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Physiological and Germination Responses of Muskmelon (*Cucumis melo* L.) Seeds to Varying Osmotic Potentials and Cardinal Temperatures via a Hydrothermal Time Model

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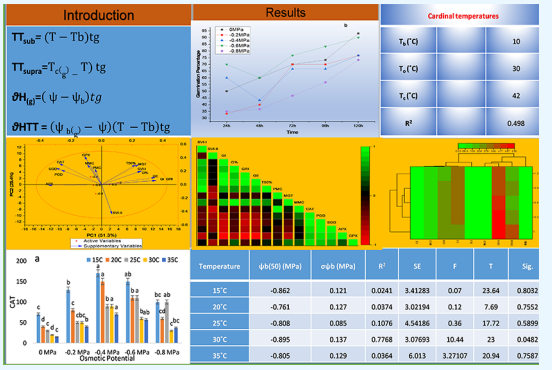
ABSTRACT: Climatic changes have a direct negative impact on the growth, development, and productivity of crops. The water potential (ψ) and temperature (T) are important limiting factors that influence the rate of seed germination and growth indices. To examine how the germination of seed responds to changes in water potential and temperature, the hydrotime model and hydrothermal model (HTT) have been employed. The HTT calculates the concept of germination time across temperatures, between T_b−T_o, with alteration, and between T_b−T_c, in supra-optimal ranges. The seeds of *Cucumis melo* L. were germinated in the laboratory for a hydro-thermal time experiment. Seeds were sown in Petri dishes containing a double-layered filter paper at different osmotic potentials (0, −0.2, −0.4, −0.6, and −0.8 MPa) by providing PEG 6000 (drought stress enhancer) at different temperatures (15, 20, 25, 30, and 35 °C). The controlled replicate was treated with 10 mL of distilled water and the rest with 10 mL of PEG solution. Results indicated that the seed vigor index (SVI−II) was highest at 15 °C with 0 MPa and lowest at 30 °C with −0.2 MPa. However, the highest activity was shown at 15 °C by catalase (CAT) and guaiacol peroxidase (GPX) at (−0.6 MPa), while the lowest values of CAT and GPX were recorded for control at 35 °C with −0.8 MPa at 35 °C, respectively. Germination energy was positively correlated with germination index (GI), germination percentage (G%), germination rate index, seed vigor index-I (SVI−I), mean moisture content (MMC), and root shoot ratio (RSR) and had a negative correlation with mean germination rate, percent moisture content of shoot and root, CAT, superoxide dismutase, peroxidase ascorbate peroxidase, and GPX. In conclusion, thermal and hydrotime models correctly predicted muskmelon germination time in response to varying water potential and temperature. The agronomic attributes were found to be maximum at 30 °C and minimum at 15 °C.



1. INTRODUCTION

Climatic shifts result in temperature increases across the globe;[1] according to a meta−analysis of 17006 simulation, every 2 °C increase in temperature substantially reduces crop yield in both tropical and temperate regions.[2] High temperature has detrimental effects on the growth, development, and productivity of economically important crops, including muskmelons.[3] In this regard, adaption techniques are indispensable to be proposed and incorporated to optimize sustainable production for fulfilling the increasing food demands of the rapidly growing population.[4,5] In order to cope with the dire consequences of constantly changing environmental regimes, scientific efforts are needed at a more rapid pace to control food insecurity and ensure a sustainable agricultural system and sustainable management of soils.[6−891011]

Biotic and abiotic stresses including drought, heavy metals,[12−1415161718] heat or temperature, salinity,[19] and nutrients[20,21] have a huge impact on seed germination and growth rate.[22] The key drivers of germination are temperature and water potential.[23,24] Using seedling development models, it is possible to comprehend how and why germination is impacted by numerous environmental factors.[25] Many researchers have used thermal, hydrothermal, and hydrotime models to analyze how T (temperature), ψ (water potential), and the interplay between $\psi \times T$ affect the germination of seeds.[26] Osmotic regulation is a vital physiological process, which acclimatize plants to stress regimes.[27] Under abiotic

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stress conditions, accumulation of organic solutes in the cytoplasm takes place, which mainly includes glycine betaine and proline that assist stressed plants in the osmotic adjustment of organic solutes.[28] Oxidative stress leads to the production of reactive oxygen species (ROS) that adversely affect plant growth.[29−31]

ROS caused by water deficit-stressed environments is the major concern of the plant defense system.[32,33] An antioxidant system that provides water deficit stress tolerance must be effective, powerful, and fast.[34] The ROS damage is reduced and repaired through enzymatic activity, hormones, and other biochemicals.[35,36] By improving the antioxidant system, ROS is absorbed to decrease lipid peroxidation and electrolyte leakage, maintaining cellular organelles and membrane integrity and vitality.[37−39] Researchers have found that water-stressed conditions enhance the activities of antioxidant enzymes including ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD).[40] The antioxidant system reduces the accumulation of reactive oxygen species to avoid cellular damage by increasing the activity of ROS-scavenging enzymes, including APX and guaiacol peroxidase (GPX).[41]

Water potential (ψ) and temperature (T) are the two key environmental parameters influencing the rate of seed germination, emergence of seedlings, germination percentages, and various physio-biochemical parameters.[42] Using various models to foretell how seeds will germinate, how seedlings will emerge, and what will be the biochemical response to various abiotic stress conditions is generally effective. To examine how the germination of seed responds to the change in T and ψ, for instance, various research studies have employed the HT (hydrotime model) and HTT (hydrothermal model).[43]

The HTT calculates the concept of germination time across T, between T_b (base temperature)-To (optimum temperature), with alteration, and between T_b−T_c (T_c-ceiling temperature), in the supra-optimal range.[3] As far as we are aware, few studies have been done on the validity of hydrothermal time in forecasting germination of *Cucumis melo* L. in response to various ψ and T levels. To better understand this relationship, an HTT model is used to analyze the impact of both T and ψ on *Cucumis melo* seed germination, as well as its cardinal Ts value.

Muskmelon (*Cucumis melo* L.) belongs to the family Cucurbitaceae.[44,45] One of the most extensively grown and devoured fruits on the globe is the melon. It is the primary member of the Cucurbitaceae family[46] and thrives in all subtropical and tropical climates around the world. Melons contain high quantities of carotenoids, vitamin A, and vitamin C, which are the key antioxidants that serve as a reducing agent to reverse oxidation in liquids.[47]

This study aimed to forecast the responses of germination and physiological attributes of *Cucumis melo* seeds at different osmotic potentials and temperatures using the HTT. keeping in mind the effects of water availability and temperature on Muskmelon cultivation in semidesert regions, the research objectives were as follows: to determine the germination rate of seed and its related parameters and seedling length in responses to varying ψ and T, synergistically or independently ($\psi \times T$); to evaluate osmotic tolerance threshold; and to calculate the cardinal temperatures of muskmelons via HTT. According to our knowledge, it is the first research study from Pakistan associated with the application of the hydrothermal time model concept to predict the germination patterns of *Cucumis melo* L. (variety-NIFA 2022) under various Ts and ψs.

2. MATERIALS AND METHODS

2.1. Petri Dish Experiment Protocol. The seeds of *Cucumis melo* L. (Cultivar-NIFA 2022) were germinated in the lab for a hydrothermal time concept experiment. The seeds of the melon plant were collected from (NIFA) Peshawar, Pakistan, sterilized with 95% alcohol, and thoroughly rinsed with distilled water three times. The experiment was carried out at various water potentials and temperatures in an incubator at the laboratory of the Botany Department, University of Peshawar. The experiment was conducted in Petri dishes. The seeds were sown in Petri dishes containing a double layer of filter paper at different osmotic potentials (0, −0.2, −0.4, −0.6, and −0.8 MPa) by providing PEG 6000, at different temperatures (15, 20, 25, 30, and 35 °C). The seeds (15) of each variety were sown in each replicate providing 10 mL of distilled water in the first treatment and 10 mL of PEG solution in the remaining treatments. At the end of the last day, agronomic parameters were measured, including fresh weight of radical and plumule, plumule length, radical length, and dry weight of radical and plumule, along with quantifying the activities of antioxidant enzymes in plumule.

2.2. Data Analysis. According to Alvarado and Bradford[48] and Khan et al.,[3] the Hydrothermal time (HTT), thermal time (TT), and hydrotime (HT) models were used to build a probit regression analysis for germination data. The germination rate, which is the inverse of the time of germination, was calculated for each T.

2.2.1. Hydrotime Model. The hydrotime model was developed to characterize the population of seed germination (SG) in response to osmotic potential by applying the formula of Gummerson,:[49]

$$\theta H_{(g)} = (\Psi - \Psi_b)t_g \tag{1}$$

$$\mathrm{GR}_{(g)} = 1/t_g = (\Psi - \Psi_b)/\theta H \tag{2}$$

where θH *is the* hydrotime constant, Ψ is the osmotic potential, Ψ_b is the base osmotic potential, t_g is the actual time to germination percentage, and GR is the growth rate.

2.2.2. Thermal Time Model. This model was utilized, which characterizes the temperature-induced germination pattern and forecasts the GR for a certain GP of seeds as a linear function of the temperature difference from the baseline temperature T_b.

$$\mathrm{TT}_{\mathrm{sub}} = (T - T_b)t_g(\text{at sub} - \text{optimal}T) \tag{3}$$

$$\mathrm{TT}_{\mathrm{supra}} = T_{c(g)} - T)t_g(\text{at supra} - \text{optimal}T) \tag{4}$$

Thus, seed emergence is indirectly proportional to germination rate, and eqs 1 and 2 may be combined to get eq 3:

$$\mathrm{TT}_{\mathrm{sub}} = 1/t_g = (T - T_{b(g)})/t_g \tag{5}$$

where $\mathrm{TT}_{\mathrm{sub}}$ is the thermal time constant at suboptimal temperature and $\mathrm{TT}_{\mathrm{supra}}$ is the thermal time constant at supra-optimal temperature.

2.2.3. Hydrothermal Model. The hydrothermal−time model was used, which describes the combined effects of both (hydrotime and thermal time) on the germination process.

$$\theta\mathrm{HTT} = (\Psi_{b(g)} - \Psi)(T - T_b)t_g \tag{6}$$

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ACS Omega 2023, 8, 33266−33279

Eq 7 is equation's modified form of (5) for further analysis.

$$\theta\mathrm{HTT} = [\Psi\mathrm{b(g)} - \psi - (k\mathrm{T}(T - T\mathrm{o}))](T - T\mathrm{b})\mathrm{tg} \quad (7)$$

where θHTT is the hydrothermal time constant and k_T is the constant of equation.

2.3. Germination and Agronomic Parameters. Radical length and plumule length were measured with the help of a scale in centimeters (cm) of all replicates after completion of the germination time.

2.3.1. Percent Moisture Contents. The PMC of each plant sample was calculated avidly. The PMC index was calculated by knowing the fresh and dry weight. It was calculated by the following method of Mubeen et al.[50]

$$\mathrm{SPMC} = (\text{wet weight of sample-dry weight of sample} \div \text{wet weight of sample}) \times 100 \quad (8)$$

2.3.2. Time to 50% Germination (T50%). T50% was determined using a mathematical formula that was suggested by Alvarado and Bradford.[48] T50% was developed to determine the time required for 50% seed germination.

$$\mathrm{T50\%} = \frac{ti + \left(\frac{N}{2} - ni\right)(tj - ti)}{(nj - ni)} \quad (9)$$

2.3.3. Germination Energy. GE was measured by following the method of Swaminathan and Revathy[51]

$$\text{Germination Energy} = XI/Y1 + (X2 - XIY2\cdots + (Xn - Xn - 1)/Yn \quad (10)$$

2.3.4. Germination Index. The GI was measured by applying the formula devised by Kader and Kumar.[52]

$$\mathrm{GI} = (10^{*}n_1) + (9xn_2) + \cdots\cdots + (1xn_{10}) \quad (11)$$

2.3.5. Mean Germination Time. MGT represents how rapidly the germination of the seed population occurs. The rate of MGT is inversely proportional to the number of seeds germination.[52]

$$\mathrm{MGT} - \Sigma 2fx/\Sigma f \quad (12)$$

2.3.6. Mean Germination Rate. The MGR was measured using the method of Mubeen et al.[50]

$$\text{Mean germination rate} = 1/\text{mean germination time} \quad (13)$$

2.3.7. Germination Rate Index. The maximum value of GRI indicates a greater and high germination rate, and GRI denotes the% age of SG regularity during the time of germination.

$$\mathrm{GRI} = \mathrm{G1}/2 + \mathrm{G2}/2 \ldots + \mathrm{G}n/2 \quad (14)$$

2.3.8. Seed Vigor Index-I. SVI was calculated by the formula of Bina and Bostani[53]

$$\mathrm{SVI} - \mathrm{I} = \text{seedling length(cm)} \times \text{seed germination\%age} \quad (15)$$

2.3.9. Seed Vigor Index-II. SVI−II was measured by the formula of Sharma et al.[54]

$$\mathrm{SVI} - \mathrm{II} = \text{seedlingdryweight(mg)} \times \text{seed germination\% age} \quad (16)$$

2.3.10. Mean Moisture Content. Mean moisture content has been calculated using the formula of Karimi et al.[55]

$$\mathrm{M.\ M.\ C} = \text{fresh weight} - \text{dry weight} \div \text{dry weight} \quad (17)$$

2.4. Antioxidant Enzymes Activities. *2.4.1. Catalase Activity.* The CAT activity was analyzed using the standard protocol of Aebi,[56] 0.5 g plant fresh tissues were mixed with 10 mL phosphate buffer followed by filtration and centrifugation for 15 min. A 0.1 mL supernatant was collected and treated with 0.5 mL of H_2O_2. The absorbance was noted at 240.0 nm.

2.4.2. Peroxidase Activity. The method of Vetter et al.[57] was followed for the peroxidase activity analysis in fresh plant tissues. A 0.5 g of plant fresh material was chopped in 2 mL of 2-(*N*-Morpholino) ethanesulfonic acid (MES), the samples were centrifuged for 15 min, and the supernatant was collected. A 0.1 mL supernatant was mixed up with 1.5 mL of 100 mM MES, 0.1 mL phenylenediamine, and 0.04 mL of H_2O_2 was added. Absorbance was recorded at 485.0 nm.

2.4.3. Superoxide Dismutase Activity. A 0.5 g amount of plant fresh tissues was chopped with 5 mL of phosphate buffer, the samples were centrifuged, and the supernatant was collected. 0.1 mL of supernatant was treated with EDTA (3 mM), 25 μL of nitro tetrazolium blue chloride, 5 mL of methionine, and Na_2CO_3 by adding 1 mL of riboflavin and then incubated at room temperature. The absorbance was recorded at 560.0 nm.[58]

2.4.4. APX Activity. A 0.5 g plant fresh material was mixed with 10 mL of phosphate buffer, centrifugation was done for 15 min, and 0.1 supernatant was collected from the samples. 0.5 mM ACA (ascorbic acid) and 0.1 mM EDTA were added to the supernatant, and deionized water was poured into the solution to adjust the final volume to 3.0 mL. The mixture was treated with H_2O_2 (0.1 mL), and the absorbance was noted at 290.0 nm by using the protocol of Nakano and Asada.[59]

2.4.5. GPX Activity. Plant fresh tissues (0.5 g) were mixed with 10 mL of phosphate buffer, the solution was spun in the centrifuge machine for 15 min, and the supernatant was collected. A 0.1 mL supernatant was combined with 16 mM guaiacol and 50 mM phosphate buffer, followed by mixing 2 mM H_2O_2. The V (volume) of the reaction mixture was adjusted to 3.0 mL by adding deionized water. The absorbance was measured at 470 nm and expressed according to the protocol of Castillo et al.[60]

2.5. Statistical Analysis. Using IBM SPSS Statistics, linear regression was used to examine the outcome of thermal time, hydrotime, and their relations to germination characteristics and seed germination rate. Analysis of variance, principal component analysis (PCA), correlation (which showed the correlation between each parameter), heatmap histogram correlation, and LSD tests were done for the analysis of data. The fundamental statistical computation was carried out using Microsoft Excel. Plotting several graphs of accelerated aging duration vs germination fraction and germination parameters against osmotic potentials and temperatures was done using the ORIGIN 2021 PC Corporation.

3. RESULTS

3.1. Germination Response to Changing Temperature and Water Potentials. The differences in seed germination time against water potential were studied separately at each T using the hydrothermal time model. θH, Ψ, Ψb, and germination rate parameters for the experimental

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data at each constant T and different Ψ were analyzed by this HTT concept. Temperature, osmotic potential, and their interactions were found to have a substantial impact on the germination rate and other characteristics in our research. Our results indicated that varying water potentials (ψs) and temperatures (T) values influenced the germination rate and germination percentage of *Cucumis melo* ($p < 0.05$) individualistically and interactively (Ψ × T). Furthermore, as assessed from the osmotic tolerance threshold using the HTT, the high θH and R^2 values were obtained at 25 °C optimal temperature and minimum at 15 °C, respectively (Table 1).

Table 1. Estimated Germination and Cardinal Temperature Values for *Cucumis melo* L. var. NIFA 2022 Using the Hydrothermal Time Model

variables	*Cucumis melo* var. NIFA 2022
hydrothermal time model parameters	
Ψ_b (50) (MPa)	−0.825
$\sigma\psi_b$ (MPa)	0.114
θH (MPa Ch-1)	78.7872
k_T (MPa Ch-1)	0.104
cardinal temperatures	
T_b (°C)	10
T_0(°C)	30
T_c(°C)	42
R^2	0.498

The increase in temperature magnitude initially favored the percentage and germination rate of seeds, but this dropped when T exceeded a particular limit. According to Table 2, temperature had a significant ($p < 0.05$) influence on the GR of *Cucumis melo* NIFA-2022. The maximum GR was seen at 25 °C and the minimum at 15 °C with 0 MPa. In general, the lowest germination rates (10 and 16.3%) were found at 15 and 35 °C under −0.8 MPa, respectively, and the highest (75.5%) at 25 °C under 0 MPa. It signifies that germination grew from 40.1 to 93.2% as the temperature increased up to 25 °C and then declined to 40.1%, as the temperature reached beyond 25 °C (optimum T) to 35 °C. In light of the results, a very high θT1/TT1 value was recorded at 35 °C in −0.4 MPa (Table 2). The θT2/TT2 value was also greatest in 0 MPa at 15 °C when compared to the control group. The TT model fits germination percent data in distilled water well, with R^2 improving to 0.7768 (Table 2). The germination percentage was plotted against various temperature percentiles, and the results showed that there was a linear rise in the GR pattern above and below the To (Table 2; Figure 1). With increasing temperature, the base water potential (Ψ_b (50)) displayed an erratic pattern. The T- and F-tests displayed a similar lopsided tendency with no linear trend, with the exception that both values peaked at 30 °C (Table 3). The θHTT values dropped with falling osmotic potential (ψ) across a range of water potentials. The HTT concept's predicted cardinal temperatures in the control were 10 °C for T_b, 25 °C for To, and 42 °C for T_c (Table 1).

The study of the current hydrothermal time model showed that water potential and temperature had a substantial impact on the agronomic parameters of *Cucumis melo* L. Our results reported that applying different water potential and temperature under PEG stress had markedly affected the GP. The GP value was maximum at 30 °C in 0 MPa. While the minimum value of GP was recorded at 20 °C in −0.8 MPa.

Table 2. Estimated Parameters of the Hydro and Thermal Time Models To Describe *Cucumis melo* L. var. NIFA 2022 Seed Germination under Different Temperatures (Ts) and water potentials (ψs)

temperature (°C)	treatment (MPa)	TT_{sub}/θT1	TT_{supra}/θT2	θH (MPa h)	θHTT (MPa h)	TT GR	HT GR
15	0	276.8	1660.8	83.04	415.2	0.0180	0.0180
	−0.2	266.4	1598.4	80.22	319.68	0.0188	0.0150
	−0.4	271.2	1627.2	80.76	244.08	0.0185	0.0112
	−0.6	263.2	1579.2	78.06	157.92	0.0191	0.0077
	−0.8	286.4	1718.4	85.52	85.92	0.0150	0.0035
20	0	512	1280	76.80	768.00	0.0196	0.0196
	−0.2	473.6	1184	71.04	568.32	0.0212	0.0170
	−0.4	478.4	1196	71.76	430.56	0.0212	0.0127
	−0.6	577.6	1444	86.64	346.56	0.0177	0.0071
	−0.8	425.6	1064	63.84	127.68	0.0236	0.0047
25	0	876	1168	87.60	1314.0	0.0271	0.0231
	−0.2	801.6	1068.8	80.16	961.92	0.0187	0.0150
	−0.4	763.2	1017.6	76.32	686.88	0.0199	0.0119
	−0.6	835.2	1113.6	83.52	501.12	0.0181	0.0072
	−0.8	816	1088	81.60	244.80	0.0183	0.0036
30	0	1059.2	794.4	79.44	1588.8	0.0189	0.0189
	−0.2	1046.4	784.8	78.48	1255.6	0.0191	0.0153
	−0.4	1078.4	808.8	80.88	970.56	0.0189	0.0113
	−0.6	1203.2	902.4	90.24	721.92	0.0167	0.0066
	−0.8	1190.4	892.8	89.28	357.12	0.0168	0.0033
35	0	1182	472.8	70.92	1773	0.0211	0.0211
	−0.2	1206.667	482.6667	72.40	1448	0.0207	0.0165
	−0.4	1288.667	515.4667	77.32	1159.8	0.0194	0.0116
	−0.6	1166	466.4	69.96	699.6	0.0216	0.0086
	−0.8	1231.333	492.5333	73.88	369.4	0.0103	0.0040

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ACS Omega 2023, 8, 33266−33279

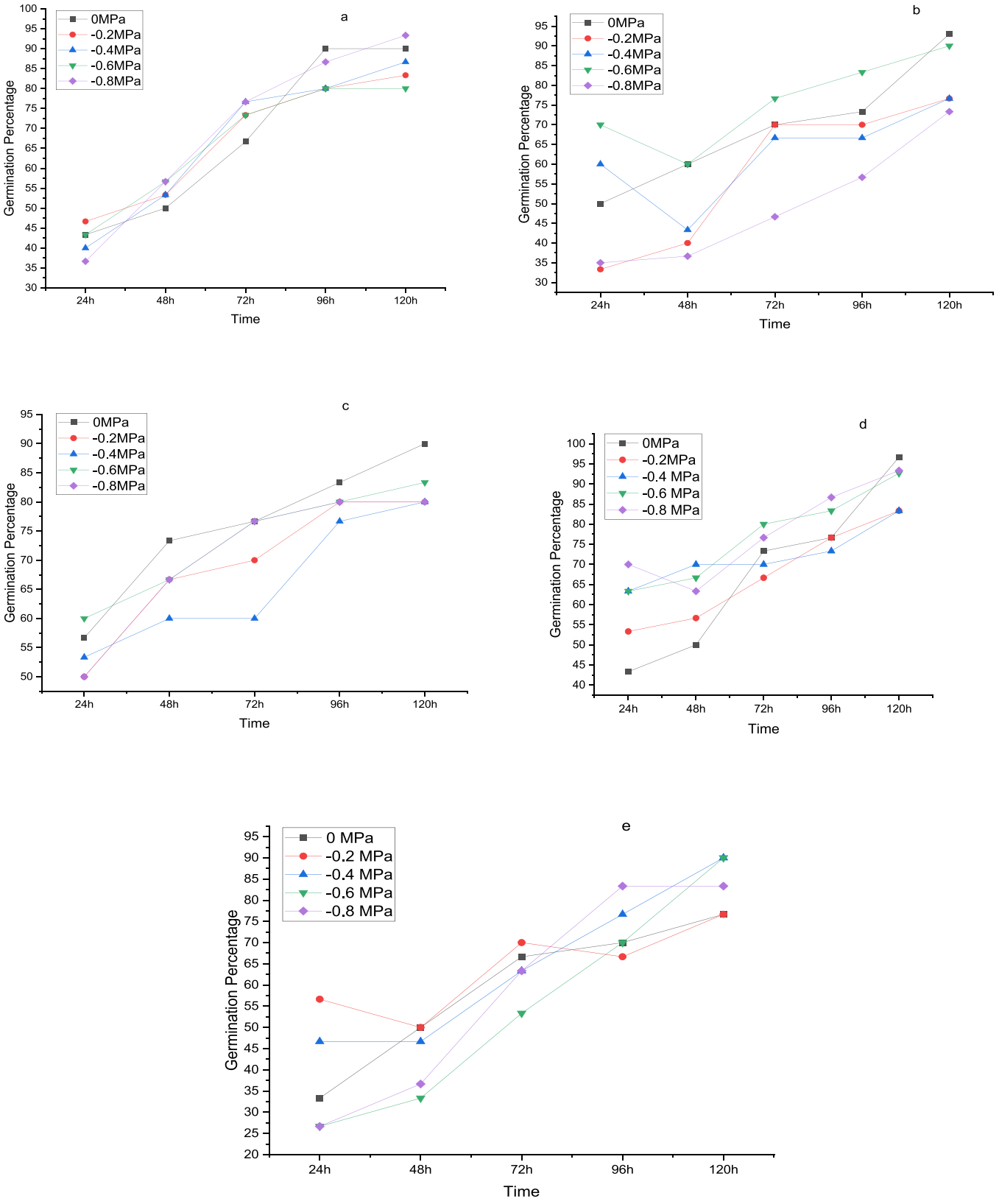

Figure 1. Germination for *Cucumis melo* L. var. NIFA 2022 at (a) 15 °C, (b) 20 °C, (c) 25 °C, (d) 30 °C, and (e) 35 °C having different water potentials (0, −0.2, −0.4, −0.6, and −0.8 MPa).

According to the results documented in (Figure 2) the seed vigor index (SVI−I) was reported to be highest up to a significant level ($p < 0.05$) at 30 °C under PEG with osmotic potential −0.4 MPa. These results showed that the seed vigor index (SVI−II) was highest at 15 °C at 0 MPa and lowest at 30 °C at −0.2 MPa (Figure 2). Results in (Figure 2) reported that applying different water potential and temperature under PEG stress had markedly affected the germination index. Germina-

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Table 3. Estimation of Hydrotime Model Parameters for *Cucumis melo* L. var. NIFA 2022 Using Nonlinear Regression

temperature (°C)	Ψ_b (50) (MPa)	$\sigma\psi_b$ (MPa)	R^2	SE	F	T	Sig.
15	−0.862	0.121	0.0241	3.41283	0.07	23.64	0.8032
20	−0.761	0.127	0.0374	3.02194	0.12	7.69	0.7552
25	−0.808	0.085	0.1076	4.54186	0.36	17.72	0.5899
30	−0.895	0.137	0.7768	3.07693	10.44	23	0.0482
35	−0.805	0.129	0.0364	6.013	3.27107	20.94	0.7587

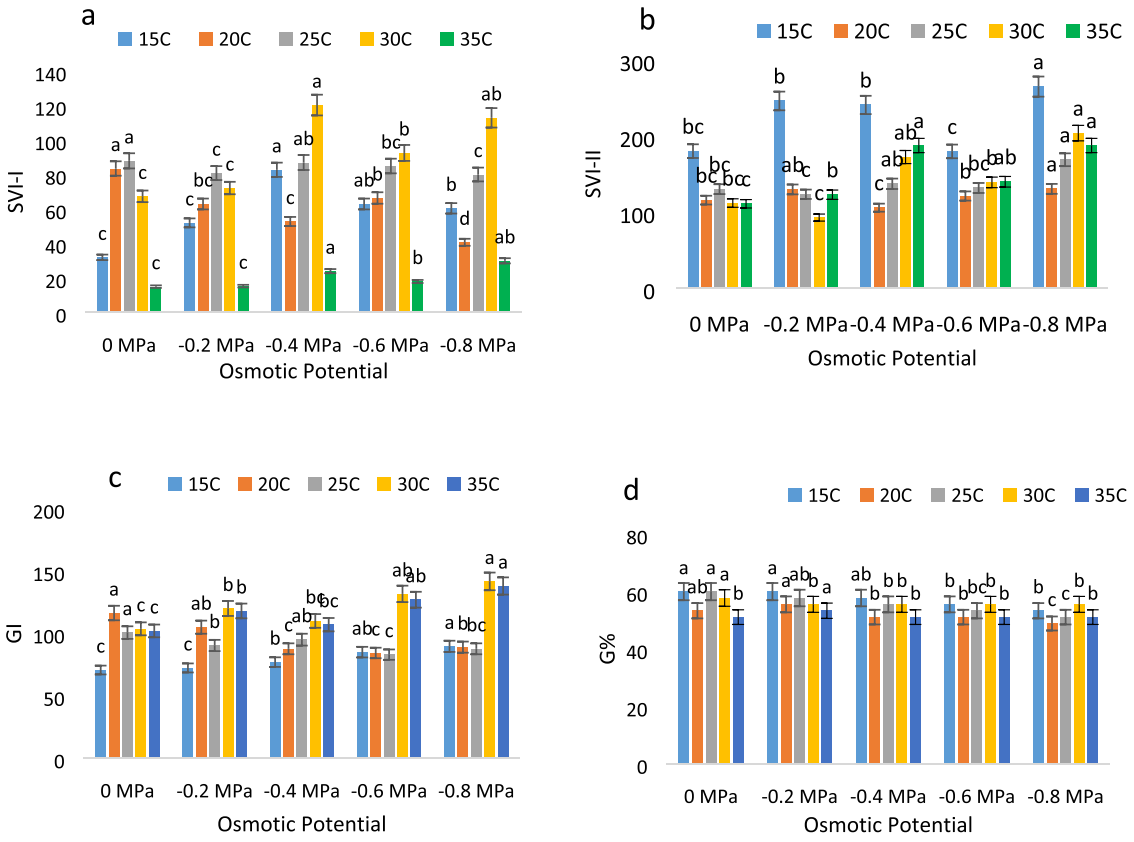

Figure 2. Interactive effect of water potential and temperature on (a) seed vigor index (SVI−I) and (b) seed vigor index (SVI−II). (c) Germination index. (d) Germination percentage of *Cucumis melo* L. var. NIFA 2022. The treatments exhibit dissimilar letters within rows that represent significance ($p \leq 0.05$) level.

tion index (GI) was reported to be highest to a significant level ($p > 0.05$) at 30 °C under PEG with osmotic potential −0.8 MPa. However, the germination index has the lowest value at 15 °C under osmotic potential 0 MPa. Moreover, the germination percentage was maximum in 0 MPa at 15 °C.

The results in Figure 3 reported that the germination rate index has the highest value at −0.8 MPa at 30 °C and the lowest value recorded at −0.2 MPa at 35 °C. The germination energy has a maximum value at 0 MPa at 20 °C and has the lowest value at −0.2 MPa at 35 °C (Figure 3). The Time to 50% germination has a maximum value at −0.2 MPa at 30 °C and a minimum value at 15 °C at 0 MPa (Figure 3). The shoot percent moisture content has a maximum value at −0.4 MPa at 30 °C and a minimum value at 0 MPa at 15 °C (Figure 3). The maximum value of the mean moisture content was recorded for −0.4 at 20 °C and has a minimum value at 0 MPa at 15 °C (Figure 4). The mean germination time was highly affected by osmotic potential and temperature. The maximum value of mean germination time has been recorded for −0.2 MPa at 20 °C and has a minimum value at −0.8 MPa at 30 °C (Figure 4).

3.2. Effect of Cardinal Temperature and Water Potential on Antioxidant Enzymes. Results of the antioxidant enzymes showed that osmotic stress and temperature fluctuations significantly affected the amount of antioxidant enzymes in fresh plant tissues. Results in Figure 5 showed that the highest activity at 15 °C was shown by CAT and GPX at −0.6 MPa, while the lowest value of CAT and GPX was recorded at 0 MPa at 35 °C and −0.8 MPa at 35 °C, respectively. Similarly, at 20 °C, the highest activities were shown by APX and SOD at −0.6 MPa and the lowest activities were recorded in the control group at 15 °C, as shown in Figure 5, and at 15 °C, the highest activity was shown by POD at −0.2 MPa and the lowest value was recorded at −0.8 MPa at 30 °C, as shown in Figure 5. It has been observed that all enzymes responded normally at 0 MPa between 25 and 30 °C. However, the effect was adverse at the highest and lowest treated temperatures. Comparing all the osmotic and thermal responses, the remarkable response was shown by APX and GPX at 15 and 20 °C under −0.4 and −0.6 MPa, respectively, as shown in Figure 5. In addition, the lowest response was shown by all antioxidant enzymes at 0 and −0.8 MPa.

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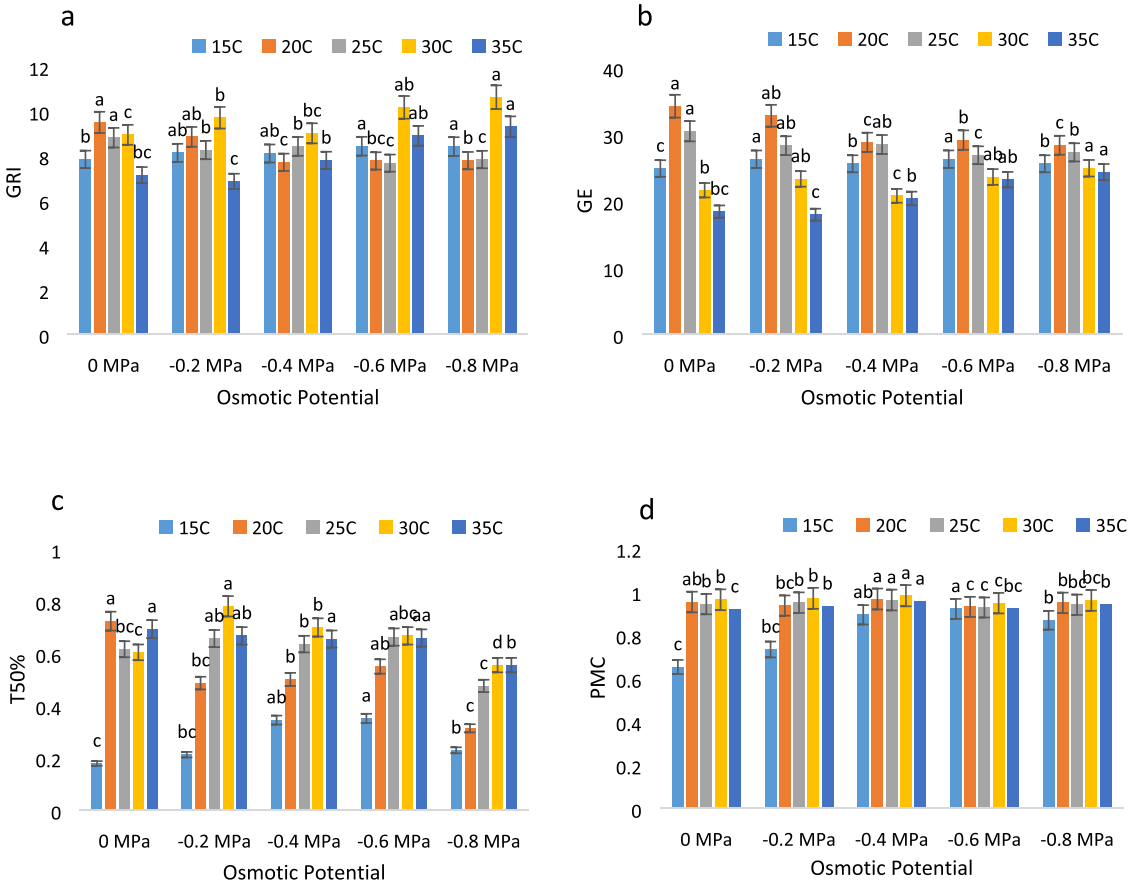

Figure 3. Interactive effect of water potential and temperature on (a) germination rate index (GRI), (b) germination energy (GE), and (c) Time to 50% germination (T50%). (d) Percent moisture content (PMC) of *Cucumis melo* L. var. NIFA 2022. The treatments exhibit dissimilar letters within rows that represent significance ($p \leq 0.05$) level.

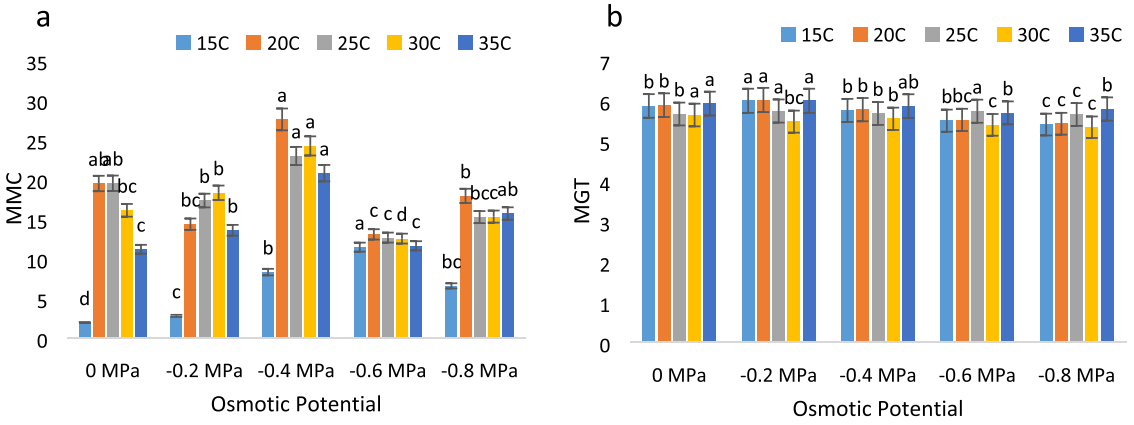

Figure 4. Interactive effect of water potential and temperature on (a) mean moisture content (MMC) and (b) mean germination index (MGT) of *Cucumis melo* L. var. NIFA 2022. The treatments exhibit dissimilar letters within rows that represent significance ($p \leq 0.05$) level.

3.3. Correlation and PCA Results of Germination and Antioxidants Enzymes to Fluctuating Temperatures and Water Potentials. The results in Figure 6 showed that GE was positively correlated with GI, G% GRI, SVI−I, MMC, and RSR and had a negative correlation with MGR, SVI−II, PMCS, PMCR, CAT, SOD, POD, APX, and GPX. All the antioxidant enzymes were positively correlated with each other. According to the results shown in Figure 7, two different clusters were formed between treatments. The first cluster consisted of 0 MPa, −0.2 MPa, and −0.4 MPa treatments, while the second cluster consisted of a control group, −0.6, and −0.8 MPa. For the germination data set, PCA was employed. According to the results, all treatments were widely spread throughout the data set. The treatment distribution reveals that the osmotic potential had a considerable impact on the germination properties. The PCA findings revealed that the first two components described 76% of the overall variance. A PCA base biplot is shown in Figure 8.

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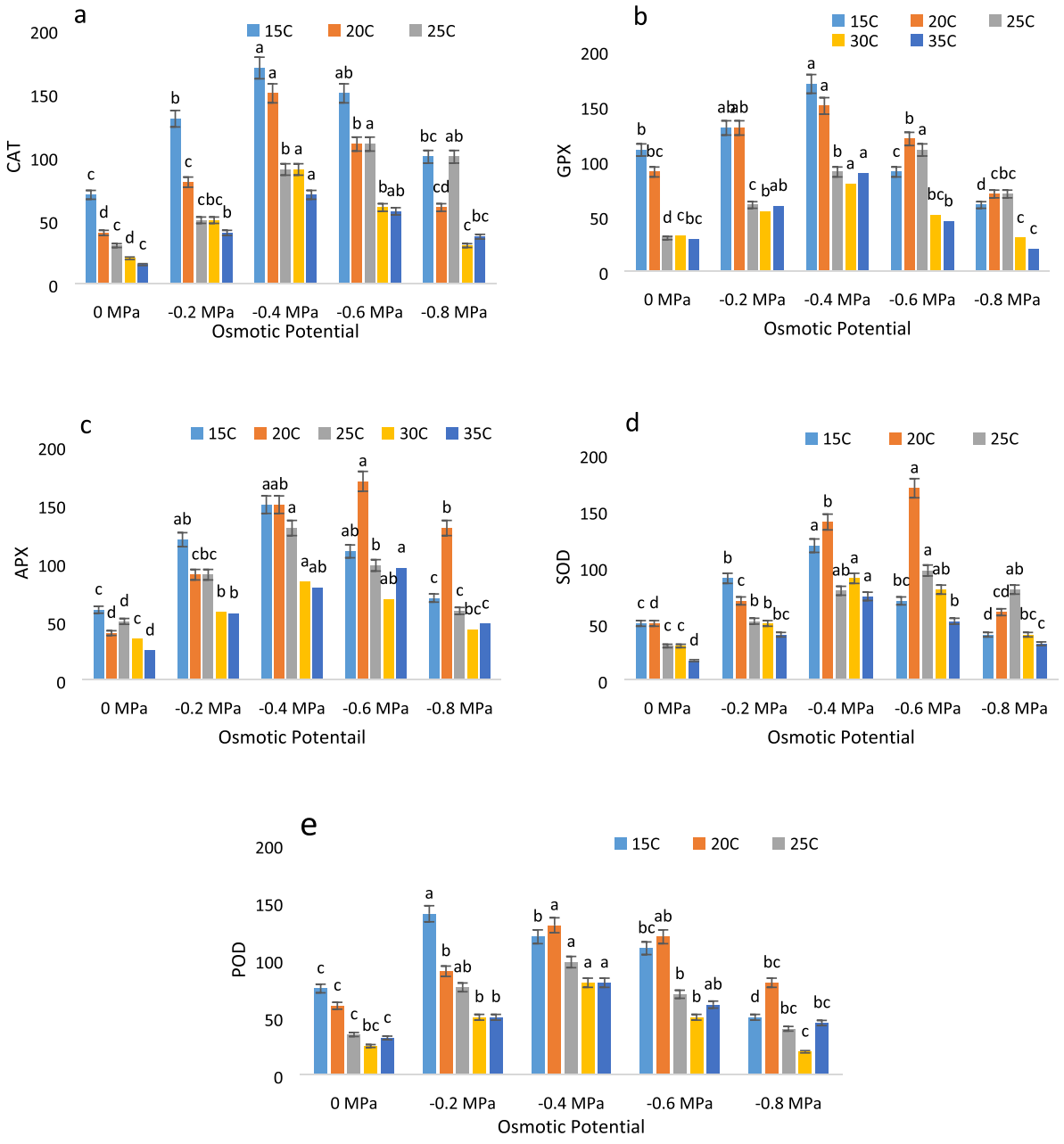

Figure 5. Interactive effect of water potential and temperature on antioxidant enzymes (a-CAT, b-GPX, c-APX, d-SOD and e-POD) of *Cucumis melo* L. var. NIFA 2022 under PEG induced stress at (a) 15 °C (b) 20 °C (c) 25 °C (d) 30 °C (e) 35 °C. The treatments exhibit dissimilar letters within rows that represent significance ($p \leq 0.05$) level.

4. DISCUSSION

Analyzing germination characteristics under different environmental conditions is necessary to pinpoint the best location for a species to flourish and germinate. To measure how these stresses affect seed germination in this way, mathematical models such as the HT, TT, and HTT models are helpful. Patterns of germination are evaluated under various environmental conditions to determine the species' optimal geographical habitat for a species. Based on population, the threshold model, commonly known as the hydrothermal time model, shows the noticed responses of germinating seed to stress, hormones, age, and other factors. The HTT model reflects the detected response of germinating seeds to stress, such as water availability and temperature. Another abiotic stress that prevents young seedlings and seeds from sprouting is water stress. Hydrothermal time (HTT), thermal (TT), and hydrothermal time (HT) models were created by several researchers as efficient ways to characterize and forecast seed population germination reactions under various environmental variables. These models are frequently employed as tools in both agronomical and basic research, since they are simple to use and provide a clear biological interpretation of the parameters.

Declines in plant growth characteristics such as LAI and LAR have been mainly caused by stomatal closure during osmotic stress conditions.[61] Polyethylene glycol-induced osmotic stress reduced the hypocotyl length and fresh and dry weight of maize root (*Zea mays* L.), while improving root length. Occasionally, moderate drought has no obvious harmful impact on root growth.[62,63] The germination process

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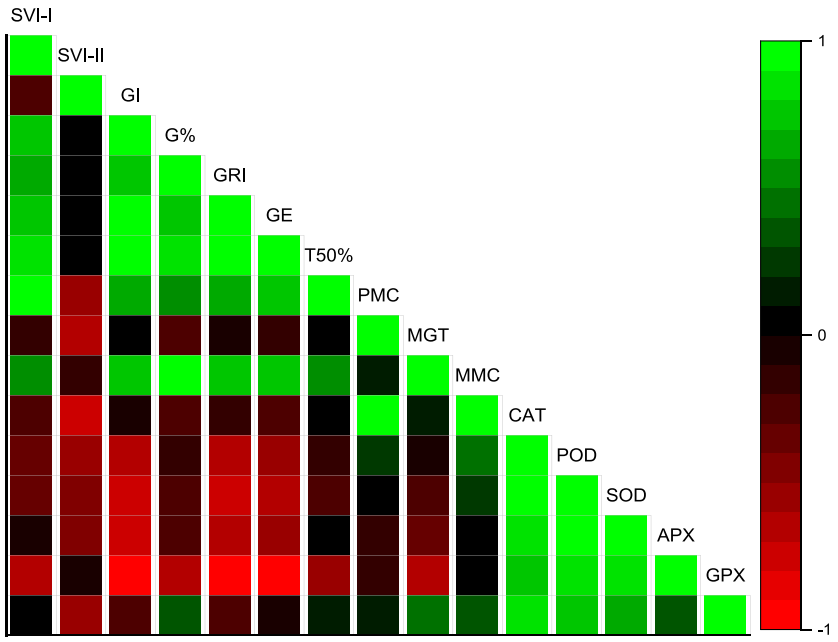

Figure 6. Correlation between various germination attributes of *Cucumis melo* L. var. NIFA 2022 using hydrothermal time model.

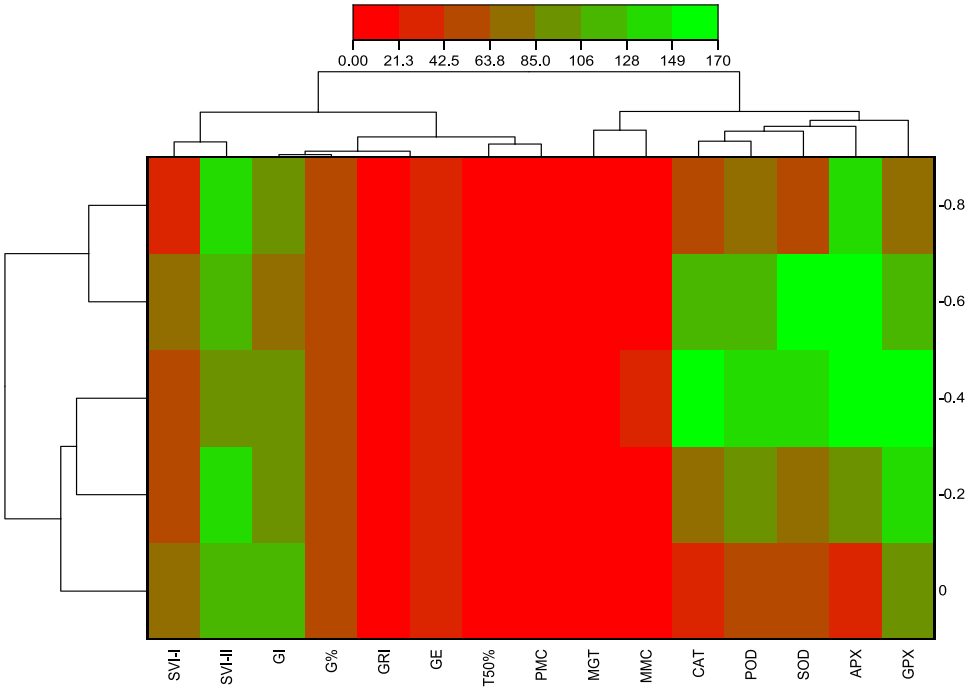

Figure 7. Heat map histogram correlation between various germination attributes of *Cucumis melo* L. var. NIFA 2022 using hydrothermal time model.

depends on many factors such as water, temperature, and time. Every area in the world has environmental factors. Water and temperature are important elements in the germination of seeds. The HT and TT models are frequently used to characterize the influence of temperature on germination and water potential (Ψ)on germination, respectively.[64] The influence of temperature and water potential on seed germination may be described and quantified using the HTT model.[23,48] The germination rate index and germination energy were the highest at 30 °C (MPa) and the lowest at 35 °C. The results of this study were similar to the findings made by Biligetu and Coulman,[65] Gul and Weber,[66] and Bewley et al.,[67] who documented that agronomic parameters were significantly affected by ψ and temperature. The rate of seed imbibition decreases as Ψ becomes more negative (decreased ψ), and as a result, the rate of germination and the percentage of germination both diminish (and may even stop altogether).[68] Reduced ψ has already been shown to have a deleterious influence on seed germination in numerous species as well as some commercial crops such as safflower,[69] *Solanum tuberosum* L.(potato),[48] *Cucurbita pepo* L.(zucchini),[43] and *Sesamum indicum* L. (sesame).[70] This section of the study focuses on how temperature, water potential, and their interaction affect seed germination in each experiment when

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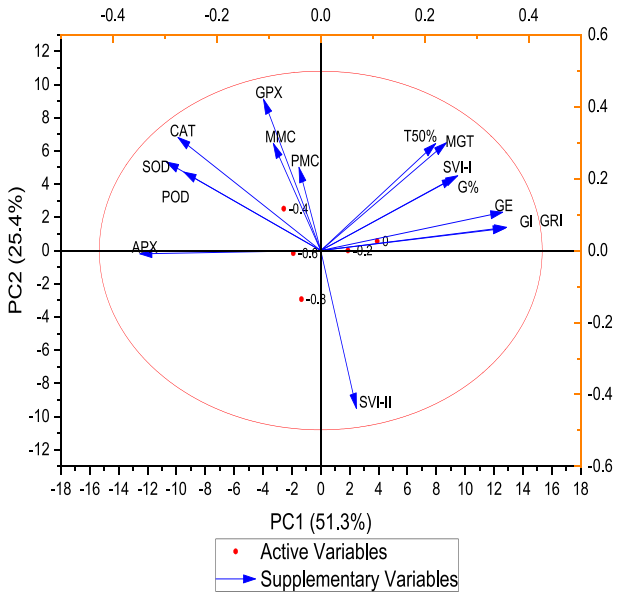

Figure 8. Loading plot of principal component analysis (PCA) on various germination attributes of *Cucumis melo* L. var. NIFA 2022 using a hydrothermal time model.

PEG stress is applied to each treatment. Within the temperature range of 15−40 °C, germination increased as the temperature climbed from 30 to 35 °C. According to Atashi et al.,[43] lowering water potential reduces seed germination due to temperature.[71] The hydrotime model, in general, could be utilized in reproducing real germination data, particularly at higher temperatures. The intricate enzymatic and nonenzymatic antioxidative system has developed in plants under stress conditions that reduce the detrimental effects of osmotic stresses by scavenging the reactive oxygen species produced, including APX, catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). Alongside activating antioxidant systems, oxidative stress instigates the stressed plants to accumulating soluble sugars, proline and soluble proteins to maintain osmoregulation.[72,73] Quantification of antioxidant enzymes indicated that osmotic stress and temperature variations significantly affected the concentration of antioxidant enzymes in fresh plant tissues. The results showed that maximum activity was shown at (15 °C) by CAT and GPX at (−0.6 MPa) while the lowest values of CAT and GPX were recorded at 0 MPa at 35 °C and −0.8 MPa at 35 °C respectively. Similarly, at (20 °C) the highest activities were shown by APX and SOD at (−0.6 MPa) and the lowest activities were recorded in the controlled group at 15 °C while the highest POD activity was recorded at (15 °C) in (−0.2 MPa) and the lowest value was noted in −0.8 MPa at 30 °C. It has been observed that all the antioxidant enzymes responded normally in (0 MPa) between (25 and 30 °C) temperature. However, the effect was adverse at the highest and lowest temperatures. Comparing all the osmotic and thermal responses a notable response was shown by GPX and APX at (15 and 20 °C) and −0.4 and −0.6 and −0.6 MPa osmotic stress, respectively.

Water stress tends to increase the levels of antioxidant enzymes such as SOD, peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT).[74,75] To prevent cellular damage, the antioxidant system neutralizes the ROS produced and finally scavenges through a series of biochemical and hormonal pathways.[76,77] SOD is important in the antioxidant defense system because it serves as the first line of defense in scavenging superoxide radicals.[78] ROS dismutation catalyzed by SOD produces H_2O_2 as a reaction product, which is then scavenged by CAT and APX activities.[79,80] Under water-limited regimes, the levels of APX and guaiacol peroxide (GPX) were reduced. APX is a crucial antioxidant that scavenges ROS during oxidative stress.[81] APX undergoes the catalysis of H_2O_2 and converts it to normal water using ascorbate as a donor of electrons. APX expression is differently regulated in response to environmental stresses as well as during normal plant development and growth.[82,83]

In order to determine an appropriate geographic location for the germination and establishment of a species, various abiotic factors had to be considered. The mathematical model (TT, HT, and HTT models) can aid in quantifying the effect of these abiotic factors on the seed germination time course within a seed lot in a practical sense by calculating the effect of these abiotic factors. Several abiotic factors affect seed germinations in several plants, with temperature being one among them (very high and very low), which is now known to be one of the most devastating limiting factors. It is also important to note that water stress is a major factor limiting the early germination of seeds.[23,42] As a result of our research, we also found that the germination of seeds was significantly affected by the temperature and changing water potential. A reduction in the germination characteristics of seeds upon exposure to a low osmotic potential has also been reported. These reductions can be attributed to the effects of high temperature and low osmotic potential on cellular mechanisms and chemical reactions within the seeds.[43] There is a strong correlation between these stresses and crop agronomy, since the attributes most closely related to these stresses are those that represent the component of highest concern to both farmers and consumers. Based on the statistical analyses of cardinal temperatures, the HTT and the results of the germination studies, insights into how temperature and osmotic potential interact to affect seed germination were provided.

5. CONCLUSIONS

The results of the current study validated that thermal and hydrotime models correctly predicted *Cucumis melo* L. germination time in response to varying levels of the water potential and temperature. The agronomic parameters were found to be maximum at 30 °C and minimum at 15 °C. Germination percentage and germination rate were found to be dependent upon water potential and PEG concentration. In conclusion, the hydrothermal time model concept could be employed in figuring out the quantitative description of distinct seed lots' germination and their relation to water potential and varying temperatures. Employing the hydrothermal time model concept, no investigation has been made so far to figure out the enzymatic activities under fluctuating osmotic and temperature treatments in growing muskmelon seedlings.

■ ASSOCIATED CONTENT

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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Notes

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