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Seed biochemical and germination behavior of stored soybean seeds as influenced by different packaging materials and storage conditions

Meena, M. K*[1] Amaregouda[1], M. Chandra Naik[1], Afsana Manik[1], Vijaykumar K[2]

[1]Department of Crop Physiology, University of Agricultural Sciences, Raichur-584 104, India
[2]Department of Seed Sciences and Technology, University of Agricultural Sciences, Dharwad-584 104, India

ABSTRACT

A lab experiment was conducted to study the Seed biochemical and germination behavior of stored soybean seeds as influenced by different packaging materials and storage conditions. Soybean seeds (JS-335) were stored in different packaging materials viz., gunny bags , high-density polythene bags and vacuum-packed bags stored at room temperature (25 ± 2º C) and cold storage (4 ± 1º C) for a period of 12 months during 2020-21. The treatments having eight combinations and consisting of different containers viz., Cloth bags, high-density polythene bags Aluminium bag and vacuum-packed bags were replicated four times in both cold and ambient storage conditions in a completely randomized design (CRD). The results of the study revealed that the seed biochemical viz., moisture content, amyalase , lipases, and proteases enzyme activities and seed germination values were higher in vacuum-packed seeds than in cloth bags, HDPE bags , Aluminium bag for soybean seeds stored under cold storage compared to room temperature throughout the storage period. Among the containers, the seeds stored in vacuum-packed bags maintained the seed biochemical and seed germination with the least deterioration compared to seeds stored in cloth bags and high-density polythene bags.

Keywords: *Seed quality, Moisture content, electrical conductivity, mineral content, vacuum packaging and cold storage.*

Introduction

Soybean [*Glycine max* (L.)] is one of the most important protein and oil seed crops throughout the world. Its oil is the largest component of the world's edible oils. Soybean seed contains 20 % oil and 40 % protein. The world production of edible oils consists of 30 % soybean. It is an ingredient of more than 50% of the world's high-protein meal. The United States of America has the largest area under soybean cultivation with the highest yield and production (Anon., 1996). Soybean is globally grown over an area of 91.40 m ha, with a production of 218.00 m tons and productivity of 2455 kg per ha (Anon., 2020).

In India soybean is grown over an area of 9.33 m ha with a production of 9.50 m tons and productivity of 1089 kg per ha which is much below the average productivity of the world (2455 kg/ha). The major soybean-producing states are Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, Uttar Pradesh, Andhra Pradesh and Gujarat. In Karnataka state also, soybean is becoming popular as an oil seed crop. The increase in area was from 0.16 lakh ha during 1991-92 to 1.92 lakh ha during 2019-20 with a production of 1.84 lakh tons and productivity of 1025 kg per ha which is less than the average productivity (1188 kg/ha) of the India (Anon., 2020). Soybean has many commercial applications due to which there has been an increasing demand for seeds to satisfy the expanding producer and consumer market. Poor germination and low seed viability are among the serious problems limiting the production of soybean. Storage of seeds at least for next sowing season is an essential concern of seed industry.

*Corresponding Author: ***Meena M. K***

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In agriculture, seed is a vehicle to deliver almost all agro-based technological innovations so that the farmers can exploit the genetic potential of new varieties. The availability, access and use of seeds of adaptable varieties are, therefore, the major determinants to attain the efficiency and productivity of other packages like irrigation, fertilizers and pesticides. This is one of the vital keys to increase crop production, enhance food security and alleviate rural poverty in the developing countries. Research on storability of seeds in India is of recent origin with the development of organized seed production and marketing. It is stipulated that 80 per cent of certified seeds produced in India require storage for one planting season and 20 per cent of seeds is carried over for subsequent sowing (Balasubramanyam *et al.,* 1983). However, when the awareness and infrastructure is developed, substantial quantity of seeds can be stored for few planting seasons as a safeguard against monsoon failure and as a precaution against production of poor quality seeds. Seed deterioration has been ascribed to physical, physiological, bio-chemical and pathological detrimental changes occurring in seeds leading to death and has been characterized as inexorable, irreversible, inevitable, and minimal at the time of physiological maturity and variable among kinds of seeds, varieties and seed lots (Delouche *et al.,* 1973). The poor storability of the seeds of soybean is accounted for its high oil content, physiological fragility and thin seed coat, which leads to rapid loss of viability and vigour in storage, which inturn results in poor establishment of the crop in the field and low productivity. Research concerning to these aspects is very meagre. Keeping above these aspects in the view and considering their importance in maintaining viability for longer period the present investigation was carried out study that Seed biochemical and germination behavior of stored soybean seeds as influenced by different packaging materials and storage conditions.

Materials and Methods

A storage experiment was carried out for a period of 12 months i.e. from October-2021 to October-2022 at the Department of Crop Physiology (Vacuum Packaging Laboratory), University of Agricultural Sciences, Raichur. In this research work, seeds of the soybean variety i.e. JS-335 collected from APMC, Raichur were dried under sun and stored under different storage conditions and containers. The temperature maintained in the cold storage was around (4 °C ± 1°C) and relative humidity was 85 to 90 percent throughout the storage period while, for ambient storage, bags were stored in the laboratory at room temperature (25 ± 2 °C). Soybean seeds were packed in 500 g vacuum-packed bags and 5 kg to gunny and high density polythene bags. After packaging of all the seeds in different containers, 50 % bags were stored properly in the iron racks without stacking so that all the bags were uniformly exposed to the particular treatment condition; while 50 % bags were stored under cold storage. The treatments having six combinations and consisting of different containers viz., gunny bags, high-density polythene bags and vacuum packed bags were replicated four times in both cold and ambient storage conditions in a completely randomized design. The observations on various seed biochemical parameters was estimated by standard methods devised by [Bernfeld,1955], [Jayaraman,1981], and [Poulle and Jones,1988], respectively at bimonthly interval upto12 months were recorded. The analysis and interpretation of the experimental data was done as suggested by Panse and Sukhatme (1967) with the level of significance used as P = 0.01.

Results and Discussion

Influence on Germination (%)

Germination (%)

It was revealed from the data (Table 1), that the containers used for storage had significant effect on soybean seed germination during all the periods of storage. At beginning of storage the seed germination was 94.37 percent.Up to 3 months of storage no significant differences between storage container and storage conditions. However, it goes on decreasing with the advancement of storage period up to 12 months. Regardless of cold storage or room temperature, vacuum-packed seeds maintained a good germination rate throughout the storage process, even up to 12 months. At the end of the storage period (12 months), the germination rate was above 91.88 percent, which is much greater than the minimum seed certification criterion.In cold storage, seeds stored in cloth bags, HDPE bags and aluminum bags could maintain minimal seed certification criteria for up to 10 to 12 months (75.06, 77.67, and 77.01 %) respectively, While seeds stored in cloth bags, HDPE bags and aluminum bags at room temperature could only be kept up to 8 to 10 months (74.11, 77.11and 76.14 %), respectively. At the end of 12 months, the vacuum-packed bags held in cold storage had the highest germination rate (91.88 %), followed by vacuum packed bags stored in ambient storage (91.65 %). Both, however, did not differ much from one another. Significantly lower germination per cent (66.53 %) was observed in cloth bags followed by HDPE bags (68.45 %) stored under ambient storage, which was significantly lower compared to all other treatments. The results show that seed storability is greater in cold storage in cloth, HDPE and aluminum bags than in room-temperature storage. The vacuum-packed seeds can be stored for up to 12 months at room temperature or in cold storage. This is mainly because of the maintenance of lower moisture content (8.52 %) during the storage period. Containers *viz.,* aluminum foil and polythene acted as moisture proof barriers. This is evidenced by lower moisture content (8.52 %) in the seeds packed in vacuum packed seeds. This lower moisture content resulted in lower respiration rate, lower metabolic activity and maintenance of higher seed vigour during storage. This is in accordance with the findingsof Saxena *et al*., (1987) in onion, cabbage, radish, cauliflower, okra, pea and Padma andReddy (2000) in onion, Rao *et al.*(2002) in sorghum, Krishnappa *et al.* (2003) in groundnut and Nataraj*et al.* (2011) in sunflower.

Table 1. Influence of packaging and storage conditions on germination (%) at different time intervals of storage in soybean seeds

Treatments	Storage period (months)						
	0	2	4	6	8	10	12
T_1 - Cloth bag (RT)	94.37	91.73	90.34	83.42	74.11	69.85	66.53
T_2 - Cloth bag (CS)	94.37	92.37	90.33	88.68	81.54	75.06	70.75
T_3 - HDPE (RT)	94.37	92.38	91.06	86.98	77.59	70.92	68.42
T_4 - HDPE (CS)	94.37	93.22	91.96	89.37	82.62	77.67	71.73
T_5 - Aluminium bag (RT)	94.37	93.36	92.23	90.45	85.55	76.14	74.18
T_6 - Aluminium bag (CS)	94.37	93.48	92.18	91.31	88.01	79.29	77.01
T_7 - Vaccume bag (RT)	94.37	94.27	94.16	93.61	92.90	91.72	91.65
T_8 - Vaccume bag (CS)	94.37	94.33	94.27	93.99	93.12	92.25	91.88
S.Em(±)	0.50	0.63	0.55	0.54	0.44	0.61	0.49
C. D. (1%)	NS	NS	1.66	1.65	1.33	1.86	1.49

HDPE - High density polythene *CS - Cold storage*
NS - Non significant *RT - Room temperature*

2. Moisture content (%)

The moisture content (%) of soybean seeds presented in Table 2 indicated significant differences between the treatments at all the stages of storage up to 12 months except at the initial stages (i.e., 0 months). In general, these was no change in the moisture content of vacuum packed as well as aluminium-packed seeds during storage for 18 months however there was a slight decline in the moisture content with progress in the storage time. More fluctuations were seen in the moisture content of soybean seeds stored in cloth bags, irrespective of storage at room temperature or cold storage throughout the storage period.

Among the different packaging materials used in the study, the seeds packed in vacuum packed bag recorded significantly lower moisture content (9.40 %) which was on par with aluminium bag(11.9 %) and highest is seeds were packed in cloth bag (15.9%). But better storability of seeds in vacuum packed bag compared to aluminum bag, cloth bag and HDPE bags could be ascribed to the fact that the seeds stored in vacuum polythene bag are moisture impervious and had got very less fluctuations in their moisture content which is very important factor in maintaining the viability of seeds during storage. Lower moisture content of seeds results in lower respiration rate, lower metabolic activity and maintenance of higher seed vigour during storage.

This is in accordance with the findings of Khalequzzaman*et al*. (2012) in French bean. Similar results are also reported by Davidson *et al.* (1982) in shelled peanuts, Malekar *et al.* (2008) in wheat, Rajendraprasad *et al.* (1998) in groundnut kernels, Dadlani and Veena (2006), Gurmitsingh and Hari Singh, (1992), Remya (2007) in chili powder, Roshany (2007) in whole chilli and Monira *et al.* (2012) in soybean storability for longer period.

Table 2. Influence of packaging and storage conditions on moisture content (%) at different time intervals of storage in soybean seeds

Treatments	Storage period (months)						
	0	2	4	6	8	10	12
T_1 - Cloth bag (RT)	9.30	14.6	14.9	15.1	15.3	15.7	15.9
T_2 - Cloth bag (CS)	9.30	13.2	13.8	14.6	14.7	14.9	15.0
T_3 - HDPE (RT)	9.30	11.4	13.7	14.4	14.6	14.7	14.9
T_4 - HDPE (CS)	9.30	11.5	12.8	13.1	13.4	13.6	13.8
T_5 - Aluminium bag (RT)	9.30	10.6	11.9	12.3	12.6	12.8	12.9
T_6 - Aluminium bag (CS)	9.30	10.8	10.9	11.1	11.5	11.8	11.9
T_7 - Vaccume bag (RT)	9.30	9.36	9.33	9.31	9.32	9.33	9.35
T_8 - Vaccume bag (CS)	9.30	9.41	9.37	9.34	9.35	9.38	9.40
S.Em(±)	0.20	0.16	0.13	0.07	0.09	0.12	0.23
C. D. (1%)	NS	0.45	0.36	0.22	0.25	0.38	0.65

HDPE - High density polythene *CS - Cold storage*
NS - Non significant *RT - Room temperature*

Influence on hydrolytic enzymes (α-amylase, Lipase and Protease)

The data on hydrolytic enzymes activity of soybean seeds presented in (Table 3, 4 and 5) indicated significant differences among the treatments hydrolytic enzymes activity was considerably declined in cloth bag seeds and high density polythene bagsin 10 and 12 months of storage. While, the hydrolytic enzymes activity in vacuum packed seeds were found to be stable and slow decline was observed with progress in storage period.

At the end of storage period, significantly higher values of hydrolytic enzymes activity (α-amylase (4.21mg maltose released/min), lipase (16.32 milliequivalent free fatty acid /min/g) and protease(24.82 mg amino acid released /min/ml)) were recorded in vacuum packed seeds stored under cold storageand it was on par with those stored under room temperature (α-amylase (4.16 mg maltose released/min), lipase(16.27 milliequivalent free fatty acid /min/g) and protease(24.77 mg amino acid released /min/ml)) and followed by aluminium packed bag α-amylase (4.06 and 4.12mg maltose released/min), lipase(16.21 and 16.23 milliequivalent free fatty acid /min/g) and protease(24.51 and 24.58 mg amino acid released /min/ml)). Significantly lower values of hydrolytic enzyme activity (α-amylase (3.89mg maltose released/min), lipase(16.24 milliequivalent free fatty acid /min/g) and protease(24.27 mg amino acid released /min/ml)) was observed cloth bag seeds under room temperature and it was also onpar with high density polythene bags (α-amylase (3.91 mg maltose released/min), lipase(16.13 milli equivalent free fatty acid /min/g) and protease(24.27 mg amino acid released /min/ml)).It is clear from the results that the seeds stored in cloth bag and high density polythene bag seeds were on par with each other when stored under either cold storage or room temperature followed by aluminium packed bag. The vacuum packed seeds could maintain higher Hydrolytic enzymes throughout the storage period.

In the present investigation, hydrolytic enzymes activity decreased with an increased storage period. Decreased α - amylase activity with an increase in storage period may bedue to reduction of free sugars and amino acids. Similar observations were alsoreported in wheat seeds (Chauhan *et al.,* 2011) and in naturally aged gram, chickpeaand wheat seeds (Agarwal and Kharlukhi, 1987), in pea (Davis, 1977) and in mustard(Sana *et al*., 2009).

Lipase and protease activity were found to be slightly less in vacuum packed bags compared to aluminium, HDPE and cloth bags. There was higher lipase activity in cloth bags followed by HDPE and aluminium bags. There was increase in the activity of these enzymes with advancement in storage period. Our results are in good agreement with results of Dhaliwal *et al.* (1991) and Chaitanya *et al.* (2000), Mohamed (2011) in wheat. Increase inprotease activity during storage may be due to decline in protein content (Chaitanya *etal.,* 2000). Similar results were observed by Chauhan *et al.* (2011) in wheat.

Table 3. Influence of packaging and storage conditions on α-amylase activity (mg maltose released/min) in soybean seeds

Treatments	Storage period (months)						
	0	2	4	6	8	10	12
T_1 - Cloth bag (RT)	**4.42**	**4.17**	**4.15**	**4.11**	**4.05**	**4.01**	**3.89**
T_2 - Cloth bag (CS)	**4.42**	**4.20**	**4.17**	**4.15**	**4.10**	**4.06**	**4.02**
T_3 - HDPE (RT)	**4.42**	**4.27**	**4.20**	**4.16**	**4.13**	**4.05**	**3.91**
T_4 - HDPE (CS)	**4.42**	**4.29**	**4.22**	**4.19**	**4.15**	**4.10**	**4.01**
T_5 - Aluminium bag (RT)	**4.42**	**4.30**	**4.27**	**4.21**	**4.16**	**4.11**	**4.04**
T_6 - Aluminium bag (CS)	**4.42**	**4.33**	**4.29**	**4.24**	**4.20**	**4.17**	**4.12**
T_7 - Vaccume bag (RT)	**4.42**	**4.39**	**4.31**	**4.28**	**4.21**	**4.19**	**4.16**
T_8 - Vaccume bag (CS)	**4.42**	**4.40**	**4.36**	**4.33**	**4.30**	**4.26**	**4.21**
S.Em(±)	**0.08**	**0.11**	**0.11**	**0.13**	**0.12**	**0.14**	**0.02**
C. D. (1%)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**0.50**

HDPE - High density polythene *CS - Cold storage*
NS - Non significant *RT - Room temperature*

Table 4. Influence of packaging and storage conditions on lipase activity (milliequivalent free fatty acid /min/g)at different time intervals of storage in soybean seeds

Treatments	Storage period (months)						
	0	2	4	6	8	10	12
T_1 - Cloth bag (RT)	16.51	16.28	16.21	16.18	16.16	16.12	16.04
T_2 - Cloth bag (CS)	16.51	16.31	16.29	16.22	16.20	16.18	16.10
T_3 - HDPE (RT)	16.51	16.34	16.27	16.21	16.18	16.16	16.13
T_4 - HDPE (CS)	16.51	16.38	16.31	16.28	16.22	16.20	16.18
T_5 - Aluminium bag (RT)	16.51	16.39	16.33	16.29	16.26	16.25	16.21
T_6 - Aluminium bag (CS)	16.51	16.41	16.38	16.32	16.30	16.28	16.23
T_7 - Vaccume bag (RT)	16.51	16.46	16.42	16.39	16.37	16.31	16.27
T_8 - Vaccume bag (CS)	16.51	16.49	16.45	16.41	16.40	16.37	16.32
S.Em(±)	0.07	0.06	0.08	0.07	0.09	0.11	0.03
C. D. (1%)	NS	NS	NS	NS	NS	NS	0.09

HDPE - High density polythene *CS - Cold storage*
NS - Non significant *RT - Room temperature*

Table 5. Influence of packaging and storage conditions on protease activity (mg amino acid released /min/ml) in soybean seeds

Treatments	Storage period (months)						
	0	2	4	6	8	10	12
T_1 - Cloth bag (RT)	25.03	24.57	24.51	24.40	24.38	24.32	24.27
T_2 - Cloth bag (CS)	25.03	24.61	24.55	24.43	24.40	24.36	24.31
T_3 - HDPE (RT)	25.03	24.64	24.65	24.61	24.56	24.46	24.40
T_4 - HDPE (CS)	25.03	24.77	24.70	24.66	24.61	24.57	24.48
T_5 - Aluminium bag (RT)	25.03	24.91	24.74	24.72	24.63	24.60	24.51
T_6 - Aluminium bag (CS)	25.03	24.93	24.83	24.81	24.79	24.71	24.58
T_7 - Vaccume bag (RT)	25.03	25.01	24.96	24.93	24.91	24.85	24.77
T_8 - Vaccume bag (CS)	25.03	25.02	25.00	24.98	24.95	24.90	24.82
S.Em(±)	0.08	0.09	0.07	0.06	0.09	0.08	0.05
C. D. (1%)	NS	NS	NS	NS	NS	NS	0.15

HDPE - High-density polythene *CS - Cold storage*
NS - Non-significant, *RT - Room temperature*

Conclusion

The soybean grains generally deteriorated with storage and deterioration was particularly strong for grains stored in cloth bags and HDPE Bags. High temperature, high relative humidity, and moisture in the storage environment are the principal factors involved in the deterioration of soybean seed quality during storage. Loss of germination capacity was the final manifestation of seed deterioration. When seed moisture content was increasing the rate of germination percentage was decreased. The deterioration rate was also the highest in seeds of cloth bag and HDPE. Seed biochemical parameters deterioration is an inexorable and irreversible process. The quality and viability of soybean seeds are subjected to variations during storage conditions and it has been found that the life span of seeds depends on the moisture content of the seeds, relative humidity, temperature, light and oxygen content under which the seeds are stored. It has been found in the present study that it is possible to extend the shelf life of soybean seeds up to 12 months without deterioration in seed quality parameters *viz.,* Seed germination and moisture content, , while seed biochemical parameters such as hydrolytic enzymes (α-amylase, Lipase and Protease) by storing them under vacuum packaging. Since seed is an important input in agriculture which determines not only the production but also the productivity, it is essential to maintain the seed quality as well as seed vigor.

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Authors' Contribution

All authors had direct participation in the execution of the experiment, writing the paper script and adequate review of this research article.

Practical Application: Farmers, and traders normally pack the seeds of various crops in either polythene bags, aluminum bags or cloth bags before being used for propagation in the next season. Many seeds lose viability during storage due to their sensitivity to oxidation and variation in moisture content during the storage period. It has been found that storing the crop seeds under vacuum-packed bags enhances the shelf life while maintaining the quality. Since the seed is an essential input in agriculture, it is of utmost necessity to maintain the viability and Vigour of the seed. Many times, it so happens that good quality seed is not available to the farmers in time due to various reasons, the average productivity of most of the crop plants has gone down considerably in the last decade and one of the reasons for such decline is the poor quality of seeds being used by the farmers. Vacuum packaging has been found to be superior technology in preserving the seed quality i.e. Germination and biochemical aspects of different field crops.

Deaclration of conflict Interest: The authors decaler(s) that there is no conflict interest of with respect this research paper, authorship and OR / publication of this research artcle.

Future scope of study: The investigation on influence of vacuum packaging on seed physiology and quality of soybean has thrown light on several aspects that need to be worked at in detail in the future. To enumerate the more probable ones; The storage studies can further be extended beyond 18 months to know the influence of vacuum packaging and storage conditions

on field crops *viz*., onion, cotton, soybean and groundnut under both ambient and cold storage and also similar storage studies may be conducted on other field crops.There is a scope for evaluating some more storage containers, which are domestically available in rural areas such as earthen pots, polythene lined gunny bags and also similar storage studies may be conducted on other agricultural as well as on horticultural crops. Vacuum packaging technology has huge potential and therefore it needs to be explored for different dehydrated products, food grains and other dry spices and food products, so that post harvest losses can be minimized to a greater extent and food hygiene can be maintained.Further studies should also focus on the influence of packaging and storage conditions on macro and micro- elements under both ambient and cold storage in other agricultural crops. Studies can also be directed towards the use of oxygen scavenger films while packing and its influence on long term storage in different food commodities.There is need to study the combined effect of vacuum packaging and use of oxygen scavengers. Measures to eliminate oxygen from the pack may be taken up. The use of active packaging methods like use of oxygen absorbents may be resorted to. The study has revealed that to oxidation occurred in vacuum packed bags to a similar extent. It is therefore imperative that work needs to be taken upto to quantify the amount of oxygen as well as carbon dioxide in the package, so that appropriate measures can be taken to avoid oxidation.

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