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Examine the Effects of Various Chemicals and Different Environmental Conditions on the Breakdown of Dormancy of Dragon Fruit (*Hylocereus undatus* var. White Fleshed Pitaya)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The dragon fruit, has attracted a lot of interest lately due to its eye-catching appearance and exceptional nutritional value. Despite its widespread use, little is known about the ideal circumstances for seed germination and the early stages of growth, such as the length of the

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seedling shoot, which are critical for the best crop production. By assessing the effects of different growing media on dragon fruit germination and early growth stages, this study seeks to close this gap. The experiment was laid out in the GPB (Genetics & Plant breeding) PG lab, Maxwell block, School of Agriculture (SOAG), ITM University, Gwalior, M.P. during 2024 in Completely Randomized Design with 9 treatments and 4 replications, i.e., T_0 (Hydropriming in room temperature at 20-24˚C), T_1 (Hydropriming 28˚C), T_2 (Sand+ water mixture room temperature 20-24˚C), T_3 (Sand+ water mixture in seed germinator 28˚C), T_4 (Blotting sheet+ water in room temperature 20-24˚C), T_5 (Blotting sheet+ water in seed germinator 28˚C), T_6 (Citric acid treatment in room temperature 20-24℃), T_7 (GA_3 treatment 100 ppm Seed germinator at 28℃), T_8 (TiO_2 NPs 200 ppm Seed germinator at 28℃). Seeds were sown in petri plates on 26th February, 2024 in seed germinator and room temperature. The highest germination efficiency or percentage with 96.67%, followed by T_8 (TiO_2 NPs treatment in seed germinator at 28˚C) with 80% and T_7 (GA_3 treatment in seed germinator at 28˚C) with 78.33%, while T_0 (Hydropriming in room temperature at 20-24^{0}C) has the lowest germination efficiency or percentage with 8.67%. The significantly highest seedling vigor index was recorded in T_6 (Citric acid treatment room temperature at 20-24˚C) with 462.67 followed by T_8 (TiO_2 NPs treatment in seed germinator at 28˚C) with 353.67 and T_7 (GA_3 treatment in seed germinator at 28˚C) with 343.57 mg, while lowest seedling vigor index was recorded in T_0 (Hydropriming in room temperature at 20-24˚C) with 32.67.

Keywords: Hydropriming; TiO2; citric acid; germination; dragon fruit; GA3.

1. INTRODUCTION

The tropical fruit known as a dragon fruit *(<u>Hylocereus</u> <u>undatus</u>*), often called a strawberry pear or pitahaya /pitaya or kamalam is distinguished by its bright red exterior and delicious flesh that is scattered with seeds. The hylocereus species are diploid having a chromosome no.- 2n=2x=22, which are members of the cactaceae family, are the source of a large range of dragon fruits with appealing and intriguing functional qualities. Because of its nutritional and therapeutic qualities, dragon fruits have become more and more well-known in recent years. Dragon fruit is one of the superfoods of the tropics because of its high nutritional value [1-4].

The fruit comes in three varieties: pink pitaya (white flesh with pink skin: *Selenicereus undatus*), red pitaya (red flesh with red skin: *Selenicereus costaricensis*), and yellow pitaya (white flesh with yellow skin: *Selenicereus megalanthus*) [5].

The dragon fruit seed, which originated in Mexico and central and south America, is an edible black seed that resembles kiwi seeds in appearance. It is embedded in the fruit pulp and has exceptional nutritional value, drawing growers from all over India to cultivate this fruit crop [6]. About 82.5 to 83.0% moisture, 0.16 to 0.23% protein, 0.21 to 0.61% fat, and 0.7 to 0.9% fiber are found in fresh dragon fruit. 6.3–8.8 mg of calcium, 30.2–36.1 mg of phosphorus, 0.5-0.61 mg of iron, and 8–9 mg of vitamin-C is found in 100g of fresh fruit pulp (Kabir *et al*. 2022).

A physiological state known as seed dormancy prevents germination under ideal circumstances. Dragon fruit seed dormancy is a physiological state that limits germination under ideal circumstances and establishes the spectrum of circumstances that permit germination. The degree of "whole-seed" dormancy can be determined by the interaction between an embryo and a coat component of the intrinsic molecular mechanisms governing dormancy [7]. Dragon fruit cultivation has faced significant challenges due to poor seed germination and seed dormancy, impacting overall production and profitability. Seed germination rates for dragon fruit are notably low, often resulting in uneven and delayed plant establishment. This inconsistency can lead to a prolonged cultivation cycle, increasing labor and resource inputs for farmers. Furthermore, seed dormancy—where seeds fail to germinate even under optimal conditions—compounds the issue, necessitating additional treatments such as stratification or chemical applications to break dormancy and enhance germination rates. These practices can be both time-consuming and costly, reducing the efficiency and economic viability of dragon fruit farming.

Present research used various chemicals like, GA_3, Citric acid and TiO_2 Nano particles for stimulating the seed germination. It has been observed from the previous research work that nanoparticles will help to stimulate the RoS enzymes and promote the seed germination. GA_3 is a growth regulator which promotes the seed metabolism to enhance the germination percentage and seedling attributes. Different growth condition helps to encourage the seed germination by providing the favourable condition. Henceforth keeping the above facts in view, the present study entitled "Examine the effects of various chemicals and different environmental conditions on the breakdown of dormancy of Dragon fruit (*Hylocereus undatus* var. White fleshed pitaya)" was conducted.

2. MATERIALS AND METHODS

2.1 Experimental Unit

The present experiment was laid out in the GPB (Genetics & Plant breeding) PG lab, Maxwell block, School of Agriculture (SOAG), ITM University, Gwalior, during 2024.

2.2 Location and Climate

Gwalior is situated in Gird Zone at the latitude of 26^0.13' North and longitude 76^0.14' east with an altitude of 211.52 meters from mean sea level, in Madhya Pradesh. The maximum temperature goes up to 45^0C during summer and minimum as low at 5^0C during winter.

2.3 Experimental Details

Design: CRD (Completely Randomized Design)
Replications: 4 (As per ISTA, 1924)
No. of Treatments: 9
Total no. of petri plates: 36
Total no. of seed/ plate: 20
Date of experiment conduct: 26^{th} February 2024
Variety: Red skin with white flesh
Season of experiment conduct: (Feb- April)
Lab temperature: 20-24^0 C
Seed germinator temperature: 28^0C
Relative humidity: 85%

2.4 Treatment Applications

The raw material- Dragon fruit i.e., pink skin with White flesh variety, from a KVK of Gwalior. Seeds was extracted from a dragon fruit. For breaking the seed dormancy different chemicals and media used, such as: - Citric acid in which lemon is used was bought from local market of Gwalior. GA_3 (Gibberellic acid), Blotting sheet, Tissue paper, TiO_2NPs, Sand, Soil, Vermicompost & Seed tray was available in the Department of Horticulture, School of Agriculture, ITM university, Gwalior.

2.5 Preparation of GA_3 Solution

The stock solution of 100ppm GA_3 solution was prepared by dissolving 0.1g or 100mg in little quantity of acetone and then made up the volume of 1l by adding distilled water.

2.6 Preparation of TiO_2 NPs Solution-

The stock solution of 200ppm $Ti0_2$ NPs solution was prepared by dissolving 0.2g or 200mg in 1l of distilled water.

Seeds were extracted from a dragon fruit i.e., pink skin with white flesh (*Selenicereus undatus*). The fruit was then cut into small pieces with a cutting knife and the seeds were extracted from it with the help of Forceps. Thus, we treated the seeds in different conditions which are as follows:

Hydropriming in room temperature: Firstly, we added normal tap water in 4 different sets of petri plates and then we took a total of 60 fresh dragon seeds, out of which each 20 seeds were embedded in 4 different petri plates with the help of forceps. Then, kept all the three petri plates in light in the normal room temperature of 20-24^0C.

List 1. Treatment applications

Treatments	Treatment Details
T_0	Hydropriming in normal water in room temperature at 20-24℃
T_1	Hydropriming in seed germinator at 28℃.
T_2	Seeds in Sand + water mixture in room temperature at 20-24℃
T_3	Seeds in Sand + water mixture in Seed germinator at 28℃
T_4	Seeds in Blotting sheet + water in room temperature at 20-24℃.
T_5	Seeds in Blotting sheet + water in Seed germinator at 28℃
T_6	Seeds with Citric acid treatment in room temperature at 20-24℃.
T_7	Seeds with GA_3 treatment with 100ppm GA_3 in Seed germinator at 28℃.
T_8	Seeds with TiO_2 NPs treatment with 200ppm TiO_2 NPs in Seed germinator at 28℃.

Hydropriming in Seed germinator at 28^{0}C: In 4 different petri plates, we added water and 20 seeds each with the help of forceps and kept them in seed germinator at 28^{0}C.

Seeds in Sand+ water mixture at room temperature: Firstly, Sand was sterilized for an investigation with the help of Autoclave and after sterilization, a mixture of sand and water was added in 4 different petri plates in which 20 seeds each were placed in 4 different petri plates with the help of forceps. Then, all the three petri plates were kept in light in the normal room temperature of 20-24^{0}C.

Seeds in Sand+ water mixture in Seed germinator at 28^{0}C: 80 dragon seeds were divided into 4 different petri plates in which the mixture of Sand and water were added to petri plates. Then, these petri plates were kept in seed germinator at 28^{0}C.

Seeds in Blotting sheet + water at room temperature: In 4 different petri plates, 20 seeds were divided each into it and after that blotting sheet with some drops of water were added to it. Then, all four petri plates were placed in light in room temperature of 20-24^{0}C.

Seeds in Botting sheet+ water in Seed germinator at 28^{0}C: 20 dragon extracted seeds were divided each into 4 different petri plates, blotting sheet with some drops of water were added to it. Then, all the petri plates were kept in Seed germinator at 28^{0}C.

Seeds with Citric acid treatment: As we know that lemon is a good source of Citric acid, therefore, lemon was bought from the local market, Gwalior. Juice was extracted from the lemon and distributed into two Eppendorf tubes. Then, 80 seeds were extracted from a dragon fruit with the help of forceps and were transferred them into the Eppendorf tubes containing juice of lemon. Then, the tubes were kept in Deep freezer (4^{0}C) for 1 weeks. After 1 weeks, the seeds kept in the Eppendorf tubes were transferred in the petri plates containing tissue paper and water. Then, kept in the normal room temperature of 20-24^{0}C.

Seeds with 100ppm GA_3 treatment: 80 dragon extracted seeds in which 20 seeds were distributed into 4 different petri plates in which tissue paper was placed into each and after that 100ppm GA_3 prepared solution were added into petri plates. Then, kept in Seed germinator at 28^{0}C.

Seeds with 200ppm TiO_2 NPs treatment: 80 seeds were extracted from dragon fruit and divided each into 4 different petri plates in which tissue paper was placed into each and after that 200 ppm TiO_2 NPs prepared solution were transferred into petri plates. Then, kept in Seed germinator at 28^{0}C.

After performing all the treatments in different environmental conditions with different chemicals, the cotyledons which became greenish in colour and after that these are transferred in a mixture of (2:1:0.5) of Cocopeat: Vermicompost: Soil, within a Seed tray for hardening purpose.

2.7 Preparation of Media

- Take 5kg Cocopeat, 2.5kg Vermicompost and 1.25kg Soil.
- Mix all the raw material (Cocopeat + Vermicompost + Soil).
- The media was sealed in transparent Polythene and was placed in Autoclave for sterilization at 121^{0}C and 15psi for 30 min.
- Then, media was prepared for transferring into Seed tray.

2.8 Details of Observations

The following observations were recorded in respect of Germination and vigour of seedlings:

2.8.1 Final germination percentage (FGP %)

This were recorded by taking observations in 24 hrs and seeing percent of sprouting in seeds from the date of sowing. Final germination percentage were calculated by-

$$Final\ Germination\ Percentage = \frac{\text{Total no. of seeds germinated}}{\text{Total no. of seeds sown}}$$

2.8.2 Mean germination time (MGT)

It is an accurate estimate of the time it takes for a large number of seeds to germinate, but it does not correspond well with time spread or germination uniformity.

Mean germination time were calculated by-

$$\boxed{Mean\ Germination\ Time = \frac{\sum f.x}{\sum f}}$$

Where; f = Seeds germinated on day x & x= no. of days

It is expressed in Day, hour or other time unit.

2.8.3 Germination index (GI)

The Germination Index is the analytical approach that best depicts the link between germination percentage and speed.

The Germination Index were calculated by-

Germination Index = (10×n1) + (9×n2) +·+ (1×n10)

Where; n1, n2 . . . n10 = No. of germinated seeds on the first, second and subsequent days until the 10th day; 10, 9 . . . and 1 are weights given to the number of germinated seeds on the first, second and subsequent days, respectively.

2.8.4 Coefficient of velocity of germination (CVG)

Coefficient of Velocity of Germination prioritizes the time it takes to achieve germination above the final percentage.

Coefficient of Velocity of Germination were calculated by-

CVG=N1 + N2 + · · · + N_x/100 × N_1T_1 + · · · + N_xT_x

Where: N=No. of seeds germinated each day,

T=No. of days from seeding corresponding to N

2.8.5 Germination rate index (GRI)

The GRI measures the percentage of germination on each day throughout the germination phase.

Germination Rate Index were calculated by-

GRI=G_1/1 + G_2/2 +· · ·+ G_x/x

Where; G1=Germination percentage × 100 at the first day after sowing,

G2=Germination percentage × 100 at the second day after sowing

It is expressed in (%/day) unit.

2.8.6 First day of germination (FDG)

FDG= Day on which the first germination event occurred

It is expressed as a unit in “Day”.

2.8.7 Last day of germination (LDG)

LDG=Day on which the last germination event occurred

It is expressed as a unit in “Day”.

2.8.8 Time spread of germination (TSG)

The difference in germination speed between the ‘fast’ and ‘slow’ germinating members of a seed lot.

TSG= The time in days between the first and last germination events occurring in a seed lot

2.8.9 Shoot length

The Shoot length of observational seedling was measured with the help of Scale after 36 days of sowing. The shoot length was measured in Centimetres.

2.8.10 Root length

The Root length of observational seedling was measured with the help of Scale after 36 days of sowing. The Root length was measured in Centimetres.

2.8.11 Shoot (fresh weight)

Fresh weight of shoot was measured in an experiment with the help of electronic weighing balance in milligrams and the average fresh weight was worked out.

2.8.12 Root (fresh weight)

Fresh weight of root was measured in an experiment with the help of electronic weighing balance in milligrams and the average fresh weight was worked out.

2.8.13 Chlorophyll

Chlorophyll content present in leaves was measured with the help of Chlorophyll meter in SPAD value.

2.8.14 Shoot (dry weight)

Dry weight of shoot was measured after drying for 24hrs at 40˚C. Then, dried shoot was measured with the help of electronic weighing balance in milligrams.

2.8.15 Root (dry weight)

Dry weight of root was measured after drying for 24hrs at 40˚C. Then, dried root was measured with the help of electronic weighing balance in milligrams. The average dry weight was worked out.

2.8.16 Vigor index

Vigor index was calculated by

Vigor index = % Seed germination x Total seedling length

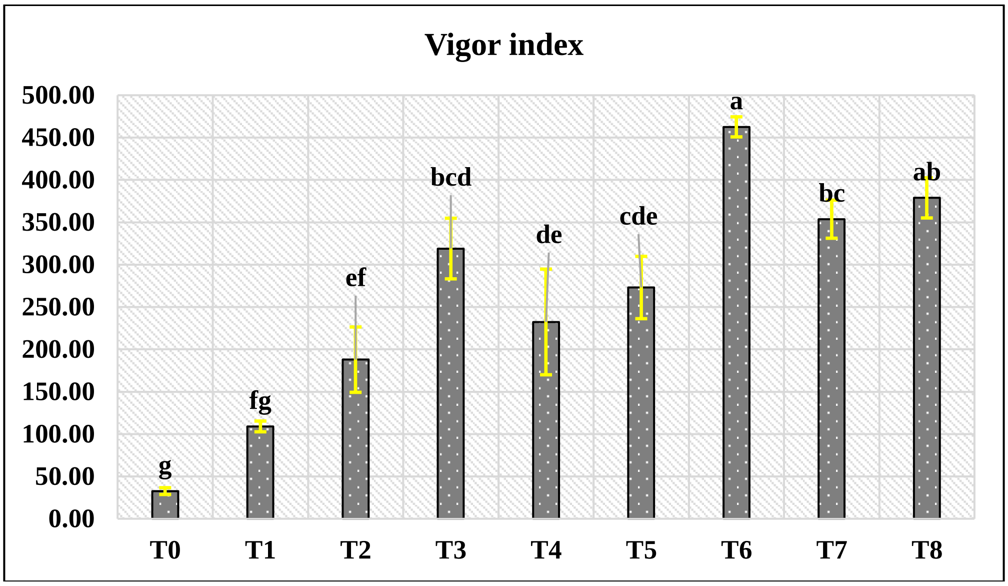
2.9 Statistical Analysis

The data obtained from all the above experiments were tabulated in 4 replications and subjected to statistical analysis (ANOVA) with completely randomized design (CRD) and results were tested at a level of 1% significant level using OPSTAT, WASP & Duncan's test. The significance of treatments was worked out by comparing the difference between two treatments mean using CD at 1% level of significance.

3. RESULTS AND DISCUSSION

3.1 Comprehensive Evaluation of Germination Attributes

Experiment has been conducted; data were collected in every 24 hours to determine the overall percentage of seeds germinated in how many seeds were sowed. T_6 (Citric acid treatment in room temperature at 20-24^{0}C) in Table 1 has the highest germination efficiency or percentage with 96.67%, followed by T_8 (TiO_2 NPs treatment in seed germinator at 28˚C) with 80% and T_7 (GA_3 treatment in seed germinator at 28˚C) with 78.33%, while T_0 (Hydropriming in room temperature at 20-24^{0}C) has the lowest germination efficiency or percentage with 8.67% (Fig. 1). T_0 (Hydropriming in room temperature at 20-24^{0}C) has the lowest germination due to the possibility that seeds may absorb water more slowly at room temperature, which will result in decreased germination rates as per observed by Ashraf et al., [8]. T_6 has the highest germination percentage because Citric acid, a natural organic acid, helps in the maintenance of an acidic environment required for the activation of specific enzymes involved in the germination process, as well as the breakdown of stored food reserves in the seed, which provides energy and nutrition to the growing embryo. Similar findings have been noted by Nonogaki et al., [9]. T_1 (Hydropriming in a seed germinator at 28^{0}C) with 4 days germinated the quickest and had the best mean germination time, followed by T_3 (seeds in sand and water mixture in seed germinator at 28^{0}C) with 5.05 days and T_8. T_6 required the longest time to germinate, which is why it is high in final germination % but not in mean germination time. T_0 had the lowest mean germination time with 11.31 days. T_6 (Citric acid treatment in room temperature at 20-24˚C), shown the best results with 30.03%, followed by T_8 with 13.51% and T_7 with 12.14%. T_0 had observed the lowest coefficient of velocity of germination of seeds with 0.12% CVG (Coefficient of velocity). %. According to Bailly et al*.* [10], the coefficient of velocity of germination (CVG) is a measure of the rate at which germination occurs. It evaluates how rapidly a seed lot germinates during a specific time period. Citric acid treated seeds considerably improves the coefficient of germination due to its antioxidant qualities, which serve to alleviate oxidative stress, protect the seed's cellular machinery, and promote quicker germination. The highest germination index was recorded in T_6 (Citric acid treatment in room temperature at 20-24˚C) with 142.33, followed by T_8 (TiO_2 NPs treatment in seed germinator at 28˚C) with 131.33 and T_7 (GA_3 treatment in seed germinator at 28˚C) Blotting sheets effectively retain moisture, and their porous structure allows for proper air circulation. It gives a quantitative measure of how quickly and uniformly seeds germinate [11]. The lowest germination index was noted in T_0 (Hydropriming in room temperature at 20-24˚C) with 16.33. T_3 (seeds in sand water mixture in seed germinator at 28^{0}C) exhibited the best first day of germination with 2.00 days, followed by T_1 (hydropriming in seed germinator at 28^{0}C) with 2.33 days and T_8 with 2.67 days. Meanwhile, T_0 has shown least in first day of germination or took maximum days to germinate with 11 days. In Table 1. it was noted that T_2 (seeds in a sand + water mixture in room temperature at 20-24˚C) showed the best results on the last day of germination with 13.00 days, followed by T_7 (GA_3 treatment in a seed germinator at 28^{0}C) with 12.00 days, and T_4 (seeds in a blotting sheet + water in room temperature at 20-24˚C) with 11.33 days. T_7 (GA_3 in seed germinator at 28⁰C) has shown maximum efficiency of average days of germination with 9.33 days, followed by T_4 (seeds in a blotting sheet + water in room temperature at 20-24˚C) with 7.67 days and T_5 (seeds in a blotting sheet + water in seed germinator at 28˚C). Similar findings were noted by Nonogaki et al. [9], who concluded that T_7 (GA_3 in the seed germinator at 28⁰C) has demonstrated maximum efficiency of average days of germination because Gibberellic acid helps break seed dormancy by inducing the production of enzymes that break down the seed's stored food reserves. This enzyme activity maintains a consistent supply of energy throughout a period of time resulting in a longer germination phase. According to the data presented, T_3 the greatest first day of germination because sand can hold onto some moisture while letting extra water drain out. Large pore spaces provide for adequate air circulation around the seeds while also providing oxygen to the growing seeds, which is required for cellular respiration and the production of energy, allowing them to germinate more quickly [12,13].



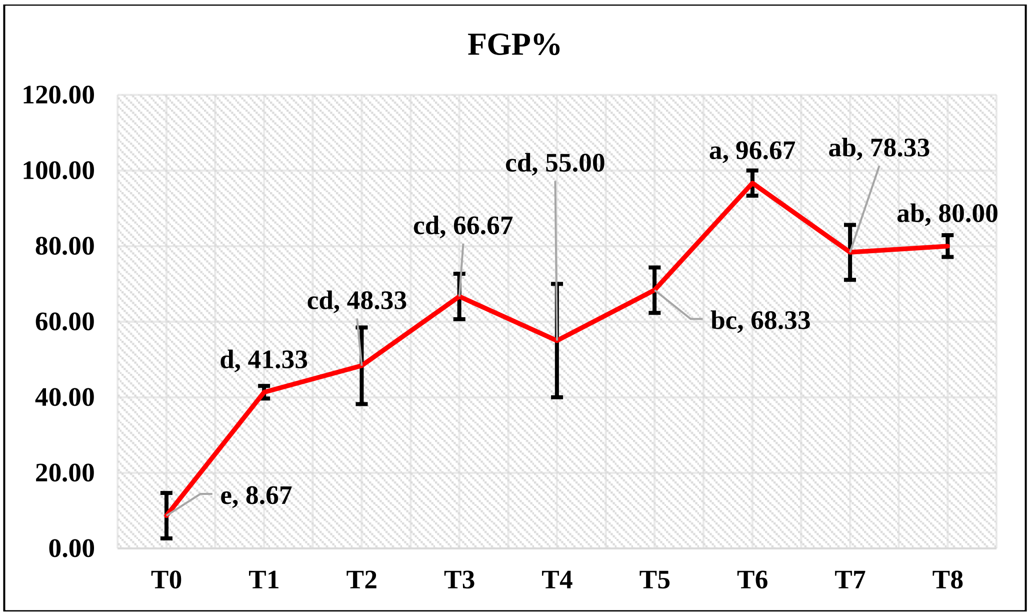


Fig. 1. Graph Represents the Final germination% and Vigour index of Dragon fruit *(Hylocereus undatus* var. White fleshed pitaya)

Table 1. Effect of various chemicals and different environmental conditions on Germination attributes of Dragon fruit (*Hylocereus undatus* var. White fleshed pitaya)

Treatment	FGP	MGT	CVG	GI	GRI	FDG	LDG	TSG
T_0	8.67 ± 6.01^{e}	11.31 ± 0.17^{d}	0.12 ± 0.123^{c}	16.33 ± 2.91^{b}	3.48 ± 0.55^{d}	11.00 ± 0.00^{a}	11.67 ± 0.33^{ab}	0.67 ± 0.33^{d}
T_1	41.33 ± 1.67^{d}	4.00 ± 1.53^{a}	8.24 ± 8.237^{b}	18.67 ± 3.93^{b}	3.66 ± 1.59^{d}	2.33 ± 0.67^{c}	5.67 ± 2.67^{d}	3.33 ± 2.40^{cd}
T_2	48.33 ± 10.14^{cd}	10.87 ± 0.13^{a}	11.14 ± 4.26^{b}	29.67 ± 5.49^{b}	4.81 ± 1.17^{cd}	7.00 ± 2.00^{b}	13.00 ± 0.00^{a}	6.00 ± 2.00^{abc}
T_3	66.67 ± 6.01^{cd}	5.05 ± 0.48^{d}	8.88 ± 1.92^{b}	58.33 ± 7.27^{b}	7.66 ± 0.96^{bcd}	2.00 ± 0.00^{c}	9.00 ± 1.00^{bcd}	7.00 ± 1.00^{abc}
T_4	55.00 ± 15.00^{cd}	9.08 ± 0.80^{ab}	11.52 ± 5.21^{b}	112.33 ± 25.44^{a}	17.86 ± 2.65^{a}	3.67 ± 1.67^{c}	11.33 ± 1.20^{abc}	7.67 ± 1.76^{ab}
T_5	68.33 ± 6.01^{bc}	5.79 ± 1.23^{cd}	10.92 ± 1.09^{b}	115.67 ± 28.99^{a}	19.67 ± 6.02^{a}	3.00 ± 1.00^{c}	10.33 ± 1.20^{abc}	7.33 ± 1.33^{abc}
T_6	96.67 ± 3.33^{a}	8.02 ± 0.06^{bc}	30.03 ± 1.98^{a}	142.33 ± 4.49^{a}	12.25 ± 0.45^{abc}	7.00 ± 0.00^{b}	10.67 ± 0.33^{abc}	3.67 ± 033^{bcd}
T_7	78.33 ± 7.27^{ab}	5.83 ± 0.67^{cd}	12.14 ± 2.00^{b}	118.33 ± 18.48a	15.42 ± 3.89^{ab}	2.67 ± 0.33^{c}	12.00 ± 1.00^{ab}	9.33 ± 1.20^{a}
T_8	80.00 ± 2.89^{ab}	5.09 ± 0.29^{d}	13.51 ± 1.50^{b}	131.33 ± 3.76^{a}	18.71 ± 1.94^{a}	2.67 ± 0.33^{c}	8.00 ± 1.00^{cd}	5.33 ± 1.33^{abc}
C.D.	**22.56**	**2.29**	**8.05**	**44.30**	**8.20**	**2.90**	**1.63**	**4.36**
S.E.(m)	**7.54**	**0.77**	**2.69**	**14.80**	**2.74**	**0.97**	**1.21**	**1.46**

Table 2. Effect of various chemicals and different environmental conditions on Seedling attributes of Dragon fruit (*Hylocereus undatus* var. White fleshed pitaya)

Treatment	Shoot length (mm.)	Root length (mm.)	Shoot (Fresh weight) (gm.)	Root (Fresh weight) (gm.)	Chlorophyll	Shoot (Dry weight) (gm.)	Root (Dry weight)	Vigor Index
T_0	2.60 ± 0.42^{b}	0.13 ± 0.03^{d}	15.00 ± 3.93^{d}	0.43 ± 0.06^{e}	2.80 ± 0.35^{e}	0.060 ± 0.042^{c}	0.011 ± 0.001^{e}	32.67 ± 4.00^{g}
T_1	3.57 ± 0.55^{ab}	0.20 ± 0.06^{cd}	23.73 ± 5.47^{cd}	0.57 ± 0.05^{de}	4.05 ± 0.79^{e}	0.200 ± 0.025^{bc}	0.013 ± 0.002^{cde}	109.00 ± 6.33^{fg}
T_2	3.70 ± 0.12^{a}	0.29 ± 0.06^{bc}	34.33 ± 4.11^{abc}	0.90 ± 0.17^{cde}	5.07 ± 1.24^{de}	0.157 ± 0.041^{bc}	0.017 ± 0.001^{cd}	187.83 ± 38.55^{ef}
T_3	3.53 ± 0.20^{ab}	0.34 ± 0.06^{abc}	33.87 ± 6.24^{abc}	1.20 ± 0.46^{bc}	9.03 ± 1.14^{bcd}	0.171 ± 0.062^{bc}	0.015 ± 0.001^{cde}	319.00 ± 35.77^{bcd}
T_4	3.93 ± 0.07^{a}	0.31 ± 0.06^{bc}	29.33 ± 3.94^{bc}	1.07 ± 0.01^{cd}	4.27 ± 0.61^{e}	0.189 ± 0.039^{bc}	0.016 ± 0.001^{cde}	232.33 ± 62.34^{de}
T_5	3.87 ± 0.20^{a}	0.32 ± 0.06^{abc}	25.10 ± 2.43^{cd}	1.13 ± 0.06^{cd}	6.40 ± 1.32^{cde}	0.229 ± 0.024^{ab}	0.012 ± 0.001^{de}	273.00 ± 36.80^{cde}
T_6	4.53 ± 0.27^{a}	0.47 ± 0.06^{a}	46.40 ± 8.31^{a}	1.93 ± 0.18^{a}	15.80 ± 2.28^{a}	0.370 ± 0.067^{a}	0.024 ± 0.003^{a}	462.67 ± 11.85^{a}
T_7	4.23 ± 0.33^{a}	0.40 ± 0.09^{ab}	34.47 ± 5.65^{abc}	1.50 ± 0.03^{abc}	10.43 ± 3.51^{bc}	0.261 ± 0.052^{ab}	0.018 ± 0.002^{bc}	353.67 ± 22.50^{bc}
T_8	4.37 ± 0.49^{a}	0.42 ± 0.06^{ab}	40.30 ± 3.10^{ab}	1.74 ± 0.26^{ab}	12.47 ± 0.90^{ab}	0.287 ± 0.027^{ab}	0.022 ± 0.002^{ab}	378.83 ± 23.71^{ab}
C.D.	**0.99**	**0.18**	**15.24**	**0.59**	**4.89**	**0.13**	**0.005**	**96.11**
S.E.(m)	**0.33**	**0.06**	**5.09**	**0.20**	**1.63**	**0.05**	**0.002**	**32.10**

3.2 Comprehensive Evaluation of Seedlings Attributes

Data recorded in Table 2 shown each treatment used in the trial. T_6 (Citric acid treatment in room temperature at 20-24˚C) had the highest shoot length of 4.53cm, followed by T_8 (TiO_2 NPs treatment in seed germinator at 28˚C) with 4.37 cm and T_7 (GA_3 treatment in seed germinator at 28˚C) with 4.23cm. Meanwhile, T_0 has a minimum shoot length with 2.60 cm. Similarly, T_6 (Citric acid treatment in room temperature at 20-24˚C), shown the best results with 0.47cm followed by T_8 with 0.42 cm and T_7 (GA_3 treatment in seed germinator at 28˚C) with 0.40 cm. T_0 was observed the lowest in root length with 0.13 cm. T6 (Citric acid treatment in room temperature at 20-24˚C) shown the highest chlorophyll content with 15.80 SPAD, followed by T_8 (TiO_2 NPs treatment in seed germinator at 28˚C) with 12.47 SPAD, and T_7 (GA3 treatment) with 10.43 SPAD. while, T_0 had a minimum chlorophyll content with 2.80 SPAD. The same conclusion was attained by Kalaji et al*.* [14], who concluded that T_6 (citrus acid treatment at room temperature, 20–24˚C) had the highest chlorophyll content because it made it easier for the plant to absorb vital micronutrients like iron and magnesium, which are crucial building blocks of chlorophyll molecules. Citric acid (T6) has demonstrated positive effects on various seedling attributes, significantly enhancing their growth and development. Studies have shown that citric acid improves seed germination rates, likely by breaking down seed coats and facilitating water and nutrient uptake. Additionally, it acts as a chelating agent, which helps in the mobilization and absorption of essential nutrients like calcium, magnesium, and iron. This improved nutrient availability promotes stronger root development, increased seedling vigor, and enhanced overall plant health. Citric acid also helps in maintaining a balanced pH in the soil, creating a more favourable environment for seedling growth [15]. The fresh weight of shoot was significant varied from 15.00 mg to 46.40 mg. Data in Table 2. significantly highest fresh weight of shoot was recorded in T_6 with 46.40 mg followed by T_8 with 40.30 mg and T_7 with 34.47 mg, however lowest fresh weight of shoot was recorded in T_0 (Hydropriming in room temperature at 20-24˚C with 15.00 mg. The similar outcome was observed by Poorter et al*.* [16], who concluded that citric acid increases cell expansion and turgor pressure and aids in supplying appropriate water availability, thus increasing shoot fresh weight. Cell hydration promotes metabolic activities and nutrition transfer, which contributes to shoot development. The dry weight of the shoot varied significantly, from 0.06 mg to 0.37 mg. Seeds are exposed to citric acid, which initiates metabolic processes in seeds, resulting in the creation of growth-promoting chemicals and enzymes involved in root elongation and nutrient absorption, eventually leading to increased root fresh weight [17]. Data in Table 2. Observed that the highest dry weight of root was recorded in T_6 with 0.024 mg, followed by T_8 with 0.022 mg and T_7 (GA_3 treatment in seed germinator at 28˚C) with 0.018 mg. Treatments showed that the significantly highest seedling vigor index was recorded in T_6 (Citric acid treatment in room temperature at 20-24˚C) with 462.67 followed by T_8 with 353.67 and T_7 with 343.57 mg, while lowest seedling vigor index was recorded in T_0 with 32.67. Metabolic processes like as, oxidation, and protein hydrolysis are triggered by enzymatic and hormonal mechanisms, resulting in germination and elongation of root and shoot lengths, which promotes seedling vigour [18-25].

4. CONCLUSION

Based on the above discussion, it is possible to conclude that treatment T_6 produced the greatest results for germination features such as final germination percentage (96.67%), germination index, and coefficient of germination velocity. From on the findings, treatment T_6 (citric acid at room temperature) emerged as the most effective overall. While T_6 exhibited a slightly slower germination time, it achieved a remarkably high final germination percentage. Moreover, seedlings from T_6 displayed superior growth in terms of shoot length, root length, fresh and dry weight, chlorophyll content, and vigor index. These results suggest that citric acid treatment effectively overcomes seed dormancy, promotes successful germination, and enhances early seedling development.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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