Original Research Article

Study of Cryopreservation and Non- Cryopreservationon seeds of Dragon Fruit (*Hylocereuscostaricensis*)

Comment [ALC1]: Consider revising the topic…
Studies on cryopreservation and Non-cryopreservation of Dragon fruit (*Hylocereuscostaricensis)* seeds.

ABSTRACT

This research paper focuses on the germination process of dragon fruit ~~(*Hylocereus costaricensis)*~~seeds under different treatments and desiccation time intervals. The fruits of the Lisa variety, commonly referred to as *Pitaya roja* or red-fleshed *pitaya*, were gathered from plants cultivated in fields for this investigation.The study used a factorial design with treatments including distilled water, GA_3, and KNO_3, and desiccation time intervals ranging from 0, 2, 4, 6, 8, 10, and 12 hours. The percentage of germination, moisture content, and air-desiccation duration were measured and analyzed. The findings provided insights into the optimal conditions for seed germination and the effects of different treatments on the germination process.The factorial design to evaluate the interaction between treatments and desiccation time intervals on the germination of dragon fruit seeds.The moisture content of the seeds decreased during the drying process, indicating the removal of moisture.The air-desiccation duration varied across different treatments and time intervals, with Experiment 1 showing higher mean values compared to Experiment 2.Best germination and seed viability was observed in the desiccated seeds as well as post-thaw seeds when treated with 0.1% KNO_3. The research findings provide valuable insights into the speed of germination, the effectiveness of different treatments, and the impact of desiccation time intervals on the germination process of dragon fruit seeds.

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Subscript

Formatted: Subscript

Keywords – Dragon Fruit, Potassium nitrate (KNO_3), Gibberellic acid (GA_3), Air-desiccation, TTC test.

Introduction:

Dragon fruit or *pitaya* is the name which refers to the fruit of several different tropical climbing species of the genus *Hylocereus*, belonging to the family Cactaceae. Plants are native to America and the family contains around 1500 and 2000 species that are spread from N~~n~~orthern Canada to

Formatted: Font: Italic

~~s~~Southern Argentina. Within this family Cactaceae, the genus *Hylocereus* includes most plants that establish themselves on the ground and cling to trees while about 14 species of epiphytic climbing vine cacti are also known (**Bauer 2003**). Although the *pitaya* is native to the tropical areas of North, Central and South America, it is now cultivated worldwide due to its commercial interest and desirable traits of cultivation *i.e.*, high drought tolerance, easy adaptation to light intensity and high temperature, and a wide range of tolerance to different soil salinities (ref).

Formatted: Font: Italic

Hylocereus spp. exhibit unique growth habits distinct from the typical cactus archetype. These cacti are epiphytic or lithophytic and grow as climbing, clambering, or crawling plants. Remarkably, they can extend their branches up to 30 feet (9 meters) in length under favorable conditions. Some *Hylocereus* spp. demonstrate impressive vertical growth, with plants achieving heights of nearly 3 meters (10 feet) within a single year. This rapid growth is facilitated by the presence of numerous, thick, and smooth branches.

The flowers of *Hylocereus* spp. are a notable feature, often referred to as the "Queen of the Night" or "Moonflower." These flowers emerge from the stem margins, typically along the edges of the ribs, creating a striking visual display. An intriguing aspect of these flowers is their nocturnal blooming behavior. Dragon fruit flowers unfurl their large, captivating blooms during the night hours, and most of them close and wilt before dawn, often lasting less than 24 hr.

The flowers of *Hylocereus* spp. are characterized by their considerable size, with an approximate length of around 29 cm. They exhibit a two-tiered perianth structure, with an outer perianth that can vary in colour from green to yellowish-green. In contrast, the inner perianth is consistently pure white, creating a striking colour contrast. The flowers of *Hylocereus* spp. bear a strong resemblance to those of dragon fruit (pitaya), although differences in the coloration and fragrance of the outer perianth are notable distinctions.

Pollination and Fruit Development: The intriguing nocturnal blooming of dragon fruit flowers is closely tied to their pollination mechanism. These flowers are primarily pollinated at night, often by moths or bats, which are attracted to the large, fragrant blooms. Successful pollination results in the formation of fruit, which is a fleshy berry characterized by its leathery, scaly skin. The fruit, known as dragon fruit or pitaya, is highly valued for its nutritional and culinary qualities. (**Hart *et al.*, 2005; Le Bellec *et al.*, 2006).**

In accordance with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), all species of the genus *Hylocereus* are listed in Appendix I. This listing signifies that *Hylocereus* cacti are subject to regulation in international trade to ensure their sustainability and prevent over-exploitation. The CITES treaty aims to protect endangered species and regulate their international trade to mitigate the negative impacts of commercial activities on these species.

The Imperative of Seed Preservation: Seeds are the fundamental units of plant life, encapsulating the genetic diversity and potential for future plant generations. They are the culmination of intricate biological processes and adaptations that have evolved over millennia. However, seeds are not immune to the challenges of time, environmental stressors, and pathogens. Without effective preservation methods, this genetic wealth can be lost forever.

Cryopreservation is a critical technique in plant germplasm conservation, particularly for the long-term storage of vegetatively propagated species and non-orthodox seed species. The main challenge in cryopreservation is the removal of intracellular water, which has the potential to form ice crystals during freezing and rewarming, leading to cell injury. The goal of cryopreservation is to minimize both dehydration-induced and intracellular freezing injuries to ensure the survival of cells and tissues (ref).

New Cryo-techniques

The success of cryopreservation, as you mentioned, is often based on the vitrification phenomenon, which involves the formation of a glass-like state instead of ice crystals within the cells or tissues being preserved. This is achieved through a carefully controlled process of desiccation, where the removal of water is a critical step. There are two primary methods for desiccation in cryopreservation:

Comment [ALC2]: Mentioned by who?

1**. Exposure to Concentrated Cryoprotective Solutions**: In this method, plant samples are exposed to cryoprotective solutions with high concentrations of specific chemicals. These solutions help protect the cells from damage during the freezing and thawing processes. The exposure to concentrated cryoprotective solutions typically involves a gradual increase in solution concentration to avoid osmotic shock to the cells. This process also dehydrates the cells and helps prevent ice crystal formation.

2. **Air Desiccation**: Air desiccation involves the controlled drying of plant samples using dry air or a desiccant. This method removes water from the samples by allowing it to evaporate gradually. Air desiccation is often used when working with smaller plant tissues, such as shoot tips, meristems, or embryos. The goal is to achieve a state where the samples are sufficiently dry but have not undergone freezing.

Methodology

Planting materials

The Lisa variety of red-fleshed pitaya fruit was collected from Bainsan village in the Mawana area of Meerut, Uttar Pradesh, India. The morphology and characteristics of the fruit are summarized as follows:

Fruit Collection:
Variety: Lisa variety
Location: Bainsan village, Mawana district, Meerut, Uttar Pradesh, India
Latitude: 29.1156969
Longitude: 77.875735
Harvesting Date: November 2, 2022
Harvesting Stage: The fruit was collected at the full maturity stage on November 2, 2022, indicating it was ready for harvesting.

Fruit Morphology & Description:
Shape: Oval and elliptical
Peel Color: Vibrant pink, which is characteristic of red-fleshed pitaya.
Flesh Color: Magenta to pink, reflecting the typical coloration of the red-fleshed pitaya.
Diameter: Approximately 75.51 mm
Length: Approximately 81.87 mm
Weight: The fruit weighed approximately 254.81 grams.
Total Soluble Solids (TSS): The fruit had a TSS measurement of 10º Brix at 20º C, indicating its sweetness level.

Seed Characteristics:
Seed Color: Black
Seed Shape: The seeds were about 1 mm in size and pear-shaped.

Seed Edibility: The seeds were fully edible, making them suitable for consumption along with the fruit's flesh.

Seed Moisture Evaluation

The seed moisture content (%) is calculated using the following formula:

$$\text{Moisture (\%)} = \frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \times 100$$

Comment [ALC3]: Use equation editior

Equipment and Materials: Moisture bottles with caps, Seeds to be tested, Hot air oven, charged silica gel, Weighing balance.

Drying in a hot Air Oven: The moisture bottles with seeds were placed in a hot air oven at 103°C for 17 hours to allow the moisture in the seeds to evaporate.

Desiccation intervals and media used

The moisture content (%) of fresh seeds using the air desiccation method at various time intervals was measured at different time points to track the drying process. Each time interval corresponds to a specific moisture content percentage (%), which can provide insights into how the seeds lose moisture over time.

0 Hours: This represents the initial moisture content (%) of the fresh seeds before the air desiccation process begins.

2 Hours: After 2 hours of air desiccation, the moisture content (%) of the seeds was measured.

4 Hours: After 4 hours of air desiccation, the moisture content (%) of the seeds was measured again.

6 Hours: After 6 hours of air desiccation, the moisture content (%) of the seeds was measured.

8 Hours: After 8 hours of air desiccation, the moisture content (%) of the seeds was measured.

10 Hours: After 10 hours of air desiccation, the moisture content (%) of the seeds was measured.

12 Hours: After 12 hours of air desiccation, the final moisture content (%) of the seeds was measured.

By measuring the moisture content at each of these time intervals, a drying curve was created that showed how the seeds gradually lose moisture over time when subjected to air desiccation. This information is valuable for understanding the drying kinetics of the seeds and determining the optimal drying time to achieve a specific target moisture content for storage or further processing.

Three different treatments: GA_3, KNO_3, and a Control (distilled water). These treatments were applied to the seeds after various air desiccation time intervals, and the resulting germination rates and patterns were evaluated. This approach allowed researchers to investigate how different treatments influenced the germination of dragon fruit seeds after air desiccation, providing valuable insights into the optimal conditions for seed germination in the present study.

Experimental details

The experiment involved a factorial design with two factors: different treatments (GA_3, KNO_3, and Control with distilled water) as factor A and desiccation time intervals (0, 2, 4, 6, 8, 10, and 12 hours) as factor B using the Randomized Complete Block Design (Factorial) method. The primary objective of the present study was to evaluate the interaction between these two factors and their impact on the germination of dragon fruit seeds.

Here's a breakdown of the experimental design:

Factor A: - Treatments for germination Distilled water, GA_3& KNO_3

1: Distilled water

2: 100 ppm GA_3

3: 0.1% KNO_3

Factor B: - Air-Desiccation time

0 Hrs, 2 Hrs, 4 Hrs, 6 Hrs, 8 Hrs, 10 Hrs, 12 Hrs

Experimental Procedure:

1. Dragon fruit seeds were subjected to different desiccation time intervals ranging from 0 to 12 hours.
2. During each time interval, seeds were treated with one of three media types: GA_3, KNO_3, or a control with distilled water.
3. After the specified desiccation period and media treatment, the seeds were evaluated for germination.
4. The germination rates and patterns were recorded and analyzed to determine the influence of desiccation time intervals and media treatments on seed germination.
5. This experimental procedure was repeated for both non-cryopreserved and cryopreserved dragon fruit seeds.

By conducting this factorial experiment, it can be identified that which combination of factors (desiccation time and media type) are most conducive to successful seed germination for dragon fruit seeds, both in their natural state and after cryopreservation both the experiment 1 and 2 treatments were listed in Table 1 & 2.

Seed Germination

Experiments were conducted for germination tests using different treatments for both non-cryopreserved and cryopreserved dragon fruit seeds, under control conditions.

1. Desiccated dragon fruit seeds, which were subjected to various air desiccation time intervals as part of the earlier experiment. Were kept for germination under the B.O.D at 25 ºCat 60-65 % humidity at light intervals of 8:16.
2. Half of the desiccated seeds were cryopreserved by quick freezing. These seeds were stored in vials and marked with their respective air desiccation durations, remaining half desiccated seeds were kept for direct germination (non-cryopreserved).
3. The vials containing seed labeled as 'cryo-exposed seeds' were immersed in liquid nitrogen at -196 ºC and kept there for 48 hours.
4. After the 48-hour cryopreservation period, the vials with the cryo-exposed seeds were taken out from the liquid nitrogen.
5. To thaw the cryo-exposed seeds, the vials were transferred to water at 40 ºC to bring them back to normal temperature.
6. Both fresh (non-cryopreserved) seeds and cryo-exposed seeds were then used for germination tests.
7. The germination tests were conducted in Petri plates using the same treatments:
1. Distilled water, 2. GA_3 100 ppm, and 3. KNO_3 0.1% for comparison.
8. Germination rates and patterns were recorded and compared between fresh seeds and cryo-exposed seeds for each media treatment.

Moisture content % at time intervals

After each desiccation period, the moisture content was assessed on the seeds to determine the level of moisture reduction. This was accomplished using the oven drying method, where the seeds were dried at 103°C for a period of 17 hours.

Moisture Content (%) = [(Fresh Weight - Dry Weight) / Fresh Weight] x 100

By following this method, the moisture content of the seeds after each desiccation time interval. This information is crucial for understanding how the duration of desiccation affects the moisture level in the seeds, which, in turn, can impact their germination and viability.

Germination percentage

1. Counting Germinated Seeds: Throughout the germination period, seeds were periodically counted the number of seeds that had germinated in each treatment at regular intervals, which in this case was every two days.
2. Total Germinated Seeds: After the entire germination process was completed, you summed up the total number of seeds that had germinated in each treatment.
3. Total Seeds Sown: 20 per peri plate
4. Percentage of Germination: To calculate the percentage of germination, the total number of germinated seeds was subtracted from the initial number of seeds sown and then expressed as a percentage:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total Number of seeds put for germination}} \times 100$$

Comment [ALC4]: Use equation editior

Results and Discussion

Moisture content % at time intervals

The moisture content of dragon fruit seeds decreased steadily during the desiccation process. At the start of desiccation (0 hours), the moisture content was relatively high at 35.09%. After 2 hours of desiccation, the moisture content dropped to 23.78%, indicating the removal of some moisture from the seeds with graphically mentioned in figure.1.

1. 0 Hours: At the start of desiccation 0 hours, the moisture content of the dragon fruit seeds was relatively high, recorded at 35.09%. This is expected as the seeds were initially harvested with a certain amount of moisture.
2. 2 Hours: Throughout desiccation, the moisture content steadily decreased. At 2 hours, it dropped to 23.78%, indicating that some moisture had been removed from the seeds.
3. 4 Hours: After 4 hours of desiccation, the moisture content further decreased to 19.32%. This trend continued as the desiccation time increased.
4. 6 Hours: At 6 hours, the moisture content was 13.79%, indicating significant drying of the seeds.
5. 8 Hours: By 8 hours, the moisture content had decreased even more, reaching 9.6%.
6. 10 Hours: As the desiccation process progressed, the moisture content significantly decreased to 8.6% after 10 hours. This indicates effective drying and removal of moisture from the seeds, which is generally desirable for storage and germination purposes.
7. 12 Hours: Interestingly, after reaching the low point at 10 hours, the seeds gained some moisture, with the moisture content increasing to 9.4% by 12 hours. This slight increase in moisture content suggests that there may have been some moisture uptake or rehydration during this period.

Days required for initiation of germination:

The time it took for the first seed to begin germinating varied under different conditions. This calculation was repeated for each treatment to assess the variation in germination initiation time. However, specific data on the time taken for germination initiation is not provided in the given document which mentioned in Table.3.

Experiment 1 (Non-Cryopreserved Seeds):

- The significantly shortest time required for germination initiation (2.33 days) was observed when seeds were subjected to 10 hours of air desiccation.
- Among the germination media, KNO_3 resulted in the fastest germination initiation, taking 3.86 days.
- This combination of 10 hours of air desiccation and KNO_3 was statistically superior to other desiccation durations.
- Among the germination media, GA_3 resulted in the slower germination initiation, taking 4.57 days.
- Among the germination treatments, Distilled water (control) resulted in the slowest germination initiation, taking 5.00 days.

Experiment 2 (Cryopreserved Seeds):

- In this experiment with cryopreserved seeds, 10 hours of air desiccation also resulted in the shortest germination initiation time, taking 4 days.
- Similarly, KNO_3 media led to the fastest germination initiation among the media, requiring 3.33 days.
- Among the germination media, KNO_3 resulted in the little germination initiation, taking 4.33 days.
- Among the germination media, GA_3 resulted in the slower germination initiation, taking 5.00 days.
- Among the germination media, distilled water (control) resulted in the slowest germination initiation, taking 5.24 days.
- The combination of 10 hours of air desiccation and KNO_3 media was again statistically superior in terms of faster germination initiation.

- GA_3 and distilled water media showed longer germination initiation times compared to KNO_3.

Overall, the interaction between germination media and hours of air desiccation significantly influenced the time required for germination initiation in both non-cryopreserved and cryopreserved dragon fruit seeds. The combination of 10 hours of air desiccation and KNO_3 media consistently resulted in the shortest germination initiation times, indicating its effectiveness in promoting the rapid germination of dragon fruit seeds. These findings can be important for optimizing seed germination protocols and improving the efficiency of dragon fruit seed propagation **[Arif at el.,(2016); Islam at el.,(2017); Zuo at el.,(2018); Ashraf at el.,(1996); McDonald at el.,(1997)].**

Germination percentage:

The results for the percentage of germination of dragon fruit seeds under various treatment combinations are shown in Table.4, which also identifies the experiments with the highest percentages of germination, Experiment 1 (non-cryo exposed) and Experiment 2 (cryexposed).

Experiment 1 (Non-Cryo Exposed Seeds): The best germination percentage in Experiment 1 was achieved with Distilled Water (100%) at 0 hours and 2 hours of air desiccation. This suggests that non-cryexposed dragon fruit seeds should be subjected to no desiccation or a very short desiccation period for optimal germination.

Experiment 2 (Cryo Exposed Seeds): In Experiment 2 (cryo exposed seeds), the best germination percentage was recorded with KNO_3 media (100%) at 10 hours of air desiccation. This treatment resulted in the highest germination percentage among all combinations.

Experiment 2. Table 2 shows that maximum germination was at 100.00% and 98.33% in the treatment (T20) treated with GA_3 and KNO_3, respectively and Treatments (T16 and T21) were at par. This value was achieved at 2 hours, 10 hours, and 12 hours of air desiccation in KNO_3 media.

The best results in Experiment 2 for minimizing the number of days required for germination were achieved with a combination of KNO_3 and either 10 or 12 hours of air desiccation. These conditions were highly effective in promoting germination even in the presence of cryopreservation. This finding emphasizes the importance of optimizing both germination and

desiccation time for efficient germination of dragon fruit seeds under cryopreserved conditions. These references provide further information on the use of control (Distilled water), GA_3, and KNO_3 in seed germination studies and support the findings and recommendations based on the provided data. **M at el.,(1994), Gomez-González at el.,(2014), Santana at el.,(2006).**

Conclusion

Based on the given data, the experiment aimed to compare the effectiveness of different treatments in promoting germination in dragon fruit seeds. A unique aspect of the study involved exposing a portion of the desiccated seeds to liquid nitrogen, followed by thawing, to explore its impact on germination of seeds. The germination percentage & days to germinate in Cryo exposed seeds was due to the orthodox behavior of seed in the dragon fruit seeds. Whereas in case of desiccated seeds with cryo exposed seeds loss of viability was not seen which is an ideal situation for long-term seed storage. The findings unveiled a noteworthy outcome: the seeds exhibited their highest germination rates when treated with 0.1% KNO_3 with the air desiccation duration of 10 hours. This outcome suggests that potassium nitrate may serve as a potent factor in enhancing dragon fruit seed germination, particularly following desiccation and freezing procedures. These insights hold substantial promise for the cultivation and propagation of this tropical fruit, potentially leading to improved yields and quality in Dragon fruit farming.

References

Bartholomew, D. P., & Paull, R. E. (1985). The pitahaya (Cactaceae). In Evolution and systematics of the Cactaceae (pp. 187-192).

Baskin, C. C., & Baskin, J. M. (2014). Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press.

Berjak, P., &Pammenter, N. (2008). From Avicennia to Zizania: seed recalcitrance in perspective. Annals of Botany, 101(2), 213-228. [DOI: 10.1093/aob/mcm137].

Berjak, P., &Pammenter, N. W. (2008). Desiccation and survival in plants: Drying without dying. CRC Press.

Berjak, P., &Pammenter, N. W. (2008). Desiccation and survival in plants: Drying without dying. CRC Press.

Bewley, J. D., & Black, M. (1994). Seeds: Physiology of Development and Germination.

Bewley, J. D., & Oliver, M. J. (1992). Desiccation tolerance in vegetative plant cells. In Plant desiccation tolerance (pp. 171-214). Blackwell Publishing. [DOI: 10.1007/978-3-642-84038-4_8].

Bonner, F. T. (2008). Cryopreservation: A means for conserving and promoting the use of wild plant germplasm. In Vitro Cellular & Developmental Biology - Plant, 44(5), 456-463. [DOI: 10.1007/s11627-008-9196-9].

Buitink, J., & Leprince, O. (2004). Glass formation in plant anhydrobiotes: survival in the dry

Cisneros-Zevallos, L. (2003). The use of controlled postharvest abiotic stresses as a tool for enhancing the nutraceutical content and adding-value of fresh fruits and vegetables.

Comstock Publishing Associates.

D. N., Wijaya, C. H., Vo, T. S., & Nguyen, M. H. (2013). Optimization of the extraction process of bioactive compounds from dragon fruit (Hylocereuspolyrhizus) peel using response surface methodology. International Journal of Food Science & Technology, 48(4), 761-767. [DOI: 10.1111/ijfs.12038].

Ellis, D. D., &Towill, L. E. (2008). Seed cryopreservation of orchids. In Vitro Cellular & Developmental Biology - Plant, 44(3), 209-220. [DOI: 10.1007/s11627-008-9122-0].

Ellis, R. H., & Hong, T. D. (2007). Seed desiccation tolerance and storability: Dependence on flat-lining relative humidity and other ecophysiological traits. Annals of Botany, 99(2), 228-248. [DOI: 10.1093/aob/mcl253].

Ellis, R. H., & Roberts, E. H. (1980). Improved equations for the prediction of seed longevity.

Ellis, R. H., Hong, T. D., & Roberts, E. H. (1988). Handbook of seed technology for gene banks. Volume I: Principles and methodology. International Board for Plant Genetic Resources (IBPGR).

Ellis, R. H., Hong, T. D., & Roberts, E. H. (1990). An intermediate category of seed storage behaviour? I. Coffee. Journal of Experimental Botany, 41(2), 1167-1174. [DOI: 10.1093/jxb/41.9.1167].

Engelmann, F. (2011). Use of biotechnologies for the conservation of plant biodiversity. In Vitro Cellular & Developmental Biology - Plant, 47(1), 5-16. [DOI: 10.1007/s11627- 010-9330-z].

Engelmann, F. (2011). Use of biotechnologies for the conservation of plant biodiversity. In Vitro Cellular & Developmental Biology - Plant, 47(1), 5-16. [DOI: 10.1007/s11627- 010-9330-z.

Engelmann, F., & Takagi, H. (2000). Cryopreservation of tropical plant germplasm—current research progress and application in Japan and developing countries. Plant Cell,

Engels, J. M. M., Visser, L., & Ellis, R. H. (1995). A key to the classification of seed storage types. Seed Science Research, 5(1), 1-10. [DOI: 10.1017/S0960258500002707].

Esquivel, P., Stintzing, F. C., & Carle, R. (2007). Phenolic compound profiles and their corresponding antioxidant capacity of purple pitaya (Hylocereus sp.) genotypes. Zeitschrift für Naturforschung C, 62(9-10), 636-644. [DOI: 10.1515/znc-2007-9- 1002].

Farrant, J. M., Moore, J. P., & Driouich, A. (2017). Drought-induced leaf shedding in Rumex species from contrasting habitats: occurrence and regulation. Annals of Botany, 120(5), 797-806. [DOI: 10.1093/aob/mcx063].

Finch-Savage, W. E., & Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. New Phytologist, 171(3), 501-523.

Harding, K. (2004). Cryopreservation of orthodox seeds. In Cryopreservation of tropical plant germplasm: Current research progress and application (pp. 7-17).

HarvestPlus Technical Monograph 2.

Hoekstra, F. A., Golovina, E. A., &Buitink, J. (2001). Mechanisms of plant desiccation tolerance. Trends in Plant Science, 6(9), 431-438. [DOI: 10.1016/S1360-1385(01)02052-0].

Hoekstra, F. A., Golovina, E. A., &Buitink, J. (2001). Mechanisms of plant desiccation tolerance. Trends in Plant Science, 6(9), 431-438. [DOI: 10.1016/S1360-1385(01)02052-0].

Hong, T. D., Linington, S., & Ellis, R. H. (1996). Seed storage behaviour: A compendium.

Ickert-Bond, S. M., Wen, J., & Van Buren, R. (2015). Evolution of the fruit in Cactaceae (Caryophyllales): Pericarp diversity and character evolution. American Journal of Botany, 102(2), 1-16. [DOI: 10.3732/ajb.1400468in seed bank patterns. Journal of Applied Ecology, 33(6), 1496-1508.International Plant Genetic Resources Institute (IPGRI).

Kermode, A. R. (2005). Role of abscisic acid in seed dormancy. Journal of Plant Growth

Ming, R., & Paull, R. E. (1999). Recurrent fruit drop of pitaya, Hylocereus spp. HoritScience,

Nerd, A., & Mizrahi, Y. (1997). Reproductive biology of cactus fruit crops. Horticultural Reviews, 18, 321-346. [DOI: 10.1002/9780470650732.ch7].

Panis, B., Nagel, M., Van den Houwe, I., & Swennen, R. (2007). Cryopreservation of plant germplasm using the encapsulation-dehydration technique: Review and case studies in potato, sugarcane and sweetpotato. Plant Cell, Tissue and Organ Culture, 90(1), 1- 13. [DOI: 10.1007/s11240-007-9247-9].

Penfield, S., Springthorpe, V., & West, A. (2012). Seed dormancy and germination: understanding the role of reactive oxygen species. Physiologia Plantarum, 145(1), 135-139.

Priestley, D. A. (1986). Seed aging: Implications for seed storage and persistence in the soil.

Priestley, D. A. (1986). Seed aging: Implications for seed storage and persistence in the soil. Comstock Publishing Associates. Provides insights into seed aging and its implications for storage and persistence in the soil.

Pritchard, H. W., & Dickie, J. B. (2003). Predicting seed longevity: the use and abuse of seed viability equations. In Seeds: The ecology of regeneration in plant communities (pp. 603-628). CABI. [DOI: 10.1079/9780851995546.0603].

Pritchard, H. W., & Seaton, P. T. (2002). Development of synthetic seeds using somatic embryos of temperate recalcitrant-seeded tree species. CryoLetters, 23(2), 83-90. [PMID: 12190583].

Probert, R. J., Adams, J., Coney, D., & Binnie, L. (1996). Inter- and intraspecific variation

Reed, B. M., & Denoma, J. M. (1993). Seed desiccation tolerance: Implications for cryopreservation. In Vitro Cellular & Developmental Biology - Plant, 29(2), 63-70. [DOI: 10.1007/BF02632061].

Reed, B. M., & Hummer, K. E. (2008). Cryopreservation of clonal germplasm: Plant performance and status of the National Plant Germplasm System. In Vitro Cellular & Developmental Biology - Plant, 44(3), 235-242. [DOI: 10.1007/s11627-008-9138-5].

Roberts, E. H., & Ellis, R. H. (1989). Water and seed survival. Annals of Botany, 63(1), 39-52. [DOI: 10.1093/oxfordjournals.aob.a087807]

Rodriguez-Amaya, D. B., & Kimura, M. (2004). HarvestPlus handbook for carotenoid analysis.

Sarasan, V., Cripps, R., Ramsay, M. M., Atherton, C., & McMichen, M. (2006). Conservation in vitro of threatened plants—progress in the past decade. In Vitro Cellular & Developmental Biology - Plant, 42(3), 206-214. [DOI: 10.1079/IVP2006774].

Siti Azizah, M. N., & Mohamad Osman, H. (2013). Dragon fruit production, postharvest handling and marketing in Malaysia. Acta Horticulturae, 985, 183-187. [DOI: 10.17660/ActaHortic.2013.985.26].

Towill, L. E., & Ellis, D. D. (2008). Cryopreservation of seeds. In Plant cryopreservation: A practical guide (pp. 111-144). Springer. [DOI: 10.1007/978-0-387-72276-9_5].

Vertucci, C. W., & Farrant, J. M. (1995). Acquisition and loss of desiccation tolerance. In Seed development and germination (pp. 237-271). Springer. [DOI: 10.1007/978-94- 011-0354-3_10].

Vertucci, C. W., & Leopold, A. C. (1984). The relationship between the physical state of water in seed and its ability to survive. In Plant Cold Hardiness and Freezing Stress (pp. 251-272). Springer [DOI: 10.1007/978-1-4612-5309-5_15].

Walters, C., & Farrant, J. M. (1989). Principles of desiccation tolerance. In Seeds: The ecology of regeneration in plant communities (pp. 287-305). CABI. [DOI: 10.1079/9780851986094.0287].

Wang, Q. C., & Valkonen, J. P. (2008). Cryotherapy of shoot tips: Novel pathogen eradication method. Trends in Plant Science, 13(1), 8-12. [DOI: 10.1016/j.tplants.2007.10.003].

Table.1: Treatment Combination for Experiment 1 *i.e.* (without cryo-preservation)

S. No.	Treatments No.	Treatments details
1	T_1	Distill water +Time: 0 Hrs
2	T_2	Distill water +Time: 2 Hrs
3	T_3	Distill water +Time: 4 Hrs
4	T_4	Distill water +Time: 6 hrs
5	T_5	Distill water +Time: 8 hrs
6	T_6	Distill water +Time: 10 hrs
7	T_7	Distill water +Time: 12 hrs
8	T_8	GA_3 +Time: 0 Hrs
9	T_9	GA_3 +Time: 2 Hrs
10	T_{10}	GA_3 +Time: 4 Hrs
11	T_{11}	GA_3 +Time: 6 hrs
12	T_{12}	GA_3 +Time: 8 hrs
13	T_{13}	GA_3+Time: 10 hrs
14	T_{14}	GA_3+Time: 12 hrs
15	T_{15}	KNO_3 +Time: 0 Hrs
16	T_{16}	KNO_3 +Time: 2 Hrs
17	T_{17}	KNO_3 +Time: 4 Hrs
18	T_{18}	KNO_3 +Time: 6 hrs
19	T_{19}	KNO_3 +Time: 8 hrs
20	T_{20}	KNO_3+Time: 10 hrs
21	T_{21}	KNO_3+Time: 12 hrs

Table.2: Treatment Combination for Experiment 2 i.e. (with cryo-preservation)

S. No.	Treatments No.	Treatments details
1	T_1	Distill water +Time: 0 Hrs +Cryo
2	T_2	Distill water +Time: 2 Hrs+Cryo
3	T_3	Distill water +Time: 4 Hrs+Cryo
4	T_4	Distill water +Time: 6 hrs+Cryo
5	T_5	Distill water +Time: 8 hrs+Cryo
6	T_6	Distill water +Time: 10 hrs+Cryo
7	T_7	Distill water +Time: 12 hrs+Cryo
8	T_8	GA_3 +Time: 0 Hrs+Cryo
9	T_9	GA_3 +Time: 2 Hrs+Cryo
10	T_{10}	GA_3 +Time: 4 Hrs+Cryo
11	T_{11}	GA_3 +Time: 6 hrs+Cryo
12	T_{12}	GA_3 +Time: 8 hrs+Cryo
13	T_{13}	GA_3+Time: 10 hrs+Cryo
14	T_{14}	GA_3+Time: 12 hrs+Cryo
15	T_{15}	KNO_3 +Time: 0 Hrs+Cryo
16	T_{16}	KNO_3 +Time: 2 Hrs+Cryo
17	T_{17}	KNO_3 +Time: 4 Hrs+Cryo
18	T_{18}	KNO_3 +Time: 6 hrs+Cryo
19	T_{19}	KNO_3 +Time: 8 hrs+Cryo

20	T_{20}	KNO_3+Time: 10 hrs+Cryo
21	T_{21}	KNO_3+Time: 12 hrs+Cryo

Table 3:Days required for initiation of germination

Air drying duration	Experiment 1				Experiment 2			
	Non-Cryopreservation				Cryo-presevation			
	Distilled water	GA_3	KNO_3	Mean B	Distilled water	GA_3	KNO_3	Mean B
0 Hrs	7.33	7.00	4.67	6.33	6.33	5.33	5.00	5.56
2 Hrs	6.00	5.33	4.33	5.22	5.67	5.33	4.67	5.22
4 Hrs	5.67	4.67	4.33	4.89	5.33	5.67	4.67	5.22
6 Hrs	4.67	4.67	3.33	4.22	5.33	5.33	4.33	5.00
8 Hrs	4.00	4.33	3.67	4.00	4.67	4.67	4.00	4.44
10 Hrs	3.00	2.33	2.33	2.56	4.33	4.33	3.33	4.00
12 Hrs	4.33	3.67	4.33	4.11	5.00	4.33	4.33	4.56
Mean A	5.00	4.57	3.86		5.24	5.00	4.33	
Factors	**C.D.**	**SE(d)**	**SE(m)**		**C.D.**	**SE(d)**	**SE(m)**	
A	**0.352**	**0.174**	**0.123**		**0.409**	**0.202**	**0.143**	
B	**0.538**	**0.266**	**0.188**		**0.625**	**0.309**	**0.218**	
Interaction A x B	**0.932**	**0.460**	**0.325**		**N/A**	**0.535**	**0.378**	

Table.4: Seed Germination percentage

Treatment	Experiment 1				Experiment 2			
	Seed germination				Seed germination			
	Distilled water	GA_3	KNO_3	Mean B	Distilled water	GA_3	KNO_3	Mean B
0 Hrs	100.00	98.33	96.67	98.33	86.67	86.67	91.67	88.33
2 Hrs	100.00	98.33	96.67	98.33	88.33	85.00	98.33	90.56
4 Hrs	100.00	93.33	91.67	95.00	93.33	86.67	96.67	92.22
6 Hrs	98.33	95.00	93.33	95.56	85.00	83.33	96.67	88.33
8 Hrs	91.67	95.00	98.33	95.00	88.33	93.33	93.33	91.67
10 Hrs	83.33	93.33	100.00	92.22	86.67	96.67	100.00	94.44
12 Hrs	81.67	93.33	96.67	90.56	85.00	88.33	98.33	90.55
Mean A	93.57	95.24	96.19		87.62	88.57	96.43	
Factors	**C.D.**	**SE(d)**	**SE(m)**		**C.D.**	**SE(d)**	**SE(m)**	

A	1.804	0.891	0.630		1.761	0.869	0.615	
B	2.756	1.361	0.962		2.690	1.328	0.939	
Interaction A x B	4.773	2.357	1.667		4.658	2.300	1.627	

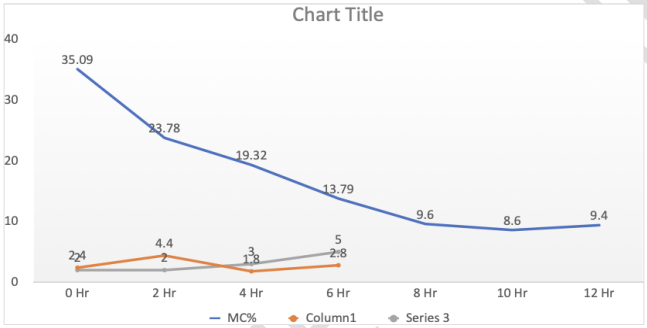

Fig: 1 Shows the interaction of moisture content to time intervals