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Standardization of Storage Containers and Storage Conditions for Longer Viability of Seeds in *Rheum australe* D. Don: An Endangered Medicinal Plant of High altitude Himalayas

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ABSTRACT

The experiment on *Rheum australe* was conducted at Dr YS Parmar UHF, Nauni, Solan Himachal Pradesh and describes the seed germination and storage behavior of *Rheum australe* D. Don an endangered medicinal herb of high altitude Himalaya. Fresh mature and healthy seeds were stored at room temperature as well as at 0 to -5º C in different types of containers viz., open in trays, paper bags, polythene bags and cloth bags. Freshly collected seeds were tested for germination parameters in petridishes placed in seed germinator maintained at 25ºC.Thereafter germination studies in seed germinator was carried out after 3,6,9 and 12 months and effect of different storage containers on seed germination as well as on viability of seeds under room temperature and refrigerated stored seeds was determined. The significant effect of storage temperatures, storage periods and storage containers on seed germination, initiation of germination and completion of germination was observed. Comparatively high seed germination, minimum time taken for germination and minimum germination period was in seeds stored at 0 to -5 ^{0}C. The seed stored at room temperature exhibit lesser viability after 12 months storage as compared to that stored under refrigerated conditions. Maximum seed viability was recorded in polythene bags stored seeds at 0 to -5^{0}C.

Keywords Endangered, Medicinal plants, Seed germination, Storage containers.

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INTRODUCTION

Storage of seeds as *ex- situ* germplasm is an essential step for the long-term conservation of plant genetic resources. Maintaining seed viability for longer period is very essential to preserve the genetic integrity in stored samples. Since very early days, simple techniques have been adopted to maintain the seed viability in both domesticated and wild sources (Onyekwelua and Fayose 2007 and Pradhan and Badola 2008). Inappropriate storage medium such as room temperature storage often results in low seed germination, seed deterioration, and loss of viability, which are natural phenomenon during storage (Muller *et al.* 2011 and Butola and Badola 2004). Several factors,

namely, temperature, nature of the seeds, seed moisture content, relative humidity, and so forth, influence the seed longevity during storage (Butola and Badola 2004 and Yang *et al*. 2005). There is a close relationship between the loss of seed viability during storage and the accumulation of genetic damage in the surviving seeds (Ellis and Roberts 1981). Seed moisture content, temperature, and storage periods are among the main factors affecting above relationship.

Slight increase in temperature and moisture may promote fungal growth and insect development in seeds. Depending on the duration and method adopted, drying and long-term storage may lead to considerable reduction in germination or to eventual death of the seeds. Before storage, if the seeds are not properly dried, the high moisture content may reduce the seed viability by promoting fungal growth. Such deterioration could further result in decline of seed germination capacity. Proper storage conditions, however, may effectively retain substantial viability in seeds over a considerable storage period. Such approaches are especially crucial in case of endangered species, where judicial use of seeds as valuable genetic material through standardizing proper storage mechanism is a precondition to strengthen species conservation program

Rheum australe D. Don commonly known as Gandhini, Revandcheni, Archa, Rhubarb, Chukri, Tukshu is a perennial stout herb, 1.0-3.0 m in height, distributed in the temperate and sub tropical regions of the world between 2800-3600m altitudes. In the Garhwal Himalayas it is generally found between 2800-4500 m on rocky soil surfaces, between boulders and near streams (Nautiyal and Nautiyal 2004 and Anonymous 1972).

Endangered standing of *Rheum australe* necessitates its propagation and mass multiplication, for which a protocol targeting an appropriate and relatively longer period of storage of seeds would be vital and urgent need of the time. This will strengthen both *in situ* as well as *ex situ* conservation of the species. The present study was undertaken with an aim to test the effect of long-term storage of seeds under different storage conditions on seed germination in *Rheum australe*.

MATERIALS AND METHODS

The experiment was conducted to study the effect of storage temperature, storage containers and storage periods on germination and viability of *Rheum australe*. Seeds were collected in the month of October – November, 2012 from natural ecological zones of *Rheum australe* in Himachal Pradesh and stored at room temperature (average 18±3 ^{0} C) for at least two weeks. Fresh mature and healthy seeds were stored at room temperature as well as at 0 to -5º C in different types of containers viz., open in trays, paper bags, polythene bags and cloth bags. Freshly collected seeds were tested for germination parameters in petridishes placed in seed germinator maintained at 25ºC.Thereafter germination studies in seed germinator was carried out after 3,6,9 and 12 months and effect of different storage containers on seed germination as well as on viability of seeds under room temperature and refrigerated stored seeds was determined. The experiment was conducted with 32 treatments and 3 replications having two temperature conditions, four storage containers and four storage months at Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan under completely Randomized block Design. Observations were recorded on germination percentage, initiation of germination and completion of germination. Data was recorded for initiation of germination, the time/date/day when the first plumule leaf emerges, Germination percentage; The total number of seeds germinated was counted and germination percent was worked out by formula:

$$\text{Germination (\%)} = \frac{\text{Total cumulative germination}}{\text{Total number of seed sown}} \times 100$$

Germination period (days); the date of sowing and date of last seed germination.The data recorded was subjected to statistical analysis.

RESULTS AND DISCUSSION

Seeds were stored immediately after harvest in polythene bags, paper bags, cloth bags, and in open trays for different duration (3, 6,9,12 months) at different storage conditions viz., 0 to -5^{0}C and room temperature.

Table 1. Effect of storage temperature and storage duration on germination percentage, initiation of germination and germination period.

Storage temperature / Storage duration (Months)		Germination percentage: Room temperature (T_1)	Germination percentage: 0 to -5^{0}C (T^2)	Germination percentage: Mean	Initiation of germination (days): Room temperature (T_1)	Initiation of germination (days): 0 to -5^{0}C (T^2)	Initiation of germination (days): Mean	Germination period (days): Room temperature (T_1)	Germination period (days): 0 to -5^{0}C (T^2)	Germination period (days): Mean
3	M_1	73.33 (58.97)	78.36 (62.64)	75.84 (60.80)	3.75	3.75	3.75	6.75	6.58	6.66
6	M_2	69.99 (56.79)	74.44 (59.77)	72.21 (58.28)	4.16	3.75	3.95	7.75	7.08	7.41
9	M_3	61.10 (51.42)	64.44 (53.40)	62.77 (52.41)	4.75	4.50	4.62	8.25	7.50	7.87
12	M_4	42.21 (40.47)	47.77 (43.70)	44.99 (42.08)	6.41	6.08	6.25	8.83	8.66	8.75
	Mean	61.66 (51.91)	66.25 (154.88)		4.76	4.52		7.89	7.45	

	CD (0.05)		CD (0.05)		CD (0.05)	
	Storage temperature (T)	132	(T)	0.23	(T)	0.43
	Storage month (M)	187	(M)	0.43	(M)	0.62
	Storage temperature × Storage month	2.64	T × M	0.61	T × M	0.87

*Figures in parenthesis are arc sine transformed values.

Germination percentage

Germination percentage was decreased with the increase in storage duration under all storage containers and temperature.The mean germination percentage irrespective of storage containers (polythene bags, paper bags, cloth bags, open trays) and storage temperature (0 to -5^{0}C) and room temperature decreased continuously from 75.84% (after 3 months storage) to 44.99% after 12 months storage (Table 1).

Under different storage temperature the rate of decrease of germination percentage was different (Table 1). Under room temperature maximum germination percentage (73.33) was observed after 3 months which decreased to 69.99 after 6 months and 61.10 after 9 months and minimum was after 12 months storage (42.21). All these values were statistically different from each other. At 0 to -5^{0}C maximum germination percentage (78.36) was after 3 months storage. It was followed by storage after 6 months storage after 9 months and minimum was after 12 months storage. These values were statistically different from each other. Maximum mean germination percentage was after 3 months (75.84) which decreased to 44.99 after 12 months storage. The mean seed germination was maximum (66.25) at 0 to -5 ^{0}C storage and minimum (61.66) under room temperature.

Initiation of germination (days)

Under different storage temperature the minimum time taken for initiation of germination (3.75 days) was recorded after 3 months of storage under T_1 and T_2 (Table 1). Maximum days taken for initiation of germination were in T_1 (6.41 days after 12 months of storage) and the mean initiation of germination was minimum (4.52 days) at 0 to -5^{0}C storage and maximum (4.76 days) under room temperature. Minimum mean time taken for initiation of germination was after 3 months (3.75 days) and maximum mean time taken for initiation of germination was after 12 months 6.25 days (Table 1).

Germination period (days)

Under different storage temperature the minimum time taken for completion of germination (6.58 days) was after 3 months of storage at 0 to -5^{0}C storage. Maximum days taken for completion of germination was in T_1 i. e., 8.83 days after 12 months of storage. The minimum time taken for completion of germination (7.45 days) was 0 to -5^{0}C storage and maximum

Table 2. Effect of storage containers and storage duration on germination percentage, initiation of germination and Germination period.

Storage containers / Storage duration (Months)		Germination percentage: Open in trays (S_1)	Paper bags (S_2)	Polythene bags (S_3)	Cloth bags (S_4)	Mean	Initiation of germination (days): Open in trays (S_1)	Paper bags (S_2)	Polythene bags (S_3)	Cloth bags (S_4)	Mean	Germination period (days): Open in trays (S_1)	Paper bags (S_2)	Polythene bags (S_3)	Cloth bags (S_4)	Mean
3	M_1	69.99 (56.79)	76.73 (61.19)	83.33 (66.30)	73.33 (58.94)	75.84 (60.80)	4.33	3.66	3.33	3.66	3.75	8.16	6.50	6.00	7.00	6.91
6	M_2	67.77 (55.40)	73.33 (58.94)	77.77 (62.00)	69.99 (56.79)	72.21 (58.28)	4.50	4.00	3.33	4.00	3.95	7.16	7.50	7.00	7.00	7.16
9	M_3	57.77 (49.46)	64.44 (53.38)	67.77 (55.40)	61.11 (51.40)	62.77 (52.41)	5.16	4.16	4.33	4.83	4.62	7.00	8.50	8.00	8.00	7.87
12	M_4	38.88 (38.53)	46.60 (43.06)	51.10 (45.61)	43.33 (41.14)	44.99 (42.08)	6.50	6.16	5.83	6.50	6.25	9.33	8.66	8.00	9.00	8.75
	Mean	58.60 (50.05)	65.29 (54.14)	69.99 (57.33)	61.94 (52.07)		5.12	4.50	4.20	4.75		7.91	7.79	7.25	7.75	

*Figures in parenthesis are arc sine transformed values.

CD (0.05)		CD (0.05)		CD (0.05)	
Storage month (M)	1.87	Storage month (M)	0.43	Storage month (M)	0.62
Storage containers (S)	1.87	Storage containers (S)	0.43	Storage containers (S)	0.62
M × S	0.94	M × S	0.86	M × S	1.24

time taken for completion of germination (7.89 days) was observed under room temperature (Table 1).

The rate of decrease of germination percentage under different containers decreased to 38.88% in open trays, 46.60% in paper bags, 51.10 % in polythene and 43.33% in cloth bags after 12 months of storage. The mean germination percentage was maximum 69.99% under polythene bags storage and minimum 58.60% in open trays. Maximum germination percentage was in polythene bags after 3 months (83.33) which reduced after 3, 9 and 12 months (Table 2).

The minimum days taken for initiation of germination was 3.33 days in polythene bags after 3 months storage and maximum under different storage germination 6.50 days was under open trays and cloth bags after 12 months storage. The mean initiation of germination was minimum under polythene bags storage and maximum days in open trays. Minimum mean time taken for initiation of germination was recorded after 3 months (3.75 days) and maximum mean time taken for initiation of germination was recorded after 12 months 6.25 days (Table 2).

Under different storage containers the minimum days taken for completion of germination 6.00 days was recorded in polythene bags after 3 months storage and maximum time taken for completion of germination 9.33 days was observed under open trays after 12 months storage. The mean completion of germination was minimum 7.25 days under polythene bags storage and maximum 7.91 days in open trays. Minimum mean time taken for completion of germination was recorded after 3 months (6.91 days) and maximum mean time taken for initiation of germination was recorded after 12 months 8.75 days (Table 2).

The maximum germination percentage was under polythene bags stored seeds under 0 to – 5 ^{0}C (73.33%) which was significantly different from all other treatments. Minimum germination percentage was recorded in open trays stored seeds at room temperature. Minimum mean germination percentage (58.61) was in open trays and maximum was observed in polythene bags (70.00). Mean germination was maximum under 0 to -5 ^{0}C and minimum was at room temperature (Table 3).

Table 3. Effect of storage temperature and storage containers on germination percentage, initiation of germination and germination period.

StoragE temperature / Storage containers (Months)		Germination percentage			Initiation of germination (days)			Germination period (days)		
		Room temperature (T_1)	0 to -5^{0}C (T_2)	Mean	Room temperature (T_1)	0 to -5^{0}C (T_2)	Mean	Room tmperature (T_1)	0 to -5^{0}C (T_2)	Mean
Open in trays	S1	56.66 (48.88)	60.55 (51.21)	58.61 (50.05)	5.17	5.08	5.13	7.50	7.67	7.58
Paper bags	S2	63.33 (52.93)	67.25 (55.35)	65.29 (54.14)	4.54	4.45	4.50	8.33	7.83	8.08
Polythene bags	S3	66.66 (54.95)	73.33 (59.70)	70.00 (57.33)	4.25	4.14	4.19	7.42	7.83	7.63
Cloth bags	S4	60.00 (50.88)	63.89 (53.25)	61.94 (52.07)	4.80	4.72	4.75	8.33	6.50	7.42
Mean		61.66 (51.91)	66.26 (54.88)		4.76	4.52		7.90	7.46	

*Figures in parenthesis are arc sine transformed values.

CD (0.05)			
Storage temperature (T)	1.32	0.23	0.43
Storage containers (S)	1.87	0.43	0.62
T× S	2.64	0.61	0.88

The minimum mean time taken for initiation of germination (4.19) days was in Polythene bags and maximum was in open trays (5.13) days. Minimum mean time taken for initiation of germination 4.52 days was at 0 to - 5 ^{0}C and maximum was recorded under room temperature. However with regard to storage temperature and storage duration minimum days taken for initiation of germination was recorded in polythene bags stored seeds at 0 to -5 ^{0}C and maximum time taken for initiation of germination was recorded in open trays at room temperature (Table 3).

The minimum mean time taken for completion of germination days was recorded in Polythene bags and maximum was observed in paper bags. Minimum mean time taken for completion of germination was recorded at 0 to - 5 degree C and maximum was recorded under room temperature. However with regard to storage temperature and storage duration minimum days taken for completion of germination was recorded in polythene bags stored seeds at 0 to -5 degree C and maximum time taken for completion of germination was recorded in paper bags and cloth bags at room temperature (Table 3).

Data presented in Table 4 revealed that among interaction effect between storage temperature, storage duration and storage containers maximum germination percentage was observed in treatment combination $T_2M_1S_3$ (88.88) i.e. when seeds were stored for three months at 0 to -5 degree C in polythene bags and this treatment is statistically superior to all other treatments. Minimum germination percentage was recorded in $T_1M_4S_1$ (35.55%) i.e., when seeds were stored for 12 months at room temperature in open trays.

Data presented in Table 4 revealed that among interaction effect between storage temperature, storage duration and storage containers minimum time taken for initiation of germination was observed in treatment combination $T_2M_1S_3$ (3.33 days) and maximum was recorded in $T_1M_4S_1$ 6.66 days.

Data presented in Table 4 revealed that among interaction effect between storage temperature, storage duration and storage containers minimum time taken for completion of germination was observed in treatment combination $T_2M_1S_3$ (5.00 days) and maximum was recorded in $T_1M_4S_1$, $T_1M_4S_4$, $T_2M_1S_1$, $T_2M_4S_2$ and $T_2M_4S_3$ (9.33) days.

Testing seeds for long-term storage, confirms that the storage temperature/condition significantly

Table 4. Effect of storage temperature, storage duration and storage containers on germination percentage, initiation of germination and germination period.

Treatments	Germination percentag (%)	Initiation of germination (days)	Germination period (days)
$T_1M_1S_1$	68.88 (56.10)	4.33	7.00
$T_1M_1S_2$	75.55 (60.39)	3.66	7.00
$T_1M_1S_3$	77.77 (61.90)	3.33	6.00
$T_1M_1S_4$	71.10 (57.49)	3.66	7.00
$T_1M_2S_1$	66.66 (54.71)	4.33	8.00
$T_1M_2S_2$	71.10 (57.49)	3.66	8.00
$T_1M_2S_3$	73.33 (58.88)	3.33	7.00
$T_1M_2S_4$	68.88 (56.10)	3.66	8.00
$T_1M_3S_1$	55.55 (48.17)	5.33	7.00
$T_1M_3S_2$	62.22 (52.06)	4.33	9.00
$T_1M_3S_3$	66.66 (54.71)	4.33	8.00
$T_1M_3S_4$	60.00 (50.74)	5.00	9.00
$T_1M_4S_1$	35.55 (36.57)	6.66	9.33
$T_1M_4S_2$	44.44 (41.78)	6.33	8.00
$T_1M_4S_3$	48.88 (44.34)	6.00	8.66
$T_1M_4S_4$	40.00 (39.21)	6.66	9.33
$T_2M_1S_1$	71.10 (57.49)	4.33	9.33
$T_2M_1S_2$	77.91 (61.98)	3.66	7.00
$T_2M_1S_3$	88.88 (70.70)	3.33	5.00
$T_2M_1S_4$	75.55 (60.39)	3.66	7.00
$T_2M_2S_1$	68.88 (56.10)	4.66	6.33
$T_2M_2S_2$	75.55 (60.39)	4.33	7.00
$T_2M_2S_3$	82.22 (65.12)	3.33	7.00
$T_2M_2S_4$	71.10 (57.49)	4.33	6.00
$T_2M_3S_1$	60.00 (50.74)	5.00	7.00
$T_2M_3S_2$	66.66 (54.71)	4.00	8.00
$T_2M_3S_3$	68.88 (56.10)	4.33	8.00
$T_2M_3S_4$	62.22 (52.06)	4.66	7.00
$T_2M_4S_1$	42.22 (40.50)	6.33	8.00
$T_2M_4S_2$	48.88 (44.34)	6.00	9.33
$T_2M_4S_3$	53.33 (46.89)	5.66	9.33
$T_2M_4S_4$	46.66 (43.06)	6.33	8.00
CD (T×M× S)	5.30	1.22	1.75

*Figures in parenthesis are arc sine transformed values.

affects the seed germination capacity as indicated by Bradbeer (1988), nonetheless, it varies greatly by species and storage conditions (Siddique and Wright 2003). The standard for judging seed viability is always a germination test under optimum conditions (Phartyal *et al.* 2002). In the present investigations seeds were collected during October- November from its natural habitat of Himachal Pradesh and stored at room temperature for at least two weeks and then seed germination studies were carried out by keeping the seeds in open trays (S_1), Paper bags (S_2),Polythene bags (S_3), and cloth bags at room temperature (T_1) and 0 to -5 ^{0}C (T_2) temperature. The studies revealed that after 3 months storage 75.84% 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 73.33 to 42.21% under room temperature and from 78.36 to 47.77 under refrigerated conditions after 12 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 and Sharma and Sharma 2017). Alvarez *et al.* (1994) reported 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 4º C, because the seeds rapidly lose viability when left at room temperature. Also there is early initiation of germination as well as early completion under refrigerated condition and under polythene bags storage as compared to room temperature storage and other storage containers.

The present study corroborates the findings of Verma *et al.* (2009) who carried out seed germination and seed storage studies in *Aconitum heterophyllum* and *Podophyllum hexandrum* and found maximum germination per cent, germination energy, germination speed and viability percentage under polythene bags storage under complete darkness for both the species. Thakur *et al*. (2004) also reported the same results. They carried out comparative studies on seed viability status of *Achillea millefolium, Gentiana kurroo* and *Podophyllum hexandrum* and revealed substantial loss of 20,75 and 25% 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. Chauhan and Nautiyal (2007) also reported lesser viability under room temperature than at low temperature storage for *Nardostachys jatamansi.*

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 required for the good germination and growth of seedlings. Low viability in open trays stored seeds is due to the reduction in seed moisture during storage which reduced the seed longevity. The low germination ability and viability of seeds in storage in open trays, cloth bags and paper 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).

Study indicates that the seed deterioration rates may vary depending on the storage conditions, and the germination percent or seed viability gradually declines with increase in storage period as reported by Yilmaz and Aksoy (2007), irrespective of different storage conditions; Nevertheless, Wang *et.al (*2010), reported an increase in seed germination with the increase in duration of seed storage time in some subalpine species. Chauhan and Nautiyal (2007), reported much faster loss of seed viability at room temperature (10-35 ^{0}C) and retaining of seed viability for more than two years (storage at 0 to -5 ^{0}C in refrigerator) in *Nardostachys jatamansi.* Onyekwelua and Fayose (2007) stated that the seeds cannot be stored at subzero temperature probably due to freezing injury resulting from ice formation, which can alternatively be controlled by placing them in airtight sealed containers. In several other species, loss of seed viability is observed within a few months of storage at room temperature (Douglas 1995, Verma *et al.* 1996). In refrigerator both temperature and the relative humidity are properly maintained thereby retaining the seed viability for longer period, relatively.

CONCLUSION

The present study stressed on the high seed germination potential in *Rheum australe* collected from natural ecological zones from Himachal Pradesh. The storage condition highly affects the seed germination percentage in *R. australe,* which declines with increasing storage duration irrespective of storage condition. The storage at 0 to -5 ^{0}C temperature can retain seed viability for the longer period than other storage conditions and suggests it as the best/effective storage condition for the seeds of *R. australe.* However, the room temperature storage can be opted for short-term storage.

Present findings suggest that seed viability decreased with the increase in storage duration irrespective of storage containers and storage temperature. The storage containers have significant effect on seed viability. Maximum seed viability was in plastic bag storage. The storage temperature also had significant effect on seed viability. The plastic bags under refrigerated conditions proved to be the best containers for retaining maximum seed viability of *R. australe.*

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