Examine the effects of various chemicals and different environmental conditions on the breakdown of dormancy of Dragon fruit (*Hylocereus undatus* var. White fleshed pitaya)

Abstract

Dragon fruit has exceptional nutritional value, drawing growers from all over India to cultivate this fruit crop with 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. 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 in seed germinator at 28˚C), T_2 (Seeds in Sand+ water mixture in room temperature at 20-24˚C), T_3 (Seeds in Sand+ water mixture in seed germinator at 28˚C), T_4 (Seeds in Blotting sheet+ water in room temperature at 20-24˚C), T_5 (Seeds in Blotting sheet+ water in seed germinator at 28˚C), T_6 (Seeds with Citric acid treatment in room temperature at 20-24℃), T_7 (Seeds with GA_3 treatment with 100 ppm GA_3 in Seed germinator at 28℃), T_8 (Seeds with TiO_2 NPs treatment with 200 ppm TiO_2 NPs in 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%. 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 (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; TiO_2, Citric acid, Germination, Dragon fruit; GA_3

1. Introduction

The tropical fruit known as a dragon fruit *(Hylocereus undatus*), 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.

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***) (Pradhan *et al*., 2022).

Commented [SS1]: Start with a clear research question: Briefly state the question about improving seed germination in dragon fruit.

Commented [SS2R1]: Remove the sentence about the fruit's nutritional value.

Commented [SS3]: Condense methodology details: Briefly mention the experimental design (CRD) and the number of treatments without specific locations and dates.

Commented [SS4]: Combine repetitive phrases: Use a single phrase like "seed germinator (28°C)" and "room temperature (20-24°C)" to represent the conditions.

Commented [SS5]: Briefly state the statistical test used to determine significant differences.

Commented [SS6]: Focus on key findings: Briefly state the highest and lowest germination percentages and seedling vigor index values with corresponding treatments

Commented [SS7]: Start with a clear research question. State a specific question about the effects of various chemicals and environmental conditions on breaking seed dormancy in dragon fruit (e.g., "Can specific chemicals or environmental conditions improve germination rates in dormant dragon fruit seeds?").

Commented [SS8]: Briefly mention the different varieties of dragon fruit and their increasing popularity, and condense general information

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 (Perween & Mandal, 2018).

Dragon fruit is a superfood with few calories but lots of fiber, probiotics, antioxidants, vitamins, and minerals, as well as good fats and phytonutrients. 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 are found in 100g of fresh fruit pulp (Kabir *et al*. 2022). Because of their dormant state, pitaya seeds germinate poorly. 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 (Hilhorst *et al.*, 2010). Seasonal variations in temperature, whether high or low, are signals that can unsettle or move the water gap that indicates the end of dormancy in natural habitats. Nevertheless, in lab settings, dry or wet heat, mechanical scarification, and even dry storage can shatter seeds or enable them susceptible to treatments that break dormancy.

The seed coat, which often comprises sclerenchyma, or sclereids, must first be penetrated by the NMs during seed germination. Because of its physical-chemical integrity, this seed coat may serve as a barrier to the NMs. NMs exploit the intercellular gaps in tissues or produce pores mostly via up regulating the synthesis of aquaporin. Certain metal oxide nanoparticles (TiO_2 NPs) have the ability to penetrate the seed coat and induce embryonic differentiation by activating the enzymes responsible for breaking seed dormancy.

Henceforth keeping the above facts in view, the present study entitled “Investigate the effects of various chemicals and different environmental conditions on the breakdown of dormancy of Dragon fruit (*Hylocereus undatus* var. White fleshed pitaya)” will be conducted.

Commented [SS9]: Briefly summarize the nutritional value without excessive detail.

Commented [SS10]: Focus on seed dormancy. Briefly mention the economic importance of dragon fruit cultivation and the challenge of low germination rates due to seed dormancy.

Commented [SS11]: Explain how the chosen treatments (chemicals and environmental conditions) haven't been extensively studied for breaking dormancy in dragon fruit or offer a more efficient/cost-effective approach compared to existing methods.

Commented [SS12R11]: This will provide an overview of the research objectives regarding the reasons for the treatment given regarding dormancy.

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. This region comes under semi-arid sub-tropical climate with extreme weather condition having hot and dry summer and cold winter. Generally, monsoon sets in during the last week of June. Annual rainfall ranges from 700 to 800 mm, most of which falls during last June to the middle of September. In this area winter rains are occasional and uncertain. The maximum temperature goes up to 45^0C during summer and minimum as low at 5^0C during winter.

Commented [SS13]: Focus on relevant details. Briefly mention the location (e.g., Gwalior, India) and controlled lab conditions (temperature, humidity).

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	:	26th February 2024
Variety	:	Red skin with white flesh
Season of experiment conduct	:	(Feb- April)
Lab temperature	:	20-24^0 C
Seed germinator temperature	:	28^{0}C
Relative humidity	:	85%

2.4 Treatment Applications

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℃.

Commented [SS14]: Explain the reasoning behind using deep freezing with citric acid and other procedures that may not be standard practice.

The raw material that is Dragon fruit i.e., pink skin with White flesh was bought 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.

Commented [SS15]: Condense seed source description, and state that seeds were obtained from a commercially available red-skinned white-fleshed dragon fruit variety.

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^{0}C

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 121^0C 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:

Commented [SS16]: Clearly describe how data for each germination and seedling vigor parameter will be collected.

Commented [SS17R16]: It is important to provide information about expanding data collection.

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-

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

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-

$$Germination\ Index = (10\times n1) + (9\times n2) + \cdots + (1\times n10)$$

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-

$$CVG = N1 + N2 + \cdots + N_x/100 \times N_1T_1 + \cdots + N_xT_x$$

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-

$$GRI=G_1/1 + G_2/2 + \cdots + G_x/x$$

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. Result and Discussion

3.1 Germination attributes-

Commented [SS18]: Combine explanations for similar treatment effects across different germination attributes.

Commented [SS19]: Consider reorganizing the discussion.
Instead of discussing each germination attribute separately, group related attributes together (e.g., FGP, MGT) and discuss the overall trends observed for seed dormancy breaking and germination speed.

3.1.1 Final Germination Percentage (FGP%)-

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). As per data recorded, T_6 (Citric acid treatment in room temperature at 20-24^{0}C) 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.*, (2010). Whereas, 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.*, (2005).

3.1.2 Mean Germination Time (MGT)

The data acquired for observing the mean germination time of dragon fruit seeds was considerably impacted by all treatments. Data in Table 1. stated that the treatment 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 (TiO_2 NPs treatment in seed germinator at 28˚C) with 5.09 days, respectively. 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 (Hydropriming in room temperature at 20-24˚C) had the lowest mean germination time with 11.31 days. According to the observed results, T1 (hydropriming in a seed germinator at 28^{0}C) exhibits the best mean germination time. Seed germinators provide accurate temperature control, offering the ideal thermal environment for enzymatic activities that are essential for seed germination and facilitating rapid germination within a day. However, failing to maintain optimal moisture levels results in a low germination percentage. Bradford *et al.* (1986) observed a similar

Commented [SS20]: Discuss non-significant findings, can briefly mention why some treatments did not perform as well and if there are potential explanations.

finding. Conversely, due to a long germination period of seeds, T_0 (Hydropriming at room temperature, 20–24˚C) had the lowest mean germination time.

3.1.3 Coefficient of Velocity of Germination (CVG)

Trial has been conducted, observing all treatments with respect to rate or Coefficient of Velocity of Germination, which is notably documented. Data in Table 1. found that the treatment T_6 (Citric acid treatment in room temperature at 20-24˚C), shown the best results with 30.03%, followed by T_8 (TiO_2 NPs treatment in seed germinator at 28˚C) with 13.51% and T7(GA_3 treatment in seed germinator at 28˚C) with 12.14%. T_0 (Hydropriming in room temperature at 20-24˚C) had observed the lowest coefficient of velocity of germination of seeds with 0.12%. According to Bailly *et al.* (2004), 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.

3.1.4 Germination Index (GI)

The findings reported in Table 1. were obtained through an experiment. The germination index as impacted by several seed treatments shown that Citric acid treatment and other treatments considerably increased the germination index in comparison to the control. 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). The lowest germination index was noted in T_0 (Hydropriming in room temperature at 20-24˚C) with 16.33.

As per result obtained, the Germination Index takes into account both the growth of the seedlings that are produced as well as the proportion of seeds that germinate. Seeds were exposed to citric acid and pre-chilled for one week, resulting in. The cold treatment stimulates enzymes that break down food stores in the seed, alters cell membrane permeability, and allows water to enter the seed and start the germination process, leads to higher germination percentage. Wang *et al.* (2020) found a similar result.

3.1.5 Germination Rate Index (GRI)

The results shown in Table 1. were acquired through conducted trial. T_5 (Seeds in blotting sheet + water in seed germinator at 28˚C) had the highest germination index (19.67%/day), followed by T_8 (TiO2 NPs treatment in seed germinator at 28˚C) with 18.71%/day and T_4 (Seeds in blotting sheet + water in room temperature at 20-24˚C) with 17.86%/day. T_0 (Hydropriming in room temperature at 20-24˚C) had the lowest germination index at 16.33% per day.

T_5 (seeds in a blotting sheet with water in a seed germinator at 28˚C) had a high germination rate index, as per the data that were collected. 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 (Copeland *et al*. 2012).

3.1.6 First Day of Germination (FDG)

Examining each treatment from the study that was performed, it was found that in Table 1. T_3 (seeds in sand+water mixture in seed germinator at 28^0C) exhibited the best first day of germination with 2.00 days, followed by T_1 (hydropriming in seed germinator at 28^0C) with

2.33 days and T_8 (TiO_2 NPs treatment in seed germinator at 28˚C) with 2.67 days. Meanwhile, T_0 (Hydropriming in room temperature at 20-24^{0}C) has shown least in first day of germination or took maximum days to germinate with 11 days. According to the data presented, T_3 (seeds in a combination of sand and water in a seed germinator at 280°C) showed 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 (Bewley *et al.* 2013).

3.1.7 Last Day of Germination (LDG)

It has been observed every treatment in the study, 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. while, T_0 (Hydropriming in room temperature at 20-24^{0}C) has demonstrated the least in last day of germination with 11.67 days. The findings indicates that T_2 seeds, which are sown in a mixture of sand and water at room temperature (20–24˚C), germinate for an extended period of time because the sand retains sufficient moisture in its pores to facilitate seed germination.

3.1.8 Time Spread of Germination-

The data obtained to monitor the Time spread of germination of dragon fruit seeds was significantly influenced by every treatment. According to data in Table 1. treatment T_7 (GA_3 in seed germinator at 28^{0}C) 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). whereas, T_0 (Hydropriming in room temperature at 20-24^{0}C) shown the minimum efficiency of average days of germination with 1.00 days. Similar findings were noted by Nonogaki *et al*. (2010), who concluded that T_7 (GA_3 in the seed germinator at 28^{0}C) 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.

3.2 Seedlings attributes

3.2.1 Shoot length

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 (Hydropriming in room temperature at 20-24^{0}C) has a minimum shoot length with 2.60 cm. T_6 (Citric acid treatment at room temperature, 20-24˚C) results in increased shoot length due to better nutrient absorption, cell proliferation, and elongation in the cambium tissue. This promotes metabolic activity and shoot elongation. Zhang *et al.* (2008) found similar findings. T_0 (Hydropriming at room temperature between 20-24^0 degrees Celsius) has a minimal shoot length because extended seed soaking in water during hydropriming might cause oxygen deprivation, resulting in lower shoot length.

Commented [SS21]: Describe statistical analysis: Indicate how statistical significance was determined (e.g., $p < 0.05$) and mention any non-significant findings.

Commented [SS22]: Briefly mention why other treatments resulted in lower seedling growth compared to T6. Are there potential explanations based on the treatment methods?

3.2.2 Root length

Trial has been conducted, observing all treatments with respect to length of root which is notably documented. Data in Table 2. found that the treatment T_6 (Citric acid treatment in room temperature at 20-24˚C), shown the best results with 0.47cm followed by T_8 (TiO_2 NPs treatment in seed germinator at 28˚C) with 0.42 cm and T_7 (GA_3 treatment in seed germinator at 28˚C) with 0.40 cm. T_0 (Hydropriming in room temperature at 20-24˚C) was observed the lowest in root length with 0.13 cm. T_6 treatment with citric acid at room temperature (20-24˚C) results in the longest root length. Exogenous application of citric acid during the early stages of seed germination alters the pH of the soil or growth medium, strengthening the solubility of specific nutrients and producing an optimal environment for root growth. Anjum *et al*. (2011) obtained similar findings to above recorded result.

3.2.3 Chlorophyll Content

The results obtained from the study that was conducted are shown in Table 2. T_6 (Citric acid treatment in room temperature at 20-24˚C) shown the highest chlorophyll content with 15.80 SPAD, followed by T_8 (TiO2 NPs treatment in seed germinator at 28˚C) with 12.47 SPAD, and T_7 (GA_3 treatment in seed germinator at 28˚C) with 10.43 SPAD. while, T_0 (Hydropriming in room temperature at 20-24˚C) had a minimum chlorophyll content with 2.80 SPAD. The same conclusion was attained by Kalaji *et al.* (2018), 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 promotes chlorophyll stability and protects chloroplasts from oxidative damage, resulting in higher chlorophyll concentration. T_0 (Hydropriming in room temperature at 20-24˚C) had a minimal chlorophyll content because to insufficient moisture or limited nutrition availability.

Commented [SS23]: Create a consolidated explanation for the positive effects of citric acid (T6) on various seedling attributes, highlighting its impact on nutrient uptake, cell division, and overall growth.

Commented [SS24]: It is important to discuss why other treatments are not significant.

3.2.4 Shoot (Fresh weight)

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 (Citric acid treatment in room temperature at 20-24˚C) with 46.40 mg followed by T_8 (TiO2 NPs treatment in seed germinator at 28˚C) with 40.30 mg and T_7 (GA_3 treatment in seed germinator at 28˚C) 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.* (2012), 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.

3.2.5 Root (Fresh weight)

The fresh weight of seedling was significant varied from 0.43 mg to 1.93 mg. Data in Table 2. significantly highest fresh weight of root was recorded in T_6 (Citric acid treatment in room temperature at 20-24˚C) with 1.93 mg followed by T_8 (TiO_2 NPs treatment in seed germinator at 28˚C) with 1.74 mg and T_7 (GA_3 treatment in seed germinator at 28˚C) with 1.50 mg, however lowest fresh weight of root was recorded in T_0 (Hydropriming in room temperature at 20-24˚C) with 0.43 mg. Considering the data collected, during the early stages, 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. Istifidah *et al*. (2013) reported similar findings.

3.2.6 Shoot (Dry weight)-

The dry weight of the shoot varied significantly, from 0.06 mg to 0.37 mg. Data in Table 2. Indicated that the highest dry weight of shoot was recorded in T_6 (Citric acid treatment in room temperature at 20-24˚C) with 0.37 mg, followed by T_8 (TiO_2 NPs treatment in seed germinator at 28˚C) with 0.29 mg and T_7 (GA_3 treatment in seed germinator at 28˚C) with 0.26 mg. The lowest dry weight of shoot was recorded in T_0 (Hydropriming in room temperature at 20-24˚C) with 0.06 mg. Applied exogenously citric acid, dragon fruit seeds resulted in the greatest dry weight of the shoot; this may be related to the seedling's overall growth. Increased seedling development has resulted in food absorption and redistribution inside the seedling. Higher nutrient mobilization and accumulation has resulted in cell elongation, and shoot length contributes to increased fresh weight of the shoot. Thus, seedling development leads to an increase in dry weight (Chen *et al.* 2016).

3.2.7 Root (Dry weight)

The dry weight of the root varied significantly, from 0.011 mg to 0.024 mg. Data in Table 2. Observed that the highest dry weight of root was recorded in T_6 (Citric acid treatment in room temperature at 20-24˚C) with 0.024 mg, followed by T_8 (TiO_2 NPs treatment in seed germinator at 28˚C) with 0.022 mg and T_7 (GA_3 treatment in seed germinator at 28˚C) with 0.018 mg. The lowest dry weight of root was recorded in T_0 (Hydropriming in room temperature at 20-24˚C) with 0.011 mg. Abdin *et al.* (2011) found the same aspect, indicating that seeds exposed to citric acid at room temperature had better nutrient mobilization and metabolic activity, which enables them to make better use of the resources available during germination and early seedling growth. This effective resource usage leads to increased root biomass and consequent dry weight.

3.2.8 Vigor Index

The data furnished in Table 2. regarding vigor index of seedling as influenced by different seed 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 (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 (Fig-1). Owing to increased respiration rates, greater metabolite consumption, and metabolite transport to the growth point, seeds treated with Citric acid exhibited the highest level of seedling vigour index. 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 (Kokmaz *et al*. 2015).

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. T_6 took the longest to germinate but had the highest germination percentage, hence it did not perform well in Mean Germination Time (Day), Germination Rate Index (%/day), First Day of Germination, Last Day of Germination, and Time Spread of Germination (Day). In contrast, attributes in respect to seedlings, T_6 (Citric

acid treatment in room temperature at 20-24˚C) resulted in greatest performance and get highest in shoot length (cm), root length (cm), shoot (fresh weight) (mg), root (fresh weight) (mg), chlorophyll content (SPAD), and vigor index.

Commented [SS25]: Based on the findings, treatment T6 (citric acid at room temperature) emerged as the most effective overall. While T6 exhibited a slightly slower germination time, it achieved a remarkably high final germination percentage. Moreover, seedlings from T6 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.

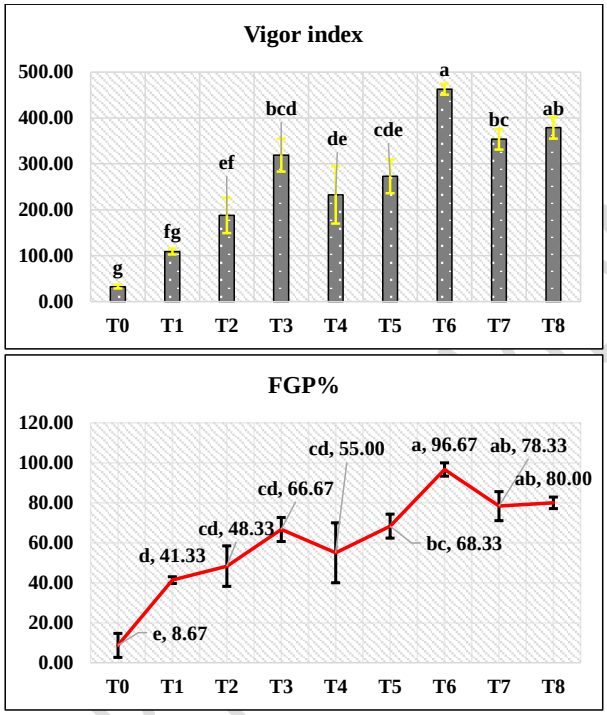

Fig- 1 Graph Represents the Final germination% and Vigour index of Dragon fruit (*Hylocereus undatus* var. White fleshed pitaya)

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Commented [SS26]: Consider recent findings: While the list covers relevant topics, including a few recent studies (2022-2024) on dragon fruit germination or seed dormancy breaking could strengthen the reference list.

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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**