An Evaluative Study on Dormancy Breakage of Dragon Fruit Seeds Under Different Environmental Conditions

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Abstract- **Seed germination is the sprouting of a seed, spore or any other reproductive body usually after a period of dormancy. Thus, Dragon fruit (pitaya) of genus *Selenicereus undatus* which belongs to the family of Cactaceae, a white-fleshed and the most common dragon fruit available in local farmers, were used to observe the germination efficiency of the dragon fruit seeds. Since the germination time of dragon fruit takes much longer and is also expensive, therefore, we have tried to minimize or shorten the time of the seed germination and its efficiency rate by using various types of treatments like dry heating seeds for 24 hours and 48 hours using lemon extract, wet heating at 100° C for 30 minutes and 60 minutes, chemical scarification was done with Sulphuric acid (H_2SO_4) for 5-30 minutes respectively, using sand and water mixture, using 0.1% Mercuric chloride were found to germinate in less time as compared to other provided conditions. Whereas, the 24hrs dehydrated seeds showed higher germination efficiency.**

Keywords: **Sprouting, Germination efficiency, Dormancy**

1. INTRODUCTION

Dragon Fruit (*Hylocereus undatus*) is a perennial, epiphytic, climbing cactus (Ahmed A. ELOBEIDY, 2019), it is also known as Pitaya or Pitahaya belonging to the family of Cactaceae. The fruit comes in three varieties, and among them, the most famous ones are Pink Pitaya (white flesh with pink skin: *Selenicereus undatus*), Red Pitaya (red flesh with red skin: *Selenicereus costaricensis*), Yellow Pitaya (white flesh with yellow skin; *Selenicereus megalanthus*) (Pandya Prutha Hitendraprasad, Karunakar Hegde, 2019). In recent years, Dragon Fruit has drawn much attention, not only because of its attractive red or pink colour and its economic value as a fruit, but also it is valued for its high antioxidant potential along with vitamin and mineral contents. The dragon fruit seed is an edible black coloured seed that are similar in appearance to kiwi seeds, which is embedded in the pulp of the fruit along with its tremendous nutritive property which attracts the growers from different parts of India to cultivate this fruit crop, which is originated in Mexico and Central and South America (Tamanna Perween, KK Mandal 2018). Dragon fruit is now cultivated worldwide due to its commercial interest, not demanding cultivation requirements, i.e., high drought tolerance, easy adaptation to light intensity and high temperature, a wide range of tolerance to different salinities, and benefits to human health (Nobel and La Barrera 2004; Nie et al. 2015; Crane et al. 2017; Mercado-Silva 2018). The texture of the dragon fruit is much alike to kiwi or watermelon, the flavour is mildly sweet and the dragon fruit pulp can also be used to make juice, wine, jam and jelly; seeds may be utilized to extract the oil (about 50% essential fatty acids) or used directly as an ingredient in many food products such as syrup, ice cream, sherbet, candy, yogurt and pastries (Pawel Pasko, Agnieszka Galanty, 2021). Since, Dragon fruit is a fairly hardy species, so it can survive in any type of climatic conditions which are favourable for flowering and fruiting purpose and the soil condition is provided with good drainage, so, therefore we can say that the climate for our country is quite suitable for the cultivation of dragon fruit (G. Karunakaran1, M. Arivalagan and S. Sriram 2019). The sandy loam soils, that are rich in excellent organic matters, plays a very crucial role for pitaya in order to obtain a good yield (Mabberley, 2008, Scherson et al., 2008). Seed germination means the growth of embryo into seedling and plant. As germination begins with an increase of metabolic activity within the seed, so the first visible sign of germination in angiosperms, that is generally an enlargement of the seed. Seeds show low germination percentage and extended period of time to complete germination due to unique structure of the ovule (Vishnupriya J, 2019). The soil temperature is kept consistent at 70-85F. Cool soils significantly delay seed germination time can inhibit germination completely. Maximum temperature for *S. undatus* was estimated to be 39°C, and the minimum temperature for this species is 7°C (Mizrahi and Nerd, 1999). Maximum net CO_2 uptake is at 30ppm/20ppm day or night, but is inhibited at 42ppm/32ppm (Nobel and De la Barrera, 2000). Dragon fruit seeds are usually easy to germinate but show variable germination rates. The Germination of pitaya is poor because of dormancy. Seed dormancy is a physiological condition that inhibits germination in favourable conditions. The dormancy of dragon fruit seed is a physiological condition that restricts germination in favourable conditions and determines the range of conditions allowing for germination. The intrinsic molecular mechanisms determining dormancy can have an embryo or a coat component that can interact to determine the degree of 'whole-seed' dormancy (Hilhorst, Cohn 1995). Vegetative propagation is the production of plants from vegetative parts of the plant like stem, root, leaf etc.

In vegetative propagation, a portion is detached from the body of the parent plant and is grown independently. In case of dragon fruit, the vegetative propagation is done by cutting the stems of the mature dragon fruit into 3-4 pieces. Thus, when the cutting becomes firm and form a root system, it is transferred into the soil. The dragon fruit can be propagated from both vegetative propagation and seeds. Since, a dragon fruit consist of numerous seeds, so, if we could break the dormancy of the seeds then from a single fruit, we would be able to get the maximum germination which will be very beneficial for us, as compared to the vegetative propagation. Mucilage is present in some parts of the dragon fruit plant such as the stem, leaf, and fruit (Oanh Thi Hoang Le, 2020). So, in this study, we will be using the dragon fruit seeds and will try to remove the slimy layer (mucilage) from the seeds by performing different treatments like dry heat, wet heat, acid scarification, surface sterilization.

2. MATERIALS AND METHOD

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

2.1 Seeds in normal water

Firstly, we added normal tap water in 3 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 3 different petri plates with the help of the forceps. Then, we kept all the three petri plates in light in the normal room temperature.

2.2 Seeds in water incubated at 37 °C until germination

In 3 different petri plates, we added water and 20 seeds each with the help of the forceps and kept them in the incubator at 37°C until germination.

2.3 Seeds in mixture of sand and water

A mixture of sand and water was added in 3 different petri plates in which 20 seeds each were placed in 3 different petri plates with the help of the forceps. Then, all the three petri plates were kept in light in the normal room temperature.

2.4 Seeds in mixture of Sand and Water incubated at 37 °C until germination

60 fresh seeds were divided into 3 different petri plates in which the mixture of sand and water were added, where 20 seeds each were embedded respectively. Then, these petri plates were kept in an incubator at 37°C until germination.

2.5 Heat treatment

2.5.1 Dry heat

Two treatments were performed under dry heat, seeds which were treated in a dehydrating condition for 24 hours and citric acid treatment which was done for 48 hours.

- **Seeds in 24 hours dehydrating Condition**: We took 3 different filter papers and then placed them in 3 different petri plates. Then, we simply added 20 seeds each in the petri plates with the help of the forceps and kept them in the incubator at 37℃ for overnight or exactly for 24 hours. These seeds are called dehydrated seeds which were then transferred in water and were kept in constant darkness in the normal room temperature.

2.5.2 Wet Heat:

40 seeds were extracted from a fresh dragon fruit. Out of the 40 seeds, 20 seeds were divided in 5 Eppendorf tubes, each Eppendorf tube containing 4 seeds. The rest of the 20 seeds were also divided in the 5 Eppendorf tubes. Then, the first 5 Eppendorf tubes were placed in a water bath for 30 mins and the rest 5 Eppendorf tubes were also placed in a water bath for 60 mins at 100℃ with the help of a stand. After the completion of water bath, the 30 min water bathed seeds were transferred into a petri plate containing the mixture of sand and water and also the 60 min water bathed seeds were also transferred in another petri plate containing the same mixture of sand and water in constant darkness in the normal room temperature. This process was also repeated twice.

2.6 Citric Acid treatment

As lemon juice is a good source of citric acid, so therefore, fresh lemon was collected from the market; juice was then extracted from the lemon and distributed or divided in two Eppendorf tubes. Then, we extracted 60 seeds from a fresh dragon fruit and transferred them into the Eppendorf tubes containing juice of lemon. Then, the tubes were kept in refrigerator (4°C) for 48 hours. After 48 hours, the seeds kept in the tubes were transferred in the petri plates containing tissue paper and water in constant darkness in normal room temperature.

2.7 Seeds treatment with 0.1% $HgCl_2$

0.1% $HgCl_2$ was divided into 3 petri plates and 20 each seed was placed in each of the 3 petri plates respectively and was placed or kept in constant darkness in the normal room temperature.

2.8 Acid treatment

Acid treatment was also carried out for the seeds in respective time interval. Here, we used 98% Sulfuric acid (H_2SO_4) to treat the seeds which can be seen in the following Table 1:

After the treatment of H_2SO_4, all the seeds were firstly washed with 70% ethyl alcohol for 5 minutes and then with water and was kept in constant darkness in the room temperature. This process was also repeated twice.

Table 1: Details of sulfuric acid treatment

Quantity of seeds	Treatment	Incubation time
20 seeds	98% H_2SO_4	5 min
20 seeds	98% H_2SO_4	10 min
20 seeds	98% H_2SO_4	15 min
20 seeds	98% H_2SO_4	20 min
20 seeds	98% H_2SO_4	25 min
20 seeds	98% H_2SO_4	30 min

After performing all the treatments, the cotyledons which became greenish in color, were transferred in a mixture (1:1 ratio) of coco peat and sand within a paper cup, for hardening purpose.

3. RESULTS

We performed a total of 9 different types of seed conditions and treatments on 3 sets of petri plates, each consisting of 20 seeds. After performing every single condition and treatment, we have observed as follows:

3.1 Seeds and water in normal temperature: In the 1st set, 7 seeds were germinated out of 20 seeds after 30 days, in the 2nd set, 6 seeds were germinated after 25 days and in the 3rd set, 4 seeds were germinated after 28 days. The overall germination efficiency showed a moderate result but the time taken by the seeds to break its dormancy was much more, so therefore the outcome was not good (Fig.1).

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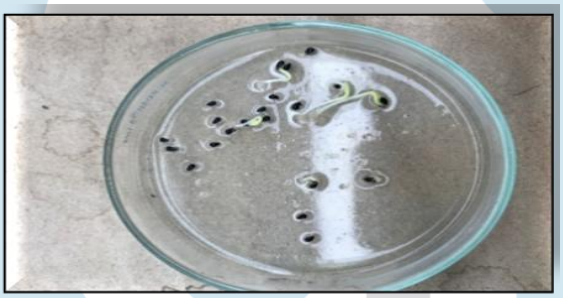


Figure 1: Few sprouts observed in water in normal room temperature

3.2 Seeds and water incubated at 37°C until germination: In the1st set, 4 seeds were germinated out of 20 seeds after 28 days, in the 2nd set 10 seeds were germinated out of 20 seeds after 31 days and in the 3rd set 2 seeds were germinated out of 20 seeds after 31 days (Fig. 2). In this treatment, the outcome of the germination efficiency was good whereas the time taken to for the seeds to germinate was longer than expected.

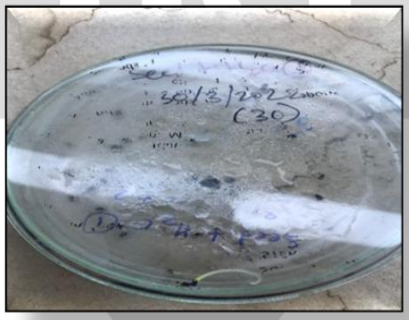


Figure 2: Few sprouts observed in water in at 37 °C

3.3 Dry heat treatment:

24 hours dehydrated seeds: In the 1st set, 17 seeds were germinated out of 20 seeds after 8 days, in the 2nd set, 16 seeds were germinated out of 20 seeds after 10 days and in the 3rd set, 18 seeds were germinated out of 20 seeds after 9 days. In Fig 3 we can see that the maximum dormancy of these seeds was broken and therefore the maximum efficiency has been observed in this particular treatment. Also, the time taken was lesser than most of the other treatments.

Figure 3: Multiple sprouts observed after treatment in 24 hours of dehydrated condition

3.4 Treatment with 0.1% $HgCl_2$: In the 1st set, 8 seeds were germinated out of 20 seeds after 4 days, in the 2nd set, 9 seeds were germinated out of 20 seeds after 7 days and in the 3rd set, 7 seeds were germinated out of 20 seeds after 5 days (Fig. 4). In this treatment we observed that, the seeds took the least days to break its dormancy but the germination efficiency was satisfactory since only a few seeds were able to break its dormancy.

Figure 4: Few sprouts observed after treatment with 0.1% $HgCl_2$

3.5 48 hours lemon treatment: In the 1st set, 3 seeds were germinated out of 20 seeds after 12 days, in the 2nd set, 5 seeds were germinated out of 20 seeds after 14 days and in the 3rd set, only1 seed germinated out of 20 seeds after 11 days (Fig. 5). The germination efficiency in this treatment was less whereas the time taken for the seeds to germinate was less.

Figure 5: A single sprout observed after 48 hours of lemon treatment

3.6 Treatment with 98% H_2S0_4: Out of all the time intervals (5 min, 10 min, 15 min, 20 min, 25 min, 30 min), we observed that the seeds treated for 15 min showed the best result for both the germination efficiency as well as it took less time compared to the other time intervals. So, therefore we have only analyzed the result for the seeds treated for 15 min. Thus, in the 1st set, 2 seeds were germinated out of 20 seeds after 7 days, in the 2nd set, 4 seeds were germinated out of 20 seeds after 8 days and in the 3rd set, not a single seed germinated (Fig. 6).

Figure 6: Few sprouts observed after treatment with 98% H_2SO_4

3.7.1 Seeds in sand and water mixture: In the1st set 4 seeds were germinated out of 20 seeds after 53 days, in the 2nd set, 4 seeds were germinated out of 20 seeds after 54 days and in the 3rd set, 3 seeds were germinated out of 20 seeds after 53 days (Fig. 7). The germination efficiency as well as the time taken by the seeds to germinate showed a satisfactory result.

Figure 7: Few sprouts observed after treatment with sand + water

3.7.2 Seeds in sand and water mixture incubated at 37°C until germination: In the1st set, 10 seeds were germinated out of 20 seeds after 31 days, in the 2nd set, 12 seeds were germinated out of 20 seeds after 28 days and in the 3rd set, 9 seeds were germinated out of 20 seeds after 29 days. The result for the germination efficiency was good (Fig. 8) but the time taken was much longer than expected.

Figure 8: Few sprouts observed after treatment with sand + water in incubation until germination

3.8 Wet heat (sand + water): Out of the two-time intervals (30 min and 60 min), we observed that the seeds treated for 60 min showed better germination efficiency than 30 min. So, therefore we have only analyzed the result for the seeds treated for 15 min (Fig 9). Thus, in the 1st set, 1 seed was germinated out of 20 seeds after 49 days, in the 2 nd set, 2 seeds were germinated out of 20 seeds after 44 days and in the 3 rd set, only 1 seed was germinated out of 20 seeds after 50 days.

Fig 9. Few sprouts observed after transferring seeds from water bath to sand + water

All the observation for various condition have been performed in triplicate and divided into two sets where the raw data for germination efficiency study is provided in Table 1 and the graphical representation have been shown in Fig. 10; raw data for days taken for germination have been shown in Table 2 and the graphical representation have been shown in Fig. 11

TABLE: 1 Observation of Germination Efficiency at various conditions

GERMINATION EFFICIENCY RATE

SL NO	PER PLATE COUNT	SEED + WATER IN NORMAL TEMPERATURE	SEED + WATER INCUBATED AT 37°C	DRY HEAT TREATMENT	TREATMENT WITH 0.1% HgCl2	TREATMENT WITH 98% H2SO4						SEEDS IN SAND + WATER	SEEDS IN SAND + WATER MIXTURE IN INCUBATED AT 37°C UNTIL GERMINATION	WET HEAT (SAND + WATER)		48 HRS LEMON TREATMENT
				24 HRS DEHYDRATED SEEDS		5 MIN	10 MIN	15 MIN	20 MIN	25 MIN	30 MIN			30 MIN	60 MIN	
S1	20	7	4	17	8	2	1	2	1	0	0	4	10	1	1	3
S2	20	6	10	16	9	1	0	4	0	0	0	4	12	1	2	5
S3	20	4	2	18	7	0	0	0	1	0	0	3	9	1	1	1

Figure 10: Graphical representation of germination efficiency under different conditions

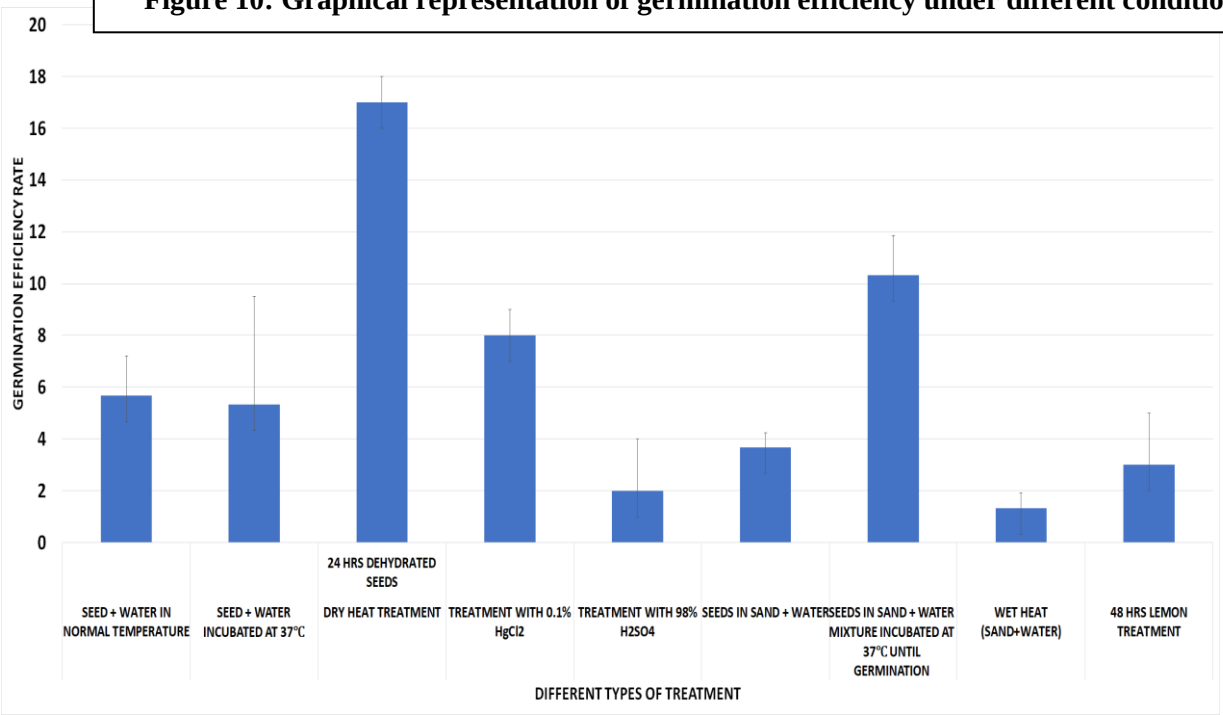
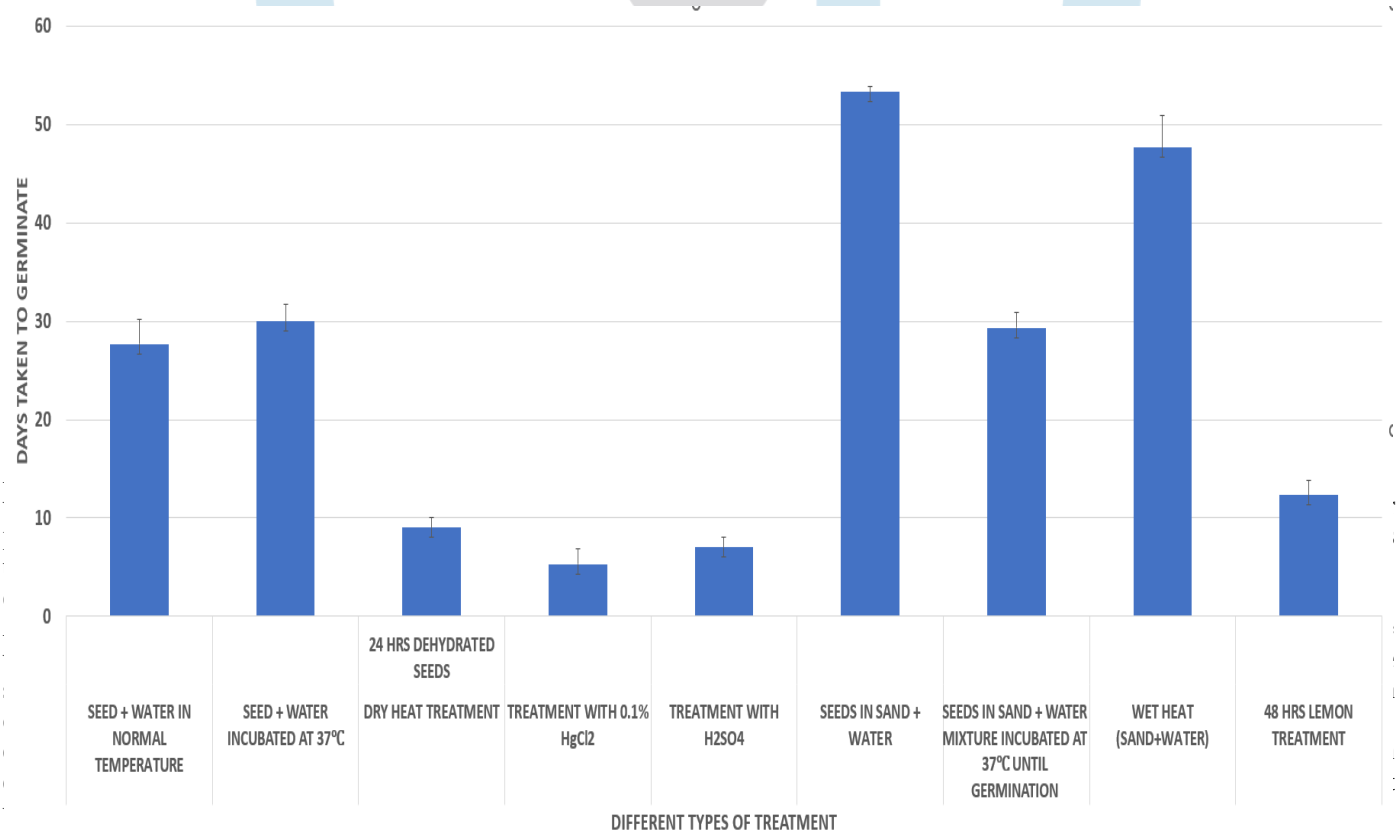


TABLE: 2 Observation for number of days taken to germinate

DAYS TAKEN TO GERMINATE

SL NO	NUMBER OF DAYS	SEED + WATER IN NORMAL TEMPERATURE	SEED + WATER INCUBATED AT 37°C	DRY HEAT TREATMENT	TREATMENT WITH 0.1% HgCl2	TREATMENT WITH 98% H2SO4						SEEDS IN SAND + WATER	SEEDS IN SAND + WATER MIXTURE IN INCUBATED AT 37°C UNTIL GERMINATION	WET HEAT (SAND + WATER)		48 HRS LEMON TREATMENT
				24 HRS DEHYDRATED SEEDS		5 MIN	10 MIN	15 MIN	20 MIN	25 MIN	30 MIN			30 MIN	60 MIN	
S1		30	28	8	4	10	9	7	12	0	0	53	31	51	49	8
S2		25	31	10	7	10	6	8	12	0	0	54	28	49	44	10
S3		28	31	9	5	13	7	6	10	0	0	53	29	53	50	9

Figure 11: Graphical representation for number of days taken to germinate



Seeds which were treated with citric acid i.e., the seeds which were exposed for 48 hours with lemon, after a maximum period of 13 days which were kept in constant darkness in the normal room temperature, the dormancy of the seeds were broken but the rate of germination efficiency was satisfactory since only a few seeds were able to germinate.

McDonell et al, 2012 reported that, hot water softens the seed coat for embryo to emerge out with less energy for a good germination to take place. But according to Y. Long a, 2012, the highest germination (31.0 ± 1.2%) was obtained for seeds exposed to boiling heat (100°C) for 10 min. When the time in boiling water exceeded 10 min, germination percentages decreased.

Regardless of the exposure time to 70, 80 or 90°C, only 1.3% to 8% of the seeds germinated. Similar to Y. Long a, 2012, the germination efficiency in seeds treated for 60 min showed a better result than the seeds treated for 30 min as well as it took slightly less time as compared to seeds treated for 30 min, but the overall results observed in this case was not very satisfactory.
Treatment with concentrated H_2SO_4 was a very effective way to break seed dormancy, and 30 and 40 min of acid scarification resulted in the highest (94–96%) germination at 20/10 °C. 50 min of acid-scarification caused a significant decrease in germination percentage (Y. Long a, 2012). Kim et al., 2008, discussed that the dormancy of (*Hylocereus undatus*) was effectively broken when treated with 90% sulphuric acid (H_2SO_4), but the results observed for both the gemination efficiency rate as well as the time taken to germinate was not good especially in case of seeds which were treated with 98% sulphuric acid (H_2SO_4) treated in 25 and 35 minutes respectively since not a single seed germinated in these two cases. The seeds treated for 15 min showed both, greater germination efficiency as well as the seeds took lesser amount of time as compared to the other time intervals. Therefore, these seeds treated for 15 min in 98% H_2SO_4 are the second fastest method or treatment to break the dormancy of the dragon fruit seeds.
The seeds which were treated with 0.1% $HgCl_2$ took a maximum of 5 days for the seeds to break its dormancy which is the least time taken by the seeds to germinate compared to all the other treatments.
So, therefore by comparing all the results of the treatments done on the dragon fruit seeds, we conclude that the treatment done under dry heat i.e., the 24 hrs dehydrated seeds showed the most germination efficiency whereas the seeds which were treated with 0.1% Mercury chloride ($HgCl_2$) took the least time to break the seed dormancy of the dragon fruit, both under dark condition in the normal room temperature.

CONFLICT OF INETEREST:
The authors declare that there are no conflicts of interest regarding the publication of this paper.

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