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Effect of cold storage on dormancy acquisition and dissipation in bitter gourd (*Momordica charantia* L.)

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Abstract
The current studies deal with the effect of cold storage on dormancy acquisition and dissipation in bitter gourd Vivek F1 hybrid. Even though the bitter gourd seed had been classified as orthodox, earlier studies reported its sensitivity to sub-zero temperature. Hence, the present study was carried out to investigate the seed germination and dormancy behaviour of bitter gourd seeds after storing at different cold storage conditions (4, 0, -20, -80 °C). In ambient condition, higher germination (71.33%) was noticed up to six months but it decreased to 36 per cent after nine months of storage. Whereas seeds stored at 0, -20, -80 °C temperatures recorded zero per cent of germination throughout the storage period. Further acquisition of secondary dormancy was confirmed by subjecting these seeds to tetrazolium viability test. Cold stored seeds require dry heat treatment (60 °C) for a period of 2 h in order to overcome cold induced secondary dormancy.

Keywords: Bitter gourd, cold storage, dormancy, germination, lipid crystallization

1. Introduction

Vegetables are important sources of proteins, vitamins, micronutrients, minerals, dietary fibers, antioxidants and phytochemicals in daily diet. *Momordica charantia* L. is a member of the cucurbitaceous family originated in the Indo Burma region of tropical Asia. It is a tropical and sub-tropical vegetable majorly grown in India, Indonesia, Malaysia, Singapore, Thailand, Japan, tropical Africa and South America and having economic, nutritional and medicinal use. It is cultivated for its immature fruits and tender shoot tips (Vogel, 1996) [12] and is also used in traditional medicine (Grover and Yadav, 2004) [7], as well as in modern medicine in the treatment of diabetes (Ahmed *et al*., 2004) [2].

For very long-term storage of seeds, cold storage is an effective approach among the different storage methods. Even though the bitter gourd seed had been classified as orthodox, earlier studies reported its sensitivity to sub-zero temperature (Doijode, 2001; Royal Botanic Gardens Kew, 2016) [5, 9]. Interestingly few gene banks reported no germination of bitter gourd seed samples stored at sub-zero temperatures for six months, whereas unchanged germination percentages could be obtained after storage at 5 °C (Ebert and Huang, 2015) [6]. Deterioration in germination of bitter gourd seed samples occurs if stored at sub-zero temperatures for more than six months (Zhang *et al*., 1990) [13]. The germination percentage decreased drastically to 43.18 per cent at fifth month and then germination percentage was reduced to zero after 180 days of cryo-storage (-196 °C) in the bitter gourd cultivar Preethi (Sharmila *et al*., 2019) [11].

Cold-induced dormancy during seed storage is evidence that seeds failure to germinate after cold storage is not necessarily due to loss of viability but seeds pass into a state of secondary dormancy during storage at sub-zero temperatures and low seed moisture content (Sebastian and Michael, 2018) [10]. Sensitivity to subzero temperature could be a major consequence for safe germplasm conservation and storage. Further seed industries faces the problem of post seed dormancy after cold storage of seeds. Hence, an attempt was made to study the behaviour of bitter gourd seeds stored at different cold storage conditions (4, 0, -20, -80 °C) and to identify the best dormancy breaking treatment.

2. Materials and Methods

2.1 Experimental site

Freshly harvested one-week old seeds of bitter gourd Vivek F1 hybrid were obtained from the Seed Unit, University of Horticultural Sciences, Bagalkot, India. The laboratory experiment was conducted by adapting completely randomized design with three replications in the Department of Seed Science and Technology, University of Agricultural Sciences, Dharwad during 2020-21. Seeds were stored at ambient storage, 0, 4, -20, -80 °C temperatures and tested periodically at first, third, sixth and nine months after storage for seed quality attributes.

2.2 Treatment details
The seeds of the bitter gourd were stored in four different cold storage environments in moisture proof sealed 700-gauge polythene bags. Seeds were kept in refrigeration at 0, 4 °C temperatures and in deep freezers at -20, -80 °C temperatures at Biotechnology laboratory, University of Agricultural Sciences, Dharwad. The required number of seeds from each cold storage environment were taken at 1, 3, 6 and 9 months of storage period to study the physiological parameters.

Further cold induced dormancy was broken by different dormancy breaking treatments such as T-1: dry heat treatment at 60 °C for 2 h, T-2: soaking in KNO3 (0.3%) solution of for 2 h and T-3: Seed priming with gibberellic acid (300 ppm) for 24 h. Among the above dormancy breaking treatment, dry heat treatment (60 °C for 2 h) was found suitable for further studies (Figure 1). After required period of cold storage as per the storage treatments, seeds were taken out from the respective cold storage conditions and dried back to original moisture content. Further exposed to dry heat in hot air oven for 2 h at 60 °C temperature than seeds were taken out from oven and kept in ambient condition till it reaches normal temperature and these seeds were used for testing seed quality parameters.

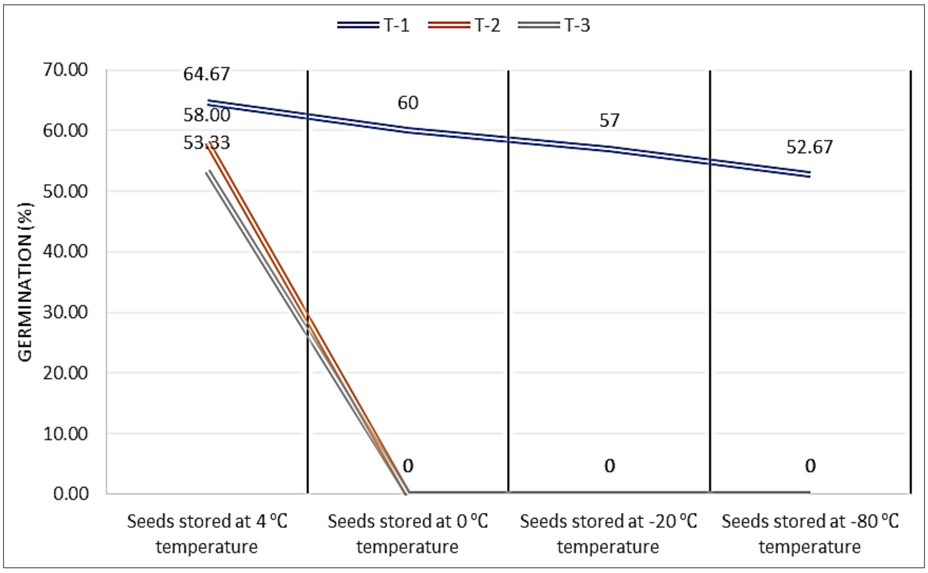

Fig 1: Effect of different dormancy breaking treatments on seed germination of bitter gourd Vivek F1 hybrid (T-1: Dry heat treatment at 60 °C for 2 h, T-2: Soaking in KNO_3 (0.3%) solution of for 2 h and T-3: Seed priming with gibberellic acid (300 ppm) for 24 h).

Standard germination test was conducted as per the procedure given by International Seed Testing Association (Annonymous, 2017) [3] by following between paper method (25 ± 1 °C temperature and 90 ± 5% relative humidity). The final count was taken on 14th day of germination test. Further observation on seed quality parameters such as abnormal seedlings (%), hard seeds (%), dead seeds (%), seedling shoot length (cm), root length (cm), seedling dry weight (mg/seedling), seedling vigour index values were measured and analysed statistically. The seedling vigour index was calculated as per the standard procedure (Abdul-Baki and Anderson, 1973) [1], multiplying the germination per cent with seedling shoot length and root length (cm).

2.3 Data analysis
The analysis and interpretation of the experimental data was done as suggested by (Panse and Sukhatme, 1985) [8]. The critical differences were calculated at one per cent level of probability wherever 'F' test was found significant for various seed quality parameters under study.

3. Results
3.1 Seeds stored at ambient temperature
Bitter gourd seeds stored at ambient, 4, 0, -20, -80 °C temperatures were analyzed for seed quality parameters at 1, 3, 6 and 9 months of storage (Table 1, 2, 3 and 4). Seeds stored at ambient storage condition recorded higher germination (71.33%) up to six months of storage period (Table 1). Initially in the freshly harvested one-month old seeds it recorded 64 per cent of germination but consequently germination per cent increased up to 84.67 per cent after three months and decreased to 71.33 per cent at sixth and 36.00 per cent after nine months of storage (Table 1). In contrast abnormal seedlings increased to 6.33 per cent after nine months of storage (Table 1). As the after ripening period increased, hard seed per cent also decreased to 12.00 per cent after 3 months of storage and again hard seeds percentage increased to 50.33 per cent after nine months of storage (Table 2). Shoot length, root length, dry weight and vigour index also increased up to three months of storage and decreased in the subsequent period of storage (Table 3 and 4).

3.2 Seeds stored at cold temperatures
While seeds stored at 0, -20, -80 °C temperatures recorded no germination but seeds stored at 4 °C temperature showed better germination at first (56%), third (64%) and sixth (61.33%) months after storage (Table 1). Interestingly after nine months of seed storage it reduced to 32.67 per cent (Table 1). Contrastingly shoot length, root length, dry weight and vigour index also increased up to six months of storage but decreased after nine months of storage. (Table 3 and 4).

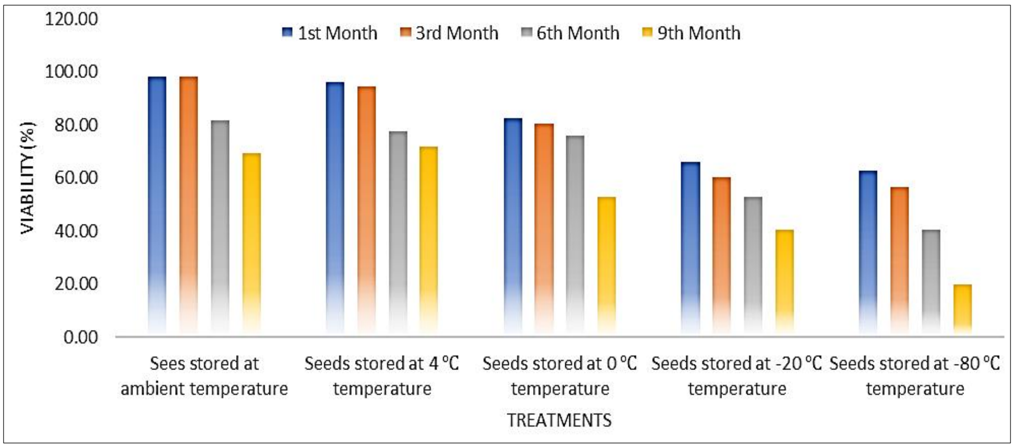

Fig 2: Effect of different cold storage temperatures on seed viability percentage of bitter gourd Vivek F_1 hybrid.

Seeds stored at 0, -20, -80 °C temperatures showed zero per cent of germination and tetrazolium test confirmed the presence of seed viability in stored seeds (Figure 2). These cold stored seeds were not germinated even though they are viable but germinated only after dry heat treatment (Table 1). These results confirmed the presence of secondary dormancy which was induced by cold storage. Similarly, along with germination, root length, shoot length, dry weight and vigour index were increased in dry heat (60 °C for 2 h) treated seeds (Table 3 and 4). Seeds stored at 0 °C recorded enhanced germination (60%) when seeds were dry heated (60 °C for 2 h) in the first month unfortunately it reduced to 50.67 per cent after six months of storage. In the same way seeds stored at -20 °C were dry heated and enhanced the germination by 57 per cent at one month after storage and it reduced to 33.33 per cent after six months of storage (Table 1). While seeds stored at -80 °C also showed similar findings and the germination reduced to 25.33 per cent after six months of storage by dry heat treatment (Table 1).

Abnormal seedlings and dead seeds were increased in all the cold storage treatments throughout the storage period (Table 2 and 3). But hard seeds per cent had reduced when seeds were dry heated and recorded minimum per cent in stored seeds (0, -20, -80 °C) (Table 2). Shoot length, root length, dry weight and vigour index were found to be decreased over period in all the treatments (Table 3 and 4).

The important observation that we found while studying the above experiment was seeds stored at 0, -20, -80 °C temperatures recorded no germination even though seeds were dry heated at nine months after storage (Table 1) and the dead seeds per cent was maximum (14.67%) in -80 °C temperature followed by -20 °C and 0 °C temperatures (Table 2). Hard seeds per cent decreased after nine months of storage due to increased dead seeds (Table 2).

Table 1: Effect of duration of cold storage on germination and abnormal seedlings percent in bitter gourd Vivek F_1 hybrid.

Treatments	Germination (%)				Abnormal seedlings (%)			
	1st month	3rd month	6th month	9th month	1st month	3rd month	6th month	9th month
T-1 Sees stored at ambient temp.	64.00 (53.13)*	84.67 (66.95)*	71.33 (57.63)*	36.00 (36.87)*	2.00 (8.13)*	2.00 (8.13)*	4.33 (12.01)*	6.33 (14.58)*
T-2 Seeds stored at 4 °C	56.00 (48.85)	64.00 (53.13)	61.33 (51.55)	32.67 (34.86)	4.00 (11.54)	4.67 (12.48)	5.67 (13.35)	13.33 (21.42)
T-3 Seeds stored at 0 °C	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)
T-4 Seeds stored at -20 °C	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)
T-5 Seeds stored at -80 °C	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)
T-6 Seeds stored at 4 °C + DH[a]	64.67 (53.53)	68.67 (55.96)	66.00 (54.33)	64.00 (53.13)	5.33 (13.35)	5.33 (13.35)	6.33 (14.58)	11.33 (19.67)
T-7 Seeds stored at 0 °C + DH[a]	60.00 (50.77)	57.33 (49.22)	50.67 (45.38)	0.00 (0.00)	5.33 (13.35)	6.33 (14.58)	7.67 (16.07)	0.00 (0.00)
T-8 Seeds stored at -20 °C + DH[a]	57.00 (49.02)	53.33 (46.91)	33.33 (35.26)	0.00 (0.00)	5.67 (13.77)	7.33 (15.71)	7.67 (16.07)	0.00 (0.00)
T-9 Seeds stored at -80 °C + DH[a]	52.67 (46.53)	48.00 (43.85)	25.33 (30.22)	0.00 (0.00)	6.33 (14.58)	8.33 (16.78)	8.33 (16.78)	0.00 (0.00)
Mean	39.37 (33.59)	41.78 (35.21)	34.22 (30.58)	14.74 (14.06)	3.19 (8.39)	3.78 (9.09)	4.45 (10.01)	3.45 (6.37)
S.Em±	0.79	0.53	0.73	0.35	0.27	0.30	0.32	0.25
CD 1%	3.25	2.17	2.98	1.43	1.12	1.20	1.30	1.02
CV (%)	4.12	2.62	4.15	4.37	5.66	5.64	5.33	6.80

*Figures in Parenthesis are arc sign values, [a]DH- Dry heat treatment at 60 °C for 2 h.

Table 2: Effect of duration of cold storage on hard seeds and dead seeds per cent in bitter gourd Vivek F_1 hybrid.

Treatments	Hard seeds (%)				Dead seeds (%)			
	1st month	3rd month	6th month	9th month	1st month	3rd month	6th month	9th month
T-1 Sees stored at ambient temp.	33.00 (35.06)*	12.00 (20.27)*	20.67 (27.04)*	50.33 (45.19)*	1.33 (6.63)*	1.00 (5.74)*	3.67 (11.04)*	7.33 (15.71)*
T-2 Seeds stored at 4 °C	36.67 (37.27)	27.67 (31.73)	26.67 (31.09)	44.33 (41.75)	3.67 (11.04)	3.67 (11.04)	6.67 (14.96)	9.67 (18.11)
T-3 Seeds stored at 0 °C	96.67 (79.48)	95.67 (77.99)	92.67 (74.29)	88.67 (70.33)	4.33 (12.01)	4.33 (12.01)	7.33 (15.71)	11.33 (19.67)
T-4 Seeds stored at -20 °C	96.00 (78.46)	94.67 (76.65)	91.67 (73.22)	88.00 (69.73)	4.00 (11.54)	5.33 (13.35)	8.33 (16.78)	12.00 (20.27)
T-5 Seeds stored at -80 °C	94.67 (76.65)	92.67 (74.29)	91.00 (72.54)	85.67 (67.75)	5.33 (13.35)	7.33 (15.71)	9.00 (17.46)	14.33 (22.25)
T-6 Seeds stored at 4°C + DH[a]	26.33 (30.87)	20.33 (26.80)	19.33 (26.08)	13.67 (21.70)	3.67 (11.04)	5.67 (13.77)	8.33 (16.78)	11.00 (19.37)
T-7 Seeds stored at 0 °C + DH[a]	30.33 (33.42)	30.00 (33.21)	32.67 (34.86)	87.00 (68.87)	4.33 (12.01)	6.33 (14.58)	9.00 (17.46)	13.00 (21.13)

T-8 Seeds stored at -20 °C + DH[a]	31.67 (34.24)	32.00 (34.45)	49.67 (44.81)	86.67 (68.58)	5.67 (13.77)	7.33 (15.71)	9.33 (17.79)	13.33 (21.42)
T-9 Seeds stored at -80 °C + DH[a]	33.67 (35.47)	35.00 (36.27)	55.33 (48.06)	85.33 (67.48)	7.33 (15.71)	8.67 (17.12)	11.00 (19.37)	14.67 (22.52)
Mean	53.22 (49.00)	48.89 (45.74)	53.26 (47.97)	69.96 (57.94)	4.26 (11.62)	5.56 (13.30)	8.07 (16.36)	11.85 (20.04)
S.Em±	0.97	0.71	0.88	0.62	0.41	0.47	0.50	0.53
CD 1%	3.94	2.90	3.58	2.54	1.67	1.92	2.05	2.15
CV (%)	3.42	2.70	3.18	1.86	6.10	6.14	5.32	4.57

*Figures in Parenthesis are arc sign values, [a]DH- Dry heat treatment at 60 °C for 2 h.

Table 3: Effect of duration of cold storage on shoot length and root length in bitter gourd cv. Vivek F_1 hybrid.

Treatments	Shoot length (cm)				Root length (cm)			
	1st month	3rd month	6th month	9th month	1st month	3rd month	6th month	9th month
T-1 Sees stored at ambient temp.	20.0	20.8	19.3	16.7	18.6	19.3	18.9	15.9
T-2 Seeds stored at 4 °C	16.8	16.6	16.5	15.1	15.5	15.2	15.1	13.3
T-3 Seeds stored at 0 °C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T-4 Seeds stored at -20 °C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T-5 Seeds stored at -80 °C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T-6 Seeds stored at 4 °C + DH[a]	19.2	19.2	19.0	18.9	18.6	18.0	17.7	17.2
T-7 Seeds stored at 0 °C + DH[a]	19.0	19.8	17.8	0.0	17.7	17.7	15.1	0.0
T-8 Seeds stored at -20 °C + DH[a]	18.9	19.2	16.2	0.0	16.7	16.2	14.5	0.0
T-9 Seeds stored at -80 °C + DH[a]	18.7	19.1	15.5	0.0	16.3	15.9	14.2	0.0
Mean	12.51	12.74	11.56	5.64	11.5	11.37	10.62	5.15
S.Em±	0.32	0.21	0.29	0.24	0.19	0.27	0.23	0.2
CD 1%	1.29	0.84	1.18	0.97	0.78	1.12	0.95	0.83
CV (%)	4.39	2.79	4.35	7.33	2.88	4.18	3.8	6.86

Table 4: Effect of duration of cold storage on seedling dry weight and seedling vigour index I in bitter gourd Vivek F_1 hybrid.

Treatments	Seedling dry weight (mg/seedling)				Seedling vigour index			
	1st month	3rd month	6th month	9th month	1st month	3rd month	6th month	9th month
T-1 Sees stored at ambient temp.	70.52	73.21	63.49	50.49	2474	3392	2726	1174
T-2 Seeds stored at 4 °C	61.01	59.36	58.55	49.60	1812	2037	1932	927
T-3 Seeds stored at 0 °C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T-4 Seeds stored at -20 °C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T-5 Seeds stored at -80 °C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T-6 Seeds stored at 4 °C + DH[a]	69.66	66.60	62.42	58.60	2443	2556	2424	2313
T-7 Seeds stored at 0 °C + DH[a]	60.17	65.76	55.26	0.00	2202	2150	1667	0.00
T-8 Seeds stored at -20 °C + DH[a]	58.35	59.59	54.38	0.00	2029	1888	1026	0.00
T-9 Seeds stored at -80 °C + DH[a]	57.21	54.16	53.66	0.00	1843	1677	753	0.00
Mean	41.88	42.07	38.64	17.63	1422.67	1522.5	1168.3	490.7
S.Em±	0.90	0.68	0.80	0.41	57.33	41.38	44.02	45.31
CD 1%	3.67	2.78	3.25	1.67	233.35	168.45	179.19	184.43
CV (%)	3.73	2.81	3.58	4.02	6.98	4.71	6.53	6.84

[a]DH- Dry heat treatment at 60 °C for 2 h.

4. Discussion

The per cent of germinated seeds after cold storage resulted in no germination with decreasing storage temperature i.e., 0, -20, -80 °C temperatures. On the other hand, seeds which were stored in 4 ℃ resulted in enhanced germination per cent from first month (56%) to six months (61.33%) after storage but it was decreased after nine months (32.67%) after storage (Table 1). Same trend was observed in other physiological parameters (root length, shoot length, dry weight and vigour index). The above studies suggest that even though the bitter gourd seeds were classified as orthodox (Doijode, 2001) [5] and they are sensitive to sub-zero temperature and it can be stored up to six months in cold storage (4 °C). Similar findings (Ebert and Huang, 2015) [6] were observed in bitter gourd. Gene banks reported no germination of bitter gourd seed samples stored at sub-zero temperatures for six months, whereas unchanged germination percentages could be obtained after storage at 5 °C (Ebert and Huang, 2015) [6]. While seeds stored at ambient conditions enhanced germination per cent and seed quality parameters up to six months of storage period, it can be concluded that bitter gourd seeds can be stored up to six months without losing germination per cent and vigour.

Germination per cent decreased in cold storage conditions as the storage period increased up to six months. After nine months of storage, except in seed stored at ambient temperature, 4 °C, and 4 °C + dry heat treatment got zero per cent of germination and increased hard and dead seeds were observed. It was mainly due to the freezing injury caused by long term cold storage (Sharmila *et al*., 2019) [11] and seeds might have been entered into a deep physiological dormancy.

During sub-zero temperature storage, the lipid solidification occurred in the seeds and it need to be melted before germination could commence in the dry seeds (Sebastian and Michael, 2018) [10]. This could be the possible reason for physiological dormancy in sub-zero temperature stored seeds which results in decreased mobilization of stored material ultimately results in blocking of metabolic activities required for seed germination and seed might undergo dormancy. Seeds of Cuphea species did not germinate after storage at -18 °C and further dry heat treatment of 45 °C could unblock the germination process. This phenomenon was attributed to the unique lipid composition of seeds (high concentrations of lauric acid or myristic acid) (Crane *et al*., 2003) [4]. In bitter gourd, extracted crude fat of the embryos showed crystallization of triterpene glycoside during sub-zero temperature as like Cuphea species (Crane *et al*., 2003) [4], so this could be the possible reason for delayed germination and cold stored seeds requires dry heat stimulatory effect for breaking physiological dormancy (Sebastian and Michael, 2018) [10]. Further biochemical and molecular studies are required to know the dormancy switch and its breaking methods for long term storage of seeds.

Thermo-dormancy induced by high temperature can be broken by chilling at low temperatures for many species. However, in bitter gourd just the opposite is observed. Low temperatures induced the dormancy and high temperature dissipated the dormancy. Therefore, in bitter gourd we found new dormancy type that could be called cold-induced dormancy, which can be broken by warm-stratification, which was already described as a method of artificial after-ripening for breaking a primary dormancy in grasses or rice (Annonymous, 2017) [3].

5. Conclusions

The above results confirmed the sensitivity of bitter gourd to sub-zero temperature. In ambient conditions higher germination was noticed up to six months but it decreased after nine months of storage. Whereas seeds stored at 0, -20, -80 °C temperatures recorded zero per cent of germination but it requires dry heat treatment (60 °C) for a period of 2 h in order to overcome cold induced secondary dormancy.

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