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SPECIAL ARTICLE

Phyto-diversity and its Conservation Assessment in Permafrost Condition at Extreme Altitude of Trans-Himalaya in Leh-Ladakh, India

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Efforts have been made to identify the suitable natural condition for long-medium term storage of horticultural crops and rare threatened species of the region, seeds cost effectivity, by comparing different natural storage conditions including controlled chamber maintained temperature at -18ºC and the other at ambient natural conditions at different locations i.e., permafrost are Chang-La, situated at the height of 17,600 ft. in trans-Himalayan region with subzero temperature for most part of the year, Leh location where temperature also remains at-subzero for half the year and Chandigarh having a tropical climate. The 16 important horticulture crops and four medicinal plants representing the dicotyledonous seeds were assessed for different storage conditions for their germination percentage and longevity. The germination of stored seeds was monitored after five years. The results showed that all the accessions were able to maintain their seed germination percentage very close to their original values in the two locations i.e., Chang-La and Leh. Suggesting that some horticulture crops and rare medicinally important species could be stored *in-situ* for long and medium-term conservation at natural low temperature at Leh and at extreme altitude 17,600 ft above sea level permafrost facility at Chang-La.

Key Words: Chang-La, Leh, Conservation, Horticulture, Germplasm, Locations, Permafrost.

Introduction

Conservation of seeds ex-situ underpins many of the recovery efforts being undertaken to help preserve rare threatened and poorly known plant species. The international standard for long-term storage for seed conservation is at -18ºC, with seed moisture content between 3 and 7% depending on the species (Genebank Standards, 1994). These storage conditions apply only to seeds of orthodox species (Roberts, 1973), which are able to survive desiccation to such low moisture levels.

Storage of plant genetic resources (PGR) at large scale as per a standard protocol would be a very expensive and more over malfunctioning of such facilities results in loss of valuable PGR, but still maintenance of such storage conditions is essential for future security of food and nutrition. As far as expense and technical problems of such facilities is concerned, experiments were carried out by Singh *et al*. (2008) on storage of seeds at natural permafrost conditions in Himalayan region for long-term storage to facilitate cost effective conservation of plant genetic resources (PGR). In the present study, experiments were conducted in the natural permafrost location at Chang-La in Ladakh trans-Himalayan region situated extreme north of India (Jammu and Kashmir), 90 km from Leh town at the elevation of 17,600 feet above mean sea level where ambient temperature remain (-12.49±7.86 ºC), in a cold desert conditions especially at higher altitudes where temperature remain sub-zero and low relative humidity for most part of the year except in summer months. By taking an advantage of such natural low temperature, a storage facilities has been built for long-term storage of seeds, including control where the facility is given a low temperature of -18 ºC (L-I) following standard protocol for long-term storage and other locations built at ambient conditions: Chang-La (L-II), Leh (L-III) and Chandigarh (L-IV) conditions.

Materials and Methods

Seed samples of 20 representative accessions from horticultural crops and medicinal plants were taken. Seed material was processed as per the established genebank standards (IBPGR, 1994) i.e., checked for physical purity, germination percentage and dried to a moisture level ranging between 5 to 7 per cent depending on the type of crop/species. 500 seeds were kept in each packets in three replication and stored at different locations i.e., L-I, L-II, L-III and L-IV. The

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packets were vacuum sealed in aluminium doubled layered sheets. The aluminium packets containing the seed material were kept in perforated plastic baskets and stored for five years. The seed materials were retrieved for monitoring of germination after five year storage, the seed quality (germination percentage) was monitored following ISTA rules (Anonymous, 1999) for standard germination test, with a modification of using three replications of 100 seeds each. The initial seed germination test was conducted after cleaning and drying the accession within two-three months after receipt of the sample in the genebank. The initial germination tests were done by following genebank standards with modification of 100 seeds (Genebank Standards, 1994).

In the present study, out of 20 species representing the dicotyledonous seeds 16 are horticulture crops and 4 are medicinal plants. Sample collection and their storage was completed in 2011 from field of Defence Institute of High Altitude Research, Leh and from the wild, including French bean var. Pusa Parvati, chickpea var. Amazing, okra var. Varsa, cucumber var. Pusa Sanyog, parsley var. Petra, brinjal var. Mukta, tomato var. Sultan, coriander var. Caribi, Summer squash var. Pusa alankaar, water melon var. Ayashi tamato, vegetable mustard var ARU black, carrot var. Nantes, celery var. triumph, true potato seeds, cabbage var. Gonzals, sunflower, brinjal var. Mukta, *Verbascum thapsus, Hypericum perforatum, Inula racemosa, Achillea millefolium*. The local taxa were identified by flora of Ladakh (Polunin and Stainton, 1989; Kachroo, 1997). All seeds were cleaned and dried to avoid weed seeds, pests and associated diseases. To achieved the moisture level of 3-7%, the seeds were dried at 10-15% relative humidity at 10-15% ºC, following (FAO, 2014).

Longevity of the seeds was calculated following standards of ICAR-National Bureau of Plant Genetic Resource (ICAR-NBPGR) on the basis of critical value of 85% viability (Trapa *et al.,* 2012; Sharrock *et al.,* 1998) (Table 3).

All the samples were analysed for ANOVA using SPSS version 17. The level of significance was tested at 5% among locations on the basis of seed performance for germinations in all species (Table 2).

Results and Discussion

Significant variations in seed quality were recorded in location L-I, L-II, and L-III when compared to initial values, except for Chandigarh during the five years storage of among vegetable crops and medicinal plants presented as per ANOVA estimation for each locations on the basis of different groups of plant germination percentage P=0.05. Details of accessions used, seed moisture content at the time of seed storage and mean germination percentage registered in seed samples of different accessions, at different locations over five years are presented in Table 2.

A total of 19 accessions were stored in different conditions, out of which 16 accessions were of major horticulture crops and 4 were of medicinal plants. The monitoring of seed germination in accessions of these crops showed the following trend during their storage. Out of 16 horticulture crops, cabbage, celery, cucumber, tomato, chick pea, parsley maintained the seed quality near to their original values in the three locations except at Chandigarh. Vegetable mustard: the stored accession at different locations showed initially increase in germination percentage up to 5%, in L-1 and L-II and then showed decreases in germination percentage to 51% when compare to initial germination. Carrot: the accession stored showed increased 4% germination at L-I and decreased to 1%, 9% at L-II and L-III respectively. True potato seeds: The stored seeds showed increase in germination by 15% from original value at L-I and L-II, 10% at L-III. French bean: accessions showed decrease in germination by 37%, 70% and 89% at L-I, L-II and L-III respectively from the original value. Water melon: accessions showed drastic increase in germination from 63% to 99%, 99%, 98% in L-I, L-II and L-III respectively. Summer squash: accessions showed 100% germination at L-I, L-II and L-III, an increase of 4% in germination from the original value. Sunflower: accessions showed very poor initial germination and increase by 12% in L-I and gradual decrease by 21% and 23% in L-I and L-II, respectively. Brinjal: accessions stored showed increase in germination percentage from 96% to 99%, 98%, 98% at L-I, LII and L-III respectively. Coriander: accessions showed increase from 71% to 90%, 90%, 88% at L-I, L-II and L-III respectively. Medicinal plants: *Inula racemosa* showed significant increase in germination percentage from 48% to 99%, 99%, 97% at L-I, L-II and L-III respectively. *Hypericum perforatum* showed slight increase from 41% to 49%, 47%, 47% at L-I, L-II and L-III respectively. *Achillea millefolium* showed drastic increase from 58% to 93%, 89%, 81% at L-I, L-II and L-III respectively and last accession *Verbascum thapsus* also showed drastic increase from 42% to 87% at L-I, L-II and L-III (Fig 2).

Table 1. Temperature and relative humidity at various locations surveyed for identification of site for zero energy based conservation 2011

Months	Changla RF Temperature (ºC)	Changla NRF Temperature (ºC)		Leh Temperature (ºC)		Chandigarh Temperature (ºC)	
		Min	Max	Min	Max	Min	Max
January	-18	-25.4	-22.2	-17	-5	12.8	18.9
February	-18	-22.0	-19.1	-15	-1	14.8	21.0
March	-18	-20.1	-15.7	-13	0	19.4	26.0
April	-18	-14.7	-10.2	-9	16	26.7	34.6
May	-18	-11.7	-6.7	9	31	31.1	38.8
June	-18	-6.2	-2.0	13	32	33.0	39.6
July	-18	-0.8	4.2	14	33	30.5	34.9
August	-18	0.3	4.5	8	29	28.8	32.9
September	-18	-4.5	-0.9	7	27	28.5	33.4
October	-18	-14.2	-10.9	-7	14	24.9	32.0
November	-18	-15.3	-11.3	-11	12	19.0	26.4
December	-18	-15.3	-11.7	-20	-6	14.1	20.7

Table 2. Seed germination at five year intervals in seed samples of various crops stored at different locations in 2011-2016

S. No.	Horticulture crops	Moisture Content (%)	Initial germination %	Avg germination % of Changla RF P=0.04	Avg germination % of Changla NRF P=0.02	Avg germination % of DIHAR Leh P=0.02	Avg germination % of DIHAR Chandigarh P=0.46
1	Cabbage	4.9	98.67	97.33	95.67	94.17	31.33
2	Vegetable mustard	4.1	92	97.33	97.33	96	41.33
3	Carrot	4.4	81.33	85.33	80	72	42
4	Celery	5.46	82	81.33	78.67	77.33	51.12
5	True potato seed	7.4	82.5	97.33	97.33	92	24
6	French beans	6.4	100	62.67	30.12	20.23	10.67
7	Cucumber	5	92	91	90.67	90.67	22.33
8	Water melon	4.35	62.67	99.33	99.33	98	21.33
9	Summer squash	6.3	96	100	100	100	32.5
10	Okra	5.7	85.33	97.33	91	96.33	21.67
11	Tomato	5.86	96	92.67	89.33	87	21.12
12	Sunflower	4.2	53.33	64.67	32	29.67	27.33
13	Chick pea	7.5	91	90	85	84.67	39.33
14	Brinjal	5.8	96	98.67	98.33	98.33	22.67
15	Coriander	7.8	70.6	89.67	89.67	87.67	21.67
16	Parsley	5.4	76	74	72.67	71.65	26
Medicinal Plants							
1	*Inula racemosa*	5.8	48	98.5	98.67	97.42	37.33
2	*Hypericum perforatum*	6	41	49.33	46.67	46.53	10.27
3	*Achillea millefolium*	5.6	58	93.33	89.33	81.33	32.67
4	*Verbascum thapsus*	6.9	42	87	86.67	86.67	33.33

As expected all the accessions showed drastic decreases in germination percentage at L-IV, Table 2 (Fig 1). It has been suggested that there would be degradation of alleles of germplasm after it reached upto 85% of critical viability (Trapa *et al.,* 2012; Sharrock *et al.,* 1998) (Table 3). It is therefore important to regenerate the base sample when it reached to its critical value. We evaluated the longevity of the each samples stored at different locations, accordingly in refrigerated module the longevity of the celery is highest 112 years (yrs), cucumber 75 yrs, chickpea 75 yrs, cabbage 56 yrs, parsley 38 yrs and tomato 23 yrs till they reach to critical value at 85% deterioration. In outside natural module the maximum survivability is of carrot, cucumber 56, followed by cabbage 25, parsley, celery 20, chickpea 13 and tomato 11 years. In Leh condition the highest

Table 3. Longevity of the seeds at different locations.

Name of crop	Test treatments											
	Initial Germination % 2011	Chang-la inside module			Chang-La outside module			DIHAR Leh			DIHAR, Dett. Chandigarh		
		Germmination % 2016	Deterioration % in 05 yrs	Longevity @ 85%	Germ. % 2016	Deterioration % in 05 yrs	Longevity @ 85%	Germination % 2016	Deterioration % in 05 yrs	Longevity @ 85%	Germ. % 2016	Deterioration % in 05 yrs	Longevity @ 85%
Cabbage	98.7	97.33	1.34	56	95.7	3	25	94.2	4.5	16.7	31.3	67.3	1.1
Vegetable mustard	92	97.33	ND	ND	97.3	ND	ND	96	ND	ND	41.3	50.7	1.5
Carrot	81.3	85.33	ND	ND	80	1.3	56.4	72	9.3	8.03	42	39.3	1.9
Celery	82	81.3	0.67	111.9	78.7	3.8	19.6	77.3	5.2	14.5	51.1	24	3.1
True potato seed	82.5	97.33	ND	ND	97.3	ND	ND	92	ND	ND	24	58.5	1.3
French beans	100	62.67	37.33	2.00	30.1	69.9	1.07	20.3	79.8	0.94	10.7	89.3	0.8
Cucumber	92	91	1	75	90.7	1.3	56.4	81.3	10.7	7	22.3	69.7	1.1
Water melon	62.7	99.33	ND	ND	99.3	ND	ND	98	ND	ND	21.3	41.3	1.8
Summer squash	96	100	ND	ND	100	ND	ND	100	ND	ND	32.5	63.5	1.8
Okra	85.3	97.33	ND	ND	91	ND	ND	96.3	ND	ND	21.7	75.7	0.9
Tomato	96	92.7	3.33	22.5	89.3	6.7	11.2	87	9	8.3	21.1	74.9	1.0
Sunflower	53.3	64.7	ND	ND	32	21.3	0.0	29.7	23.6	4.7	27.3	25.9	2.5
Chick pea	91	90	1	75	85	6	12.5	84.7	6.3	11.8	39.3	51.7	1.5
Brinjal	96	98.7	ND	ND	98.3	ND	ND	98.3	ND	ND	22.7	73.3	1.0
Coriander	70.6	89.7	ND	ND	89.7	ND	ND	87.7	ND	ND	21.7	48.9	1.5
Parsley	76	74	2	37.5	72.7	3.3	22.4	71.7	4.3	17.24	26	50	1.5
Inula racemosa	48	98.5	ND	ND	98.7	ND	ND	97.4	ND	ND	37.3	10.7	7.03
Hypericum perforatum	41	49.3	ND	ND	46.67	ND	ND	46.5	ND	ND	10.3	30.7	2.4
Achillea millefolium	58	93.3	ND	ND	89.3	ND	ND	81.3	ND	ND	32.7	25.3	2.9
Verbascum thapsus	42	87	ND	ND	86.7	ND	ND	86.7	ND	ND	33.3	8.7	8.7

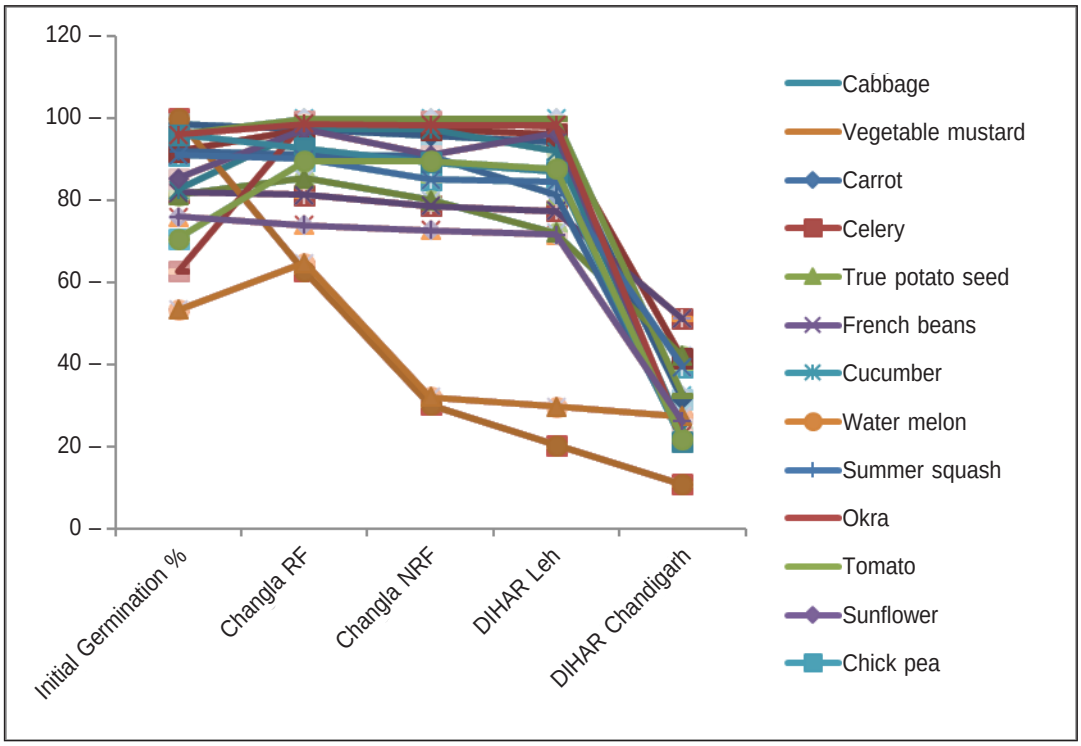

Fig 1. Seed germination of different vegetable crops stored under different storage conditions including controlled at -18 °C, Chang-la, Leh and Chandigarh.

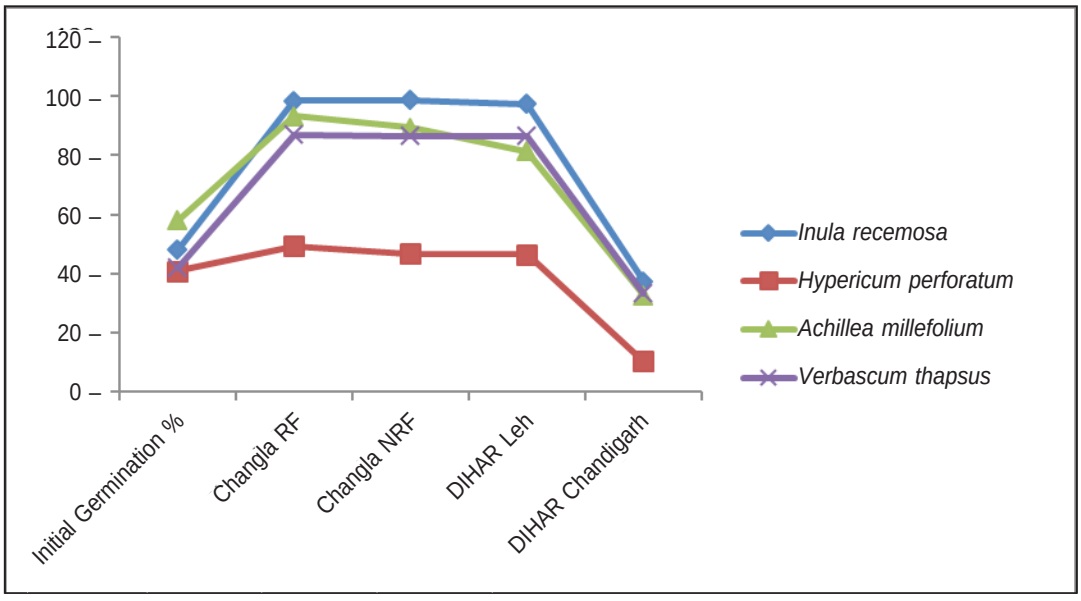

Fig 2. Seed germination of medicinal plants stored under different storage conditions including controlled at -18 °C, Chang-la, Leh and Chandigarh.

survivability was recorded in cucumber 56, followed by parsley 17 yrs, cabbage 17 yrs, chickpea 12 yrs, carrot 8 yrs and tomato 8 yrs and at Chandigarh longevity of all the seed were recorded very low except for *Verbascum thapsus* (Table 3). Some crops such as true potato seeds, water melon, summer squash, okra, brinjal, coriander and medicinal plants shows more or less increase in germination percentage in L-I, L-II and L-III from

their initial values, therefore they were not determine (ND) for the longevity, the increase in germination of the crops may be due to the physiological dormancy of the seeds, therefore the longevity of these crops will be analyse after another five years of storage. However, all the samples were drastically decrease in longevity in L-IV. Vegetable mustard showed increase in germination from initial value in L-I and L-II and gradual decrease, crops such as carrot and sunflower showed increase in germination percentage only in controlled module and observed considerably decreases in germination in L-II, L-II and L-IV (Table 3).

The study was conducted to explore the feasibility of long-term storage of orthodox seeds under natural low temperature conditions in the region, to facilitate cost-effective conservation of plant genetic resources. The critical evaluation of meteorological data using (tiny tag data logger) (Table 1), indicated that Chang-la pass situated at the height of 17,380 on a way to Pangong lake is the most appropriate place with sub-zero temperatures (–25^{o} to 0.4 ºC) most of the year, with mean temperature of (-12 ºC). Monitoring of the seed quality (germination percentage) in 16 and 4 different plants, belonging to horticulture crop and medicinal plants group respectively, stored under the four locations, three natural conditions, prevailing at Chang-La, Leh and Chandigarh and the controlled maintained-18 ^{o}C. Storage, starting from year 2011 to 2016 registered various trends in maintaining the seed quality between the accessions of the crop and wild species. This could either because of the genotypic differences among accessions or due to differences in physiological maturity of the seed materials, at the beginning of the seed storage. Most crop species are able to maintain the seed quality near to their initial values or increase in germination percentage from the initial value. The results were similar to those of the seed material stored under artificial low temperature conditions of -18 ºC in refrigerated chambers at Chang-la. Thereby, suggesting that in general all the crops could be stored under the natural low temperature conditions (permafrost) of Himalayan Region with similar level of success. However, differences may occur, mostly due to the differences in quality of seed samples at the start of seed storage or may be due to fluctuation of temperature in natural conditions. According to the longevity of each sample it may be recommended that, celery, parsley, cabbage, cucumber, and carrot can be stored at natural permafrost condition at Chang-la for long term storage at 20-56 years without any energy inputs. Tomato and parsley can be preserved for medium-term at natural low temperature at Leh conditions for 8-12 years. Some sensitive seeds can be stored in the refrigerated condition by utilization of small amount of energy at Chang-la.

Among various crops, the parsley, cabbage, cucumber, carrot, tomato and parsley depicted a consistent trend in maintaining the seed quality, very near to their original values at two natural locations, L-I and L-II. Vegetables which includes mustard, carrot, true potato seeds, water melon, summer squash, okra, brinjal and coriander and medicinal plants in fact showed a sharp increase in the germination, which perhaps continued to increase in subsequent monitoring. This may be because of variations in the physiological maturity or due to physiological dormancy of the seeds. On the other hand, French bean and sunflower, registered low seed germination during storage and showed decreases in germination in L-II L-III and almost nil in L-IV (Fig. 1).

Most of the vegetables, as solanceous, cucurbitaceous, cole crops, tuber crops, including medicinal plants depicted a consistent behavior in maintaining the seed quality in natural low temperature in L-I and L-II closer to their original values and showed almost similar trend with seeds stored in controlled chamber at -18 ^{o}C during the five years of conservation with an exception sunflower and French bean which need further evaluation.

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