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Effect of storage temperature and period on seed germination of *Rheum australe* D Don: an endangered medicinal herb of high altitude Himalaya

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ABSTRACT

Rheum australe D Don is a high altitude endangered medicinal perennial herb with thick and long roots that grows on rocky lands and near streams. The experiment on the species was conducted to study its seed germination and storage behaviour at Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, HP. Effect of different storage conditions (room temperature and 0 to -5ºC) and different storage periods over 12 months on seed germination in *R australe* collected from natural ecological zone in Himachal Pradesh was determined. Statistical analysis revealed significant effect of storage temperature and storage period on seed germination, initiation of germination and completion of germination. Comparatively high seed germination, minimum time taken for germination and minimum germination period were recorded in seeds stored at 0 to -5ºC. Results also showed that seed stored at room temperature exhibit lesser viability after 12 months storage as compared to storage under refrigerated conditions.

Keywords: Endangered; perennial; medicinal plants; germination

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. Inappropriate storage medium (Van Hezewijk et al 1993, Müller et al 2011) such as room temperature storage often results in low seed germination, seed deterioration and loss of viability which are natural phenomenon during storage (Nasreen et al 2000, Schmidt 2002). Several factors namely temperature, nature of the seeds, seed moisture content, relative humidity and so forth influence the seed longevity during storage. 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, Roos 1981, Rao et al 1987). Roberts (1988) reported that 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 (Christensen 1972). 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. Romanas (1991) found that 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 (Chen et al 2007). Such approaches are especially crucial in case of endangered species where judicious use of seeds as valuable genetic material through standardizing proper storage mechanism is a precondition to strengthen a species conservation programme.

Rheum australe D Don commonly known as Gandhini, Revandcheni, Archa, Rhubarb, Chukri and 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 2,800-3,600 m altitudes. In Himachal Pradesh it is found growing in Chhota Bhangal and Bara Bhangal of Kangra, Pangi Barmour of Chamba, Parvati valley of Kullu, Dodrakwar, Khashadhar and Rohru ranges of Shimla district, Kinnaur, Lahaul and Spiti districts (Chauhan 1999).

Endangered standing of *R australe* further 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 *R australe* .

MATERIAL and METHODS

The experiment was conducted to study the effect of storage temperature on germination and viability of *Rheum australe*. Seeds were collected in the month of October-November from natural ecological zone of *R australe*. Fresh mature and healthy seeds were stored at room temperature as well as at 0 to -5ºC 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 petri dishes

placed in seed germinator maintained at 25ºC. Thereafter germination studies in seed germinator were carried out after 3, 6, 9 and 12 months and effect of temperature ie under room temperature and refrigerated stored seeds was determined. Experiment was conducted in laboratory of Department of Forest Products, College of Forestry, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, HP under completely randomized block design. Observations were recorded on germination percentage, initiation of germination and completion of germination. The data recorded weere 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 3, 6, 9, 12 months at 0 to -5^{0}C and room temperature. Germination percentage was found to decrease with the increase in storage duration. The mean germination percentage irrespective of storage containers (polythene bags, paper bags, cloth bags and open trays) and storage temperature 0 to -5^{0}C and room temperature was found to decrease continuously from 75.84 per cent after 3 months storage to 44.99 per cent after 12 months storage.

Germination percentage

Data presented in Table 1 reveal that under different storage temperatures the rate of decrease of germination percentage was different. Under room temperature germination percentage decreased to 42.21per cent and at 0 to -5^{0}C germination percentage decreased to 47.77 per cent after 12 months storage. The mean seed germination was maximum 66.25 per cent at 0 to -5^{0}C storage and minimum 61.66 per cent under room temperature irrespective of storage duration and storage containers.

Initiation of germination

Pursual of data presented in Table 2 reveal that under different storage temperatures the minimum days (3.75) taken for initiation of germination were under room temperature and 0 to -5^{0}C after 3 months and both the treatments are statistically at par with each other. However after 6 months under T1 and T2 days taken for initiation of germination were found to be 4.16 and 3.75 respectively and both the treatments being statistically at par with each other. Maximum days taken for initiation of germination were observed in T1 ie 6.41 days after 12 months of storage. 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 irrespective of storage duration and storage containers and both the treatments were statistically at par with each other.

Germination period

Data presented in Table 3 reveal that under different storage temperatures minimum time taken for completion of

Table 1. Effect of storage temperature and duration on per cent germination

Storage temperature/ storage duration	Room temperature (T1)	0 to -5^{0}C (T2)	Mean
3 Months M1	73.33 (58.97)	78.36 (62.64)	75.84 (60.80)
6 Months M2	69.99 (56.79)	74.44 (59.77)	72.21 (58.28)
9 Months M3	61.10 (51.42)	64.44 (53.40)	62.77 (52.41)
12 Months M4	42.21 (40.47)	47.77 (43.70)	44.99 (42.08)
Mean	61.66 (51.91)	66.25 (54.88)	
	$CD_{0.05}$	SE±	
Storage temperature (T)	1.328	0.665	
Storage month (M)	1.878	0.940	
T x M	2.64	1.329	
Storage containers (S)	1.878	0.940	
T x S	2.64	1.329	
M x S	3.71	1.880	

Table 2. Effect of storage temperature and duration on initiation of germination

Storage temperature storage duration	Room temperature (T1)	0 to -5^{0}C (T2)	Mean
3 Months M1	3.75	3.75	3.75
6 Months M2	4.16	3.75	3.95
9 Months M3	4.75	4.50	4.62
12 Months M4	6.41	6.08	6.25
Mean	4.76	4.52	
	$CD_{0.05}$	SE±	
Storage temperature (T)	3.04	0.153	
Storage month (M)	0.433	0.217	
T x M	0.431	0.306	
Storage containers (S)	0.433	0.217	
T x S	0.60	0.306	
M x S	0.86	0.433	

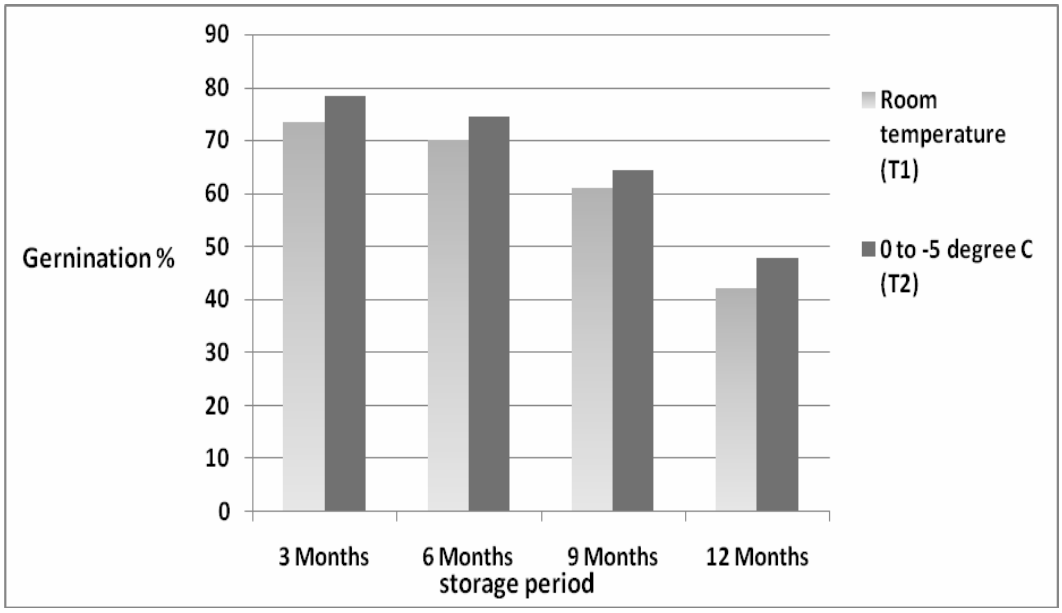

Fig 1. Effect of storage temperature and duration on per cent germination

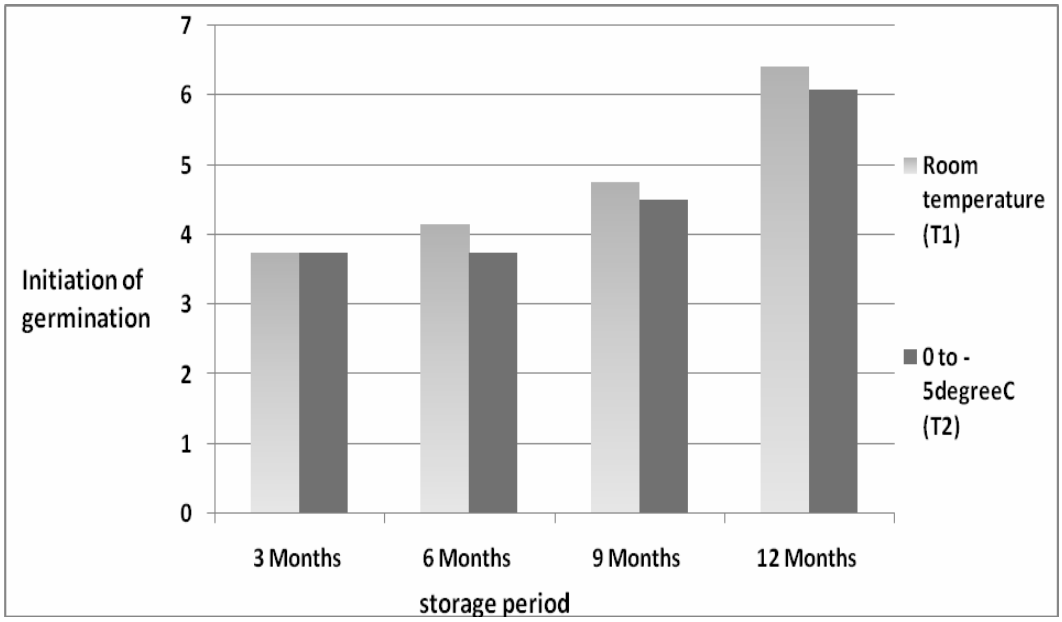

Fig 2. Effect of storage temperature and duration on Initiation of germination

germination was observed under T2 after 3 months ie 6.58 days followed by T1 ie 6.75 days and both the treatments were statistically at par with each other. With increase in storage duration time taken for completion of germination also increased. Maximum days taken for initiation of germination were after 12 months under T1 (8.83 days) followed by T2 (8.66 days) and both the treatments were statistically at par with each other. The mean completion of germination was minimum 7.45 days at 0 to -5^{0}C storage and maximum 7.89 days under room temperature irrespective of storage duration and storage containers and both the treatments differed significantly from each other.

The present study shows that the storage temperature/condition significantly affects the seed germination capacity. It varies greatly by species and storage conditions. The study indicates that the seed deterioration rates may vary depending on the storage conditions and the germination per cent and/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 sub-alpine 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*. In several other species loss of seed viability is observed within few months of storage at room temperature (Douglas 1995, Verma et al 1996). Seed moisture content is adjusted as per the relative humidity of the surrounding air which changes with the air temperature and seeds differ in the way they adjust their moisture content to humidity. In refrigerator both temperature and the relative humidity are properly maintained thereby retaining the seed viability relatively for longer period.

The standard for judging seed viability is always a germination test under optimum conditions (Phartyal et al 2002).The present investigations reveal that after 3 months storage 75.84 per cent seeds 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 per cent under room temperature and from 78.36 to 47.77 per cent 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

Table 3. Effect of storage temperature and duration on germination period

Storage temperature storage duration	Room temperature (T1)	0 to -5^{0}C (T2)	Mean
3 Months M1	6.75	6.58	6.66
6 Months M2	7.75	7.08	7.41
9 Months M3	8.25	7.50	7.87
12 Months M4	8.83	8.66	8.75
Mean	7.89	7.45	
	$CD_{0.05}$	SE(±)	
Storage temperature (T)	0.437	0.220	
Storage month (M)	0.623	0.312	
T x M	0.877	0.441	
Storage containers (S)	0.620	0.312	
T x S	0.881	0.441	
M x S	1.246	0.624	

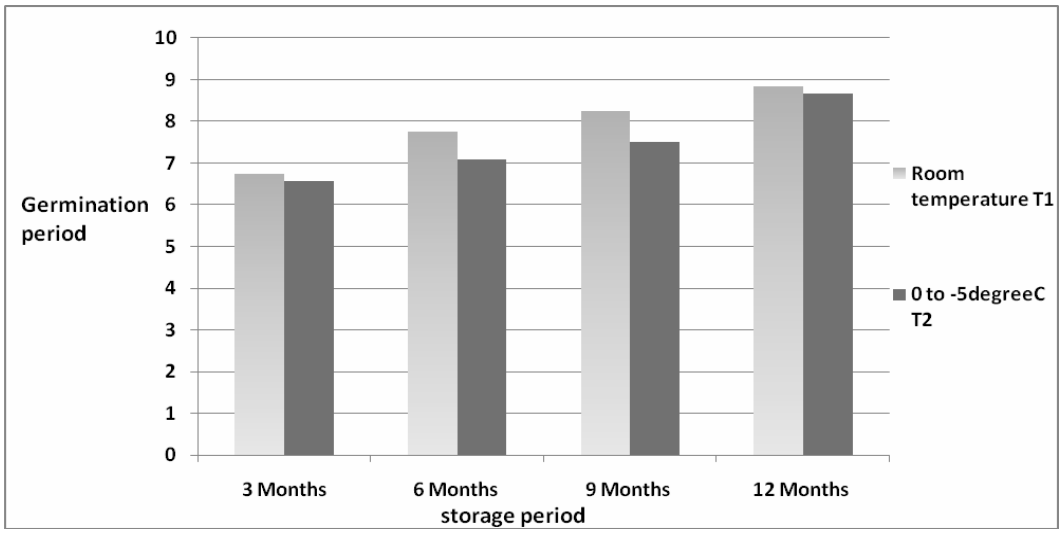

Fig 3. Effect of storage temperature and duration on germination period

due to loss of moisture which is the chief cause of seed deterioration under open conditions (Chauhan and Nautiyal 2007).

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 and 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 *G kurroo* in comparison to *A millefolium* and *P hexandrum* even under similar storage conditions. Chauhan and Nautiyal (2007) also reported lesser viability under room temperature than at low temperature storage for *Nardostachys jatamansi.*

CONCLUSION

The study emphasized on the high seed germination potential in *Rheum australe* collected from natural ecological zone from Himachal Pradesh. It concludes convincingly that the storage condition highly affects the seed germination percentage in *Rheum australe* which declines with increasing storage duration irrespective of storage condition. The study indicated that 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. Equally important is the recommendation on practicing sustainable harvesting taking care of precise time and exact spot in the site where earlier collections were made using potential populations for conservation programmes in case of endangered species.

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