

## Effect of storage condition and its duration on seed quality of chickpea (*Cicer arietinum* L.)

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### ABSTRACT

The present study was conducted to evaluate the effect of types of storage and duration on seed quality of extra large seeded kabuli chickpea (Kripa). The seeds were stored for 20 months in four different types of seed storage *i.e.* gunny bag, silo, polypropylene sacks, hermetic polyethylene bag. The study showed that types of storage had significant effect on chickpea seed quality. The moisture content, electrical conductivity, proportion of abnormal and dead seeds gradually increased along with storage period, while the proportion of normal seedlings (germination%) and seed vigour decreased gradually. Irrespective of storage condition seeds were able to maintain the germination percentage, up to eight months after which it declined sharply. After 20 months seeds stored in jute gunny bag, silo, conventional propylene sack and hermetic polyethylene bag recorded 6, 36, 20 and 48% germination, respectively. The reduction in germination upon storage could be attributed to change or alteration in the seed storage protein as a number of peptides with molecular weight ranging between 14 to 45 kDa were lost after 16 months of storage as compared to fresh seeds.

**Keywords** Chickpea, Electrical conductivity, Germination, Proteins, Types of storage

Chickpea (*Cicer arietinum* L.) is an important pulse crop which is grown in over 50 countries throughout the world and consumed in over 120 countries. India contributes about 68% share in global chickpea production and is a leading chickpea producing country (FAO 2014). Chickpea is of two types, desi and kabuli. Desi types are generally small seeded with thick and coloured seed coat, while kabuli types are usually large seeded with thin and non-pigmented seed coat. Phenolic compounds (tannin) imparts seed coat colour (Calder and Blair 2009), which are considered to also have antifungal, antimicrobial properties at the same time is also reported to protect the seed from insect pests and precocious germination. The seed coat and chemical composition of seed coat are physical and chemical defensive mechanisms, respectively for seed to survive through various biotic and abiotic stresses. Therefore, the desi type with thick seed coat coupled with high tannin content are reported to have better seed vigour than kabuli type (Lamichaney *et al.* 2016, 2017a, 2017b). Seed vigour differences in pigmented and un-pigmented seeds are reported in other legumes as well (Kantar *et al.* 1996; Peksen *et al.* 2004; Saedi, 2008). High seed vigour implies to greater ability of seeds to survive in harsh environmental condition, to perform better in wide environments and also to have better longevity (Finch Savage and Bassel 2016).

Differences in longevity due to seed coat colour has been reported in soybean (Sooganna *et al.* 2016) whereby, dark coloured soybean are considered a better storer than yellow seeded soybean. Desi and kabuli chickpea behaves similar to black and yellow coloured soybean, whereby kabuli chickpea, especially extra large seeded, loses viability rapidly if stored under poor condition. Infestation by bruchid during storage is the main reason that affects quality of the seed, which can be overcome by storing the seed under moisture proof conditions and other modern seed storage types. In India, about 60-70% of food grains produced are stored in traditional storage structures like bamboo baskets, mud structures and gunny bags (Kanwar and Sharma 2003; Channal *et al.* 2004), which leads to reduction in the quality of the produce during storage. Considering the importance of storage condition on maintaining the quality of seeds and availability of very few storage alternatives in small farms, this work evaluated four types of storage (jute gunny bags, hermetic polyethylene bag, silo and conventional propylene sacks) for 20 months, aiming to minimize the loss of seed quality.

### MATERIALS AND METHODS

The seed of kabuli chickpea variety Kripa was used in this experiment. After harvesting the crop in 2015-16 *rabi* season, the moisture content of the seed was adjusted by sun drying to about 10-11%. The seeds were kept in jute gunny bags, hermetic polyethylene bag, silo and conventional propylene sacks at normal laboratory condition and were stored for 20 months. At a periodic interval of 4 months *i.e.* [0 (before storing), 4, 8, 12, 16 and 20 months after storing] seeds were sampled and tested for different seed quality parameters. The moisture content (%) was determined using the standard high temperature oven method ( $130 \pm 2^\circ\text{C}$ ) for 1 h, according to ISTA (2015).

For electrical conductivity determination, fifty seeds were weighed and soaked in 250 ml of distilled water and kept inside dark incubator maintained at  $20^\circ\text{C}$  for 24 hrs, after which, electrical conductivity was measured using electrical conductivity meter and expressed as  $\text{Scm}^{-1} \text{g}^{-1}$  of seed.

The protocol available at ISTA rules (ISTA 2015) was followed to conduct germination test. Briefly, one hundred seeds were placed in pre-moistened germination towels, which was rolled and covered with butter paper to avoid moisture loss. The germination towels were then kept for germination in a dark incubator maintained at  $20^\circ\text{C}$ . After 7 days of incubation, seedlings were categorized as normal seedlings, abnormal seedlings, freshly un-germinated seeds

(FUS) and dead seeds. Proportion of normal seedlings only was considered for determining germination percentage. Ten random and similar looking seedlings were selected; their root and shoot lengths were measured and dried for 24 hrs in an oven maintained at 80 °C to determine the seedling dry weight. Vigour indices *i.e.* VI-I and VI-II were calculated according to the formula of Abdul Baki and Anderson (1973).

Vigour index I = germination (%) X Seedling length (cm)

Vigour index II = germination (%) X Seedling dry weight (g)

For SDS PAGE analysis, total protein was extracted from seed (200 mg) by grinding it in chilled extraction buffer (0.1M Tris (pH7.5), 1% Triton X-100). Lowry's method (Lowry, 1951) was used to quantify the amount of total protein. 20 g of protein mixed with 4X loading buffer was resolved over 12% gel and electrophoresis was carried out at 80 V for fractionation of proteins. After the electrophoresis was over, the gel was kept for staining overnight in a staining solution comprising of 10% glacial acetic acid, 50% methanol and 0.1% Coomassie brilliant blue R-250. After washing several times in destaining solution comprising of 10% glacial acetic acid and 40% methanol, the gel was scanned, and standard protein markers was used to predict the molecular mass of the expressed peptides.

## RESULTS AND DISCUSSION

The seed moisture content of chickpea was significantly influenced by the interaction of storage duration and storage condition, and the variability can be observed in Fig.1. The moisture content of seeds stored in jute gunny bag, silo and conventional propylene sacks reduced upto 4 months of storage, thereafter a gradual increase in moisture content can be observed with time. However, in hermetic polyethylene bag, the moisture content reduced up to 16 months, and presented the lowest values. Seeds stored in gunny bag had the highest moisture content, reaching over 11%, while hermetic polyethylene bag recorded lowest (9.8%) after 20 months of storage. Higher moisture content in seeds stored in gunny bag then other airtight containers may be due to free

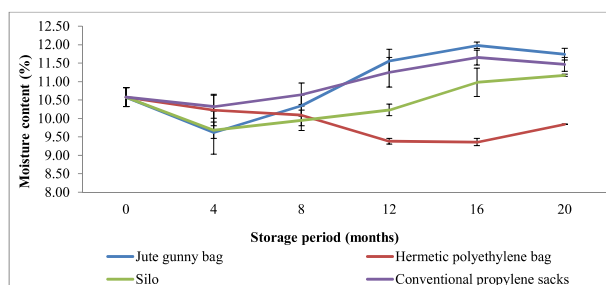


Fig 1. Mean moisture content percentage of chickpea seeds during 20 months of storage in different storage condition

Table 1. Mean percentages of normal seedling, abnormal seedling and dead seed for Kripa seeds stored for 20 months in different storage conditions

Time (months)	Types of storage				Mean
	Gunny bag	Hermetic bag	Silo	Propylene sack	
<b>Normal seedling (%)</b>					
0	80	80	80	80	80.0
4	78	80	78	74	77.5
8	74	80	86	72	78.0
12	66	68	66	62	65.5
16	24	46	38	40	37.0
20	6	48	36	20	27.5
Mean	54.7	67.0	64.0	58.0	
<b>LSD (5%) Storage duration: 3.49, Type of storage: 2.85, Interaction: 6.99</b>					
<b>Abnormal seedling (%)</b>					
0	19	19	19	19	19.0
4	18	20	12	20	17.5
8	20	17	4	16	14.3
12	24	22	25	26	24.3
16	36	40	44	38	39.5
20	17	25	29	36	26.8
Mean	22.3	23.8	22.2	25.9	
<b>LSD (5%) Storage duration: 3.84, Type of storage: NA, Interaction: 7.68</b>					
<b>Dead seed (%)</b>					
0	1	1	1	1	1.0
4	4	0	10	6	5.0
8	6	3	10	12	7.8
12	10	10	9	12	10.3
16	40	14	18	22	23.5
20	77	27	35	44	45.8
Mean	23.0	9.2	13.8	16.2	
<b>LSD (5%) Storage duration: 3.85, Type of storage: 3.14, Interaction: 7.70</b>					

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LSD (5%) Storage duration: 3.85, Type of storage: 3.14, Interaction: 7.70

exchange of gases and/or water vapour between the seed and the environment(De Domenico, 2015).

The process of seed ageing, though, starts immediately after its development and maturation within plants (Anderson and Baker, 1983; McDonald, 2004), chickpea seeds usually, have above 90% fresh seed germination following processing. The rate by which the seed deteriorates increases with passage of time during prolong storage eventually leads to fall in germination percent below the prescribed 85% germination standard. Better condition during seed storage can reduce or slowdown the rate of deterioration, which otherwise is an inexorable and irreversible process. In the present investigation, physiological changes in terms of germination and related parameters were studied by storing seeds in different storage containers or materials. The germination percent (normal seedlings) significantly decreased by the interaction of storage duration and storage condition, and the variability can be observed in Table 1 and Fig. 2. The germination of Kripa seeds was 80% before storage. The reduction in germination after 4 months of storage was significant only for propylene sacks. The reduction in germination was much pronounced after 16 months of storage. After 20 months of storage, jute gunny bag, silo and conventional propylene sack and hermetic polyethylene bag recorded 48, 36, 20 and 6% germination respectively. Likewise, with storage duration the proportion of abnormal and dead seeds increased significantly. The

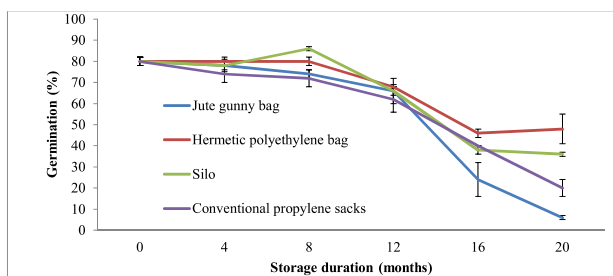


Fig 2. Mean germination percentage of chickpea seeds during 20 months of storage in different storage condition



Fig 3. Physical purity of chickpea seeds stored at gunny bag and hermetic polyethylene bag after 20 months of storage



Fig 4. Changes in germination of chickpea seed with storage

proportion of abnormal seedlings was less after 20 months of storage in all the storage types as compared to 16 months which was due to increase in the proportion of dead seeds (Table 1). Bruchid infestation was much pronounced in gunny bag as compared to other storage types, which lead to decline in seed germination (Fig. 3). Seedling length and seedling dry weight were significantly affected by storage duration and not by storage type while vigour index I and II were affected by both storage duration and storage type (Table 2). Seedling length, seedling dry weight, vigour index I and II irrespective of storage type increased after 4 months of storage, thereafter, it decreased significantly from 24.1 cm, 1.19 g, 1866.78 and 92.43 (4 months) to 7.1 cm, 0.28 g, 195.50 and 7.78 (20 months) respectively (Table 2; Fig. 4).

Electrical conductivity (EC) is a standard vigour test for kabuli chickpea (ISTA, 2015), higher the value of EC lower is the seed vigour and vice versa. The electrical conductivity of seed leachate increased by the interaction of storage duration and storage condition (Fig. 5). After 16 months of storage the seeds stored in jute bag recorded maximum conductivity of seed leachate and minimum was observed for the seeds stored in hermetic bag. Cell membrane integrity is maintained by the proteins responsible for maintaining cell structure. The low ability of seeds to germinate after storage may be due to ageing enabled inhibition in repair mechanism of cellular membrane. The EC of seeds stored in hermetic polythene bag recorded lowest EC after 20 months of storage and maximum EC was observed in seeds of gunny bag. Thus, seeds stored at gunny bag are of low vigour as compared to hermetic

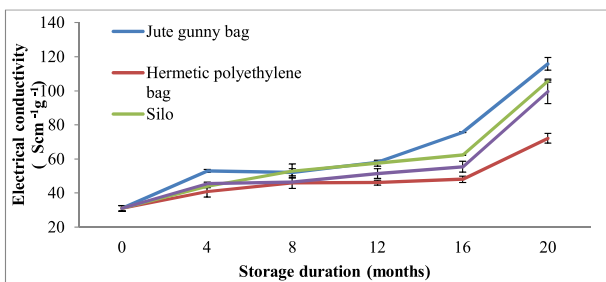


Fig 5. Mean electrical conductivity of chickpea seeds during 20 months of storage in different storage condition

**Table 2. Seedling length, seedling dry weight and vigour indices of Kripa seeds stored for 20 months in different storage conditions**

Time (months)	Types of storage				Mean
	Gunny bag	Hermetic bag	Silo	Propylene sack	
<b>Seedling length (cm)</b>					
0	21.3	21.3	21.3	21.3	21.3
4	24.3	25.2	23.2	23.7	24.1
8	13.9	15.8	16.7	13.2	14.9
12	13.6	14.8	13.2	8.8	12.6
16	7.4	9.6	9.0	8.2	8.5
20	6.4	6.7	7.5	7.7	7.1
Mean	14.5	15.6	15.2	13.8	
<b>LSD (5%) Storage duration: 4.19, Type of storage: NS, Interaction: NS</b>					
<b>Seedling dry weight (g)</b>					
0	0.84	0.84	0.84	0.84	0.84
4	1.17	1.25	1.23	1.11	1.19
8	0.73	0.62	0.72	0.63	0.68
12	0.38	0.47	0.48	0.38	0.43
16	0.32	0.37	0.40	0.32	0.35
20	0.26	0.28	0.28	0.30	0.28
Mean	0.62	0.64	0.66	0.60	
<b>LSD (5%) Storage duration: 0.062, Type of storage: NS, Interaction: NS</b>					
<b>Vigour index I</b>					
0	1704.00	1704.00	1704.00	1704.00	1704.00
4	1895.40	2012.00	1809.60	1750.10	1866.78
8	1026.40	1267.20	1431.90	951.80	1169.33
12	900.24	1006.40	873.80	545.60	831.51
16	177.60	443.40	343.50	329.20	323.43
20	38.60	321.60	268.20	153.60	195.50
Mean	957.04	1125.77	1071.83	905.72	
<b>LSD (5%) Storage duration: 134.04, Type of storage: 109.44, Interaction: NS</b>					
<b>Vigour index II</b>					
0	67.2	67.2	67.2	67.2	67.20
4	91.3	100.4	95.9	82.1	92.43
8	54.0	49.6	61.9	45.4	52.73
12	25.1	31.9	32.0	23.6	28.15
16	7.6	17.1	15.14	12.8	13.16
20	1.6	13.4	10.1	6.0	7.78
Mean	41.13	46.60	47.04	39.52	
<b>LSD (5%) Storage duration: 6.46, Type of storage: 5.27, Interaction: NS</b>					

LSD (5%) Storage duration: 4.19, Type of storage: NS, Interaction: NS

LSD (5%) Storage duration: 0.062, Type of storage: NS, Interaction: NS

LSD (5%) Storage duration: 134.04, Type of storage: 109.44, Interaction: NS

LSD (5%) Storage duration: 6.46, Type of storage: 5.27, Interaction: NS

polythene bag. Our findings are in accordance with the findings of Sacandeet *et al.* (2001), Khan *et al.* (2004), Ratajczak and Pukacka (2005), Eliud *et al.* (2010) as they also reported higher electrical conductivity of seed leachate upon storage.

SDS PAGE profiling of fresh seed (before storage) and 16 months old seed indicated significant differences in protein pattern upon ageing. Most of the changes were observed in proteins of molecular weight ranging between 14 to 45 kDa. Notably, peptide of about 45, 28, 30, 20, 16 and 14 kDa degraded as such peptides were absent in 16 months old seed (Fig 6). In general, the band intensity as well as the number of proteins were higher in fresh seed than aged seed. Most of the seed storage proteins get accumulated during early to mid seed maturation stage which acts as a

source of energy supply during seed germination and subsequent growth and development of seedling (Li *et al.* 2007). Post-translational alterations and modifications, ageing induced protein degradation may be associated with loss of protein bands (Rajjouet *et al.* 2008). The results are in accordance with that of Kapoor *et al.* (2010), Machado *et al.* (2001), Vasudevan *et al.* (2012).

In general, the seed germination percent and vigour (as revealed by EC, vigour indices and seedling dry weight) reduced with storage period. The reduction in germination and vigour may be attributed to change in the function and properties of seed storage protein as a number of peptides were lost in old seed as compared to fresh seeds. Comparing the storage types, seeds stored at hermetic polyethylene bag recorded much higher germination even after 20 months

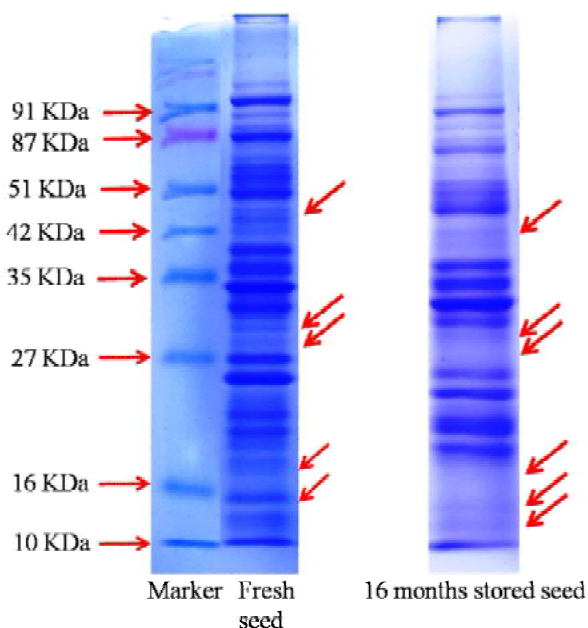


Fig 6. Effect of seed storage period on protein expression

of storage; therefore, it may be recommended for safe storage of kabuli seeds.

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