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Effect of storage conditions on germination and associated physiological attributes on seeds of *Withania somnifera* Dunal

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Abstract
The current articulation reveals the effect of storage conditions on the physiological behaviour of *Withania somnifera* seeds under different storage conditions *viz*; temperature, duration and container. Three factors were included in the experiment which was, storage temperature, duration and container. Statistically, significant results were obtained with the interaction of the above mentioned factors. Among all the treatment combinations seeds stored in plastic jars at 0ºC for 6 months showed maximum germination (74.25%), mobilization efficiency (99.75 %), α- Amylase activity (739.58 µg starch dehydrogenase/min./g), dehydrogenase activity (0.585 Δ O.D/g/ml) and peroxidase activity (28.21 Δ A_{475}/ min./g seed). Conversely, minimum solute leakage (18.62 dSm^{-1}) was observed after 12 hours of soaking in seeds stored for 2 months at 0ºC in plastic jars.

Keywords: *Withania somnifera*, physiological quality, germination percentage, mobilization efficiency, enzyme activity

Introduction
Seeds are uniquely equipped to survive as a viable regenerative organism until the time and place is right. Being a living entity, it deteriorates beyond the physiological maturity which is inevitable and will be pronounced when seeds are stored under hostile conditions. Loss of quality, viability and vigor of seeds during ageing in storage is a natural, irreversible and degenerative process occurring during the storage and leads to seed deterioration (Jyoti and Malik, 2013) [9]. Rapid loss of physiological quality of seeds during storage is one of the major constraints faced by the Indian Seed Industry and the corresponding financial implications. Maintaining seed viability for longer period is very essential to preserve the genetic and physiological integrity in stored samples (Ellis *et al*., 1991; Pradhan and Batola, 2008 and Roberts, 1973) [5, 14, 19]. Thus, storage of seeds as *ex situ* germplasm is an essential step.
Loss of seed viability during storage and the aggregation of damaged genetic compounds in the surviving seeds had a very close relationship with each other (Rao *et al*., 1987) [17]. Seed moisture content, temperature and duration are among the main factors affecting the above relationship (Roberts, 1988) [20]. Inappropriate storage medium (Hezewijk *et al*., 1993 and Muller *et al*., 2011) [7, 12] such as storage at room temperature often results in low seed germination, seed deterioration and loss of viability (Nasreen *et al*., 2000 and Schimdt *et al*., 2002) [13, 21]. Therefore, storage of seeds in ideal conditions is essential for preserving high seed quality, vigor and viability for future use by farmers and breeders (Copeland and McDonald, 2012) [3].
Withania somnifera Dunal. commonly known as Ashwagandha or Indian ginseng is an important commercial medicinal species, which is in high demand by pharmaceutical companies. The plant possesses tremendous medicinal value due to the presence of a number of alkaloids and steroidal lactones in the roots, leaves and fruits (Rayees *et al*., 2017) [18]. Due to this propensity of the herb, its demand has increased drastically with time. Since, the germination potential of the crop is very low, its propagation in nature is not sufficient to ensure the survival of the plant (Vakeswaran and Krishnaswamy, 2003) [28]. Henceforth, to conserve this valuable germplasm ideal storage conditions are required; and due to the scarce scientific information available on physiological behaviour of seeds of this commercial medicinal species under storage conditions an experiment was designed which aimed for identification of optimum seed storage conditions, alongwith underlying physiological changes affecting the seed longevity in *Withania somnifera*.

Materials and Methods

Freshly, harvested seeds of *Withania somnifera* were properly cleaned, air dried to 9 % moisture content and subjected to four storage temperatures *viz*; 0, 5, 10 ºC and ambient temperature for three durations i.e. 2, 6 and 10 months in two different types of containers i.e. plastic jars and canvas cloth bags. At regular intervals for 10 months a total of 100 seeds each were drawn from storage and allowed to germinate in petri-dishes using top paper method in seed germinator at 25±2ºC and 80% relative humidity. The evaluation of various seed attributes were done as per the following formulae:-

Germination percentage was evaluated as per ISTA 2015, after 30 days of sowing in 4 replicates of 100 seeds:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds kept for germination}} \times 100$$

Solute leakage: Electrical conductivity of seed leachate was determined by taking one gram of seeds of each species randomly in four replications and soaked in 25 ml of distilled water for 24 hours at 25 ºC. After incubation, the seed leachate was decanted and the conductivity was measured by digital conductivity meter at 25 ºC and expressed in dS/m.

Mobilization efficiency of seeds was estimated by weighing the seed remnant on the 30th day of sowing after the seedlings have emerged and was calculated as per following formulae given by Shrivastava and Sareen, 1974 [23].

$$\text{Mobilization efficiency (\%)} = \frac{\text{Dry wt of the seed} - \text{Dry wt of the seed remnant}}{\text{Dry wt of the original seed}} \times 100$$

Enzyme activity: Activity of α-Amylase and peroxidase enzymes was evaluated as per procedure given by Vimale, 1983 [29] whereas; dehydrogenase activity was evaluated as per procedure given by Kittock and Law, 1968 [11].

Result and Discussion

Germination

Effect of storage conditions on seed germination behavior can be clearly illustrated in Fig.1. Seeds stored at 0ºC in plastic jars for 6 months recorded maximum germination percentage (74.25%) as compared to minimum germination percentage (39.25 %) recorded for seeds stored under ambient condition in canvas cloth bags for 2 months. This experiment, further indicated an important observation that seeds stored for 10 months at either of the three temperatures, immaterial of storing in plastic jars or canvas cloth bags did not show germination, thus revealing the complete loss of germination ability. An overall view of germination behavior of the seeds revealed that they perform better at low temperature probably due to the slow rate of deteriorative processes in storage. Storage of seeds at low temperature could promote germination by inducing GA biosynthesis (Thakur *et al.*, 2005) [26] or by increasing its GA sensitivity (Pullock and Toole, 1961) [16]. Low temperature on the other hand exerts a stimulating effect on seed germination promoting factors other than GA`s or increase of ABA degradation (Egly and Paul, 1982) [4] or may also instigate changes in membrane permeability which are pivotal in seed germination (Francis and Coolbear, 1987) [6].

Solute Leakage

Solute leakage is a physiological evidence for determination of seed deterioration in seeds due to changes in membrane during ageing. Storage conditions *viz*; temperature, container and duration had significant effect on seed membrane (Table 1). Maximum solute leakage (102.23 dSm^{-1}) was registered after 24 hrs of soaking by seeds stored under ambient conditions for 10 months in canvas cloth bags. Conversely, the minimum solute leakage i.e (18.62 dSm^{-1}) was observed after 12 hours of soaking in seeds stored for 2 months at 0ºC in plastic jars. Solute leakage in seeds has a clear and direct relationship with membrane integrity of seeds. High solute leakage is due to membrane deterioration during storage conditions and this deterioration in seeds during ageing leads to increased electrolytes. The greater leakage from old seeds and less from young seeds implies that intergrity of plasmalemma and tonoplast is lost during ageing (Priestly, 1986) [15]. Excessive solute leakage can also be attributed to degradation of cell membrane due to moisture imbalance and metabolic activities as suggested by Schoettle *et al.* (1984) [22]. Our results on solute leakage are in concurrence with endangered medicinal species i.e *Achillea millefolium*, *Gentiana kuroo* and *Podophyllum hexandrum*, where significant negative correlation was established between solute leakage and germination potential of aged seeds indicating the loss of membrane integrity (Thakur *et al*., 2004) [25].

Mobilization efficiency

The data on mobilization of stored reserves presented in Table 2 invariably commences after radical elongation in the growth region of embryonic axis thus, it is a post germinative event. Among all the treatment combinations seeds stored for 6 months in plastic jars at 0 ºC showed maximum mobilization of seed reserves (99.75%) whereas minimum mobilization efficiency (30.30%) was registered by seeds stored for 2 months in canvas cloth bags at 0 ºC. Seeds stored at 10 months at either of temperature and containers did not show mobilization of stored reserves as no germination was observed. Maximum mobilization of solutes in germinating seeds stored at low temperature could be assigned to higher activity of α-Amylase as it is a very important hydrolytic enzyme causing solubilization of starch during germination. Some mobilization may occur before germination is completed. The product of hydrolysis of cotyledonary reserves are significant for early seedling establishment. α-Amylase is very important hydrolytic enzyme causing the solubilisation of starch during germination. Thus, higher mobilization of solutes could be assigned to higher activity of α-Amylase in seeds.

Enzyme activity

During the course of present investigation activity of enzymes i.e α-Amylase, Dehydrogenase and Peroxidase were affected by different storage conditions. Storage of seeds at 0ºC for 6 months showed maximum peroxidase activity (28.21 ΔA_{475}/min./gm) and α-Amylase activity (739.58 µg starch degradation/min./gm) whereas maximum dehydrogenase activity (0.645 O.D/gm/ml) was observed in seeds at 0ºC in plastic jars after 2 months which further decreases thereafter with increase in storage duration i.e. 10 months. While seeds kept in canvas cloth bags at ambient temperature showed minimun enzyme activity (0.12 ΔA_{475}/min./gm, 0.83 µg starch degradation/min./gm and 0.003 O.D/gm/ml) for peroxidase,

α-Amylase and dehydrogenase, respectively which can be clearly visible in Table 2 and 3. Packaging material, moisture content, storage temperature and storage period are the principle factors affecting the activity of above enzymes and viability of seeds during storage. Retention of maximum viability in seeds stored in plastic jars at 0ºC can be assigned to the higher activities of antioxidant enzymes such as peroxidase and dehydrogenase which might have reduced the process of lipid peroxidation. Dehydrogenase enzyme is a very important respiratory pathway enzyme that generates ATP in Kreb Cycle and ageing of seeds impairs the activities of respiratory enzyme particularly dehydrogenase (Throneberry and Smith, 1955) [27]. In general, decrease in dehydrogenase activity in seeds decreases its respiratory potential, so that the energy (ATP) and food supply for the germinating seeds become less (Tatipata, 2010) [24] as indicated in our studies. Similar reduction of dehydrogenase activity in ageing seeds of sunflower was detected by Kannababu and Karivaratharaju (2000) [10], who reported that the rate of reduction in dehydrogenase activity in ageing seeds was high in embryonic axis where the meristematic cells are present. Hence, decline in the activity of dehydrogenase is one of the important symptoms of seed deterioration (Copeland and McDonald, 1985) [2]. Cakmek *et al.*, 2010 [1] also reported that decrease in germination ability of the aged legume seeds are correlated with decreased activity of antioxidant enzymes. Antioxidant enzymes are considered to be the main protective enzymes engaged in the removal of free radicals and activated oxygen species thereby reducing seed deterioration with ageing. Decline in the activities of above enzymes in seeds stored under ambient conditions may have resulted in decreased viability and vigour characteristics.

Table 1: Effect of storage containers, temperatures and durations on solute leakage of seeds after 12 hours and 24 hours (dSm^{-1}).

Storage Temperature (ºC)	Plastic Jars				Canvas cloth bag				Plastic Jars				Canvas cloth bag			
	Storage Durations (months)				Storage Durations (months)				Storage Durations (months)				Storage Durations (months)			
	12 hours								24 hours							
	2	6	10	Mean	2	6	10	Mean	2	6	10	Mean	2	6	10	Mean
0	18.62	28.45	43.10	30.06	19.06	30.58	44.83	31.49	29.64	49.45	78.93	52.67	32.81	55.73	81.11	56.55
5	18.68	33.38	50.18	34.08	19.45	34.11	51.38	34.98	31.58	54.83	86.15	57.52	33.58	61.08	88.90	61.18
10	20.01	33.63	50.68	34.77	24.30	35.40	54.02	37.90	36.16	56.08	90.20	60.81	44.18	63.47	93.02	66.89
Ambient temperature	19.60	38.38	55.10	37.69	23.16	36.94	57.47	39.19	33.70	65.25	95.68	64.88	39.23	66.57	102.23	69.34
Mean	19.23	33.46	49.76	34.15	21.49	34.25	51.92	35.89	32.77	56.40	87.74	58.97	37.45	61.71	91.32	63.49
	C $D_{0.05}$ 12.09								C $D_{0.05}$ NS							

Table 2: Effect of storage containers, temperatures and durations on mobilization efficiency (%) and α- Amylase Activity of seeds (µg starch degradation/min./gm).

Storage Temperature (ºC)	Mobilization efficiency (%)								α- Amylase Activity (µg starch degradation/min./gm)							
	Plastic Jars				Canvas cloth bag				Plastic Jars				Canvas cloth bag			
	Storage Durations (months)				Storage Durations (months)				Storage Durations (months)				Storage Durations (months)			
	2	6	10	Mean	2	6	10	Mean	2	6	10	Mean	2	6	10	Mean
0	43.18	99.75	0	98.78	30.3	94.39	0	65.03	49.58	739.58	4.17	264.45	31.04	520.83	4.19	185.35
5	49.2	84.69	0	66.95	36.13	85.5	0	60.81	91.25	493.75	3.33	196.11	42.98	420.83	2.15	155.32
10	61.43	72.25	0	66.84	56.68	96.98	0	63.32	166.67	387.5	1.67	185.28	154.17	289.58	1.13	148.29
Ambient temperature	55.4	62.45	0	58.93	42.88	55.25	0	49.06	145.83	263.54	1.04	136.81	127.08	206.25	0.83	111.39
Mean	52.30	79.78	0.00	65.37	41.49	76.28	0.00	59.55	113.33	471.09	2.55	195.66	88.82	359.36	2.07	150.09
	$CD_{0.05}$: 0.28								$CD_{0.05}$: NS							

Table 3: Effect of storage containers, temperatures and durations on Dehydrogenase activity (O.D/gm/ml) and Peroxidase activity (ΔA_{475}/min./gm).

Storage Temperature (ºC)	Dehydrogenase activity (O.D/gm/ml)								Peroxidase activity (ΔA_{475}/min./gm)							
	Plastic Jars				Canvas cloth bag				Plastic Jars				Canvas cloth bag			
	Storage Durations (months)				Storage Durations (months)				Storage Durations (months)				Storage Durations (months)			
	2	6	10	Mean	2	6	10	Mean	2	6	10	Mean	2	6	10	Mean
0	0.645	0.585	0.055	0.428	0.555	0.428	0.006	0.339	21.92	28.21	1.66	17.26	15.47	18.27	0.92	11.55
5	0.558	0.523	0.048	0.376	0.468	0.390	0.005	0.287	19.76	25.28	1.46	15.50	13.92	17.56	0.76	10.75
10	0.523	0.458	0.045	0.342	0.453	0.305	0.004	0.254	14.10	16.70	0.70	10.50	10.22	15.84	0.62	8.89
Ambient temperature	0.488	0.420	0.043	0.317	0.440	0.230	0.003	0.224	12.39	15.49	0.22	9.37	8.70	13.99	0.12	7.61
Mean	0.553	0.496	0.048	0.366	0.479	0.346	0.004	0.276	17.04	21.42	1.01	13.16	12.08	16.42	0.61	9.70
	$CD_{0.05}$: NS								$CD_{0.05}$: NS							

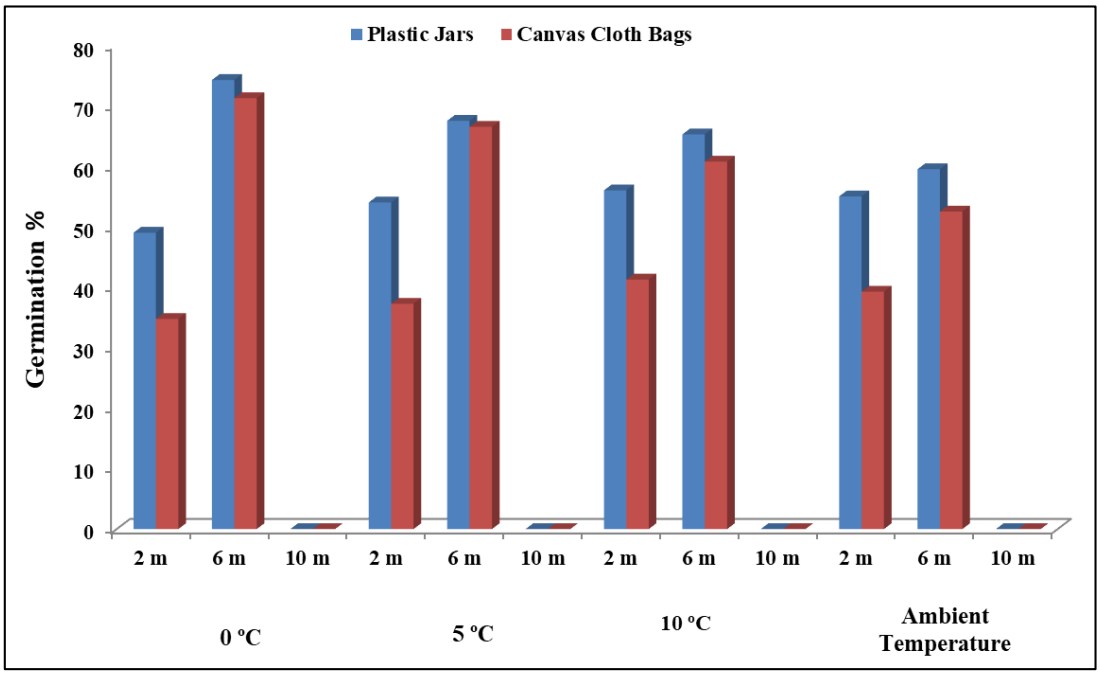

Fig 1: Effect of storage containers, durations and temperatures on germination percentage of seeds of *Withania somnifera*

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