



Comparison of seed viability in vegetable and grain type soybean (*Glycine max* L.) Merrill] as influenced by packing materials and seed treatments during storage

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Abstract

Vegetable soybean is one of the largest protein rich grain crop in the world. Maintaining high seed viability in soybean during the storage period is very important for seed industry. Therefore, the present study was conducted to evaluate the influence of packing materials and seed treatment on seed viability of vegetable soybean in comparison with grain type during the storage. To achieve this objective, seeds of two soybean cultivars *viz.*, GC-00209-4-1-1 (KARUNE, vegetable type) and RKS -18 (grain type) were treated with Thiram (3g kg⁻¹), *Trichoderma viridae* (7.5g kg⁻¹) and untreated seed as control and then they were stored in cloth bag, super grain bag and polypouches for nine months under ambient conditions of Bangalore, India. The results indicated that seeds treated with Thiram and packed in polypouches and super grain bag recorded significantly higher germination (82.0%), field emergence (74.4%), seedling vigour index (2018), total dehydrogenase activity (2.4) and lower electrical conductivity of seed leachate (0.95mS/ppt) as compared to seeds treated with *Trichoderma viridae* and untreated seeds (control). However, among the cultivars, RKS-18 (grain type) stored better and recorded higher germination (84.3%), field emergence (76.0%), seedling vigour index (2279), TDH (2.6) and low EC of seed leachate (0.70 mS/ppt) at the end of nine months storage. It is concluded that seed treatment of soybean with Thiram (3 g kg⁻¹) and using either polypouches or super grain bag could be advocated to maintain seed viability of both cultivars during the storage.

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Introduction

Soybean has gained importance in enriching the human diet and is more nutritious than vegetable green peas. In addition to domestic consumption, soybean also has export potential. In spite of its food value and industrial use, it has got many impediments. Vegetable type soybean is popular in Japan, Korea, China and Taiwan. Its importance in human nutrition is increasingly recognized in many countries; hence its consumption is increasing very rapidly. The green-shelled soybeans can be cooked to make tasty and nutritious meal or snacks. The seeds of vegetable soybean are generally larger, sweeter and tender than grain type soybean. They also contain a lower percentage of gas-producing starches. Such green seeds are commonly used in most countries. Soybean suffers from poor seed longevity and it is classified as moderate storer under tropical conditions. Seeds of soybean showed varietal differences for seed viability and vigour during storage (Gurmit Singh *et al.*, 1994, Kumar *et al.*, 2007 and Kuchlan, 2010). The seed treatment chemicals combat both pathogenic and non-pathogenic flora of seed and thereby improve the germination and enhance storage life (Ramesh, 2002, Muthuraj *et al.*, 2002, Gaythri *et al.*, 2008 and Rathod *et al.*, 2010). Soybean seeds are highly susceptible to mechanical damage and classified as a very poor storer. The seed deterioration starts right from the field levels immediately after maturation; hence, it is required to store the seeds safely to maintain the viability and vigour intact. According to an estimate, about 30-40 per cent of soybean seed lots are being rejected annually for not meeting the prescribed germination standards (Singh, 1987). Seed coat in soybean is highly fragile in nature and leads to deterioration of quality. Seed longevity is greatly influenced by storage conditions mainly the relative humidity and temperature and lowering of these two factors significantly increases storage life of the seeds (Toole, 1947). If the seeds are stored for longer time, leakage of cellular membrane increases and the enhanced leakage of electrolytes and other solutes are quite often associated with fall in germination of seeds and

therefore measurement of seed leachates is considered as one of the good indicators of seed quality in soybean.

The loss of viability impairs the biological value of seed, which is a special concern to breeders, seed producers and farmers for successful seed production and distribution program. Seed deterioration is an irreversible, inexorable and inevitable process (Delouche 1973), but the rate of seed deterioration could be slowed down either by storing the seeds under controlled conditions or along with seed treatment chemicals. Loss of seed viability and vigour in storage leads to weak stand establishment of the crop and low productivity. Seed quality, germination, vigor and viability are highly influenced by environmental factors in field and storage (Jyoti and Malik 2013). Several biotic and abiotic factors, influence soybean seed longevity in storage and their subsequent field performance (Roberts, 1972; Delouche, 1973). Further, not much work has been done on the assessment of seed viability and storage potential of vegetable type of soybean during the storage.

Keeping these factors in view, the present investigation was carried out to determine the influence of packing material and seed treatment on seed viability of vegetable type soybean in comparison with grain type with emphasis on vegetable type soybean.

Materials and methods

Seed material and preparation of seed for storage

Freshly harvested seeds of two soybean cultivars *viz.*, GC-00209-4-1-1 (KARUNE, vegetable type) and RKS -18 (grain type) were obtained from AICRP on Soybean, University of Agricultural Sciences GKVK, Bangalore, dried to safe moisture (<9%), graded manually to uniform size, and then used for the study.

The initial quality attributes *viz.*, germination percentage, field emergence percentage, seedling vigour indices, electrical conductivity of seed leachate and total dehydrogenase activity were recorded.

After recording the initial observations, seeds were treated with Thiram and *Trichoderma viridae* (Bioderma) and then packed in three packing material viz., cloth bag, super grain bag and polypouches as per the treatment details. These packed seeds were stored under ambient conditions at the postgraduate laboratory of Department of Seed Science and Technology, University of Agricultural Sciences, Bangalore for a period of nine months under ambient conditions. The details of the treatments are given in table 1.

Seed treatment methodology

Soon after recording the initial observations on various seed quality attributes, the seed material was subjected to manual treatment as indicated above. While treating with powder formulation of *Trichoderma viridae* (7.5 g kg⁻¹), a chemical sticker solution called CMC (Carboxyl Methyl Cellulose) of 3 per cent concentration has been used as binding material for uniform coating. Further, the seeds of both cultivars were treated with powder formulation of Thiram 75% WP. (3g per kg⁻¹) of seeds using polykot (5ml in 5ml of water) as binding agent. Treated seeds were shade dried and then packed in three packing materials viz., cloth bag, super grain bag and polypouches. The packed seeds were stored for a period of nine months under ambient conditions of Bangalore where the temperature and the relative humidity was ranging from 17.18 to 28.2°C and 59 to 76.50 per cent, respectively. Seed samples were drawn at bimonthly intervals (up to six months) and subsequently at monthly intervals (up to nine months) and evaluated for the following various seed quality attributes.

Standard germination test

Standard germination test was conducted following 'between paper method as per ISTA (2010). One hundred seeds of four replicates were placed equidistantly between moist germination papers, the rolled towels were incubated in walk-in germination chamber maintained at 25±1 °C and 90 per cent relative humidity.

The germination counts on 5th and 8th day were taken based on normal seedlings, and total germination percentage was calculated based on normal seedlings on 8th day of the test and expressed in percentage.

Field emergence (%)

25 seeds of four replicates were sown in the field on a well prepared raised seedbed and adequate moisture was maintained by watering regularly. Field emergence was taken on 15th day of sowing and the germination percentage was calculated considering the emergence of normal seedlings.

Seedling vigour index (SVI)

The seedling vigour index was calculated by adopting the method suggested by Abdul-Baki and Anderson (1973) and expressed in whole number.

Seedling Vigour index = Germination (%) X Mean seedling length in (cm)

Electrical conductivity (EC) of seed leachate (mS/ppt)

Ten seeds were taken randomly from each treatment with three replicates and they were washed with acetone to remove chemical residues from seed surface and air-dried overnight. Then they were soaked in 25 ml of double distilled water for 24 hours at 25±1°C. After incubation, the conductivity of seed leachate was measured using a digital conductivity meter (Systronics conductivity TDS meter 308Uc) and the EC was expressed in mS/ppt.

Total dehydrogenase activity (TDH)

The total dehydrogenase activity was determined by method described by Perl *et al.* (1978) with slight modifications. The seeds selected for estimating the EC value were used to determine the total dehydrogenase activity. Seed coat of these imbibed seeds were carefully removed and then soaked with 0.5 per cent tetrazolium solution at 30±1 °C for a period of 6-8 hours. Then they were washed thoroughly with distilled water. The red colour (Formazan) was eluted from the stained embryos by soaking in 5ml of 2- methoxyethanol (methyl cellosolve) for 24 hours in an airtight screw capped vials.

The extract was decanted and the colour intensity was measured with using Spectrophotometer (Model-Systronics UV-VIS spectrophotometer117) at 480nm. The dehydrogenase activity was expressed in terms of Absorbance at 480 nm.

Statistical analyses

The data were analyzed by using Completely Randomized Design with factorial concept and suitable ANOVA.

The critical differences between the treatments were worked out at five per cent significance (Snedecor and Cochran, 1967).

Results and discussion

Seed germination

The germination percentage of soybean seeds treated with Thiram and *Trichoderma viridae* decreased with the increase in storage period in both the cultivars but superior over untreated control (Table2). However, Thiram treated seeds recorded higher germination compared to untreated seeds. Between the tested cultivars, grain type soybean (RKS-18) had retained better germination capacity compared to vegetable type (GC-00209-4-1-1) at the end of storage period. Among the packing material polypouches recorded higher germination followed by super grain bag compared to cloth bag.

Table 1. Description of seed materials, treatments and packing materials.

Cultivars (C):	Seed treatments (T):	Packing materials (P):
C ₁ - Vegetable Soybean cv. GC-00209-4-1-1 (KARUNE)	T ₁ - Control (untreated)	P ₁ - Cloth bag
C ₂ - Grain Type Soybean cv. RKS -18	T ₂ - <i>Trichoderma viridae</i> (7.5 g kg ⁻¹)	P ₂ - Super grain bag {polypropylene (< 300 gauge)}
	T ₃ - Thiram (3 g kg ⁻¹ + Polykote)	P ₃ -Polypouches (500 gauge)

In general, among the combinations cv. RKS-18 treated with Thiram (3g kg⁻¹) and packed in polypouches was found to be superior over other treatment combinations, which ensured better storability of soybean seeds. , similarly seeds of cv. GC-00209-4-1-1(vegetable type) treated with Thiram and stored in polypouches expressed high germination compared to untreated which were packed in cloth bag. These findings are in Agreement with Omvati and Verma (2014), Tame and Elam (2015).

Field emergence

Generally field emergence decreased with the increase in storage period. Among treatments Thiram (3 g kg⁻¹) recorded higher field emergence compared to *Trichoderma viridae* and control at the end of nine month of storage. Seeds packed in polypouches recorded higher field emergence followed by super grain bag and the lowest was observed in cloth bag.

Similar finding was reported by Omvati and Verma (2014). Furthermore, cv. RKS-18 recorded higher field emergence compared to cv. GC-00209-4-1-1 irrespective of treatments.

The interaction effect due to seed treatment showed differential response on field emergence (Table 2). Seeds treated with Thiram and packed in polypouches recorded higher field emergence at the end of nine month storage. While, untreated seeds that packed in cloth bag were damaged by bruchidbeetle and thus their field emergence was nil. Similarly, Anuja Gupta, 1999, Raj *et al.* (2002), Gaythri *et al.* (2008) and Saman *et al.* (2014) in soybean reported differential response due to seed treatment and packing materials on field emergence potential.

Table 2. Germination, field emergence and seedling vigour index (SVI) of soybean as influenced by cultivars, packing materials and seed treatments after nine months of storage

Treatments	Seed germination (%)		Field emergence (%)		SVI	
	Initial	End of storage	Initial	End of storage	Initial	End of storage
Cultivars						
C ₁	86.00	58.66	82.33	50.44	1979	1270
C ₂	96.00	74.59	87.00	51.70	2905	1939
Mean	91.00	66.63	84.66	51.07	24.42	1605
SEm±	0.491	0.623	0.638	0.5581	39.97	14.79
CD(0.05P)	1.512	1.785	1.966	1.600	123.5	42.43
Packaging						
P ₁	90.83	35.05	84.67	4.44	2486	776
P ₂	90.83	82.38	84.67	73.33	2486	1959
P ₃	90.83	82.44	84.67	74.44	3486	2078
Mean	90.83	66.62	84.67	50.74	2486	1974
SEm±	0.601	0.762	0.782	0.683	48.95	18.12
CD(0.05P)	1.851	2.186	2.481	1.960	150.83	51.96
Seed Treatment						
T ₁	90.00	62.05	83.00	48.88	2375	1447
T ₂	90.00	64.50	83.00	49.27	2404	1539
T ₃	92.50	73.33	85.00	55.05	2547	1827
Mean	90.83	66.63	83.66	51.07	2442	1604
SEm±	0.601	0.762	0.782	0.683	48.95	18.12
CD(0.05P)	1.851	2.186	2.481	1.960	150.83	51.962

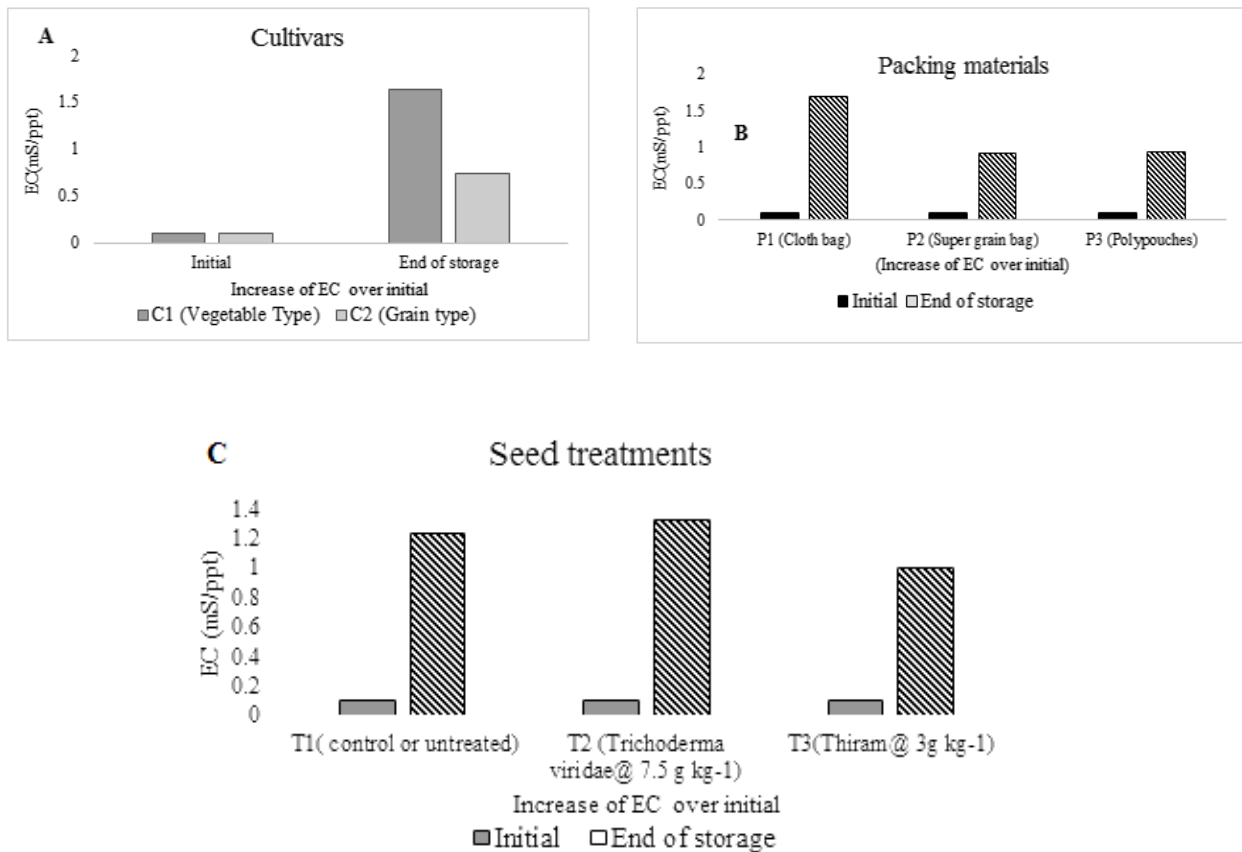


Fig. 1. Electrical conductivity of seed leachate (mS/ppt) as influenced by cultivars (A), packing materials (B) and seed treatments(C) after nine months of storage.

Seedling vigour index (SVI)

Another aspect related to germination is vigour. Since seedling length is a measure of synthesis, mobilization and growth as such, any assessment including seedling length is very much required to determine real quality of seed. The seedling vigour index (SVI) based on seedling length decreased with increase in storage period in all the treatments of

both the cultivars (Table 2). Higher SVI was observed in cv. RKS-18, which was due to higher germination at the end of storage period. However, seed treatment with Thiram (3 g kg^{-1}) resulted in maintenance of higher seedling vigour index compared to *Trichoderma viridae* (7.5 g kg^{-1}). These results are in conformity with Pardeshi *et al.* (1989), Paul and Mishra (1994), Omvati and Verma (2014).

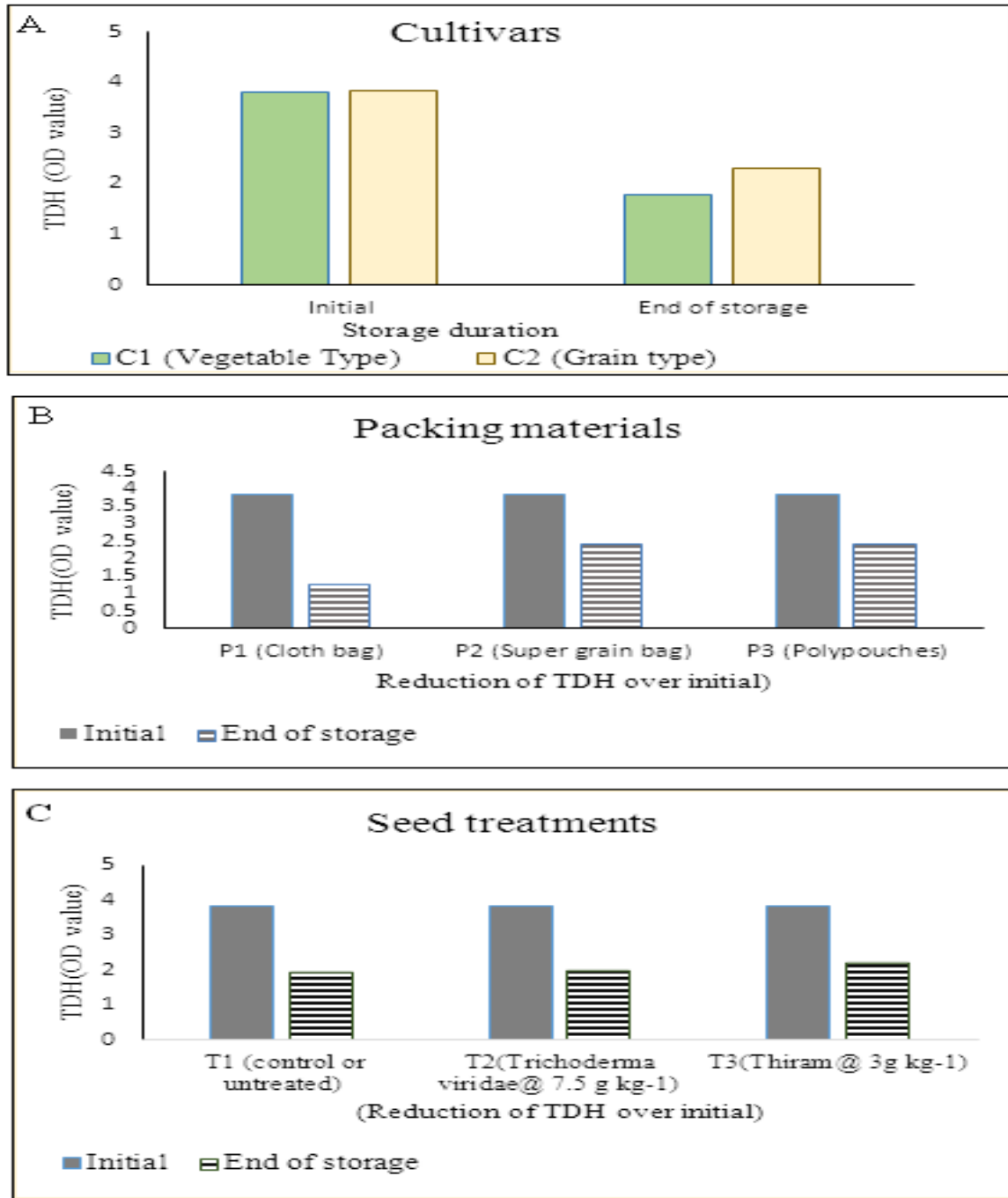


Fig. 2. Total dehydrogenase activity of soybean seeds as influenced by cultivars (A), packing materials (B) and seed treatments(C) after nine months of storage.

Electrical conductivity (EC) of seed leachate (mS/ppt)

The solute leakage measured in terms of electrical conductivity indicates the movement of ions from the seed to the imbibing medium. This solute leakage has been closely associated with the loss of viability and vigour in most of the crops. In the present study, a progressive increase in electrical conductivity (EC) of seed leachate was noticed with increase in storage period in all the treatments. Between the cultivars cv. GC-00209-4-11 (vegetable type), which had low germination, recorded highest EC indicating their susceptibility to membrane damage (Fig. 1A). Also among the packing materials super grain bag and polypouches recorded low EC of seed leachate as compared to cloth bag (Fig. 1B). EC of seed leachate was higher in untreated control throughout the storage period when compared to other treatments. Although EC increased over storage, seeds treated with Thiram (3g kg⁻¹) recorded low EC of seed leachate as compared to *Trichoderma viridae* (7.5 g kg⁻¹) at end of nine months storage (Fig.1C). Similar findings were also reported in soybean by Pavan (2000) and Ramesh (2002), which suggest that Thiram might have protected the seed coat from internal damage and packing materials *viz.*, super grain bag and polypouches also significantly helped in maintaining membrane integrity.

Total dehydrogenase activity (TDH)

Total dehydrogenase activity (TDH) decreased with increase in storage period and it is negatively associated with seed quality. The TDH activity was lower in cv. GC00209-4-1-1 compared to cv. RKS-18 (Fig. 2A). Among the packing material polypouches recorded higher TDH followed by super grain bag as compared to cloth bag (Fig. 2B). However, among the seed treatments it was higher in seeds treated with Thiram (3 g kg⁻¹) compared to untreated control even at the end of storage period (Fig. 2C). Similar reports of reduction in TDH activity during storage were made in soybean seeds (Meng, 1993; Vamadevappa, 1998; Pavan, 2000). Therefore, it is evident from the study that the reduced germination of stored seeds might be due to the low ebb of dehydrogenases and

suggested that the activity of these enzymes could be taken as one of the index in assessing the quality of treated soybean seeds.

Conclusion

The results of this experiment indicates that seed quality attributes of both cultivars decreased with increase in storage period. Therefore, the study suggested that conventional chemical seed treatment could be useful to prolong the storage life of both soybean cultivars and preserve the viability of seeds until next growing season. Polypouches and super grain bag (polypropylene both) could be used for better preservation of seed viability. Seed treated with Thiram (3 g kg⁻¹) could be stored up to nine months without any detrimental effect on seed viability. The results obtained can be supportively acknowledging the superiority of grain type soybean over vegetable type in maintaining viability during storage and the use of appropriate packing material and seed treatment to preserve the seed longevity.

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