# Effect of Storage Temperature, Storage Containers and Storage Duration on Seed Viability of *Stevia rebaudiana*

Prateek Mastana[1], Y P Sharma[2], Rajeev Dhiman[3]
[1,2]*Department of Forest Products, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, (H.P.) 173230*
[3]*Department of Silviculture and Agroforestry, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, (H.P.) 173230*

***Abstract*-The present investigation entitled, "Effect of Storage Temperature, Storage Containers and Storage Duration on Seed Viability of *Stevia rebaudiana*" was carried out in laboratory and experimental area, located at Nauni of the Department of Forest Products. The experiment comprised of treatments: (A) Storage conditions: 1) At room temperature, (2) In refrigerator (4ºC – 6ºC): B) Storage containers: (1) Plastic bags, (2) Paper bags, (3) Cloth bags; C) Storage duration: (1) 0 month (immediate after harvest) ( control), (2) Up to 1 month, (3) Up to 2 months, (4) Up to 3 months, (5) Up to 4 months, (6) Up to 5 months, (7) Up to 6 months with 3 replications in Completely Randomized Design. The results showed that the seed viability was found to decrease with the increase in storage duration irrespective of storage container and storage temperature. Seed viability was found to decrease from 83.33% (freshly harvested seed) to 52.78 % (under refrigerated condition) and 40% (under open storage) after 6 months of storage. Among different storage containers, plastic bags retain maximum viability (53.33%) and cloth bags recorded minimum (40.00%) after 6 months of storage. However, mean viability of seeds decreased to 46.39 % after 6 months of storage. Hence plastic bags under refrigerated conditions proved to be the best containers for retaining maximum seed viability of *Stevia rebaudiana.***

***Keywords*: Storage Containers, Storage Duration, Seed Viability and *Stevia rebaudiana etc***

## INTRODUCTION

*Stevia rebaudiana* Bertoni, a natural sweetener plant with zero calorie content, becomes an inevitable substitute to sugar especially with the over 346 million diabetic population across the world (WHO, 2011). Stevia is a non-caloric sweetener and that the sweet compounds pass through the digestive process of the body without chemically breaking down, hence making it a safe food substance for consumption by people who need to regulate their blood glucose level (Strauss, 1995). Stevia doesn't have any adverse effect on humans (Brandle and Rosa, 1992). The leaves could be eaten fresh or when dried and it could be boiled in tea to release the sweetener. Though agronomically it possesses above mentioned positive characteristics, it also possesses some agronomically challenging characteristics such as being day length sensitive, water logging sensitive, low to moderately resistant to drought (Jia, 1984), with poor seed germination (Duke, 1993 and Carneiro et al., 1997) and self-incompatibility (Chalapathi et al., 1997a). Due to its poor germination, this study was carried out to study the effect of Storage temperature, storage containers and duration on the viability of seeds of *Stevia rebaudiana*.

## MATERIAL AND METHODS

Freshly harvested seeds (dark colored) were stored in refrigerator (4ºC- 6ºC) and at room temperature in laboratory in three types of storage containers viz. paper bags, plastic bags and cloth bags. Seed viability was studied at monthly interval up to six months of storage through germination method. Twenty seeds in each replication were placed on germination paper in petriplates. These petriplates were then placed in seed germinator with the temperature maintained at $24^0$C – $28^0$C and relative humidity at 65-70%. The experiment comprised of Treatments: (A) Storage conditions: 1) At room temperature, (2) In refrigerator (4ºC – 6ºC): B) Storage containers: (1) Plastic bags, (2) Paper bags, (3) Cloth bags; C) Storage duration: (1) 0 month (immediate after harvest) ( control), (2) Up to 1 month, (3) Up to 2 months, (4) Up to 3 months, (5) Up to 4 months, (6) Up to 5 months, (7) Up to 6 months with 3 replications in Completely Randomized Design.

RESULTS AND DISCUSSION

Seeds stored immediately after harvest in plastic bags, paper bags and cloth bags for different duration (0, 1, 2, 3, 4, 5, 6 months) at different storage conditions viz. $4^0$C and room temperature were tested for seed viability percentage through germination method. The results were found significant and are presented in Table 1 and 2.

Table 1. Effect of storage duration and storage containers on seed viability

| Storage duration (months) \ Storage containers | Seed viability (%) | | | |
|---|---|---|---|---|
| | Plastic bags | Paper bags | Cloth bags | Mean viability (%) |
| 0 month | 83.33 (66.26) | 83.33 (66.26) | 83.33 (66.26) | 83.33 (66.26) |
| 1st month | 74.17 (59.79) | 67.50 (55.58) | 68.33 (55.86) | 70.00 (57.08) |
| 2nd month | 70.00 (56.95) | 65.00 (54.10) | 56.67 (48.88) | 63.89 (53.31) |
| 3rd month | 65.83 (54.43) | 62.50 (52.33) | 57.50 (48.88) | 61.39 (51.68) |
| 4th month | 64.17 (53.30) | 59.17 (50.35) | 48.33 (49.41) | 57.78 (49.60) |
| 5th month | 60.83 (51.35) | 51.16 (45.99) | 44.17 (44.01) | 52.22 (46.28) |
| 6th month | 53.33 (46.93) | 45.83 (42.59) | 40.00 (39.02) | 46.39 (42.85) |
| Mean viability (%) | 67.38 (55.57) | 62.14 (52.46) | 56.90 (49.90) | |

* Figures in parentheses are ARC sine transformed values

| | | $CD_{0.05}$ | S E (±) |
|---|---|---|---|
| Storage Container (T) | = | 2.14 | 1.08 |
| Storage Temperature (C) | = | 1.75 | 0.89 |
| Storage Months (I) | = | 3.28 | 1.65 |
| T X C | = | 3.00 | 1.52 |
| T X I | = | 5.64 | 2.85 |
| T X C | = | 4.61 | 2.33 |

At the time of harvest, 83.33 per cent seeds were found viable. The seed viability was found to decrease with the increase in storage duration under all the storage containers and temperatures.

The mean viability of seeds irrespective of storage containers (plastic bags, paper bags and cloth bags) and storage temperature ($4^0$C and room temperature) was found to decrease continuously from 83.33 %( at the time of storage) to 46.39% after 6 month of storage (Table 1 and 2).

Table 2. Effect of storage duration and storage temperature on seed viability

| Storage duration (months) \ Storage temperature | Seed Viability (%) | | |
|---|---|---|---|
| | $4^o$C | Room temperature | Mean Viability (%) |
| 0 month | 83.33 (66.26) | 83.33 (66.26) | 83.33 (66.26) |
| 1st month | 76.67 (61.34) | 63.33 (52.82) | 70.00 (57.08) |
| 2nd month | 70.56 (57.43) | 57.22 (49.19) | 63.89 (53.31) |
| 3rd month | 64.44 (53.48) | 58.33 (49.88) | 61.39 (51.68) |
| 4th month | 67.78 (55.53) | 47.78 (43.66) | 57.78 (49.60) |
| 5th month | 60.56 (51.19) | 43.89 (41.38) | 52.22 (46.28) |
| 6th month | 52.78 (46.61) | 40.00 (39.09) | 46.39 (42.85) |
| Mean viability (%) | 68.02 (55.98) | 56.27 (48.90) | |

* Figures in parentheses are ARC sine transformed values

| | | $CD_{0.05}$ | S E (±) |
|---|---|---|---|
| Storage Container (T) | = | 2.14 | 1.08 |
| Storage Temperature (C) | = | 1.75 | 0.89 |
| Storage Months (I) | = | 3.28 | 1.65 |
| T X C | = | 3.00 | 1.52 |
| T X I | = | 5.64 | 2.85 |
| T X C | = | 4.61 | 2.33 |

Under different storage temperature, the rate of decrease of seed viability was different. Under 40C storage temperature, seed viability decreases to 52.78% after 6 months of storage. At room temperature, seed viability decreases to 40.00 after 6 months of storage. The mean seed viability was maximum 68.02% at $4^0$C storage and minimum 56.27% under room temperature irrespective of storage duration and storage containers (Table 2).

Under different storage containers, the rate of decrease of seed viability was different. Seed viability decreased to 53.33% in plastic bags, 45.83% in paper bags and 40.00% in cloth bags after 6 month of storage. The mean seed viability was found maximum 67.38% under plastic bags storage and minimum 56.90% under cloth bags storage (Table 1).

Production of viable seeds is the culmination of all the physiological and developmental phases that a plant undergoes during its life cycle. This is to ensure perpetuation and spread of the species in larger habitats overcoming the hazards of unfavorable environmental conditions. Often the extent up to which the seeds remain viable determines to a large extent the success of establishing new populations at a location. Most of the seeds have inherent capacity to withstand adverse environmental conditions but gradually keep on loosing viability with the passage of time. For any cultivation effort to succeed it is imperative to understand the extent up to which its seeds can be stored and under what conditions.

The concept of seed viability is very intricate one and it denotes the degree to which a seed is metabolically active and capable of germinating. It is probably highest at the time of physiological maturity when most seeds are dispersed (Bedell. 1998). Conventional seed storage is believed to be a safe, effective and inexpensive method of ex-situ conservation of plant genetic resources, which not only maintains its viability but also its vigour without hampering the genetic makeup. The ideal storage conditions are always those which reduce the growth processes of respiration and transpiration to the lowest possible degree without impairing in any way the inherent vitality and strength of the seed embryo (Phartyal et al., 2002).

In the majority of species (orthodox species) the period of seed viability is dependent on temperature, moisture content and oxygen pressure, decreasing any one of these factors increases the period of viability. Moreover the conditions before and during harvest (including post harvest drying) may affect subsequent loss of viability (Roberts. 1973). In several plant species seed viability is lost within few months of storage at room temperature. Loss of viability depends upon time span and storage conditions (Chauhan and Nautiyal, 2007).

Knowledge of the fundamental factors that induce seed deterioration is thus essential for understanding of the practical aspects of seed storage (Bedell, 1998). Any stress to viability during storage results in accelerated physiological ageing which leads to loss of viability and seed deterioration. Storage stresses lead to deleterious cytological and biochemical changes including increase in the activity of various enzymes like phytases, proteases and phosphatase which result in loss of seed viability.

The standard for judging seed quality (viability) is always a germination test under optimum conditions (Phartyal et al., 2002). The present investigation reveals that at the time of harvest, 83.33 per cent seed were viable. The seed viability decreased with the increase in storage duration under both refrigerated and room temperature conditions. However, under refrigerated storage, the decrease of seed viability was less as compared to room temperature storage. Seed viability decreased from 83.33 per cent to 52.78 per cent under refrigerated conditions, upto 40 per cent under room temperature conditions and up to 27.22 percent after 6 months of storage. The slow loss of viability under refrigerated conditions may possibly be due to reduced rate of metabolic activities and inactivation of enzymes at low temperature thus helping to retain viability. The rapid decrease in seed viability under room temperature conditions may be due to high metabolic activity at higher temperature and due to loss of moisture which is the chief cause of seed deterioration under open conditions (Chauhan and Nautiyal, 2007). It has been reported that orthodox seed storage life is halved by each 5$^{o}$C increase in temperature or by each 1 % increase in seed moisture content (Harrington, 1972).

Alvarez et al., (1994) shows that in case that it is impossible to do the sowing of seeds immediately after harvest, they should be kept in tightly sealed containers and kept in refrigerator at 4$^{0}$C, because the seeds rapidly lose their viability when left at room temperature.

The present study corroborates the findings of Thakur et al. (2004) who carried out comparative studies on seed viability status of *Achillea millefolium*, *Gentiana kurroo* and *Podophyllum hexandrum* which revealed substantial loss of 20, 75 and 25 per cent seed viability, respectively after one year of storage. The loss of viability during the storage period was found to be highest in *Gentiana kurroo* in comparison to *Achillea* and *Podophyllum* even under similar storage conditions i.e. average temperature of 19$^{o}$C and relative humidity of 60 per cent.

Similar results have been reported by Chauhan and Nautiyal (2007) in *Nardostachys jatamansi*; Kumar et al. (2007) in *P. pinnata*; Barnett and Vozzo (1985) in *Pinus elliotii* and *P. echinata* and Wakeley and Barnett (1968) in Slash and Short leaf pine.

The effect of storage containers on seed viability with the progress of storage duration under this investigation recorded superior results for plastic bags. The seed viability was 83.33 % immediately after the storage of seeds in three types of containers used viz. plastic bags, paper bags and cloth bags. The seed viability was found maximum in plastic bags (53.33%) and minimum for cloth bags (40.00 %) after six months of storage. The mean viability also decreased from 67.38% in plastic bags to 56.90 % in cloth bags after six month of storage. The maximum seed viability in plastic containers may probably be due to maintenance of proper moisture content and exchange of gases in the seeds which also helps in maintaining proper level of sugar and starch etc. required for the good germination and growth of seedlings. Low viability of paper bags and cloth bags stored seeds is due to the reduction in the seed moisture during storage which reduced the seed longevity. The low germination ability and viability of seeds in storage in the paper bags and cloth bags in the present study may be due to the changes in the physiochemical state of seeds particularly the seed metabolism due to the reduction in moisture content. The changes in seed metabolism are reported as one of the major factors for low seed germination and viability (Abdul Baki, 1980).

## CONCLUSION

- Freshly harvested seed recorded 83.33 per cent viability.
- Seed viability was found to decrease with the increase in storage duration irrespective of storage containers and storage temperature.
- The storage container has significant effect on seed viability. Maximum seed viability (53.33%) was observed in plastic bag storage and minimum (40.00%) in cloth bags after six month of storage.
- The storage temperature also has significant effect on seed viability. Seed viability reduces to about 52 per cent (under refrigerated storage condition) and 40 per cent (under room temperature storage condition) after six month of storage.
- Hence plastic bags under refrigerated conditions proved to be the best containers for retaining maximum seed viability of *Stevia rebaudiana*.

## REFERENCE


[1] Abdul-Baki A A. 1980. Biochemical aspects of seed vigour. Hort. Sci. 15:765771.

[2] Alvarez L, Casaccia R and Lopez G. 1994. Production de ka'a he'e. Instituto Agronomico Nacional. S.E.A. Ministerio de Agriculturay Ganaderia. Asuncion. Paraguay : 1-47.

[3] Barnett James P and Vozzo J A. 1985. Viability and vigor of slash and shortleaf pine seeds after 50 years of storage. Forest Science 31(2): 316-320.

[4] Bedell P E. 1998. Seed Science and Technology-Indian Forestry Species. Allied Publishers Limited, New Delhi. 346p.

[5] Brandle J E and Rosa N. 1992. Heritability for yield, leaf-stem ratio and stevioside content estimated from a landrace cultivar of Stevia rebaudiana. Can. J. Plant Sci. http://dx.doi.org/10.4141/cjps92-159

[6] Carneiro J W P, Muniz A S and Guedes T A. 1997. Greenhouse bedding plant production of Stevia rebaudiana. Canadian Journal of Plant Science 77(3): 473-474.

[7] Chalapathi M V, Shivaraj B, and Parama V R R. 1997. Nutrient uptake and yield of stevia as influenced by methods of planting and fertilizer levels. Crop Research 14(2): 205-208.

[8] Chauhan R S and Nautiyal M C. 2007. Seed germination and seed storage behaviour of Nardostachys jatamansi D C. an endangered medicinal herb of high- altitude Himalaya. Current Science 92(11): 1620-1624.

[9] Duke, J 1993 Stevia rebaudiana (Bert) ///: J Duke, Ed. CRC Handbook of Alternative Cash Crops CRC Press, Boca Raton, FL. Pp. 422-424

[10] Harrington J F. 1972. Seed storage and longevity. In: Seed Biology. T T Kozlowaski (ed.), Academic Press, New York. 3: 145-245.

[11] Jia G N. 1984. An experiment on the cultivation of Stevia rebaudiana [Chinese]. Shanxi Agricultural Science (1): 20-21.

[12] Kumar Santosh, Radhamani J, Singh Anuradh K and Varaprasad K S. 2007. Germination and seed storage behaviour in Pongamia pinnata L. Current Science 93(7): 910-911.

[13] Phartyal Shyam S, Thapliyal R C, Koedam Nico and Godefroid Sandrine. 2002. Ex situ conservation of rare and valuable forest tree species through seedgene bank. Current Science 83(11) : 1351-1357.

[14] Roberts E H. 1973. Loss of seed viability: Chromosomal and genetic aspects. Seed Sci. & Tech. 1: 515-527.
[15] Strauss, S. 1995.The perfect sweetener. Technology Review 98:18-20.
[16] Thakur Anju, Mehta Richa and Thakur P S. 2004. Germination, viability and vigour of fresh and aged seeds of some endangered medicinal plant species of Western Himalayas. Indian J. Plant Physiology 9(3): 247-254.
[17] Wakeley P C and Barnett J P. 1968. Viability of slash and short leaf pine seed stored for 35 years. Indian Journal of Forestry 66: 840-841.
[18] WHO. 2011. Diabetes Fact sheet N 312 August. [Online] Available: http://www.who.int/ mediacentre/factsheets/fs312/en/%20%20%28Retrieved 08/08/2011%29.