International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 06-03-2022, Accepted: 22-03-2022, Published: 07-04-2022
Volume 7, Issue 4, 2022, Page No. 33-40

Varietal difference in physiological activities of *vigna radiata* during different seed storage container

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Abstract
The seed is that the first biological process part and also the basic variety of adaptation of upper plants to extant in unfavourable environmental conditions. Seed aging is a natural process and affected by types of storage container and varieties of seeds and decrease their viability. Aging involves progressive physiological changes over time. One of the main physiological changes is an excessive production of free radicles i.e. electrical conductivity. If the electrical conductivity is too high, it causes destruction of the structure of lipid membranes, carbohydrates, proteins, and DNA. Current research study report include different 3 type of storage containers Cotton bag (S_1), Jute bag (S_2) and Polythene bag (S_3) were utilised for the storage of *Vigna radiata* (mungbean) seeds of 3 variety Gujarat-4 (M_1), GAM-5 (M_2) and Virat (M_3) under ambient warmth and comparative dampness for a time of 1.5 years (18 month). Specific amount of the seeds from respectively storage container were taken after 90 days. Initally 0 days to 540 days intervals were denoted by T_1 to T_7 respectively and experiments were performed for physiological changes observations. The electrical conductivity of seed leachate was higher in the variety Virat (0.487 mSiemens/cm) as compared to Gujarat-4 and GAM-5 as 0.469 mSiemens/cm and 0.421 mSiemens/cm respectively after storage of 540 days in Jute bag. Maximum deviation observed in Virat varieties stored in Jute bag while minimum i.e. 0.341 mSiemens/cm in GAM-5 in polythene bag. Standard germination, Length of Seedling and Field emergence were observed inversely as compared to electrical conductivity. The seeds preserved in Polyethylene bag showed least physiological deviation as compared to Cotton and Jute bag. The objective of current research work was confirming the reasons of seed aging and determining its consequences for future.

Keywords: electrical conductivity, length of seedling, physiological studies, storage containers, *Vigna radiata* (mungbean)

Introduction
The seed is that the first natural process part and also the introductory variety of adaption of upper plants to extant in unfavourable ecological circumstances. It's the role of seeds to transfer options to offspring, in indispensable words, to insure the durability of species (Kurek *et al*. 2019) [11]. Seeds tend to age, rather like the other plant part (Lehner *et al*. 2008) [13]. The method reduces plant's capability to sprout, which is the essential role of seeds within the life span of plants (Jimenez-Alfaro *et al*. 2016) [8]. Most of the farmers keep harvested seeds in a stock for month or year before selling or next sowing season. The viability of seeds is changed by numerous factors like core physiological and biochemical (Hu *et al*. 2012a) [5] that rely on the species and external, like humidity and warmth conditions of storage of seeding and packaging material (Marcos-Filho, 2015; Pukacka and Ratajczak, 2007) [14, 23].
One of the chief factors that affect the quality of seed is vigor. Seed vigour and viability illustrated by sigmoid curve. Although literatures were explained that seed sustainability reducing throughout storage and its assessment has been standardized. *Vigna radiata* (Mungbean) is an ancient and well-known pulse crop originated from South East Asia (Nair *et. al.*, 2013) [18]. About 98% mungbean production originated from Asian countries like India, China and Thailand (Lambrides and Godwin, 2007) [12]. Mungbean is seasonable crop to increase income of farmers. It is chiefly cultivated for mankind food in the form of sprouts, floor, boiled beans as well as immature pods. The dry seeds used for birds of poultry either roasted or boiled (Winch 2006) [31]. Furthermore it helps to fix atmospheric nitrogen by rhizobium bacteria to increase the fertility of soil (Somta *et al*., 2007) [27]. Though it has great value as food and fodder, it leads to increase the demand in supply of mungbean.
The seeds of mungbean are stored for 7 to 8 month prior to next season sawing though make sure Sun dried seeds has been observed to increased viability. Still, seed isn't dried to fairly safe humidity after harvest; its storability will be declined (Gadewar *et. al*., 2009) [2]. Indeed Seeds stored under ambient conditions in a Jute bags would affect in drastically loss of germinability (Charjan and Tarar, 1992) [6]. Seed ageing responsible to reduce seed germination (Shaban, 2013) [24]. Standard germination test is mainly performed to evaluating the quality of seeds. It is perform under the optimum growth conditions of specific seed varieties (Julio, 2015) [9].

Thus it is utmost importance to understand the effect of storage on mungbean seed vigour. Literature reviews reveals that very less work on the physiological studies has been carried out with account of Mungbean seed varieties and different storage containers which farmers frequently use. Regarding to relation between seed vigour and quality this research has been performed with the objective to evaluate the effectiveness of storage container on the storage of variety of mungbean seeds and its vigour and quality.

Materials and Methods

Seeds of the following kinds and varieties i.e. Gujarat-4 (M_1), GAM-5 (M_2), and Virat (M_3) were purchased from "Gujarat State Seeds Corporation Ltd.", Gujarat. The seed samples were sawn in field and mature harvested sundried seeds collected and stored in the respective containers Cotton bag (moisture pervious), Jute bag (moisture pervious) and Polyethylene bag 700 gauge (moisture vapour proof). Cotton bag, Jute bag and Polyethylene bag, are denotes by S_1, S_2 and S_3 respectively. All the three bags will be of 25 cm x 30 cm in size. The respective storage containers were kept under ambient warmth and comparative dampness for a period of 1.5 years (i.e. 18 months).

The experiment was conducted at the Department of Botany, M.D. Science College, Porbandar,Gujarat,India. Portion of the seeds from each container were taken after every 90 days i.e., 0 days, 90 days (3 month), 180 days (6 month), 270 days (9 month), 360 days (12 month), 450 days (15 month) and 540 days (18 month) intervals are denoted by T_1, T_2, T_3, T_4,T_5, T_6 and T_7 respectively and examined for changes found in physiological characteristics.

The standard germination test was carried out by ISTA rules with the help of moist towel paper.

Nine samples of seeds were sown on paper apkins, bedewed with water equivalent to 2.7 times the weight of dry ampkins. The apkins rolls were kept in a germination chamber and temperature kept constanst 25 °C throughout experiment period. The observation were noted down after 7 and 12 days of sowing, and the observed data were presented as a percentage of normal seedlings (Oliveira *et al*., 2014a). After seven days of sowing, no. of germinated seeds were counted. The results were presented as a percentage of normal seedlings (Oliveira *et al*., 2014a).

Electrical conductivity of seeds were performed by digital auto ranging conductivity meter with magnetic stirrer. Nine sample of seeds were weighed and washed properly with 0.1M mercury chloride solution. Seeds were dry with paper towel and immersed in 100 mL of distilled water and kept in a chamber at 25 °C for a day. Next day, the electrical conductivity of the solution was determined by conductivity meter. The results were noted down in mSiemens/cm of seeds.

The field emergence test was carried out with the help of Complete Randomized Design (CRD). The length of Seedling was calculated with help of standard measuring scale. The sum of root and shoot measured in centimeter. The data obtained from the experiments were statistically analysed.

Results and Discussion

Standard germination (%)

Figure 1 and Table 1 presented the data of effect of type of storage container and time period of storage on standard germination in all three mungbean varieties Gujarat-4 (M_1), GAM-5 (M_2) and Virat (M_3).

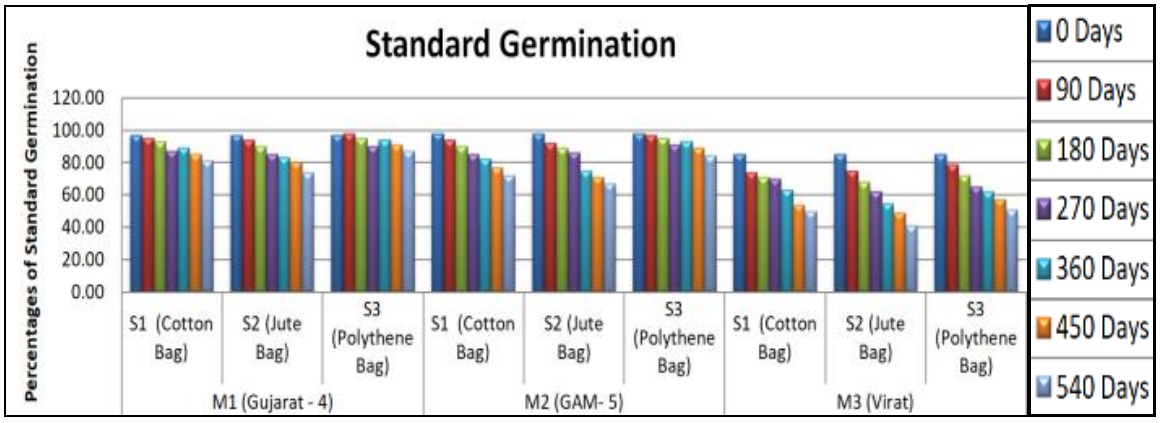

Fig 1: Effect of Mungbean Varieties (M), time Periods of Storage (T) and type of Storage Containers (S) on Standard Germination (%) of seeds of mungbean during storage.

Table 1

M X S X T	M_1 (Gujarat - 4)			M_2 (GAM- 5)			M_3 (Virat)		
	S_1 (Cotton Bag)	S_2 (Jute Bag)	S_3 (Polythene Bag)	S_1 (Cotton Bag)	S_2 (Jute Bag)	S_3 (Polythene Bag)	S_1 (Cotton Bag)	S_2 (Jute Bag)	S_3 (Polythene Bag)
0 Days	97.00	97.00	97.00	98.00	98.00	98.00	85.00	85.00	85.00
90 Days	95.00	94.00	98.00	94.00	92.00	97.00	74.00	75.00	79.00

180 Days	93.00	90.00	95.00	90.00	89.00	95.00	71.00	68.00	72.00
270 Days	87.00	85.00	90.00	85.00	86.00	91.00	70.00	62.00	65.00
360 Days	89.00	83.00	94.00	82.00	75.00	93.00	63.00	55.00	62.00
450 Days	85.00	80.00	91.00	77.00	71.00	89.00	54.00	49.00	57.00
540 Days	81.00	74.00	87.00	72.00	67.00	84.00	50.00	41.00	51.00
Average	89.57	86.14	93.14	85.43	82.57	92.43	66.71	62.14	67.29

Table 2: Effect of Mungbean Varieties (M), time Periods of Storage (T) and type of Storage Containers (S) on Standard Germination (%) of seeds of mungbean during storage.

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Rows	4440.19	6	740.0317	41.39578	2.46E-17	2.294601
Columns	8024.794	8	1003.099	56.11121	9.53E-22	2.138229
Error	858.0952	48	17.87698			
Total	13323.08	62				

The rate of standard germination is inversly propotional to the storage time periods of seeds.
Variety Virat (M_3), showed maximum decline in standard germination in compare of Gujarat-4 (M1) and GAM-5 (M2) after 540 days. However the rate of loss in standard germination varied with the type of container used. Seeds stored in Jute bag (S_2) showed significantly minimum standard germination (41.00 %) while in Cloth bag (S_1) (50.00 %) and Polythene bag (S_3) (51.00 %) after 540 days (T_7) days of the storage. In all three varieties of Mungbean and types of storage containers, M_1 variety in polythene bag showed maximum standard germination (93.14%) while 89.17% and 86.14% averagely observed in cloth bag(S_1) and Jute bag(S_2) respectively. Gujarat-4 (M_1) variety stored in polythenebag showed higher germination in compared to Virat (M_3) in the same condition. The reason to decline standard germination is due to reduction in metabolic activities of seeds stored under ambient conditions (Marcos-Filho, 2015) [14].

Field emergence (%)
Figure 2 and Table 2 presented the data of effect of type of storage container and time period of storage on field emergence in all three mungbean varieties Gujarat-4 (M_1), GAM-5 (M_2) and Virat (M_3).

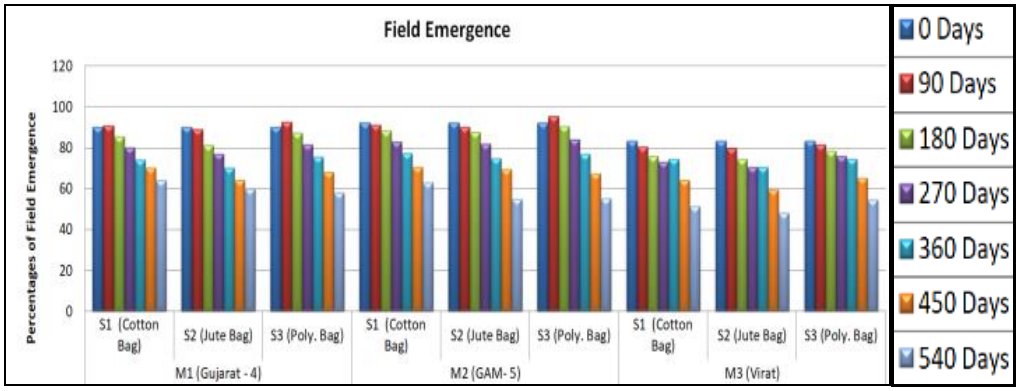

Fig 2: Effect of Mungbean Varieties (M), time Periods of Storage (T) and type of Storage Containers (S) on field emergence (%) of seeds of mungbean during storage.

Table 3

M X S X T	M_1 (Gujarat - 4)			M_2 (GAM- 5)			M_3 (Virat)		
	S_1 (Cotton Bag)	S_2 (Jute Bag)	S_3 (Poly. Bag)	S_1 (Cotton Bag)	S_2 (Jute Bag)	S_3 (Poly. Bag)	S_1 (Cotton Bag)	S_2 (Jute Bag)	S_3 (Poly. Bag)
0 Days	90.22	90.22	90.22	92.43	92.43	92.43	83.54	83.54	83.54
90 Days	90.83	89.31	92.64	91.22	90.39	95.76	80.72	80.09	81.78
180 Days	85.67	81.36	87.49	88.36	87.64	90.75	75.94	74.68	78.35
270 Days	80.12	77.08	81.52	83.12	82.18	84.36	73.22	70.65	75.86
360 Days	74.23	70.12	75.63	77.51	74.84	77.16	74.59	70.5	74.68
450 Days	70.14	64.21	68.25	70.68	69.45	67.54	64.22	59.5	65.28
540 Days	64.25	60.14	58.34	63.21	54.86	55.31	51.26	48.25	54.75
Average	79.35	76.06	79.16	80.93	78.83	80.47	71.93	69.60	73.46

Table 4: Effect of Mungbean Varieties (M), time Periods of Storage (T) and type of Storage Containers (S) on Standard Germination (%) of seeds of mungbean during storage.

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Rows	7469.834	6	1244.972	203.23	2.29E-32	2.294601
Columns	936.3437	8	117.043	19.10616	1.61E-12	2.138229
Error	294.0445	48	6.125927			
Total	8700.222	62				

Variety GAM-5 (M2) preserved in polythene bag exhibited maximun field emergence (80.47%) while those stored in Jute bag (S_2) (78.33%) up to 540 days (T_7) days of the storage. In variety Virat (M_3), the field emergence showed significantly inversonaly proportional to storage period. Among the containers Cotton bag (S_1) showed significantly higher field emergence (80.93%) in GAM-5 as compared to Virat (M_3) (71.93%) throughout the storage period.

Length of Seedling (cm)

The effect of container and storage period on Length of Seedling in all three varieties M_1, M_2 and M_3 is presented in Figure 3 and Table 3.

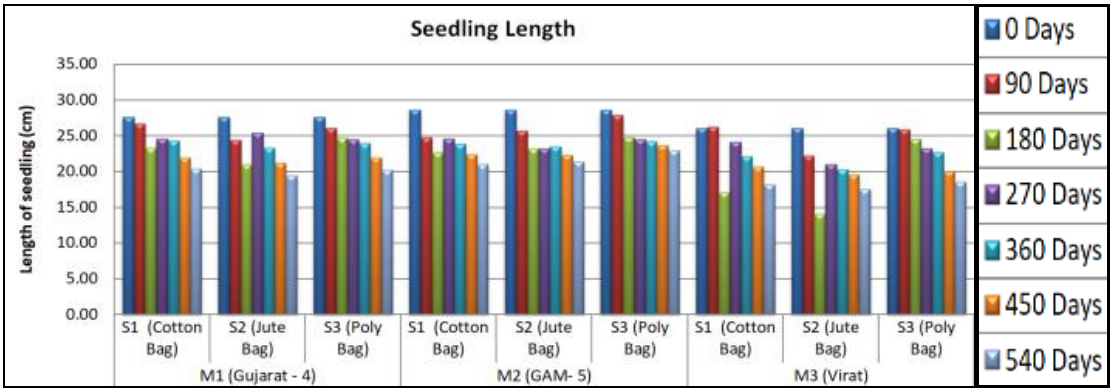

Fig 3: Effect of Mungbean Varieties (M), time Periods of Storage (T) and type of Storage Containers (S) on Length of Seedling (cm) of seeds of mungbean during storage.

Table 5

M X S X T	M_1 (Gujarat - 4)			M_2 (GAM- 5)			M_3 (Virat)		
	S_1 (Cotton Bag)	S_2 (Jute Bag)	S_3 (Poly Bag)	S_1 (Cotton Bag)	S_2 (Jute Bag)	S_3 (Poly Bag)	S_1 (Cotton Bag)	S_2 (Jute Bag)	S_3 (Poly Bag)
0 Days	27.65	27.65	27.65	28.64	28.64	28.64	26.10	26.10	26.10
90 Days	26.70	24.40	26.13	24.83	25.74	27.88	26.24	22.26	25.88
180 Days	23.32	21.04	24.73	22.67	23.21	24.83	17.08	14.14	24.53
270 Days	24.58	25.41	24.55	24.62	23.26	24.57	24.17	21.01	23.25
360 Days	24.31	23.32	23.97	23.89	23.52	24.28	22.18	20.30	22.75
450 Days	21.98	21.26	21.97	22.41	22.36	23.68	20.65	19.61	20.02
540 Days	20.41	19.47	20.19	21.07	21.41	23.01	18.21	17.54	18.58
Average	24.13	23.22	24.17	25.19	25.21	26.48	23.40	20.88	24.94

Table 6: Effect of Mungbean Varieties (M), time Periods of Storage (T) and type of Storage Containers (S) on Length of Seedling (cm) of seeds of mungbean during storage.

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Rows	354.3519	6	59.05864	35.19934	5.82E-16	2.294601
Columns	125.3862	8	15.67328	9.341378	1.17E-07	2.138229
Error	80.53602	48	1.677834			
Total	560.2741	62				

In variety Virat (M_3), the Length of seedling also showed same result as observed in standard germination and field emergence. Length of seedling significantly declined with respect increase in time period of storage. However, the rate of loss in Length of seedling varied with the type of varieties used. After 540 days (T_7) of the storage, Seeds of GAM-5 (M_2) stored in polythene bag showed maximum Length of seedling (18.58 cm) as compared to those stored in Jute bag (S_2) (17.54 cm). Among the containers Jute bag (S_2) showed significantly

minimum Length of seedling as compared to Cotton and polythene bag throughout the storage period in all varieties.

Electrical Conductivity (mSiemens/cm)

The effect of container and storage period on Electrical Conductivity in all three varieties M_1, M_2 and M_3 is presented in Figure 4 and Table 4.

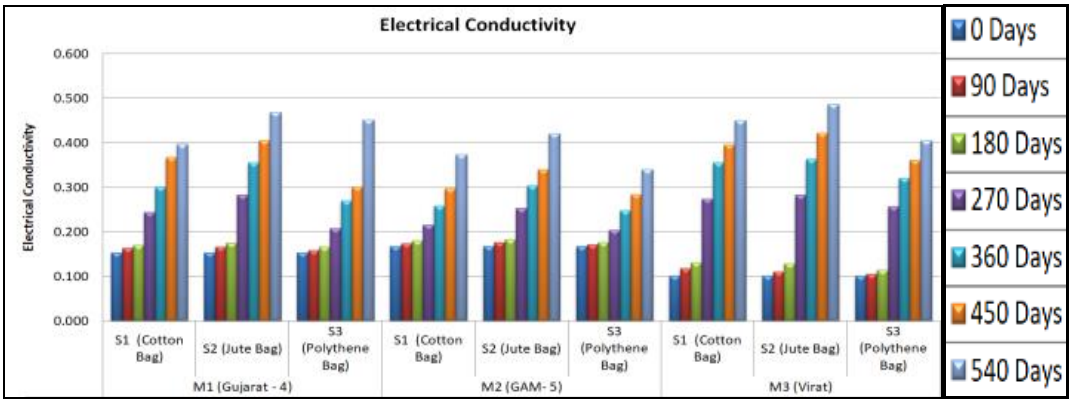

Fig 4: Effect of Varieties (M), Storage Containers (S) and Storage Periods (T) and three factor interaction on Electrical Conductivity (mSiemens/cm) of seeds of mungbean during storage.

Table 7

M X S X T	M_1 (Gujarat - 4)			M_2 (GAM- 5)			M_3 (Virat)		
	S_1 (Cotton Bag)	S_2 (Jute Bag)	S_3 (Polythene Bag)	S_1 (Cotton Bag)	S_2 (Jute Bag)	S_3 (Polythene Bag)	S_1 (Cotton Bag)	S_2 (Jute Bag)	S_3 (Polythene Bag)
0 Days	0.154	0.154	0.154	0.168	0.168	0.168	0.102	0.102	0.102
90 Days	0.164	0.167	0.159	0.174	0.177	0.172	0.118	0.111	0.106
180 Days	0.171	0.176	0.167	0.181	0.183	0.177	0.132	0.129	0.115
270 Days	0.245	0.284	0.208	0.216	0.253	0.204	0.274	0.283	0.257
360 Days	0.301	0.356	0.270	0.259	0.304	0.248	0.356	0.364	0.321
450 Days	0.368	0.406	0.301	0.298	0.341	0.285	0.396	0.423	0.362
540 Days	0.398	0.469	0.452	0.375	0.421	0.341	0.451	0.487	0.406
Average	0.257	0.287	0.244	0.239	0.264	0.228	0.261	0.271	0.238

Table 8: Effect of Varieties (M), Storage Containers (S) and Storage Periods (T) and three factor interaction on Electrical Conductivity (mSiemens/cm) of seeds of mungbean during storage.

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Rows	0.664138	6	0.11069	86.85181	4.61E-24	2.294601
Columns	0.019783	8	0.002473	1.940339	0.075316	2.138229
Error	0.061174	48	0.001274			
Total	0.745096	62				

In M_1,M_2 and M_3 all varieties, the electrical conductivity showed directly proportinal with storage period. As storage period increased, electrical conductivity increased. However, the rate of incresed electrical conductivity wide-ranging with the type of varieties used. Seeds of GAM-5 (M_2) stored in polythene bag revealed drastically lower electrical conductivity (0.341 m Siemens/cm) as related to those preserved in Jute bag (S_2) (0.421 m Siemens/cm) up to 540 days (T_7) days of the storage. Amongst the containers Polythene bag (S_3) exposed minimum conductivity as compared to Jute and Cotton bag throughout the storage period in all varieties.

Discussion

Storage of seed is responsible for seed aging. Due to Seed aging, seed became weakens which was also responsible for changes in physiological parameters like seed germination, field emergence, electrical conductivity and length of seedling (Shaban, 2013) [24]. Type of storage containers and varieties of seeds showed varietion in standard germination. Tame and Elam, (2015) [28] supported the same result. They studies on varieties of soybean with five storage containers. They observed that seed stored in polythene bag and plastic bottle showed maximum germination percentages in compared of other containers during storage.

Standard germination

According to Gadewar *et al.* (2020) [3], Standard germination test is mostly used for observing the quality of seeds. It was performed under ideal climatic growth conditions which is necessory for a growth of species. A sharp reducing sigmoid survival curve was observed in seed germination of the three varieties of mungbean seeds kept in Jute bag and cotton bag throughout storage. This result possibly due to moistness absorbant nature of storage container which absorb more moisture increases biochemical activity and growth of microorganism throughout storage. The seed stored in polythene bag shows moderate reducing curve in compare of cotton and Jute bag. It was due to resistant bareer for dampness and decline variations in seed humidity.

The experimental data recommended that mungbean seed should be kept in a polythene bag rather than cotton or Jute bag. The same results confirmatory with Gadewar *et al*., (2020) [3] and Sing *et al.*, (2016) [26]. Varietal differences for germination were also significant in the mungbean. Virat exhibited less germination percentage than Gujarat-4 and GAM-5 varieties throughout storage. It would be due to genetic composition of Virat (M3) variety of mungbean. Tamme and Elam (2015) [28] reported that genetic difference is also responsible for physiological changes like standard germination. Previous study about the preservation of *J. curcas* seeds have exhibited a declined in standard germination throughout storage, especially under ambient circumstances (Chaves *et al*., 2012; Pereira *et al*.) [7]. Furthermore, Pinto Junior *et al*. (2012) [22] didn't perceive a decline in germination for *J. curcas* seeds stored for 180 days in absorptive container (multiwall paper bags) and moisture impermeable container (high density plastic bags and glass containers) in diverse storage conditions.

Field emergence

The type of storage container directly affects the right of field emergence percentage (Gadewar *et al*., 2020) [3]. Seeds stored in polythene bag perform higher field emergence in compare of cotton and Jute bag. This reduction in field emergence could be due to sub-optimal condition which differ from the optimum growth condition which is use in standard germination experiment. Gadewar *et al*., (2020) [3] and Monira *et al*., (2012) [16] stated that the field emergent test of seed preserve in moisture resistant container was always notably greater than those stored in a moisture permiable container. Noory and Gowda (2017) [19] and Mostaghi-Khavaran *et al*., (2014) [17] also found that standard germination rate was always superior than field emergence percentages accompanying the present result.

Length of seedling

Seeds preserve in polythene bag exhibited higher length of seedling in contrary to cotton and Jute bag. The declined in length of seedling with time period of storage was also observed by Gadewar *et al*., (2020) [3], Kandil *et al*., (2013) [10], Basso *et al*., (2018) [1] and Meena *et al*. (2017) [15]. The causes for reduction in in length of seedling might be due to fluctuation in enzymatic activity and loss of vigor during storage of seeds. The seed stored in polythene bag perform noticeably extended length of seedling throughout storage. Gadewar *et al*.,(2020) [3] and Vanangamudi (1988) [29] reported that wetness proof container increase the viability of seed and exhibited higher shoot and root length.

Electrical conductivity

As seed weakening proceeds, the cell membranes reducing rigidity and flattered more water penetrable. It lets the cell matters to trickle into solution and rising electrical conductivity. It affords a swift signal of seed sustainability for seed lots. Aging involves progressive physiological changes over time. One of the main physiological changes is an excessive production of free radicles i.e. electrical conductivity. Higher electrical conductivity is causes damage of the structure of carbohydrates, DNA, lipid membranes and proteins.

Electrical conductivity is directly proportional to the storage period of seeds in all varieties of mungbeans. Mungbean stored in cotton and Jute bag shows significantly higher value of electrical conductivity of seed leachates in compared to those stored in polythene bag. The electrical conductivity of seed leachate was higher in the variety Virat (0.487 mSiemens/cm) as compared to Gujarat-4 and GAM-5 as 0.469 mSiemens/cm and 0.421 mSiemens/cm respectively after storage of 540 days in Jute bag. Maximum deviation observed in Virat varieties stored in Jute bag while minimum i.e. 0.341 mSiemens/cm in GAM-5 in polythene bag. The seeds preserved in Polyethylene bag showed least physiological deviation as compared to Cotton and Jute bag.

Seeds kept at relative humidity exhibits rised in respiration, heating, and mycological conquest resulting in diminished seed vigor and viability. Seeds are moisture absorptive in nature; they can fascinate and liberates dampness from and to the surrounding air. They absorb or lose moisture till the vapour pressure of seed moisture and atmospheric moisture reach equilibrium (Shelar *et al*, 2008) [25]. Deteriorative consequences happen more promptly in seeds at higher moisture content and successively, this circumstance constitute hazard to the durability of seed survival (Vashisth and Nagarajan, 2009) [30]. Subsequently physiological maturity the rate of seed quality demise depends on the degree of unfavorable conservational situations nearby the seed. The minimum the humidity, the extended seeds can be stored provided that the moisture level can be controlled all through the storage period.

Conclusion

The utmost rate of field emergence, standard germination and length of seedling were perceived in GAM-5 variety, respected by Gujarat-4 and Virat at the end of 1.5 years (540 days) of storage. Amongst the storage

containers, the seeds kept in Jute bag recorded the least values of three parameters followed by Cotton bag and Polyethylene bag. Correspondingly it is accomplished that for the storage of Mungbean seeds in Polyethylene bag is advanced as contrasted to Cotton bag and Jute bag. The electrical conductivity of seed leachate was opposite to related with the former seed quality parameters.

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