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Optimizing germination protocols for *Citrullus colocynthis* (L.) Schrad.: A study on seed dormancy breakthrough through chemical and physical treatments

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Optimizing germination protocols for *Citrullus colocynthis* (L.) Schrad.: A study on seed dormancy breakthrough through chemical and physical treatments

Abstract: Bitter apple (*Citrullus colocynthis* (L.) Schrad.), a desert plant with medicinal properties, typically exhibits low seed germination due to dormancy. This study investigated physical and chemical treatments to improve *C. colocynthis* seed germination. A completely randomized design with factorial arrangement and three replications was used to test the effects of gibberellic acid (GA3) at concentrations of 0, 10, 50, 100, and 150 ppm, under four diurnal temperature and light (TL) regimes: (1) 20 °C (12 h light/dark), (2) 25 °C (12 h light/dark), (3) 35 °C (12 h light) / 15 °C (12 h dark), and (4) 30 °C (12 h light) / 20 °C (12 h dark). Results showed that TL regime, GA3, and their interaction significantly affected all measured germination parameters. The highest germination percentage, relative germination percentage, mean daily germination, germination index, germination value, and seedling dry mass were achieved with 100 ppm GA3 under the 35-15 °C regime. The study concluded that average germination speed, germination time variation coefficient, germination speed coefficient, and synchronization index were unreliable for assessing seed quality under low germination conditions, suggesting that physiological dormancy in bitter apple seeds may stem from embryo immaturity, inhibitory factors, or both.

Key words: seed dormancy, physiological dormancy, diurnal temperature alternation, gibberellic acid.

Optimiziranje protokolov kalitve kolokinte (*Citrullus colocynthis* (L.) Schrad.): Študija o prekinitvi mirovanja semen s kemičnimi in fizikalnimi obdelavami

Izvleček: Kolokinta (*Citrullus colocynthis* (L.) Schrad.)), puščavska rastlina z zdravilnimi lastnostmi, ima zaradi dormance semen majhno kalivost. V raziskavi so bila preučevana fizikalna in kemična obravnavanja za izboljšanje kalitve semen te rastline. Izveden je bil popolni naključni faktorski poskus s tremi ponovitvami za preučevanje učinkov giberilinske kisline (GA3) v koncentracijah 0, 10, 50, 100 in 150 ppm, v diurnalnih temperaturno-svetlobnih režimih (TL): (1) 20 °C (12 h svetloba/tema), (2) 25 °C (12 h svetloba/tema), (3) 35 °C (12 h svetloba) / 15 °C (12 h tema), in (4) 30 °C (12 h svetloba) / 20 °C (12 h tema). Rezultati so pokazali, da so ti režimi in GA3, ter njihove interakcije značilno vplivali na vse merjene parameter kalitve. Največje vrednosti parametrov kot so največji odstotek kalivosti, relativni odstotek kalivosti, poprečna dnevna kalivost, indeks kalivosti, vrednost kalivosti in suha masa sejank so bile dosežene pri 100 ppm GA3 in režimu 35-15 °C. Na osnovi raziskave je bilo ugotovljeno, da poprečna hitrost kalitve, koeficient spreminanja časa kalitve, koeficient hitrosti kalitve in sinhronizacijski indeks niso primerni za ocenjevanje kakovosti semen v razmerah majhne kalivosti, kar nakazuje, da fiziološka dormanca semen kolokinte lahko izvira iz nezrelosti embrija ali inhibitornih dejavnikov ali obeh skupaj.

Ključne besede: dormanca semen, fiziološka dormanca, diurnalno menjavanje temperatur, giberilinska kislina

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1 INTRODUCTION

Citrullus colocynthis (L.) Schrad., commonly known as bitter apple, is a perennial herb in the Cucurbitaceae family. This plant features a small prostrate or climbing stem with perennial rootstocks and can reproduce through seeds and vegetative buds (Li et al., 2022). Bitter apples exhibit anti-inflammatory and antibacterial properties that are utilized in the treatment of various diseases, including type II diabetes (Jafarizadeh et al., 2022) and breast cancer (Perveen et al., 2021). Additionally, *C. colocynthis* produces a significant quantity of oily seeds, which can be converted into inexpensive biodiesel (Aziz et al., 2023). The essential oil derived from *C. colocynthis* has also been used in biological pest control to enhance resistance to whitefly infestations in watermelon cultivars (Kahrom et al., 2022). Seed germination and dormancy are critical factors in the cultivation and development of *C. colocynthis* (El-Keblawy et al., 2017).

Seed dormancy is a temporary block to germination, even in the presence of favorable environmental conditions. It is an adaptation that allows seeds to delay germination until conditions are suitable for the seedling's survival (Klupczyńska and Pawłowski 2021). In dry climates, germination can be hindered by adverse conditions such as drought and high temperatures (Vicente et al., 2020). Many desert plants, such as *C. colocynthis*, produce seeds that exhibit various types and levels of dormancy, which can be effectively broken by exposure to appropriate environmental cues (Al-Turki et al., 2022).

Environmental factors such as photoperiod and temperature significantly regulate the physiological processes that induce germination and break seed dormancy (Yan and Chen 2020). G.).))For many species, an optimal light duration signals the right time for seeds to awaken from dormancy, ensuring their growth aligns with favorable seasonal conditions (Kharshiing et al., 2019). Similarly, temperature influences enzymatic activity and metabolic rates within the seed, facilitating the breakdown of growth inhibitors and the activation of growth-promoting hormones (Zhang et al., 2021). Research has shown that specific combinations of light and warmth can enhance germination rates and synchronize seedling emergence, contributing to plant diversity and ecosystem stability (Bhatla and Lal 2023, Zheng et al., 2005).

Growth regulators like GA_3 are phytohormones used to enhance germination and seed establishment (Silva Edvan Costa Da et al., 2021). As a natural regulator, GA_3 significantly influences plant physiology, resulting in various agricultural and horticultural applications (Joshi et al., 2023). Notably, GA_3 promotes seed germination by being released from the embryo during the germination process, which stimulates the production of mRNA and alpha-amylase (Nedunchezhiyan et al., 2023).

Numerous studies highlight the importance of light, temperature, and GA_3 in the germination of various Cucurbitaceae species. For instance, darkness is essential for the germination of *Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Cucurbita maxima* Duchesne, *Lagenaria siceraria* (Molina) Standl., *Benincasa hispida* (Thunb.) Cogn., and *Momordica harantia* L. (Nakamura et al., 1955), as well as for *Citrullus lanatus* 'Sugar Baby' (Thanos and Mitrakos 1992) and *C. lanatus* var. citroides (Ramirez et al., 2014). Additionally, germination temperature significantly affects other Cucurbitaceae species. For example, the germination rate of melon dropped from nearly 100 % to zero at sub-optimal temperatures (Edelstein and Kigel 1990). The Sugar Baby watermelon germinated almost entirely in darkness at temperatures between 20 and 40 °C, but there was a significant decrease at 15 °C and 42.5 °C (Ramirez et al., 2014). No germination occurred in *Citrullus lanatus* var. *citroides* at day/night temperatures of 10/5 and 15/10 °C, regardless of light conditions (Ramirez et al., 2014). Mature seeds of *C. colocynthis* from the Iranian desert failed to germinate without treatments (Gharehmatrossian et al., 2014, Saberi M. et al., 2011). The dormancy of this species was attributed to a mechanical barrier in the testa (physical dormancy), rather than allelochemicals that might inhibit germination (El-Keblawy et al., 2017).

To optimize the germination and emergence of *C. colocynthis* while ensuring adequate field density, it is crucial to identify effective treatments for breaking seed dormancy. This study aimed to evaluate factors that might trigger the germination of *C. colocynthis* seeds, including light and temperature cycles, as well as GA_3 levels. The research hypotheses propose that a combination of physicochemical factors—specifically light, diurnal temperature alternation, and GA_3—can effectively break seed dormancy and enhance key parameters during germination.

2 MATERIALS AND METHODS

2.1 SEED COLLECTION

Fully ripened yellow fruits of large, uniform sizes were collected from a wild population of *C. colocyn* growing around Masjed Soleyman City in Khuzestan Province, southern Iran (48°, 24' east longitude and 31.93°, 49.30' north latitude). Immediately after collection, seeds were manually separated from the fruits, washed with water for 48 hours, dried at room temperature, and disinfected in a 10 % sodium hypochlorite solution for one minute. Fifty seeds were placed in each Petri dish, which were then transferred to the germinator. To determine seedling dry mass, samples were dried at 70 °C and weighed on a digital scale.

2.2 STATISTICAL SCHEME AND TREATMENTS

The experiment was conducted using a completely randomized design with a factorial arrangement across three replications. Seeds were exposed to different concentrations of gibberellic acid (GA_3) (0, 10, 50, 100, and 150 ppm) under alternating diurnal temperatures and light (TL) conditions. The light and temperature conditions for the seeds in this experiment were as follows:

(1) 20°C with a 12-hour light/dark photoperiod, (2) 25 °C with a 12-hour light/dark cycle, (3) a 12-hour light cycle at 35 °C followed by a 12-hour dark cycle at 15 °C, and (4) a 12-hour light cycle at 30 °C followed by a 12-hour dark cycle at 20 °C.

2.3 SOWING AND GERMINATION

Seeds were sown in 10 cm diameter Petri dishes lined with filter paper discs, each containing 10 ml of distilled water or varying concentrations of GA3, according to the respected treatment. Germination was recorded daily for 10 days at 11 AM, and seedling length was measured using a digital caliper. A seed was considered as germinated when complete germination occurred.

2.4 STUDIED TRAITS

Seedling length (SL) was measured using a digital caliper. For seedling dry mass (SDM) calculations, samples were dried in an oven at 70 °C and weighed on a digital scale with an accuracy of 0.001 g.

The germination parameters were calculated as follows:

2.4.1 Germination percentage (GP)

This criterion is "a measure of the survival of a collection of seeds" (Guragain et al., 2023).

$$GP = \frac{\sum n_i}{N} \times 100 \qquad (1)$$

where N is the total number of seeds utilized, and n_i is the number of seeds that germinated on the ith day.

2.4.2 Relative germination percentage (RGP)

As indicated in Eq. 2, "relativizing germination percentage enables comparisons between treatments that are equivalent when the quantity of dormancy disruption varies" (Guragain et al., 2023).

$$RGP = \frac{Actual\ Percentage}{the\ highest\ percentage\ among\ the\ group\ of\ data} \times 100 \qquad (2)$$

2.4.3 Mean germination time (MGT)

As expressed in Eq. 3, MGT is defined as "the average time it takes for a seed to germinate or emerge" (Guragain et al., 2023).

$$MGT = \frac{\sum n_i t_i}{\sum n_i} \qquad (3)$$

where $n_i t_i$ is the number of seeds germinated in the ith time interval, and n_i is the number of seeds germinated at the ith time.

2.4.4 Mean daily germination (MDG)

As stated in Eq. 4, MDG is defined as "the average number of seeds that germinate each day during a specified period" (Sarwar et al., 2024).

$$MDG = \frac{GP}{t_n} \qquad (4)$$

where *GP* is the final cumulative germination percentage and t_n represents the total time intervals.

2.4.5 Mean germination rate (MGR)

"*MGR* was calculated as the reciprocal of the *MGT*" (Guragain et al., 2023).

$$MGR = \frac{1}{MGT} \qquad (5)$$

2.4.6 Germination value (GV)

"The GV reflects the performance of the seed lot, indicating the health and potential growth rate of the seeds" (Sarwar et al., 2024).

$$GV = PV \times MDG \qquad (6)$$

Where *MDG* represents the mean daily germination and *PV* indicates the peak value, which is the final percentage of germination.

2.4.7 Germination index (GI)

"The germination index measures the number of days needed for a specific percentage of seeds to germinate." (Sarwar et al., 2024).

$$GI = \frac{\sum n_i}{t_i} \quad (7)$$

Where n_i and t_i are defined as described above.

2.4.8 Synchrony of the germination process (Z)

"This criterion indicates the degree of overlap among members of a specific demographic. The Z index yields a value only when two seeds complete the sprouting process simultaneously. This criterion can be estimated using Eq. 8 (Guragain et al., 2023)"

$$Z = \frac{\sum C_{ni,2}}{C_{\sum ni,2}} \quad (8)$$

$$C_{ni,2} = \frac{n_i(n_i - 1)}{2}$$

Where $C_{ni,2}$ represents the combination of seeds germinated in pairs at the ith time, n_i is the total number of seeds germinated at that time. The value of Z equals one when all seeds sprout simultaneously and equals zero when at least two seeds sprout at the same time.

2.4.9 The coefficient of the velocity of germination (CVG)

As indicated in Eq. 9, the *CVG* reflects the speed of germination and increases as the number of germinated seeds rises (Sarwar et al., 2024).

$$CVG = \frac{\sum n_i}{\sum n_i \times t_i} \times 100 \quad (9)$$

2.4.10 Coefficient of variation of germination time (CVT)

CV_t was estimated using Equation 10 (Sarwar et al., 2024).

$$CV_t = \frac{S_t}{\bar{t}} \times 100$$

$$S_t = \sqrt{\frac{\sum n_i(t_i - \bar{t})^2}{(n_i - 1)}} \quad (10)$$

where: S_t represents the standard deviation of germination time and represents *MGT*.

2.5 DATA ANALYSIS

The data were analyzed using SAS statistical software, version 9.4. A factorial analysis of variance was conducted to examine the impact of treatments on germination indices and measured traits. Means were compared using Duncan's multiple range test at a 5 % significance level. Bar charts were created using Microsoft Excel (2019). The correlation diagram was generated using the corrplot package in R Studio 2024.090 with R version 4.4.2, while the heatmap was prepared in Minitab version 22.1.

3 RESULTS

3.1 GERMINATION PERCENTAGE AND RELATIVE GERMINATION PERCENTAGE

Analysis of variance on traits related to *C. colocynthis* seed germination revealed that both TL and GA_3, along with their interaction, significantly impacted all examined indicators at the 1 % significance level (Data not shown). The treatment with 100 ppm GA_3 at an alternating temperature of 15–35 °C (12 hours at 35 °C in light and 12 hours at 15 °C in darkness) produced the highest GP of 57.33 % and RGP of 84.31 %. Additionally, GP and RGP at this alternating temperature were consistently higher across all GA_3 concentrations than at other temperature levels. In contrast, the control treatment with distilled water showed the lowest germination percentage across all conditions (Table 1).

3.2 MEAN GERMINATION TIME

The longest MGT recorded was 4.41 days, occurring with the treatment of 100 ppm GA_3 at an alternating temperature of 20-30 °C. While no significant difference was observed between the 50 ppm and 150 ppm GA_3 treatments at the same alternating temperature, the control

Table 1: Effect of temperature and gibberellic acid concentrations on the germination indices of bitter apple (*Citrullus colocynthis*) seeds.

GA3 (ppm)	T (°C)	G (%)	R (%)	MGT	MGR	CVt
0	20	0.67 ± 0.07 n	0.98 ± 0.04 k	0.67 ± 0.04 h	0.17 ± 0.01 j	0 ± 0 m
10		4 ± 0.58 kl	5.88 ± 0.03 i	2.17 ± 0.19 cdef	0.58 ± 0.05 b	20.54 ± 0.71 j
50		6.67 ± 0.67 ij	9.8 ± 0.27 h	2.03 ± 0.17 def	0.5 ± 0.01 c	55.35 ± 0.63 b
100		11.33 ± 0.67 fg	16.67 ± 0.27 f	2.69 ± 0.17 bc	0.38 ± 0.01 fg	50.13 ± 0.83 c
150		11.33 ± 1.2 fg	16.67 ± 0.2 f	2.81 ± 0.1 b	0.36 ± 0.01 g	48.09 ± 0.67 d
0	25	1.33 ± 0.67 mn	1.96 ± 0.09 k	1.67 ± 0.09 fg	0.28 ± 0.004 h	0 ± 0 m
10		4.67 ± 0.67 jk	6.86 ± 0.53 i	2.44 ± 0.29 bcd	0.42 ± 0.01 def	31.82 ± 1.07 h
50		8 ± 0.58 hi	11.76 ± 0.25 gh	1.78 ± 0.22 ef	0.58 ± 0.02 b	26.22 ± 1.24 i
100		12 ± 0.58 f	17.65 ± 0.03 f	2.39 ± 0.3 bcd	0.43 ± 0.01 de	47.97 ± 0.64 d
150		9.33 ± 0.67 gh	13.73 ± 0.1 g	2.05 ± 0.39 def	0.53 ± 0.01 c	63.05 ± 0.55 a
0	20-30	2 ± 0.58 lmn	2.94 ± 0.08 jk	1 ± 0.06 h	0.5 ± 0.03 c	0 ± 0 m
10		6.67 ± 0.88 ij	9.8 ± 0.27 h	2.67 ± 0.09 bc	0.17 ± 0.01 j	0 ± 0 m
50		30 ± 1.15 d	45.1 ± 0.88 d	4.31 ± 0.33 a	0.24 ± 0.01 hi	15.87 ± 1.07 k
100		21.33 ± 0.88 e	31.37 ± 1.01 e	4.41 ± 0.05 a	0.23 ± 0.002 i	15.94 ± 0.39 k
150		20 ± 0.58 e	29.41 ± 0.7 e	3.9 ± 0.05 a	0.26 ± 0.003 hi	13.71 ± 0.41 l
0	15-35	3.33 ± 0.33 klm	4.9 ± 0.1 ij	1.17 ± 0.09 gh	0.89 ± 0.03 a	15.71 ± 0.35 k
10		41.33 ± 0.88 b	60.78 ± 1.75 b	2.75 ± 0.03 bc	0.38 ± 0.01 efg	34.77 ± 0.7 g
50		40.67 ± 1.45 b	59.8 ± 2.47 b	2.29 ± 0.12 bcde	0.44 ± 0.01 d	38.66 ± 0.89 f
100		57.33 ± 0.88 a	84.31 ± 1.68 a	2.44 ± 0.2 bcd	0.41 ± 0.01 def	44.2 ± 0.68 e
150		38 ± 1.15 c	55.88 ± 1.63 c	2.63 ± 0.11 bcd	0.38 ± 0.01 efg	31.75 ± 0.77 h
GA3 (ppm)	T (°C)	CVG	GI	Z	MDG	GV
0	20	16.67 ± 0.34 h	0.17 ± 0.01 i	0 ± 0 g	0.05 ± 0.005 i	0.05 ± 0.005 g
10		57.78 ± 0.62 b	0.97 ± 0.04 hi	0 ± 0 g	0.29 ± 0.02 fghi	0.44 ± 0.11 g
50		50 ± 0.66 c	2.14 ± 0.09 fg	0.28 ± 0.03 e	0.48 ± 0.05 efgh	1.62 ± 0.37 fg
100		37.5 ± 2.41 e	2.69 ± 0.25 ef	0.19 ± 0.05 f	0.81 ± 0.05 e	2.81 ± 0.27 fg
150		35.73 ± 0.4 e	2.49 ± 0.26 efg	0.16 ± 0.02 f	0.81 ± 0.01 e	2.44 ± 0.67 fg
0	25	27.78 ± 0.72 f	0.28 ± 0.01 i	0 ± 0 g	0.1 ± 0.01 hi	0.08 ± 0.04 g
10		42.06 ± 1.13 d	1.11 ± 0.02 hi	0.44 ± 0.01 c	0.33 ± 0.01 fghi	0.6 ± 0.14 g
50		58.33 ± 1.01 b	2.44 ± 0.24 efg	0.54 ± 0.03 b	0.57 ± 0.03 efg	2.29 ± 0.08 fg
100		43.3 ± 1.19 d	3.23 ± 0.05 de	0.2 ± 0.004 f	0.86 ± 0.03 e	3.43 ± 0.33 f
150		52.6 ± 1.43 c	3.31 ± 0.69 de	0.36 ± 0.04 de	0.67 ± 0.01 ef	3.71 ± 0.28 f
0	20-30	50 ± 1.15 c	0.67 ± 0.04 hi	0.33 ± 0.02 de	0.14 ± 0.004 hi	0.29 ± 0.01 g
10		16.67 ± 0.38 h	0.83 ± 0.05 hi	0.67 ± 0.03 a	0.48 ± 0.01 efgh	1.24 ± 0.04 fg
50		23.52 ± 0.65 g	4.07 ± 0.32 d	0.61 ± 0.03 ab	2.19 ± 0.53 c	16.55 ± 0.24 d
100		22.68 ± 0.25 g	2.51 ± 0.12 efg	0.36 ± 0.01 de	1.52 ± 0.01 d	8.38 ± 1.39 e
150		25.67 ± 0.34 fg	2.68 ± 0.85 ef	0.54 ± 0.03 b	1.43 ± 0.04 d	6.97 ± 0.51 e
0	15-35	88.89 ± 1.25 a	1.5 ± 0.1 gh	0.33 ± 0.02 de	0.24 ± 0.005 ghi	0.67 ± 0.05 g
10		38.31 ± 1.06 e	8.58 ± 0.26 c	0.37 ± 0.02 cd	2.95 ± 0.02 b	37.97 ± 2.63 b
50		43.93 ± 1.44 d	10.86 ± 0.24 b	0.32 ± 0.01 de	2.9 ± 0.03 b	40 ± 0.38 b
100		41.47 ± 1.73 d	14.83 ± 0.62 a	0.29 ± 0.03 de	4.1 ± 0.04 a	72.51 ± 1.66 a
150		38.13 ± 0.29 e	8.07 ± 0.46 c	0.34 ± 0.04 de	2.71 ± 0.07 b	31.94 ± 0.8 c

In each column, means that share at least one common letter are not significantly different at the 0.05 probability level, according to Duncan's multiple range test.
GA3: Gibberellic acid concentration, T: Temperature, G (%): Germination percentage, R (%): Relative germination percentage, MGT: Mean Germination Time, MGR: Mean germination rate, CVt: Coefficient of variation of germination time, CVG: Coefficient of velocity of germination time, GI: Germination index, Z: Synchrony of germination process, MDG: Mean daily germination, GV: Germination value.

treatment showed the lowest average germination time of 0.67 days at a constant temperature of 20 °C (Table 1).

3.3 MEAN GERMINATION RATE

The highest MGR (0.89) was observed in the control treatment at an alternating temperature of 15-35 °C, significantly different from the other treatments. Conversely, the lowest MGR (0.17) was recorded in the control treatment at a constant temperature of 20 °C and in the treatment with 10 ppm GA_3 at an alternating temperature of 20-30 °C, both of which were significantly lower than the other treatments (Table 1).

3.4 COEFFICIENT OF VARIATION OF GERMINATION TIME

The treatment of 150 ppm GA_3 at a constant temperature of 25 °C resulted in the highest CV_t (63.05), which was significantly different from the other treatments. In contrast, the lowest CV_t, recorded as zero, was observed in the 10 ppm GA_3 treatment at an alternating temperature of 20–30 °C, as well as in the control treatment at constant temperatures of 20 °C and 25 °C (Table 1).

3.5 THE COEFFICIENT OF VELOCITY OF GERMINATION

The highest CVG (88.89) was observed in the control treatment at an alternating temperature of 15-35 °C. In contrast, the lowest CVG (16.67) was associated with the control treatment at a constant temperature of 20 °C, as well as with the 10 ppm GA_3 treatment at an alternating temperature of 20-30 °C (Table 1).

3.6 GERMINATION INDEX

The highest GI (14.83) was observed in the treatment with 100 ppm GA_3 at an alternating temperature of 15-35 °C, and this result was statistically significantly different from the other treatments. Conversely, the lowest GI was recorded in the control treatment across all temperature levels (Table 1).

3.7 SYNCHRONY OF THE GERMINATION PROCESS

The highest Z value (0.67) was observed in the treatment containing 10 ppm GA_3 at an alternating temperature of 20-30 °C. In contrast, the Z values in treatments with an alternating temperature of 15-35 °C exhibited a consistent pattern across all GA_3 concentrations. The lowest Z values were recorded in the control and 10 ppm GA_3 treatments at a constant temperature of 20 °C, as well as in the control treatment at a constant temperature of 25 °C (Table 1).

3.8 MEAN DAILY GERMINATION

The treatment with 100 ppm GA_3 at an alternating temperature of 15–35 °C exhibited the highest MDG

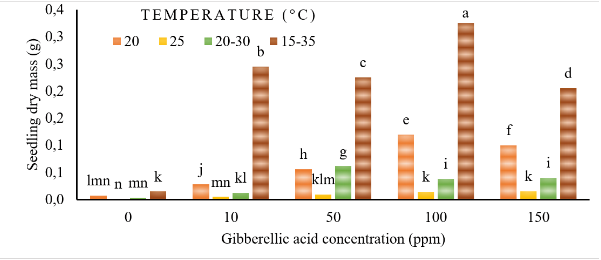

Figure 1: Effect of temperature and gibberellic acid concentrations on the seedling dry mass of bitter apple (*Citrullus colocynthis*) seeds. Columns sharing common letters are not significantly different at the 0.05 probability level, according to Duncan's multiple range test.

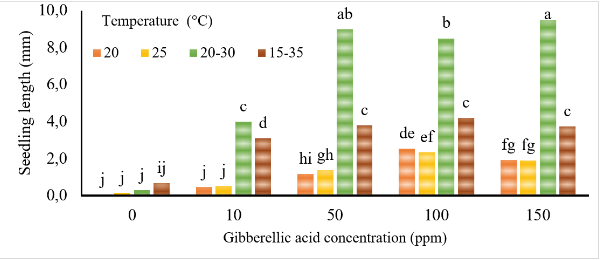

Figure 2: Effect of temperature and gibberellic acid concentrations on the seedling length of bitter apple (*Citrullus colocynthis*) seeds. Columns sharing common letters are not significantly different at the 0.05 probability level, according to Duncan's multiple range test.

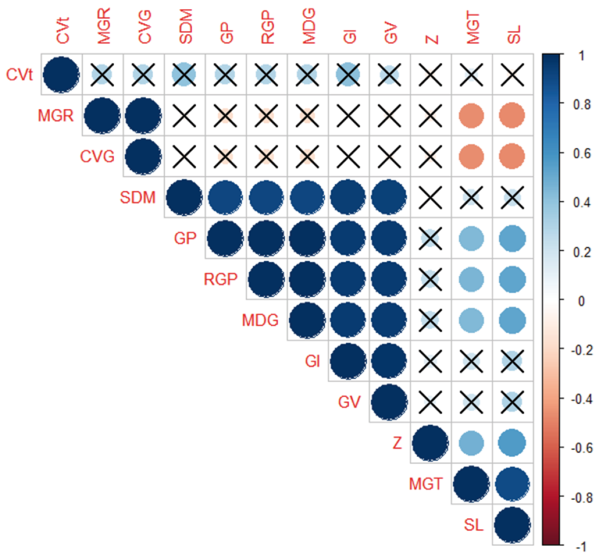

Figure 3: Graphical Pearson correlations of germination characteristics of bitter apple (*Citrullus colocynthis*) influenced by temperature periods and varying concentrations of gibberellic acid. Each circle represents a significant correlation at the 0.05 probability level. The circle's diameter reflects the correlation's magnitude, with blue indicating a positive correlation and red a negative one. The non-significant correlations, with the *p*-value above 0.05 are indicated with a cross.

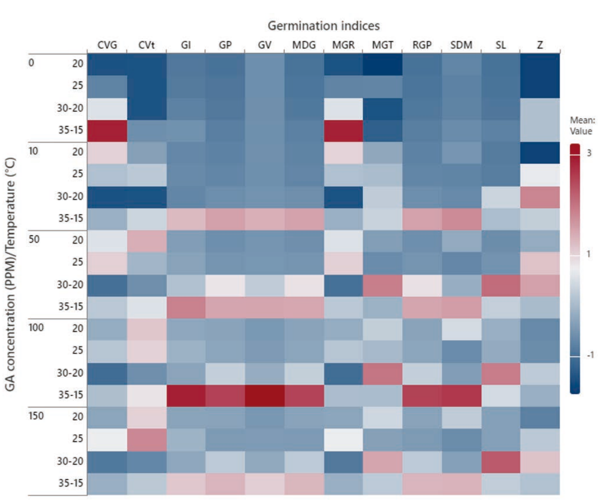

Figure 4: Heat map of germination indices of bitter apple (Citrullus colocynthis) seeds in response to temperature periods and varying concentrations of gibberellic acid. All traits were standardized for comparability. Values less than 1 appear in blue, while values greater than 1 appear in red. Color intensity reflects the magnitude of values.

(1.4), which was significantly different from the other treatments. In contrast, the control treatment, which did not contain GA_3, recorded the lowest MDG across all temperature treatments (Table 1).

3.9 GERMINATION VALUE

The highest GV of 51.72 was observed in the treatment involving 100 ppm GA_3 at an alternating temperature of 15–35 °C. In contrast, the control group and the treatment with 10 ppm GA_3 exhibited the lowest germination values across all temperature levels (Table 1).

3.10 SEEDLING DRY MASS AND SEEDLING LENGTH

The highest SDM (0.33 g) was observed in the treatment with 100 ppm GA_3 at an alternating temperature of 15–35 °C. Conversely, the lowest SDM was noted in the control treatment across all temperature levels (Fig. 1). Additionally, the maximum SL of 9.49 mm was recorded in the treatment with 150 ppm GA_3 at an alternating temperature of 20–30 °C, while the minimum SL was again found in the control treatment across all temperature levels (Fig. 2).

3.11 PEARSON CORRELATIONS BETWEEN GERMINATION INDICES

Pearson's coefficient was calculated to evaluate the correlation between various germination indices, as illustrated in Figure 3. MGT and SL were negatively and significantly correlated with MGR and CVG, but positively and significantly correlated with GP, RGP, MDG, and Z parameters. This indicates that along with healthy seedling growth, rapid and synchronized germination is essential for optimal germination.

4 DISCUSSION

One of the most critical strategies for uniform and successful crop production under agronomic conditions is the rapid and high germination of planted seeds. For certain crops exhibiting seed dormancy, it is essential to break dormancy appropriately. The findings indicated that, although the seeds exhibited dormancy, the application of appropriate treatments significantly enhanced their germination rate and effectively overcame this dormancy. Notably, the highest values for GP, RGP, MDG, GI, GV, SDM, and the shortest MGT were observed at alternating temperatures of 15-35 °C with a GA_3 concentration of 100 ppm. However, interaction effects between temperature and GA_3 have been observed on the germination indices of seeds from regions with hot, dry summers and cold winters. During the germination stage, GA_3 is one of the most important factors influencing the release of food reserves, including starch, in the seeds of these species (Baskin and Baskin 2014). A study on eggplant seeds found that the germination percentage was significantly higher at alternating temperatures (20-30 °C or 20-35 °C) compared to a constant temperature of 25 °C (Ozden et al., 2021). Specifically, alternating temperatures, which mimic natural daily fluctuations, are more effective in enhancing seed germination rates (Hung et al., 2004). Several studies have attributed the positive effects of alternating temperatures on germination to a decrease in abscisic acid (ABA) synthesis and a reduction in the ABA to GA_3 ratio (Ali-Rachedi et al., 2004, Huarte and Benech-Arnold 2010). Asaadi and Heshmati (2015) reported the highest germination percentage with 100 ppm GA_3 when breaking the dormancy of Khorasani thyme (*Thymus transcaucasicus* Ronn.), which aligns with our results. They linked this outcome to GA_3's role in the synthesis of auxins and cytokinins, necessary for inducing dormancy break. Our findings are consistent with those of Shahmoradi et al., (2015), who investigated the mechanisms of dormancy breaking in wild barley (*Hordeum spontaneum* (K. Koch) Thell.).

This study indicates that MGT is not a reliable metric for evaluating the impact of treatments on germination across all conditions. In scenarios where the seed germination rate is low, MGT fails to effectively reflect seed quality (Omidi et al., 2012). In this investigation, although the control treatment at a constant temperature of 20 °C produced the shortest MGT (0.67 days), it was considered an unsuitable treatment. Conversely, the treatment with 100 ppm GA_3 under an alternating temperature regime of 15-35 °C, which achieved the highest germination percentage, was identified as the most effective treatment, despite its lower MGT.

The GI is a significant indicator of the relationship between the percentage and rate of germination (Afzal et al., 2022). Begum et al., (2022) reported the highest GP and GI during high-temperature cycles (25-35 °C) compared to moderate-temperature cycles (20-30 °C), which aligns with the findings of the present study. Similarly, an experiment involving two cultivars of sage (*Salvia verbenaca* L.) found that increased temperatures correlated with enhanced germination (Javaid M. M. et al., 2018). Furthermore, studies on sweet sorghum seeds (*Sorghum bicolor* L. Moench) (Zhu et al., 2019) and *Allium stracheyi* Baker (Payal et al., 2014) demonstrated that GI also increased with higher GA_3 levels, confirming our findings.

Germination value serves as an index that integrates both the speed and completeness of germination (Czabator 1962). A higher GV serves to indicate a more favorable germination process (Rath et al., 2023). Consequently, the treatment involving 100 ppm GA_3 combined with an alternating temperature of 15-35 °C demonstrated a superior germination process compared to other treatments, as evidenced by the highest GV recorded.

The MDG rate is defined as the number of seeds that germinate per day concerning the maximum germination rate (Rath et al., 2023). The treatment involving 100 ppm GA_3 combined with alternating temperatures of 15-35 °C produced the highest MDG rate. A study investigating the germination of jatropha (*Jatropha curcas* Linn.) seeds demonstrated a significant positive effect of elevated temperatures and temperature fluctuations on MDG (Gairola et al., 2011). Additionally, another study indicated that increasing concentrations of GA_3 correlated with an enhancement in the MDG of yarrow (*Achillea millefolium* L.) seeds, which is consistent with the findings of the present experiment (Nejad et al., 2022).

The beneficial impact of GA_3 on the enhancement of SDM has been documented in various plant species (Banerjee and Roychoudhury 2020, Chauhan et al., 2019, Kumari et al., 2017, Tsegay and Andargie 2018). An increase in GA_3 concentration facilitates the augmentation of SDM by promoting the hydrolysis of seed starch, thereby converting it into accessible materials for the embryo (Esanejad et al., 2015). This phenomenon elucidates the observed increase in SDM under the treatment of 100 ppm GA_3 combined with an alternating temperature regime of 15-35 °C. Additionally, numerous studies have reported the positive influence of alternating temperature on the enhancement of SDM (Kumar et al., 2016, Nogueira et al., 2014, Pellizzaro et al., 2019). Seeds exhibiting high germination capacity demonstrate an enhanced ability to synthesize materials with greater efficiency and to transport these materials more rapidly to the developing embryonic axis. This process contributes to increased dry matter accumulation and greater seedling length (Omidi et al., 2012). Treatment with 150 ppm GA_3, in conjunction with alternating temperatures of 20-30 °C, significantly improved these outcomes, with the maximum SL recorded under this treatment condition. The beneficial effects of varying concentrations of GA_3 on SL have been documented in multiple studies (Amini et al., 2019, Payal et al., 2014, Saberi Morteza et al., 2020). Additionally, research on *Jeffersonia dubia* (Maxim.) Benth. & Hook. f. ex Baker & Moore seeds has indicated a positive influence of alternating temperatures on the enhancement of SL (Rhie et al., 2015). Similarly, an investigation involving sunflower seeds (*Helianthus annuus* L.) revealed that the greatest SL occurred at alternating temperatures of 20-30 °C, aligning with the findings of the present study (Yari et al., 2014).

The positive influence of GA_3 on seed germination is primarily associated with the stimulation of hydrolyzing enzyme synthesis within the seed. This mechanism aids in the degradation of starch, proteins, and other nutrients, thereby promoting the transfer of these essential substances from the endosperm to the developing embryo. Moreover, GA_3 enhances the activity of the catechol oxidase enzyme, which reduces seed phenolic compounds, further facilitating germination. Additionally, it promotes the synthesis of DNA and proteins, which consequently impacts the phospholipid composition of the embryo's cell membrane (Yousefi et al., 2021).

Among the various environmental factors influencing seed germination, temperature is recognized as the most critical determinant (Javaid Muhammad Mansoor et al., 2022). Research indicates that alternating temperatures significantly contribute to alleviating seed dormancy and stimulating germination (Ozden et al., 2021). Seeds that respond to alternating temperatures possess an enzymatic mechanism that functions optimally under varying thermal conditions, likely due to ecological adaptation to their environment (Silva Dandara Yasmim Bonfim de Oliveira et al., 2018). Furthermore, alternating temperatures enhance seed germination by reducing the concentration of growth inhibitors present in the seed coat of *C. colocynthis* (El-Keblawy et al., 2017). Under alternating temperature conditions, the levels of germination-promoting hormones increase relative to those of germination-inhibiting hormones, thereby facilitating the breakdown of the seed's physiological dormancy (Ozden et al., 2021).

The findings of the experiment indicate that the physiological dormancy observed in the *C. colocynthis* seed can be attributed to either the immaturity of the embryo, the presence of inhibitory factors within the seed, or a combination of both (Asaadi and Heshmati 2015). The mass production and economic viability of the *C. colocynthis* plant, which holds medicinal, edible, and industrial significance, depend on the implementation of appropriate treatments aimed at enhancing germination and overcoming seed dormancy (Saberi Morteza et al., 2017). The results of this study suggest that alternating temperatures of 15-35 °C, in conjunction with a gibberellic acid concentration of 100 ppm, represent the most effective treatment for alleviating the dormancy of the *C. colocynthis* seed.

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