_0 = Time interval at germination onset, k = Total number of time intervals	(Shmueli and Goldberg, 2022)
EmergenceRateIndex_ SG_mod (ERI_{mod})	$ERI_{mod} = \frac{\sum_{i=i_0}^{k-1} N_i(k-i)}{N_g}$	N_g = Total of germinated seeds at the end of assay	(Ranal and de Santana, 2006)

Table 1. Continue

Index	Formula	Description	Reference
EmergenceRateIndex_ BilbroWanjura (ERI_{bilb})	$ERI_{bilb} = \frac{\sum_{i=1}^{k} N_i}{\bar{T}}$	$\bar{T}$ = Mean germination time or mean emergence time	(Bilbro and Wanjura, 1982)
EmergenceRateIndex_ Fakorede (ERI_{fak})	$ERI_{fak} = \frac{\bar{T}}{FGP/100}$	FGP = Final germination time	(Fakorede and Agbana, 1983; Fakorede and Ayoola, 1980; Fakorede and Ojo, 1981)
PeakValue (PV)	$PV = max\left(\frac{G_1}{T_1}, \frac{G_2}{T_2}, \frac{G_3}{T_3}, \ldots \frac{G_k}{T_k}\right)$	T_n = Time from experiment start to the nth interval, G_n = Cumulative germination percentage at the nth interval, k = Total number of time intervals	(Czabator, 1962)
GermValue_Czabator (GV)	$GV = PV \times MDG$	MDG = Mean daily germination percentage from onset	
GermValue_DP (GV_{dp})	$GV_{dp} = \frac{\Sigma\, DGS}{N} \times GP \times c$	DGS = Speed of daily germination calculated by dividing the cumulative germination percentage by the number of days since germination began, N = Frequency of calculated DGS, c = Average daily germination speed	(Djavanshir and Pourbeik, 1976)
GermValue_Czabator_mod (GV_{mod})	GV_{mod}	Germination Value (GV) calculations typically consider only the time from germination onset. However, a modified GV (GV_{mod}) incorporating the full test duration	(Brown and Mayer, 1988)
GermValue_DP_mod (GV_{mod})			
GermSynchrony (Z)	$Z = \frac{\sum_{i=1}^{k} C_{N_i,2}}{C_{\Sigma N_i,2}}$	$C_{Ni,2}$ = Number of two-seed combinations possible from the N_i = Seeds germinated in the ith interval (calculated as $C_{Ni,2} = N_i\,(N_i\text{-}1)/2$), $C_{\Sigma Ni,2}$ = Same calculation for the total number of germinated seeds, assuming simultaneous germination	(Primack, 1985; Ranal and de Santana, 2006)
GermUncertainty ($\bar{E}$)	$\bar{E} = -\sum_{i=1}^{k} f_i \log 2\, f_i$	f_i = Relative frequency of germination during the ith time interval, N_i = Number of seeds that germinated during the ith time interval, k = Total number of time intervals over which germination is observed	

A principal component analysis (PCA) was conducted on the seed germination data for each plant species. For this analysis, the data were structured into a matrix format suitable for use in R. Each row represented a biological replicate (n = 15 per treatment), and each column represented a calculated seed germination index derived from the three treatments (aqua regia, ozone, and control). Confidence ellipses were generated for each treatment. PCA computations were performed using the *prcomp* function, and visualizations were generated using the *fviz_pca_ind* function from the package *factoextra*. All statistical analyses were conducted in RStudio version 2024.04.0.

RESULTS AND DISCUSSION

A comparative analysis of various treatments on the germination of tomato and roselle seeds revealed varying degrees of efficacy. Germination rates were assessed relative to a control group. The analysis of germination rate data for tomato seeds exhibited a significant disparity between the control group and ozone treatment (Kruskal-Wallis test, $p \leq 0.01$) and between aqua regia and ozone treatment (Kruskal-Wallis test, $p \leq 0.01$). Ozone treatment demonstrated significantly higher germination rates compared to the control and aqua regia in tomato seeds (Table 2).

Table 2. Descriptive statistics of seed germination rates after 10 days for tomato (*Solanum lycopersicum* L.) and roselle (*Hibiscus sabdariffa* L.) under different treatments compared to a control group.

Treatment	Control		Aqua regia		Ozone	
Plant	Tomato	Roselle	Tomato	Roselle	Tomato	Roselle
Mean*	8A	4	8A	5	9B	4
Minimum	6	2	7	2	8	2
Maximun	10	6	10	9	10	7
Sum	124	64	122	73	140	58
Median	8	4	8	5	9	3
Standard error of the mean	0.4	0.4	0.2	0.55	0.2	0.44
Standard deviation	1.4	1.4	0.8	2.13	0.7	1.72
Coefficient variance	1.92	1.92	0.69	4.55	0.52	2.98

*Group means with different letters indicate statistically significant differences ($p \leq 0.01$) as determined by Kruskal-Wallis test.

No statistically significant differences were observed between the germination rates in seeds of the control group and either aqua regia or ozonated water treatments in roselle seeds (Kruskal-Wallis test, $p \geq 0.05$). Although not statistically significant in all cases, a discernible trend towards improved germination was observed across both plant species when utilizing ozonated water and aqua regia treatments (Table 2).

Previous research has extensively documented the capacity of pre-treatments to enhance plant growth by triggering a cascade of biochemical and physiological responses within the plant. Kangasjärvi *et al.* (1994) emphasized the potential of ozone to stimulate plant growth, suggesting that microdosing and short-term exposure can effectively enhance the growth and yield of various plant species. The findings of this study align with previous research that has highlighted the effectiveness of priming techniques, including the use of ozone and acids, in enhancing germination and crop yield in diverse plant species (Sudhakar *et al*., 2008, 2011; Varela and Arana, 2011; Condori Tarqui and Martinez Flores, 2020).

Seed germination varied both within and between treatments. A general trend of increasing germinated seeds was observed, although a proportion of seeds failed to germinate. In tomato, seed germination exhibited substantial inter-treatment variation (Figure 1A). Over the 10-day experimental period, tomato seed germination ranged from 0 to 100 seeds. The ozone treatment induced a rapid increase in germination between days 2 and 3, reaching a peak of approximately 100 germinated seeds. While both control and aqua regia treatments followed similar germination patterns, their peak germination counts were lower, reaching approximately 75 and 60 seeds, respectively, by day three. Following this peak, germination declined across all treatments, with the most pronounced decrease observed in the ozone treatment. Notably, the aqua regia treatment consistently resulted in the lowest germination rates in the tomato seed germination rate.

Roselle had a seed germination on a scale of 0 to 50 seeds. The ozone treatment stimulated rapid initial germination in roselle, peaking at approximately 50 seeds by day two. The control and aqua regia treatments exhibited similar germination

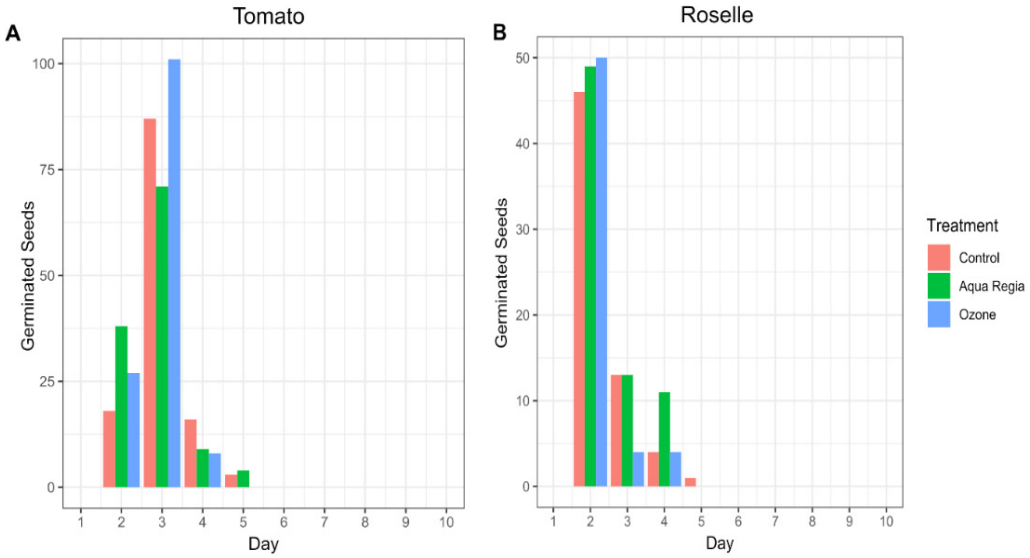

Figure 1. Histogram of seed germination frequency over 10 days in control and treated. seeds using ozonated water and aqua regia for two plant species. A: tomato (*Solanum lycopersicum* L.) seeds; B: roselle seeds (*Hibiscus sabdariffa* L.).

patterns, reaching approximately 48 and 45 seeds, respectively, also by day two. Following this peak, germination declined in both the control and ozone treatment. However, uniquely, the aqua regia treatment maintained a germination up of 10 seeds through day four.

The seeds of tomato and roselle possess distinct differences in their seed coats. Notably, tomato's seed coating is softer than roselle's due to the latter's thicker layer of irregular collapsed cells, contributing to increased hardness in the seed coating (Mabrouk *et al.*, 2016). The seed germination efficiency between both plant species is based on the effects of each treatment and the nature of the seed. On the one hand, ozone effects are well known to promote a trigger of biochemical and physiological responses after priming (Pandiselvam *et al*., 2020), which is mainly related to the high germination frequency by ozone in tomato seeds. On the other hand, treatment with aqua regia results in scarification of the roselle's coat, exposing the lumens of the macrosclereids cells. This process facilitates water imbibition and triggers seed germination (Nikoleave, 1977). Thus, the germination rate is influenced by the thickness of each type of seed when subjected to various treatments (Figure 1).

Ozone exhibited superior performance compared to control and aqua regia (Figure 2A). Seeds primed with ozone demonstrated a 95 % germination rate, surpassing the control group (83 %) and the aqua regia-primed seeds (83 %). Germination velocity also varied significantly. Ozone-primed seeds exhibited rapid

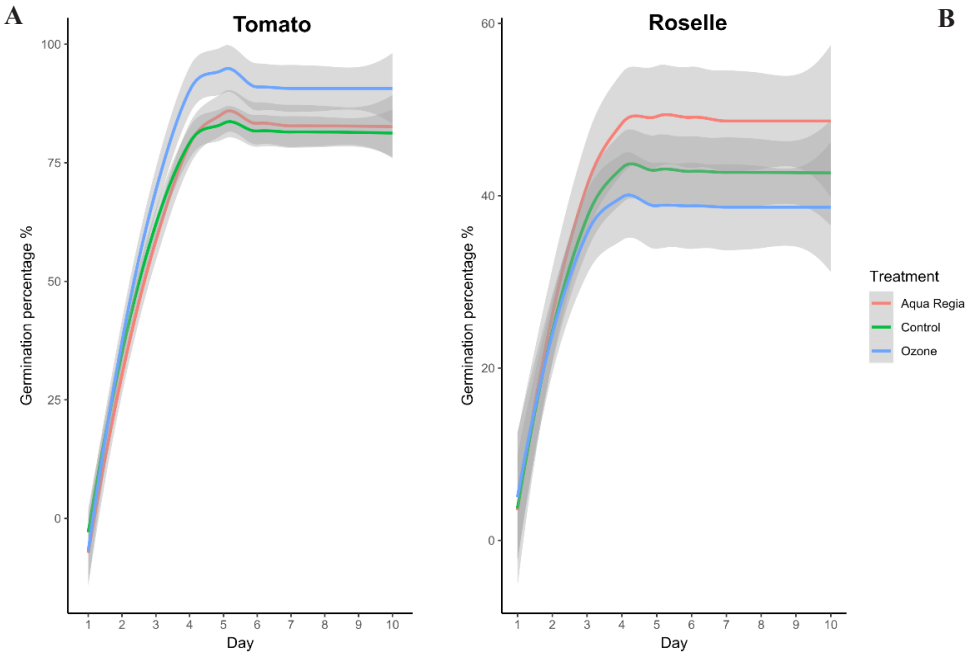

Figure 2. Germination percentages of the evaluated seeds over a 10-day period under three different treatments (control, aqua regia, and ozone). The shaded areas represent the variability or uncertainty associated with the measurements. A: tomato (*Solanum lycopersicum* L.) seeds; B: roselle seeds (*Hibiscus sabdariffa* L.).

germination, reaching maximum rates by the fourth day. In contrast, aqua regia-primed seeds displayed a delayed peak germination on the fifth day. These findings corroborate previous research by Kangasjärvi *et al.* (1994) and Pandiselvam *et al.* (2020), which demonstrated the potential of low-dose ozone to stimulate biochemical and physiological responses, enhancing germination and vigor in various plant species.

The observed increase in germination may be attributed to the stimulation of jasmonic acid production, a known promoter of root growth and seed germination in maize (*Zea mays* L.) and overall plants (Violleau *et al*., 2007). Several studies across various species have demonstrated significant differences in seed germination following ozone treatment (Dong *et al*., 2022; Tütüncü *et al*., 2024). These effects are likely mediated by the stimulation of biochemical cascades within the plant embryo and the release of volatile organic compounds (Dong *et al*., 2022). The observed enhancement of tomato seed germination is likely due to biostimulation rather than scarification of the seed coat. While these results align with those of Tütüncü *et al*. (2024) regarding the positive effects of ozone on seed germination, this study observed a more rapid response, achieved with only one minute of ozone exposure. The thin endosperm layer in tomato seeds may influence the efficacy of each priming treatment. It is possible that aqua regia treatment may have compromised the endosperm, leading to germination failure in some seeds (Madueño-Molina *et al*., 2006).

The germination of roselle seeds under ozonated water priming and aqua regia priming yielded distinct outcomes compared to those observed in tomato seeds (Figure 2B). The treatment using aqua regia priming demonstrated the most favorable germination rates for roselle seeds, reaching 48 % of the total germination. Conversely, ozonated water priming resulted in a maximum of 38 % germination (even lower than the control group, 43 %). The germination velocity exhibited a swift response when treated with aqua regia. The maximum germination was observed on the fifth day of incubation (Figure 2B). Despite the lower germination efficiency associated with ozonated water priming, this method demonstrates the quickest path to reaching maximum germination, typically observed by the fourth day.

While the use of aqua regia presents itself as the most beneficial method for seed germination under these conditions, it is noteworthy that ozonated water exhibits an interesting effect on germination velocity. Aqua regia is widely regarded as the most effective method for scarifying seeds with a hard coating and thicker endosperm, as demonstrated by its successful application in treating roselle seeds. Similar findings were documented in a study conducted by Babashpour *et al.* (2011), where enhanced germination rates were observed following imbibition in sulfuric acid of Fabaceae seeds. In the current study, seeds were subjected to a one-minute exposure to aqua regia, while other investigations have explored the effects of prolonged exposure to sulfuric acid. Youssef (2009) conducted studies that demonstrated promising outcomes in the germination of desert plant seeds following several days of exposure. However, additional research on the effects of exposure during varying time periods is required.

Subsequent to the application of ozone, there was a notable enhancement in germination

velocity, which holds promising implications for agricultural productivity. As both species of seed plants demonstrated similar germination velocities, the present work suggests the utilization of ozone as a biostimulant for seed germination and plant growth. Substantial evidence supports the observed benefits resulting from ozone treatment (Kangasjärvi *et al.*, 1994; Violleau *et al.*, 2007; Sudhakar *et al.*, 2011; Pandiselvam and Thirupathi, 2015; Pandiselvam *et al.*, 2020); the current findings further support the recognition of this agent as a stimulant for enhancing germination velocity, irrespective of its impact on efficiency.

The PCA (Figure 3) was used to analyze inter-treatment variability in tomato seed germination indices. The first two principal components, Dim1 and Dim2, captured 74.2 % of the total variance, with Dim1 accounting for 55.5 % and Dim2 for 18.7 %. This ordination facilitated interpretation of the germination data based on biological relevance.

The first quadrant of the biplot (Figure 3) revealed a cluster of germination indices, including 'EmergenceRateIndex_SG_mod', 'TimsonsIndex_Labouriau', 'GermIndex_mod', 'MeanGermRate', 'GermValue_DP', 'GermRateRecip_Coolbear', 'PeakGermPercent', 'GermSpeedCorrected_Accumulated', 'CVG', 'GermValue_Czabator', 'GermValue_Czabator_mod', and 'GermSpeedCorrected_Normal', demonstrating strong positive correlations. This clustering suggests that the ozone and, to a lesser extent, aqua regia treatments within this quadrant are associated with elevated values across these germination metrics. Consequently, these treatments appear to promote rapid, coordinated, and enhanced germination, characterized by

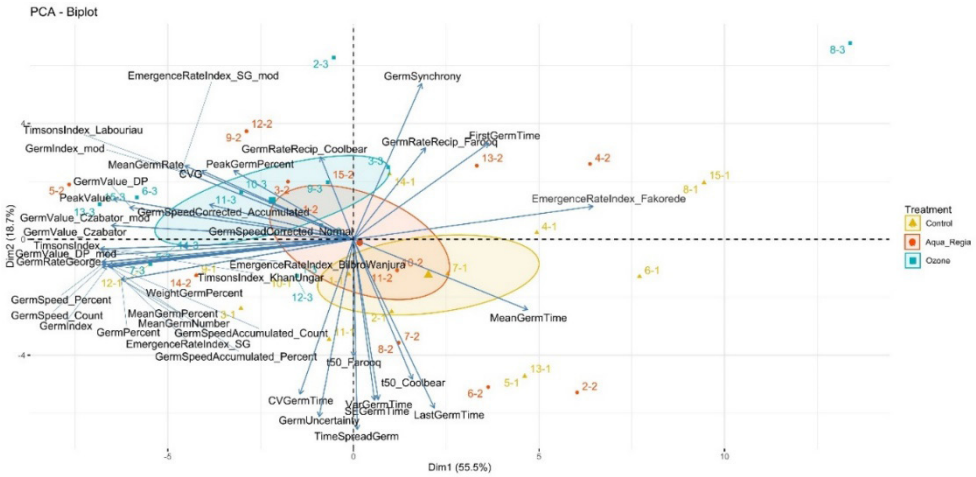

Figure 3. Principal Component Analysis (PCA) biplot of 42 tomato (*Solanum lycopersicum* L.) seed germination indices under different treatments and a control. The plot shows the relationships between germination indices (vectors) and treatments (points), with confidence ellipses representing treatment groups. Dim1 and Dim2 represent the first two principal components, explaining 55.5 and 18.7 % of the variance, respectively.

high emergence rates, peak germination percentages, and normalized germination speeds. The observed pattern indicates a synergistic effect of factors contributing to robust seed germination within this region of the PCA space.

The lower left quadrant of the PCA biplot (Figure 3) demonstrates a significant association among seed germination metrics and ozone treatment. Specifically, 'EmergenceRateIndex_SG' and 'GermSpeedAccumulated_Count/Percent' exhibit a strong positive correlation, indicating that accelerated emergence rates correspond to higher cumulative germination. Furthermore, 'MeanGermPercent,' 'MeanGermNumber,' 'GermPercent,' and 'GermIndex' cluster closely, suggesting redundancy in their measurement of overall germination success. Conversely, 't50_Farroq,' 'CVGermTime,' and 'TimeSpreadGerm' show a greater association with control and aqua regia treatments compared to ozone treatments.

In the lower right quadrant of the PCA biplot (Figure 3), control and aqua regia treatments exhibit a positive correlation with 'LastGermTime,' 'SEGermTime,' 't50_Coolbear,' 'VarGermTime,' and 'TimeSpreadGerm.' This indicates that these treatments are associated with increased variability and prolonged timing in seed germination, specifically influencing the time to reach 50 % germination (t50_Coolbear), the standard error of germination time (SEGermTime), the last germination time (LastGermTime), the variance of germination time (VarGermTime), and the spread of germination times (TimeSpreadGerm).

The upper right quadrant of the PCA biplot (Figure 3) reveals a strong association between ozone and aqua regia treatments and enhanced seed germination. This quadrant positively correlates with 'GermSynchrony,' 'GermRateRecip_Farooq,' and 'FirstGermTime,' indicating improved germination synchronicity, rate, and speed, respectively. The prominent projection of 'EmergenceRateIndex_Fakorede' along Dim1 confirms higher emergence rates for these treatments. Within this quadrant, the control and aqua regia treatments are differentiated, versus the control exhibiting the highest 'EmergenceRateIndex_Fakorede' and shortest 'FirstGermTime,' indicative of superior and faster emergence.

Variability in germination responses to seed treatments was observed in tomato seeds. This heterogeneity is attributable to a complex interplay of intrinsic and extrinsic factors, with particular emphasis on seed morphology and the specific nature of the applied stimulation. Therefore, analyzed indices on seed germination reflected a variation in their germination traits. PCA revealed distinct differentiation between control and treated seeds, particularly highlighting the divergence induced by ozone. Seed germination in control demonstrates that these seeds were delayed for seed germination because 'MeanGerminationTime' and 't50' indices reached the maximum scores for these seeds. The above reflects the baseline of the experiment and is very related to the natural dormancy of the seeds without treatment or stimulation. Gene expression plays a pivotal role in establishing phenotypic traits, encompassing size, morphology, and biochemical composition, during dormancy (Nonogaki, 2006). However, exogenous treatments can induce alterations in gene expression, primarily mediated through the modulation of hormone biosynthesis and catabolism.

The PCA (Figure 3) elucidated the integrated effects of the two treatments on seed germination, revealing a distinct separation of treatment effects characterized by increased germination quantity and rate. Aqua regia treatment induced a biphasic response in seed dormancy, characterized by the stimulation of germination initiation alongside a concomitant reduction in germination rate. This treatment effectively facilitated seed coat removal, yielding rapid results compared to prolonged acid treatments. Specifically, Sonkar *et al*. (2022) demonstrated the efficacy of hydrochloric acid (HCl) presowing treatments lasting four and six hours, underscoring the comparatively accelerated effect of aqua regia.

Results from PCA indicated that ozone treatment elicited the most significant and consistent improvement in seed germination parameters in tomato, suggesting a harmonized and accelerated germination process. Godínez-Mendoza *et al*. (2023) documented the biostimulant effects of various chemical treatments, characterizing a range of chemical stimuli. These authors emphasized the concept of hormesis, wherein a chemical stressor, when applied at an appropriate dose, elicits a beneficial response, termed "eustress." The observed hormetic effect, as demonstrated in the present study, exhibited similarities to those associated with gibberellic acid-mediated enhancement of seed germination, a crucial regulatory process (Finch-Savage and Leubner-Metzger, 2006).

Ozone priming appears to correlate with elevated gibberellic acid levels in treated plants (Kangasjärvi *et al*., 1994). Following ozone, a cascade of physiological and biochemical responses is initiated, potentially contributing to the enhanced efficiency, speed, and rate of germination in tomato seeds, as germination indices denote. Additionally, several studies have documented the beneficial effects of ozone on seed germination (Violleau *et al*., 2008; Patwardhan and Gandhare, 2013; Pandiselvam *et al*., 2020; Tütüncü *et al*., 2024), due to ozone treatment potentially activating ethylene and reactive oxygen species (ROS) signaling pathways, which contribute to the maintenance of "eustress" and the subsequent transition from dormancy in seeds (El-Maarouf-Bouteau and Bailly, 2008).

The PCA performed on roselle seeds was conducted to explore the relationships among various seed germination indices and to assess the influence of different seed treatments (control, aqua regia, and ozone). The biplot (Figure 4) revealed that the first two principal components (Dim1 and Dim2) accounted for 91.7 % of the total variance (Dim1: 52.1 %, Dim2: 39.6 %), indicating that these two components effectively captured the major patterns in the data.

The upper left quadrant of the PCA biplot (Figure 4) exhibits a strong correlation with variables including 'TimsonsIndex_Labouriau,' 'EmergenceRateIndex_SG_mod,' 'CVG,' 'GermIndex_mod,' 'MeanGermRate,' 'GermRateRecip_Coolbear,' 'GermSynchrony,' 'GermSpeedCorrected_Normal,' 'GermRateRecip_Farooq,' and 'GermSpeedCorrected_Accumulated.' This spatial distribution indicates that the ozone treatment significantly influenced germination kinetics, particularly enhancing the rate and synchrony of germination, thereby promoting a more consistent and accelerated

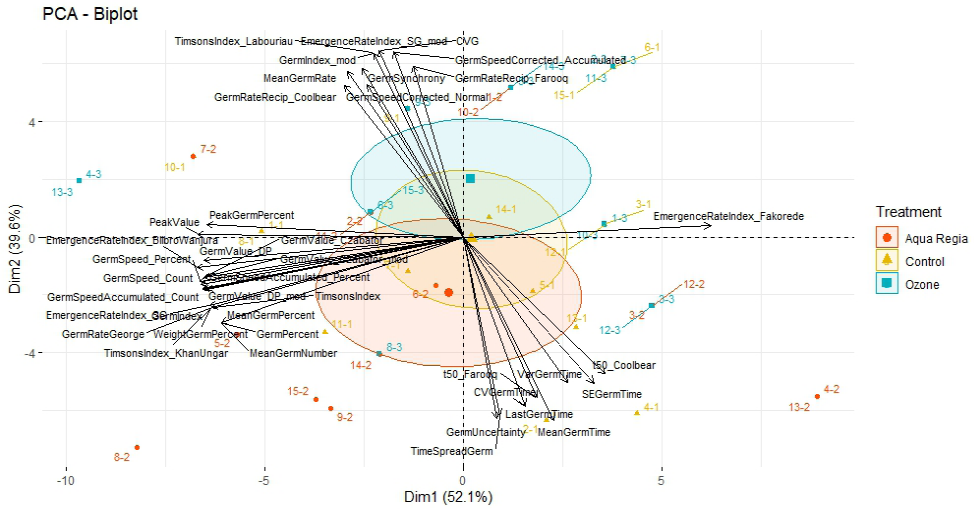

Figure 4. Principal Component Analysis (PCA) biplot of 42 roselle (*Hibiscus sabdariffa* L.) seed germination indices under different treatments and a control. The plot shows the relationships between germination indices (vectors) and treatments (points), with confidence ellipses representing treatment groups. Dim1 and Dim2 represent the first two principal components, explaining 52.1 and 39.6 % of the variance, respectively.

germination process. Furthermore, the observed correlation of 'GermPeakPercent' and 'PeakValue' with both the ozone treatment and control groups suggests that these treatments facilitated the attainment of maximum seed germination within a defined temporal window.

The lower left quadrant of the PCA biplot (Figure 4), characterized by vectors representing variables such as 'EmergenceRateIndex_BilbroWanjura,' 'GermValue_DP,' 'GermSpeed_Percent,' 'GermSpeed_Count,' 'GermValue_Czabator_mod,' 'GermSeedAccumulated_Percent,' 'GermRateRecip_Coolbear,' 'GermSpeedCorrected_Accumulated,' 'GermSeedAccumulated_Count,' 'TimsonsIndex,' 'EmergenceRateIndex_SG,' 'GermIndex,' 'MeanGermPercent,' 'GermRateGeorge,' 'WeightGermPercent,' 'GermPercent,' 'TimsonsIndex_KhanUngar,' and 'MeanGermNumber,' demonstrates a strong association with the aqua regia treatment. This spatial distribution suggests that seeds subjected to this treatment exhibited enhanced germination kinetics, characterized by elevated germination rates, increased percentages of germinated seeds, and a higher overall cumulative germination, as reflected by the aforementioned variables.

The lower right quadrant of the PCA biplot (Figure 4) revealed a clustering of variables including 'CVGermTime,' 'TimeSpreadGerm,' 'LastGermTime,' 'MeanGermTime,' 'SEGermTime,' 'VarGermTime,' 'GermUncertainty,' 't50_Farooq,' and 't50_Coolbear.' This spatial distribution reflects that both the control and aqua regia treatments were

associated with a broader distribution of germination times and increased germination uncertainty. This pattern indicates a higher degree of heterogeneity in the germination process within these treatment groups. The upper right quadrant of the PCA biplot (Figure 4) revealed a strong correlation of the 'EmergenceRateIndex_Fakorede' index with both the ozone and control treatments. This spatial proximity relates that both treatments contributed to a more rapid and synchronized emergence of roselle seeds, as indicated by the enhanced values of this specific emergence index.

The PCA of roselle seed germination indices revealed distinct effects of the different treatments, highlighting their potential for enhancing germination in species with thicker seed coats, such as roselle. This study utilized a short seed imbibition time in aqua regia to enhance germination percentage and kinetics in roselle seeds. Aqua regia's scarification effect breaks down the hard seed coat, facilitating water imbibition and promoting germination (Youssef, 2009; Nasr *et al*., 2013). In contrast, Abdel Latef *et al*. (2020) reported negative effects on roselle seed germination with long-term aluminum oxide nanopriming. This discrepancy may stem from the differing treatment durations and mechanisms: short-term aqua regia exposure facilitates seed coat disruption, while extended nanopriming may hinder germination processes. The beneficial effects of acid imbibition for seed scarification have been acknowledged. Hence, it is imperative to conduct further studies in roselle to thoroughly evaluate extended time-lapses for imbibition in acids at overall, thus several assays have taken long period times on acid soaking for enhance seed germination (Habib, 2010; Aboelgoud, 2015; Nair *et al*., 2017). PCA showed that control reflects the baseline of delayed germination such as "baseline" response due to dormancy in seeds as was discussed previously in the case of tomato seeds.

Ozone treatment demonstrated the highest efficacy in promoting the speed and uniformity of germination in roselle seeds. However, evidence of hormesis was observed, characterized by the enhanced synchrony and rate of germination. While a reduction in overall germination rate was noted, the concurrent increase in several germination indices suggests the activation of a complex biochemical response cascade. Specifically, ozone-induced oxidative stress, leading to the production of ROS, particularly hydrogen peroxide (H_2O_2), is posited to play a critical role in dormancy release (Sudhakar *et al*., 2011). This mechanism likely contributes to the accelerated and more efficient germination process observed in ozone-treated roselle seeds. Furthermore, the observed reduction in abscisic acid (ABA) levels, a known germination inhibitor, likely contributed to the enhanced germination indices. Ozone may also stimulate the production of gibberellic acid and ethylene, both of which promote germination and antagonize ABA's inhibitory effects (El-Araby *et al*., 2006). This hormonal interplay likely underlies the observed improvements in germination synchrony and vigor. Additionally, ROS, particularly H_2O_2, are implicated in endosperm weakening, facilitating radicle protrusion (Leubner-Metzger, 2005). This mechanism elucidates how ozone priming can lead to faster and more uniform germination, as evidenced in both roselle and, as well, in tomato seeds.

CONCLUSIONS

The pre-sowing treatments applied to tomato and roselle seeds revealed species-specific effects on germination, underscoring their potential to enhance crop productivity and sustainability. Ozone treatment significantly improved germination rates and velocity in tomato seeds, outperforming both the control and aqua regia treatments, likely mediated by the triggering of biochemical and physiological mechanisms. Aqua regia effectively enhanced germination in roselle seeds by scarifying their thick seed coats, facilitating water absorption and improving germination percentages and kinetics. This presents a rapid alternative to prolonged acid treatments. These findings contribute to addressing global food security challenges and advancing sustainable agricultural development. Further research is warranted to optimize treatment protocols and evaluate their long-term effects on plant growth and yield under field conditions.

ACKNOWLEDGEMENTS

The first author wants to thank the Mexico's Secretariat of Science, Humanities, Technology, and Innovation (SECIHTI) for the given master's scholarship for developing the present study. The authors thank to the Postgraduate College Montecillo Campus for the monetary and in-kind resources provided for the development of the present research.

REFERENCES

Aboelgoud S. 2015. Pre-sowing treatments for breaking seed dormancy in relation to germination, seedling vigour and yield of teosinte. Journal of Plant Production 6 (12): 2177–2186. https://doi.org/10.21608/jpp.2015.52461

Aravind J, Vimala Devi S, Radhamani J, Jacob SR, Srinivasan K. 2019. germinationmetrics: Seed germination indices and curve fitting. ICAR-National Bureau of Plant Genetic Resources. New Delhi, India. https://github.com/aravind-j/germinationmetrics

Ashraf M, Foolad MR. 2005. Pre-sowing seed treatment—A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. Advances in Agronomy (88): 223–271. https://doi.org/10.1016/S0065-2113(05)88006-X

Babashpour AM, Sharivivash R, Rahbari A. 2011. Effect of different treatments on seed germination of honey locust (*Gleditschia triacanthos*). Modern Applied Science 5 (1): 200–204. https://doi.org/10.5539/mas.v5n1p200

Baskin CC, Baskin JM. 1998. Seeds: Ecology, biogeography, and evolution of dormancy and germination. Choice Reviews Online 36 (4): 2164. https://doi.org/10.5860/choice.36-2164

Bilbro JD, Wanjura DF. 1982. Soil crusts and cotton emergence relationships. Transactions of the ASAE 25 (6): 1484–1487. https://doi.org/10.13031/2013.33750

Bradford KJ. 2017. Water relations in seed germination. Seed Development and Germination: 351–396. https://doi.org/10.1201/9780203740071-13

Brown RF, Mayer DG. 1988. Representing cumulative germination. 1. A critical analysis of single-value germination indices. Annals of Botany 61 (2): 117–125. https://doi.org/10.1093/oxfordjournals.aob.a087534

Canul-Ku J, Morán-Girón D, Barrios-Gómez EJ, Hernández-Meneses E, Rangel-Estrada SE. 2023. Cruza manual en tomate nativo (*Solanum lycopersicum* L.) de México como base para su mejora genética. Brazilian Journal of Animal and Environmental Research 6 (3): 2261–2272. https://doi.org/10.34188/bjaerv6n3-024

Casals J, Martí M, Rull A, Pons C. 2021. Sustainable transfer of tomato landraces to modern cropping systems: The effects of environmental conditions and management practices on long-shelf-life tomatoes. Agronomy 11 (3): 533. https://doi.org/10.3390/agronomy11030533

Condori-Tarqui A, Martinez-Flores Z. 2020. Tratamientos físicos y químicos en la germinación de semillas de especies nativas del altiplano. Revista de Investigación Agropecuaria y de Recursos Naturales 7 (2): 46–57.

Coolbear P, Francis A, Grierson D. 1984. The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. Journal of Experimental Botany 35 (11): 1609–1617. https://doi.org/10.1093/jxb/35.11.1609

Czabator FJ. 1962. Germination value: An index combining speed and completeness of pine seed germination. Forest Science 8 (4): 386–396.

Djavanshir K, Pourbeik H. 1976. Germination value, a new formula. Silvae Genetica 25 (2): 79–83.

Dong X, Sun L, Maker G, Ren Y, Yu X. 2022. Ozone treatment increases the release of VOC from barley, which modifies seed germination. Journal of Agricultural and Food Chemistry 70 (10): 3127–3135. https://doi.org/10.1021/acs.jafc.1c06812

Draganić I, Lekić S. 2012. Seed priming with antioxidants improves sunflower seed germination and seedling growth under unfavorable germination conditions. Turkish Journal of Agriculture and Forestry 36 (4): 421–428. https://doi.org/10.3906/tar-1110-16

Edmond JB, Drapala WJ. 1958. The effects of temperature, sand and soil, and acetone on germination of okra seed. American Society for Horticultural Science 71: 428–434.

Edwards TI. 1932. Germination studies with the seeds of *Xanthium canadense*. American Journal of Botany 19 (8).

El-Araby MM, Moustafa SMA, Ismail AI, Hegazi AZA. 2006. Hormone and phenol levels during germination and osmopriming of tomato seeds, and associated variations in protein patterns and anatomical seed features. Acta Agronomica Hungarica 54 (4): 441–457. https://doi.org/10.1556/AAgr.54.2006.4.7

El-Maarouf-Bouteau H, Bailly C. 2008. Oxidative signaling in seed germination and dormancy. Plant Signaling and Behavior 3 (3): 175–182. https://doi.org/10.4161/psb.3.3.5539

Ellis RH, Roberts EH. 1980. Improved equations for the prediction of seed longevity. Annals of Botany 45 (1): 13–30. https://doi.org/10.1093/oxfordjournals.aob.a085797

Evetts LL, Burnside OC. 1972. Germination and seedling development of common milkweed and other species. Weed Science 20 (4): 371–378. https://doi.org/10.1017/s004317450003589x

Fakorede MAB, Agbana SB. 1983. Heterotic effects and association of seedling vigor with mature plant characteristics and grain yield in some tropical maize cultivars. Maydica 28 (4): 327–338.

Fakorede MAB, Ayoola AO. 1980. Relationship between seedling vigor and selection for yield improvement in maize. Maydica 25 (3): 135–147.

Fakorede MAB, Ojo DK. 1981. Variability for seedling vigour in maize. Experimental Agriculture 17 (2): 195–201. https://doi.org/10.1017/S0014479700011455

Farooq M, Basra SMA, Ahmad N, Hafeez K. 2005. Thermal hardening: A new seed vigor enhancement tool in rice. Journal of Integrative Plant Biology 47 (2): 187–193. https://doi.org/10.1111/j.1744-7909.2005.00031.x

Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. New Phytologist 171 (3): 501–523. https://doi.org/10.1111/j.1469-8137.2006.01787.x

Godínez-Mendoza PL, Rico-Chávez AK, Ferrusquía-Jimenez NI, Carbajal-Valenzuela IA, Villagómez-Aranda AL, Torres-Pacheco I, Guevara-González RG. 2023. Plant hormesis: Revising of the concepts of biostimulation, elicitation and their application in a sustainable agricultural production. Science of the Total Environment 894: 164883. https://doi.org/10.1016/j.scitotenv.2023.164883

Goloff A, Bazzaz FA. 1975. A life history analysis of the winter annual *Gnaphalium purpureum*. Oecologia 20 (2): 107–122.

Grose RJ, Zimmer WJ. 1958. Some laboratory germination responses of the seeds of river red gum, *Eucalyptus camaldulensis* Dehn. syn. *Eucalyptus rostrata* Schlecht. Australian Journal of Botany 6 (2): 129–153. https://doi.org/10.1071/BT9580129

Gross BL, Olsen KM. 2010. Genetic perspectives on crop domestication. Trends in Plant Science 15 (9): 529–537. https://doi.org/10.1016/j.tplants.2010.05.008

Habib M. 2010. Sugarbeet (*Beta vulgaris* L.) seed pre-treatment with water and HCl to improve germination. African Journal of Biotechnology 9 (9): 1338–1342. https://doi.org/10.5897/ajb10.1460

Herrera-Arellano A, Flores-Romero S, Chávez-Soto MA, Tortoriello J. 2004. Effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertension: A controlled and randomized clinical trial. Phytomedicine 11 (5): 375–382. https://doi.org/10.1016/j.phymed.2004.04.001

ISTA (International Seed Testing Association). 2023. International rules for seed testing. In Wallisellen, Switzerland.

Kangasjärvi J, Talvinen J, Utriainen M, Karjalainen R. 1994. Plant defence systems induced by ozone. Plant, Cell and Environment 17 (7): 783–794. https://doi.org/10.1111/j.1365-3040.1994.tb00173.x

Kaya MD, Okçu G, Atak M, Çıkılı Y, Kolsarıcı Ö. 2006. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). European Journal of Agronomy 24 (4): 291–295. https://doi.org/10.1016/j.eja.2005.08.001

Khamassi K, Harbaoui K, Teixeira JA, Jeddi FB. 2013. Optimal germination temperature assessed by indices and models in field bean (*Vicia faba* L. var. minor). Agriculturae Conspectus Scientificus 78 (2): 131–136.

Khan MA, Ungar IA. 1984. The effect of salinity and temperature on the germination of polymorphic seeds and growth of *Atriplex triangularis* Willd. American Journal of Botany 71 (4): 481–489. https://doi.org/10.2307/2443323

Koop SHAA, van Leeuwen CJ. 2017. The challenges of water, waste and climate change in cities. Environment, Development and Sustainability 19 (2): 385–418. https://doi.org/10.1007/s10668-016-9760-4

Kotowski F. 1962. Temperature relations to germination of vegetable seed. Proceedings of the American Society for Horticultural Science 23: 176–184.

Kumar N, Bhardwaj ML, Sharma A, Kumar N. 2017. Assessment of genetic divergence in tomato (*Solanum lycopersicum* L.) through clustering and principal component analysis under mid hills conditions of Himachal Pradesh. International Journal of Current Microbiology and Applied Sciences 6 (5): 1811–1819. https://doi.org/10.20546/ijcmas.2017.605.197

Labouriau LG. 1983. A germinação das sementes. Secretaria Geral da OEA, Washington, DC, USA. 173 p.

Labouriau LG, Bicalho-Valadares M. 1976. On the germination of seeds of *Calotropis procera*. Anais da Academia Brasileira de Ciências 48 (2): 263–283.

Abdel Latef AAH, Zaid A, Abu Alhmad MF, Abdelfattah KE. 2020. The impact of priming with Al2O3 nanoparticles on growth, pigments, osmolytes, and antioxidant enzymes of Egyptian roselle (*Hibiscus sabdariffa* L.) cultivar. Agronomy 10 (5): 1–16. https://doi.org/10.3390/agronomy10050681

Leubner-Metzger G. 2005. B-1,3-glucanase gene expression in low-hydrated seeds as a mechanism for dormancy release during tobacco after-ripening. Plant Journal 41 (1): 133–145. https://doi.org/10.1111/j.1365-313X.2004.02284.x

Mabrouk M, Youssef A, Amin W, Abou-Samaha R. 2016. Physical properties, microstructure and proximate chemical composition of roselle (*Hibiscus sabdariffa*) seeds and kernels. Alexandria Journal of Food Science and Technology 13 (2): 19–32. https://doi.org/10.12816/0038411

Madueño-Molina A, García-Paredes D, Martínez-Hernández J, Rubio-Torres C, Navarrete-Valencia A, Bojórquez-Serrano J. 2006. Germinación de semilla de frijolillo, *Rhynchosia minima* (L.) DC., luego de someterla a tratamientos pregerminativos. Bioagro 18 (2): 101–105.

Melville AH, Galletta GJ, Draper AD, Ng TJ. 1980. Seed germination and early seedling vigor in progenies of inbred strawberry selections. HortScience 15 (6): 749–750. https://doi.org/10.21273/hortsci.15.6.749

Meyer RS, Purugganan MD. 2013. Evolution of crop species: Genetics of domestication and diversification. Nature Reviews Genetics 14 (12): 840–852. https://doi.org/10.1038/nrg3605

Montaño-Arango O, Corona-Armenta JR, Ortega-Reyes AO, Garnica-González J. 2024. La flor de jamaica como producto estratégico para la salud humana en el contexto de México. Inter Disciplina 12 (33): 117–142. https://doi.org/10.22201/ceiich.24485705e.2024.33.88242

Nair AGH, Vidya P, Sreekumar J, Mohan C. 2017. Effect of seed pre-sowing treatment on germination of sweet potato. International Journal of Applied and Pure Science and Agriculture 3 (9): 69–75. https://doi.org/10.22623/ijapsa.2017.3101.fjzzz

Nasr SMH, Savadkoohi SK, Ahmadi E. 2013. Effect of different seed treatments on dormancy breaking and germination in three species in arid and semi-arid lands. Forest Science and Practice 15 (2): 130–136. https://doi.org/10.1007/s11632-013-0209-7

Nichols MA, Heydecker W. 1968. Two approaches to the study of mass media. Proceedings of the International Seed Testing Association 33 (3): 531–540.

Nikoleave MG. 1977. Factors controlling seed dormancy pattern. *In* Khan AA. (ed.), The Physiology and Biochemistry of Seed Dormancy and Germination. North Holland Publishing Company: Amsterdam, Netherlands, pp: 52–74

Nonogaki H. 2006. Seed germination. The biochemical and molecular mechanisms. Breeding Science 56 (2): 93–105. https://doi.org/10.1270/jsbbs.56.93

Pandiselvam R, Mayookha VP, Kothakota A, Sharmila L, Ramesh SV, Bharathi CP, Gomathy K, Srikanth V. 2020. Impact of ozone treatment on seed germination – A systematic review. Ozone: Science and Engineering 42 (4): 331–346. https://doi.org/10.1080/01919512.2019.1673697

Pandiselvam R, Thirupathi V. 2015. Reaction kinetics of ozone gas in green gram (*Vigna radiate*). Ozone: Science and Engineering 37 (4): 309–315. https://doi.org/10.1080/01919512.2014.984158

Patwardhan MS, Gandhare WZ. 2013. High voltage electric field effects on the germination rate of tomato seeds. Acta Agrophysica 20 (2): 403–413.

Primack RB. 1985. Longevity of individual flowers. Annual Review of Ecology and Systematics 16 (1): 15–37. https://doi.org/10.1146/annurev.es.16.110185.000311

Quintanilla LG, Pajarón S, Pangua E, Amigo J. 2000. Effect of temperature on germination in northernmost populations of *Culcita macrocarpa* and *Woodwardia radicans*. Plant Biology 2 (6): 612–617. https://doi.org/10.1055/s-2000-16638

Ranal MA. 1999. Seed germination. Brazilian Journal of Plant Physiology 11 (3): 153–161.

Ranal MA, de Santana DG. 2006. How and why to measure the germination process? Revista Brasileira de Botanica 29 (1): 1–11. https://doi.org/10.1590/S0100-84042006000100002

Roh MS, Bentz JA, Wang P, Ercheng LI, Koshioka M. 2004. Maturity and temperature stratification affect the germination of *Styrax japonicus* seeds. Journal of Horticultural Science and Biotechnology 79 (4): 645–651. https://doi.org/10.1080/14620316.2004.11511820

Roshchina VV, Roshchina VD. 2003. Ozone and plant cell. Springer: Dordrecht, Netherlands. 267 p. https://doi.org/10.1007/978-94-017-2523-1

Scott SJ, Jones RA, Williams WA. 1984. Review of data analysis methods for seed germination. Crop Science 24 (6): 1192–1199. https://doi.org/10.2135/cropsci1984.0011183x002400060043x

Shahrokhi M, Tehranifar A, Hadizadeh H, Selahvarzi Y. 2011. Effect of drought stress and paclobutrazol-treated seeds on physiological response of *Festuca arundinacea* L. Master and *Lolium perenne* L. Barrage. Journal Biology and Environment Science 5 (14): 77–85.

Shmueli M, Goldberg D. 2022. Emergence, early growth, and salinity of five vegetable crops germinated by sprinkle and trickle irrigation in an arid zone. Horticulture Science 6 (6): 563–565. https://doi.org/10.21273/hortsci.6.6.563

Smith PG, Millet AH. 1964. Germinating and sprouting responses of the tomato at low temperatures. Journal of the American Society for Horticultural Science 84: 480–484.

Sonkar A, Love SK, Sharma A, Pahwa C, Sharma I, Biju J, Amla S. 2022. Effect of HCl pretreatment on tomato (*Solanum lycopersicum* L.) seed germination. Zenodo 7 (10): 491–494. https://doi.org/10.5281/zenodo.7269311

Sudhakar N, Nagendra-Prasad D, Mohan N, Hill B, Gunasekaran M, Murugesan K. 2011. Assessing influence of ozone in tomato seed dormancy alleviation. American Journal of Plant Sciences 2 (3): 443–448. https://doi.org/10.4236/ajps.2011.23051

Sudhakar N, Nagendra-Prasad D, Mohan N, Murugesan K. 2008. A preliminary study on the effects of ozone exposure on growth of the tomato seedlings. Australian Journal of Crop Science 2 (1): 33–39.

Tester M, Langridge P. 2010. Breeding technologies to increase crop production in a changing world. Science 327 (5967): 818–822. https://doi.org/10.1126/science.1183700

Thompson K, Bewley JD, Black M. 1995. Seeds: Physiology of development and germination. The Journal of Ecology 83 (6): 1053. https://doi.org/10.2307/2261186

Throneberry GO, Smith FG. 1955. Relation of respiratory and enzymatic activity to corn seed viability. Plant Physiology 30 (4): 337–343. https://doi.org/10.1104/pp.30.4.337

Tolhurst TN, Ker AP. 2015. On Technological change in crop yields. American Journal of Agricultural Economics 97 (1): 137–158. https://doi.org/10.1093/ajae/aau082

Tucker H, Wright LN. 1965. Estimating rapidity of germination. Crop Science 5 (5): 398–399. https://doi.org/10.2135/cropsci1965.0011183x000500050006x

Tütüncü M, Andiç M, Kebeli F, Çelikel FG, Şimşek Ö. 2024. Effects of ozone treatments on *in vitro* seed germination of *Ruscus aculeatus*, *Ruscus hypoglossum* and *Danae racemosa*. Black Sea Journal of Agriculture 7 (5): 542–547. https://doi.org/10.47115/bsagriculture.1528904

Valencia-Romero R. 2019. Soberanía alimentaria de granos básicos en México: un enfoque de cointegración de Johansen a partir del TLCAN. Análisis Económico 34 (87): 223–248. https://doi.org/10.24275/uam/azc/dcsh/ae/2019v34n87/Valencia

Vallance KB. 1950. Studies on the germination of the seeds of *Striga hermonthica*. Annals of Botany 14 (3): 347–363. https://doi.org/10.1093/oxfordjournals.aob.a083251

Varela S, Arana V. 2011. Latencia y germinación de semillas. Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Paraná. Bariloche, Argentina. 10 p.

Violleau F, Hadjeba K, Albet J, Cazalis R, Surel O. 2007. Increase of corn seeds germination by oxygen and ozone treatment. IOA Conference and Exhibition. Valencia, Spain. 6 p.

Violleau F, Hadjeba K, Albet J, Cazalis R, Surel O. 2008. Effect of oxidative treatment on corn seed germination kinetics. Ozone: Science and Engineering 30 (6): 418–422. https://doi.org/10.1080/01919510802474631

Youssef AM. 2009. Seed germination of some desert plants from Egypt. Journal of Applied Sciences Research 5 (2): 144–150.