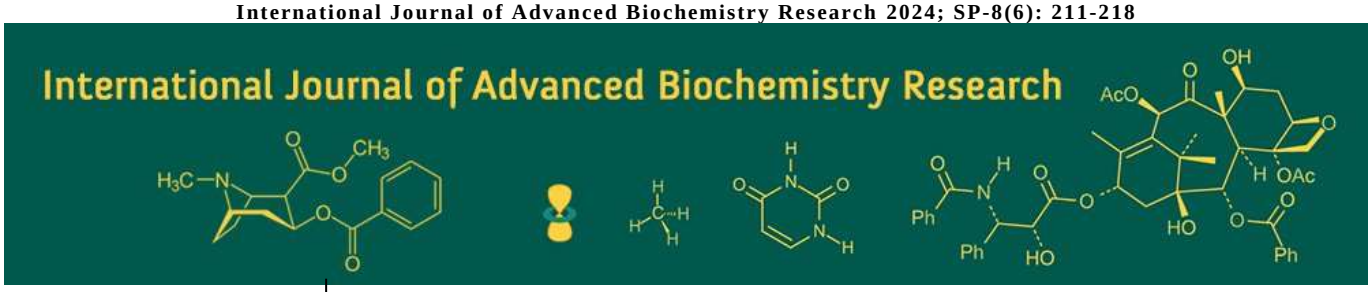


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# Assessment of biochemical parameters of different seed coat colored soybean genotypes during seed storage

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**Abstract**
20 soybean genotypes were selected for evaluating the biochemical changes on seed longevity. 5 different testa coloured soybean genotypes had used for the experiment. 20 different soybean genotypes were stored in polythene bags of 400 gauges for 10 months. Different biochemical parameters were analysed before the seed storage and on monthly intervals. Among them, Black coat seeds had showed resistant for seed longevity and yellow coloured genotypes exhibited susceptibility for seed longevity. All the soybean genotypes showed a significant difference for total soluble sugars, crude protein (%), amylase activity and peroxidase activity. Among the different genotypes, black seed coated soybean EC-76756 showed highest peroxidase activity (0.998 and 0.703), crude protein (38.89% and 27.24%), amylase activity (0.198 to 0.157) and lowest total soluble sugars (93.46 to 118.69 µg/ml of seed leachates) from initial month of seed storage to 10th month of seed storage respectively. And the lowest peroxidase activity, crude protein, amylase activity and highest total soluble sugars were recorded by yellow colour soybean seeds in both initial and 10$^{th}$ month of seed storage.

**Keywords:** Storability, peroxidase activity, total soluble sugars, crude protein and amylase activity

## Introduction
Soybean [*Glycine max* (L.) Merrill] is the important economic pulse cum oil seed crop and identified as important grain legume in the world, in view of total production and international trade. Soybean otherwise known as a "Miracle crop" with contains 40 per cent protein, 20 per cent oil, 85 per cent polyunsaturated fatty acid, 25-30 per cent carbohydrates, minerals, antioxidants, beta-carotene and isoflavonoids. The soybean [*Glycine max* (L.) Merril] was originated from eastern Asia/China, it is a member of Leguminacae family and cultivated soybean (*Glycine max* L. Merril) was derived from a wild progenitor Glycine ussuriensi. Soybean has genome size of 1.1 to 1.15 Gb.

Soybean (2n = 40) belongs to the family Fabaceae and originated from China and distributed across Asia, USA, Brazil, Argentina etc. Approximately around 85 per cent of the world's soybean processed into vegetable oil and soybean meal. The soybean producers are USA (36%), Brazil (36%), Argentina (18%), China (5.0%) and India (4.0%). In World, soybean occupies 126.64 million hectare area and production 346.31 million tones and productivity of 2,735 kg per hectare. India occupies 10.56 million hectare area and production of 11.22 million tonnes with productivity of 1,153 kg per hectare (Anon., 2018) [1].

The storability of seeds varies from species to species and also varieties of the same species. Seed storage of soybean is a big challenge to seed industry because the soybean seeds lose their viability faster below the Indian minimum Seed Standards (70%) before next growing season due to seed structure and chemical composition. This leads to a problem of non-availability of vigourous seed and high seed viability at the time of sowing which finally leads to poor field stand, production and productivity. Soybean seeds have classified as poor storer, because of their delicate endosperm, seed coat and vulnerable position of embryo. Among oilseed crops; soybean is the most extensively studied crop with respect to ageing. Soybean oil consists of approximately 60 per cent of polyunsaturated fatty acids content which is liable to rapid degradation making it a poor storer (Priestley, 1986) [12]. Some of the intrinsic factors which are believed to be closely associated with seed deterioration are

alteration of chemical composition, loss of membrane integrity, changes in enzyme activities, depletion of food reserves and chromosomal aberrations (Oliveira *et al*., 1984) [11]. The poor storability is a major problem in soybean. The cause of decline in quality of different soybean cultivars at different levels depends upon the genetic composition of the genotypes and lipid peroxidation of poly unsaturated fatty acids is the main basis for poor seed storability of soybean, which also results in the production of volatile aldehydes at high level during seed storage. Unfavourable environmental conditions and seed storage can leads to oxidative damage in plant tissue caused by oxidative stress; development of superoxide radical, hydrogen peroxide and hydroxyl radical, which are toxic and destructive products of oxidative stress. The composition of fatty acids is a prime factor which indicates susceptibility of oils to oxidation. The types of fatty acids present in oil and in particular number of their double bonds, determines the extent and type of chemical reactions which occurs during the seed storage. Lipid peroxidation (LP) causes oxidative damage lipoproteins, cell membranes and other molecules containing lipids, caused by oxidative stress. Once initiated, lipid peroxidation reaction continues auto-catalytically and progressively leads structural and functional substrate changes. Some biochemical changes strongly influencing the seed viability and seed quality that takes place in soybean seed during aging. The chemical structure of soybean seed (20-22% oil) enables some specific processes, very often degrading in nature (Lavanya, 2015) [7]. This paper represents the different biochemical parameters associated with seed longevity of soybean.

## Materials and Methods

The laboratory experiments were comprised of 20 genotypes stored for 10 months in polythene bags of 400 gauges to evaluate the biochemical changes during storage at NSP, University of Agricultural Sciences, Bengaluru (Plate 1 & 2). The experiment was carried out in complete randomised statistical design with three replications.

**Table 1:** Genotypes of different testa colour

| Testa colour of genotypes | | | | |
|---|---|---|---|---|
| **Brown** | **Variegated** | **Green** | **Black** | **Yellow** |
| DS-72-244 | RSC 10-71 | JS - 90 41 | EC-57042, TR-5, IC-501268, EC-76756, PB-5. | JS-335, JS - 9560, NRC-86, 115-B, DSB-21, RKS-45, NRC-37, RKS-24, JS - 2034, JS - 2069, AGS 29, JS – 2029 |

### Total soluble sugars (µg/ml of seed leachate)

The total soluble sugar content of seed samples was estimated by phenol sulphuric acid method according to Dubois *et al.* (1951) [4].

The procedure followed is given below:

### i. Preparation of reagents

1 Seed leachate
2 Phenol (5%) (Five gram of analytical grade phenol was dissolved in distill water to make100 ml.)
3 Sulphuric acid (96%)
4 Standard glucose solution: 100 mg of glucose was dissolved in 100 ml water. One ml of this solution was diluted to 10 ml with water to get working standard containing 10 - 100 µg/ml.

**ii. Estimation of total soluble sugars:** The required quantity (0.1 ml) of seed leachate was diluted to 1 ml with distilled water. One ml of 5 per cent phenol and 5 ml of 96 per cent $H_2SO_4$ were added. Sulphuric acid was added in such a way that it hits the reactants surface directly. The mixture was allowed to cool for 45 minutes at room temperature. After cooling to room temperature, the absorbance was read at 490 nm against the reagent blank. A standard curve was constructed with glucose as a standard with the concentration of 10 to 100 µg. The standard curve was used to estimate the total soluble sugars of the sample and the results are expressed in µg/ml of seed leachate.

### Crude protein (%)

Protein content was estimated by micro-Kjeldhal method of nitrogen estimation (Miller and Houghton, 1945) [9]. Nitrogen is one of the major elements found in living organism being an essential constituent of amino acids, protein, nucleic acid, amides, vitamins, coenzymes, hormones etc. In most proteins, nitrogen constitutes nearly 16% of the total composition and hence, the total nitrogen content of the sample is multiplied by 6.25 to calculate the protein content. The sample was digested with concentrated $H_2SO_4$ in the presence of a catalyst to convert the nitrogen in protein or any other organic material to ammonium sulphate. By steam distillation of this salt in the presence of a strong alkali, ammonia is liberated and collected in boric acid solution as ammonium borate which was estimated against a standard acid by titration. On an average most proteins have 16 per cent nitrogen in their composition i.e., 1 mg nitrogen equals 6.25 mg protein. Thus, by finding out the amount of ammonia formed from a known amount of sample, the amount of protein present in the sample was calculated.

1 Preparation of reagents weight about 0.2 g of finely powdered homogenate sample into the digestion flask.
2 Add a pinch of digestion mixture (0.1 g $K_2SO_4$ and 10 mg Hgo) and 10 ml of concentrated $H_2SO_4$ and mix well. Keep the sample overnight for pre-digestion
3 Add 10 ml of water along the sides of the digestion tube for the reaction to stop and transfer to the dictation apparatus with successive rinsing water.
4 Place a 100 ml conical flask containing 20 ml of boric acid solution with a few drops of mixed indicator in such a way that the tip of the condenser dipping inside the solution
5 Distil and collect the ammonia in boric acid. The colour change from pink to green is an indication of ammonia absorbed.
6 Collect the distillate and titrate against the standard HCl or $H_2SO_4$ (0.1 N) until the appearance of original pink colour as the end point.

The nitrogen and protein content of the sample were obtained by the following equations,

$$\text{Percent N content} = \frac{\text{Titre value} \times \text{normality of acid} \times 0.014}{\text{Weight of the sample}} \times 100$$

Percent protein content = percent N content × 6.25

**Amylase activity (mg maltose/ml/min)**

The amylase activity in germinating seeds was estimated according to the method of Bernfeld (1955) [2].

**i. Estimation of amylase activity:** The enzyme was extracted by grinding 0.5 g of seeds in 1ml of 0.1 M phosphate buffer pH 7.2. The ground material was left at 4 °C for one hour and centrifuged at 10000 rpm at 4 °C for 15 minutes. The supernatant was used as an enzyme source. To 0.5 ml of enzyme extract, 0.5 ml of starch solution was added and incubated at room temperature for 15 minutes. Immediately after incubation, 1 ml of DNS reagent was added boiled for 5 minutes over a water bath and 0.5 ml of 40 per cent sodium potassium tartarate was added to stop the reaction. The solution was cooled at room temperature and 2.5 ml of distilled water was added. Blank was prepared without adding starch solution and control without incubation for 15 minutes. The enzyme activity was calculated by measuring OD at 560 nm. Amylase activity was expressed as mg maltose released / ml/ min.

**4. Peroxidase activity (A436 / min /g of seed)**

i. Enzyme extraction One gram of seeds were extracted in 1 ml of 0.1 M Phosphate buffer with pH 7.0 by grinding with a pre cooled pestle and mortar. The slurry was transferred to eppendorf tubes and kept at 4 °C for 4 hours for enzyme extraction and then tubes are transferred to 20 °C. The homogenate was centrifuged at 10,000 rpm at 4 °C for 15 minutes. The supernatant was used as enzyme source. The enzyme extract was stored in ice box till the assay is carried out.

**ii. Preparation of reagents**

1. Phosphate buffer 0.1 M (pH 7.0)
2. Guaiacol solution (20 mM): 242 µl guaiacol was added to distil water and volume was made to 100 ml. It can be stored in frozen condition for many months.
3. Hydrogen peroxide solution (0.042%) (12.3 mM): 125 µl of 30% hydrogen peroxide was added to distilled water and volume was made to 100 ml. It should be prepared at the time of use. Absorbance of 12.3 mM $H_2O_2$ was adjusted to 0.4 by adding 20 ml of water to it, before using the solution for assay.

**iii. Estimation of peroxidase activity**

The enzyme assay was carried out according to Sadasivam and Manickam (1996) [13]. The reaction mixture was prepared in cuvette by adding 2 ml of 0.1 M phosphate buffer of pH 7.0, Guaiacol-200 µl and 12.3 mM $H_2O_2$-200 µl. Brought the mixture to 25 °C and then placed the cuvette in the Spectrophotometer set at 436 nm. Then, add 100 µl of enzyme extract mix it properly with pipette tip, immediately start the stopwatch. Read the initial absorbance at 436 nm and note increase the absorbance for 3 minutes at an interval of 30 seconds by using enzyme kinetics. Water is used as blank during the assay period and enzyme activity was expressed in terms of change in absorbance per minute per gram of seed.


**Plate 1:** Seeds with different testa colour used for seed storage

**Plate 2:** General view of soybean seeds stored under ambient condition

## Results and Discussion

The ageing of the stored seed is a natural phenomenon and the seeds tend to lose viability even under ideal storage conditions. Auto-oxidation of lipids and increase in the content of free fatty acids during storage period are the main reasons for rapid deterioration in oil seeds. The characteristics of biochemical composition of oil seeds are directly associated to processes occur during their seed ageing. Lipid auto-oxidation and increase of free fatty acid content during storage are the most often mentioned reasons for damage of seed of oily plant species (Morad Shaban, 2013) [10].

The present laboratory experiment was conducted to know the performance of soybean genotypes on biochemical changes occur during seed storage. The results obtained through the study are revealed and discussed below.

All the genotypes in soybean showed a significant difference for total soluble sugars, Peroxidase activity, Amylase activity and crude protein (%) present in seed leachate during seed ageing. Among the different soybean genotypes, black colored soybean seeds are considered as good storers and yellow colored soybean seeds are considered as poor storers. With respect to different biochemical parameters, black colored soybean genotypes showed highest peroxidase activity, crude protein, amylase activity and lowest total soluble sugars from initial month of seed storage to 10th month of seed storage followed by brown and variegated coloured genotypes. Whereas, yellow seeded genotypes showed a higher activity of TSS, lower peroxidase activity, crude protein, amylase activity from initial month of seed storage and after 10th month of seed storage followed by green coloured genotypes.

Among 20 genotypes, higher activity of TSS found in yellow coloured genotype, 115-B (135.56 to 179.87 (µg/ml of seed leachates) and lower activity of TSS recorded in EC-76756 (93.46 to 118.69 (µg/ml of seed leachates) from initial month of seed storage to 10th month of seed storage and represented in table 2. These may be due to least membrane integrity of yellow seeded genotypes and their higher permeability results in maximum leach of soluble sugars compare to black seeded genotypes. The similar results of increased in total soluble sugar content upon ageing were revealed by Bhanuprakash, *et al.* (2006) [3] and these results clearly demonstrate that the aged seeds lost integrity of cell membranes.

**Table 2:** Performance of soybean genotypes during storage on total soluble sugar (µg/ml of seed leachates)

| Total soluble sugar (µg/ml of seed leachates) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Period of storage (December, 2018 to October, 2019) | | | | | | | | | |
| **Genotypes** | **Initial** | **1** | **3** | **5** | **7** | **8** | **9** | **10** | **Percent increase** |
| **Black colour genotypes** | | | | | | | | | |
| EC-76756 | 93.46 | 95.21 | 101.47 | 104.40 | 108.84 | 111.25 | 115.50 | 118.69 | 21.28 |
| PB -5 | 95.34 | 97.77 | 103.30 | 105.54 | 109.33 | 112.40 | 117.10 | 122.25 | 22.01 |
| IC -501268 | 97.03 | 101.40 | 104.39 | 110.89 | 113.13 | 115.69 | 119.62 | 124.37 | 21.98 |
| TR-5 | 100.20 | 104.67 | 107.36 | 113.93 | 116.18 | 119.44 | 122.33 | 126.55 | 20.82 |
| EC-57042 | 96.78 | 98.02 | 101.14 | 106.26 | 109.69 | 112.62 | 118.28 | 123.04 | 21.34 |
| **Yellow colour genotypes** | | | | | | | | | |
| NRC-86 | 123.47 | 125.63 | 131.85 | 139.62 | 143.88 | 146.33 | 149.22 | 158.45 | 22.08 |
| 115-B | 135.56 | 138.04 | 143.33 | 151.66 | 154.99 | 158.17 | 163.29 | 179.87 | 24.63 |
| DSB-21 | 117.98 | 120.25 | 124.44 | 129.02 | 132.69 | 135.84 | 139.33 | 154.65 | 23.71 |
| RKS-45 | 112.34 | 115.60 | 119.52 | 126.69 | 129.49 | 133.59 | 136.17 | 147.43 | 23.80 |
| NRC-37 | 110.03 | 113.77 | 117.11 | 123.55 | 126.46 | 128.58 | 132.18 | 154.32 | 28.70 |
| JS -9560 | 118.21 | 121.73 | 127.28 | 132.36 | 135.36 | 138.28 | 143.21 | 156.43 | 24.43 |
| RKS-24 | 132.92 | 135.39 | 139.51 | 147.14 | 150.59 | 154.38 | 157.30 | 176.98 | 24.90 |
| JS – 2069 | 131.23 | 133.70 | 138.25 | 142.03 | 147.00 | 151.22 | 154.07 | 187.65 | 30.07 |
| JS – 335 | 127.00 | 129.43 | 133.25 | 141.77 | 145.29 | 149.10 | 152.70 | 187.34 | 32.21 |
| JS – 2029 | 124.30 | 126.16 | 132.44 | 140.29 | 144.03 | 148.88 | 151.47 | 163.43 | 23.94 |
| JS - 20 34 | 119.33 | 123.03 | 130.05 | 138.62 | 141.03 | 144.70 | 146.14 | 167.89 | 28.92 |
| AGS 29 | 115.27 | 122.08 | 129.88 | 136.62 | 139.06 | 142.54 | 145.03 | 153.67 | 24.99 |
| **Brown colour genotypes** | | | | | | | | | |
| DS 72-244 | 97.57 | 103.23 | 105.44 | 111.70 | 113.88 | 117.32 | 120.47 | 125.48 | 22.24 |
| **Green colour genotypes** | | | | | | | | | |
| JS - 90 41 | 107.37 | 110.81 | 114.06 | 118.40 | 121.29 | 125.65 | 128.75 | 143.32 | 25.08 |
| **Variegated colour genotypes** | | | | | | | | | |
| RSC 10 71 | 105.43 | 108.89 | 111.03 | 117.28 | 120.76 | 123.33 | 127.76 | 145.23 | 27.40 |
| Mean | 113.04 | 116.24 | 120.76 | 126.89 | 130.15 | 133.47 | 137.00 | 140.77 | |
| S.Em. ± | 1.72 | 2.01 | 2.16 | 1.91 | 2.90 | 2.02 | 1.89 | 1.78 | |
| C.D. (P= 0.01) | 6.17 | 6.71 | 6.26 | 5.79 | 9.11 | 6.73 | 5.69 | 5.81 | |
| CV (%) | 2.64 | 3.00 | 3.10 | 2.61 | 3.87 | 2.62 | 2.40 | 2.29 | |

Among the 20 genotypes, black coloured genotypes showed slight reduction the crude protein (%) over the storage period compared to yellow coloured genotypes. The maximum crude protein (%) was found in EC-76756 (38.89%) followed by EC- 57042 (38.58%). The minimal seed crude protein was observed in 11 5-B (33.60%) followed by JS - 2069 and RKS-24 (34.73%) at the initial period of storage. After 10 months of seed storage, the highest crude protein (%) was found in black coloured genotype (27.24%) followed by brown (24.19%) and variegated (22.98%). The lowest crude protein (%) was found in yellow coloured genotype (15.41%) followed by green (22.61%).The maximum per cent reduction was found in 11 5-B (54.14%) and the minimum per cent reduction were found in EC-76756 (29.96%) and depicted in table 3. This might be due to delicate seed coat of yellow coloured testa seeds absorbs more moisture and also due to increase in moisture, fluctuating temperature, relative humidity. The results were in conformity with Kakde and Chawan (2011) [6] in oil seeds and Sharma *et al*. (2013) [15] in soybean and Vijayalaksmi (2017) [19] in groundnut. Age associated reduction in activity of key enzymes (Super oxide dismutase, Peroxidase, dehydrogenase, amylase etc.,) in seeds has been reported. Thus, reduction in enzyme activity may be a reflection of changes in protein synthesis (Wilson and McDonald, 1986 and McDonald, 1999) [20, 8].

**Table 3:** Performance of soybean genotypes during storage on seed protein content (%)

| Seed protein content (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Period of storage (December, 2018 to October, 2019) | | | | | | | | | |
| **Genotypes** | **Initial** | **1** | **3** | **5** | **7** | **8** | **9** | **10** | **Percent reduction** |
| **Black colour genotypes** | | | | | | | | | |
| EC-76756 | 38.89 | 37.72 | 35.77 | 35.1 | 30.18 | 29.61 | 28.76 | 27.24 | 29.96 |
| PB -5 | 39.45 | 37.26 | 35.47 | 34.7 | 29.66 | 29.00 | 27.15 | 26.39 | 33.11 |
| IC – 501268 | 38.44 | 36.62 | 34.13 | 33.7 | 28.05 | 26.78 | 25.93 | 25.17 | 34.52 |
| TR-5 | 37.90 | 36.06 | 32.97 | 31.3 | 26.80 | 25.48 | 24.63 | 23.87 | 37.02 |
| EC-57042 | 38.58 | 37.52 | 34.87 | 34.3 | 28.80 | 27.47 | 26.62 | 25.86 | 32.92 |
| **Yellow colour genotypes** | | | | | | | | | |
| NRC-86 | 36.24 | 31.06 | 28.98 | 28.0 | 23.79 | 20.00 | 19.15 | 18.39 | 49.25 |
| 115-B | 33.60 | 29.04 | 27.00 | 26.7 | 19.03 | 17.02 | 16.18 | 15.41 | 54.14 |
| DSB-21 | 36.78 | 33.38 | 30.23 | 29.3 | 23.12 | 22.59 | 21.74 | 20.98 | 42.96 |
| RKS-45 | 37.22 | 34.07 | 30.92 | 29.9 | 23.90 | 23.03 | 22.18 | 21.42 | 42.45 |
| NRC-37 | 38.35 | 34.95 | 31.11 | 30.2 | 24.83 | 24.04 | 23.19 | 22.43 | 41.51 |
| JS -9560 | 36.78 | 32.26 | 29.79 | 29.0 | 22.69 | 21.16 | 20.31 | 19.55 | 46.85 |
| RKS-24 | 34.73 | 29.84 | 27.13 | 26.9 | 19.25 | 17.55 | 16.70 | 15.94 | 54.10 |
| JS – 2069 | 34.73 | 30.03 | 27.50 | 27.0 | 19.80 | 18.27 | 17.01 | 16.25 | 53.21 |
| JS – 335 | 35.38 | 30.17 | 28.05 | 27.3 | 20.11 | 18.68 | 17.83 | 17.07 | 51.75 |
| JS – 2029 | 35.91 | 30.41 | 28.43 | 27.7 | 20.72 | 19.20 | 18.35 | 17.59 | 51.02 |
| JS - 20 34 | 36.31 | 31.85 | 29.13 | 28.3 | 21.79 | 20.36 | 19.51 | 18.75 | 48.36 |
| AGS 29 | 36.83 | 32.17 | 29.52 | 28.8 | 22.12 | 21.03 | 20.18 | 19.42 | 47.27 |
| **Brown colour genotypes** | | | | | | | | | |
| DS 72-244 | 38.10 | 36.83 | 33.22 | 32.8 | 27.34 | 25.80 | 24.95 | 24.19 | 36.51 |
| **Green colour genotypes** | | | | | | | | | |
| JS - 90 41 | 37.69 | 34.95 | 31.45 | 30.4 | 25.78 | 24.22 | 23.37 | 22.61 | 40.01 |
| **Variegated colour genotypes** | | | | | | | | | |
| RSC 10 71 | 38.00 | 35.73 | 32.13 | 30.7 | 26.12 | 24.59 | 23.74 | 22.98 | 39.53 |
| Mean | 36.99 | 33.59 | 30.88 | 30.10 | 24.19 | 22.79 | 21.87 | 21.07 | |
| S.Em. ± | 0.27 | 0.49 | 0.40 | 0.76 | 0.39 | 0.26 | 0.48 | 0.45 | |
| C.D.(P= 0.01) | 0.94 | 1.49 | 1.53 | 2.62 | 1.41 | 0.98 | 1.55 | 1.52 | |
| CV (%) | 1.26 | 2.55 | 2.25 | 4.39 | 2.82 | 2.01 | 3.83 | 3.70 | |

Among the 20 genotypes, black coloured genotypes (EC-76756, IC-501268, EC-57042, TR-5, PB-5) and brown (DS-72-244) seeded genotype observed as resistant for seed ageing with slight reduction in amylase activity (From 0.198 to 0.157 and (From 0.192 to 0.144) followed by variegated and green testa genotype (From 0.187 to 0.139) and (From 0.186 to 0.133 respectively).Whereas yellow seeded, (NRC-86, 115-B, DSB-21, RKS-45, NRC-37, JS - 9560, RKS-24, JS - 2069, JS - 335, JS - 2029, JS - 20 34, AGS 29) genotypes observed as susceptible for longevity which showed a higher reduction in amylase activity (from 0.166 to 0.089) and depicted in figure 1. The maximum percent reduction was found in yellow coloured genotype, 11 5-B (46.39) and minimum percent reduction was found in black coloured genotype, EC-76756 (20.71). The decrease in amylase activity over storage period could be due to reduction in the antioxidants synthesis which is associated with ageing. The continuous decline of the antioxidants may be due to seed senescence which increases the activity of free radicals and scavenger enzymes like catalase and peroxidase which suppresses the activity of key antioxidants. The similar results were obtained by the Verma *et al*. (2003) [18]. Along with the constitution of seed testa (Higher lignin), the higher activity of these antioxidants (dehydrogenase, SOD, peroxidase) in black coloured genotypes is one of the reason for their higher vigour and storability (Francisco *et al*., 2008) [5]

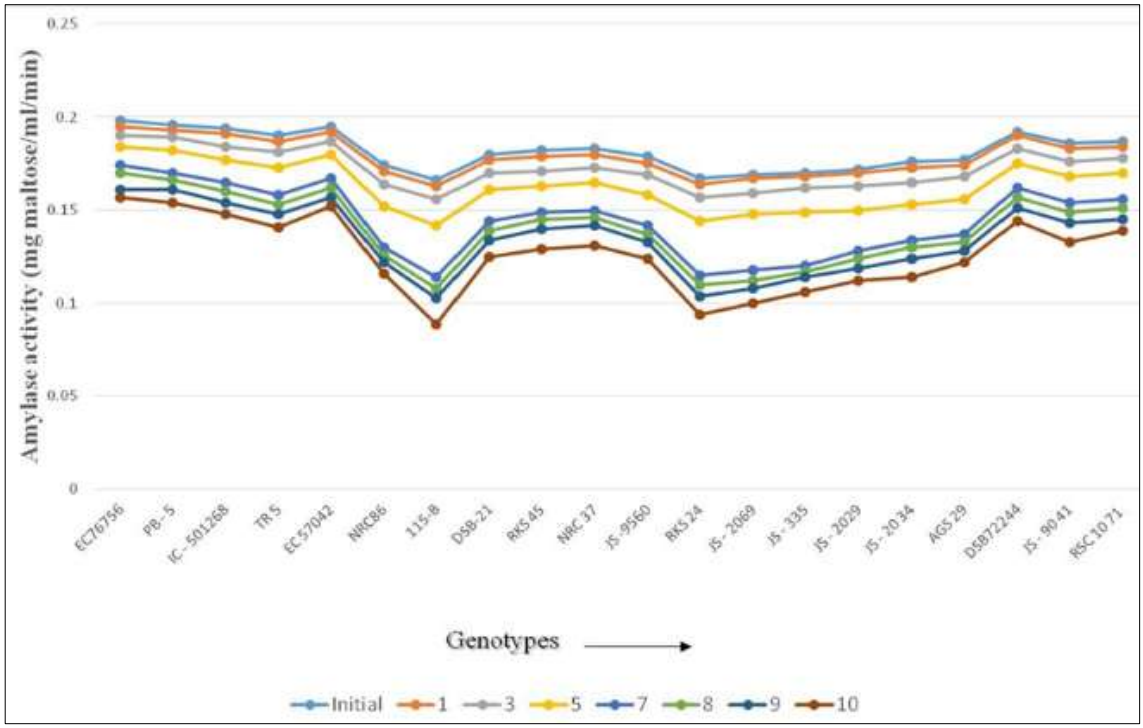


**Fig 1:** Performance of soybean genotypes during storage on amylase activity (mg maltose/ml/min)

EC-76756 showed highest peroxidase activity in the initial period of storage and after 10th month storage (from 0.998 and 0.703 respectively) followed by IC-501268, EC-57042(from 0.995 to 0.587, from 0.995 to 0.621 respectively) and PB-5 (From 0.994 to 0.687). 11 5-B recorded lowest peroxidase activity in the initial period of storage and after 10th month storage (From 0.960 to 0.264) followed by RKS-24 (From 0.963 to 0.278) and JS - 2069 (From 0.966 to 0.295). The maximum percent reduction was found in yellow coloured genotype, 11 5-B (72.50) and minimum percent reduction was found in black coloured genotype, EC-76756 (29.56) represented in table 4.

The rate of reduction in the peroxidase activity is more in yellow seeded genotypes followed by green and variegated seeded types this might be due to delicate seed coat of yellow coloured seeded genotypes which absorbs more water and causes free radicals damage. Peroxidase is the key enzyme which detoxifies the excessive hydrogen peroxide in the seeds. Increased level of peroxidase enzymes protects the cell against the oxidative damage by removal of reactive oxygen species and free radicals. In soybean ageing is associated with a decrease of total peroxidase activity as well other radical scavenging enzymes (Stewart and Bewley, 1980 and Sung and Chiu, 1995) [16, 17]. Whereas similar results were revealed by Scialabbal, *et al.* (2002) [14] that aged seed lots which maintained high viability showed an increase in peroxidase activity in two distinct parts of the seed, integument and cotyledons upon decline in viability, peroxidase activity also gets declined.

**Table 4:** Performance of soybean genotypes during storage on peroxidase activity (A436 /min/ g of seed)

| Peroxidase activity (A436 / min /g of seed) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Period of storage (December, 2018 to October, 2019) | | | | | | | | | |
| **Genotypes** | **Initial** | **1** | **3** | **5** | **7** | **8** | **9** | **10** | **Percent reduction** |
| **Black colour genotypes** | | | | | | | | | |
| EC-76756 | 0.998 | 0.995 | 0.982 | 0.955 | 0.892 | 0.851 | 0.788 | 0.703 | 29.56 |
| PB – 5 | 0.994 | 0.992 | 0.971 | 0.943 | 0.865 | 0.820 | 0.722 | 0.687 | 30.89 |
| IC – 501268 | 0.995 | 0.993 | 0.947 | 0.900 | 0.833 | 0.764 | 0.656 | 0.587 | 41.01 |
| TR-5 | 0.991 | 0.987 | 0.933 | 0.877 | 0.798 | 0.740 | 0.612 | 0.533 | 46.22 |
| EC-57042 | 0.995 | 0.992 | 0.965 | 0.917 | 0.841 | 0.770 | 0.689 | 0.621 | 37.59 |
| **Yellow colour genotypes** | | | | | | | | | |
| NRC-86 | 0.975 | 0.969 | 0.876 | 0.787 | 0.652 | 0.511 | 0.432 | 0.355 | 63.59 |
| 115-B | 0.960 | 0.949 | 0.830 | 0.739 | 0.587 | 0.424 | 0.355 | 0.264 | 72.50 |
| DSB-21 | 0.981 | 0.976 | 0.897 | 0.820 | 0.728 | 0.600 | 0.506 | 0.444 | 54.74 |
| RKS-45 | 0.984 | 0.979 | 0.905 | 0.833 | 0.746 | 0.632 | 0.537 | 0.476 | 51.63 |
| NRC-37 | 0.986 | 0.982 | 0.911 | 0.840 | 0.762 | 0.673 | 0.564 | 0.488 | 50.51 |
| JS -9560 | 0.980 | 0.974 | 0.889 | 0.816 | 0.707 | 0.588 | 0.491 | 0.402 | 58.98 |
| RKS-24 | 0.963 | 0.950 | 0.844 | 0.745 | 0.596 | 0.453 | 0.362 | 0.278 | 71.13 |
| JS – 2069 | 0.966 | 0.957 | 0.846 | 0.751 | 0.603 | 0.465 | 0.376 | 0.295 | 69.46 |
| JS – 335 | 0.969 | 0.962 | 0.856 | 0.765 | 0.627 | 0.489 | 0.388 | 0.321 | 66.87 |
| JS – 2029 | 0.973 | 0.968 | 0.863 | 0.783 | 0.632 | 0.503 | 0.421 | 0.335 | 65.57 |
| JS - 20 34 | 0.988 | 0.984 | 0.917 | 0.854 | 0.774 | 0.692 | 0.589 | 0.387 | 60.83 |
| AGS 29 | 0.977 | 0.971 | 0.881 | 0.802 | 0.669 | 0.521 | 0.464 | 0.399 | 59.16 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Brown colour genotypes** | | | | | | | | | |
| DS 72-244 | 0.993 | 0.989 | 0.941 | 0.897 | 0.824 | 0.753 | 0.623 | 0.554 | 44.21 |
| **Green colour genotypes** | | | | | | | | | |
| JS - 90 41 | 0.988 | 0.984 | 0.917 | 0.854 | 0.774 | 0.692 | 0.589 | 0.505 | 48.89 |
| **Variegated colour genotypes** | | | | | | | | | |
| RSC 10 71 | 0.990 | 0.986 | 0.924 | 0.869 | 0.780 | 0.721 | 0.609 | 0.522 | 47.27 |
| Mean | 0.982 | 0.976 | 0.903 | 0.835 | 0.730 | 0.627 | 0.533 | 0.458 | |
| S.Em. ± | 0.006 | 0.005 | 0.015 | 0.007 | 0.011 | 0.007 | 0.007 | 0.008 | |
| C.D.(P= 0.01) | 0.021 | 0.016 | 0.019 | 0.029 | 0.037 | 0.027 | 0.027 | 0.031 | |
| CV (%) | 1.21 | 1.05 | 2.98 | 1.59 | 2.67 | 2.01 | 2.31 | 3.13 | |

## Conclusion

Some biochemical changes strongly influencing the seed viability and seed quality that takes place in soybean seed during aging. The chemical structure of soybean seed enables some specific processes, very often degrading in nature. The higher vigour and storability of soybean seeds can be attributed to the combined effect of seed coat (testa) colour, physio-biochmeical changes, seed coat membrane integrity and efficiency of antioxidant system in the seed to counter free radical accumulation. Among the 20 genotypes, six genotypes *viz.*, EC-76756, EC-57042, IC- 501268, TR-5, PB-5 and DS-72-244 were identified as high vigour genotypes. Among yellow colour genotypes, 11 5-B followed by RKS-24 are susceptible for seed longevity (Poor storers) because of its delicate seed coat and minimum mechanical strength of seed coat and maximum seed coat permeability and different biochemical changes.

## Conflict of interest

The authors declare no competing interests

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## Authors Contributions

**Priyanka M:** Conducted the experiment, data analysis and drafted the article

**Parashivamurthy:** Conception of experiment, supervised and critical revision of the article

**Siddaraju M:** Design of the experiment, supervised and critical revision of the article

**Usha Ravindra:** Design of the experiment, supervised and critical revision of the article

**Ramanappa T M:** Design of the experiment, supervised and critical revision of the article

## Data Availability

Not applicable

**Research involving human and/ or animals-** Not involved

## Consent for publications

All authors agree to publish this article in International Journal of Advanced Biochemistry Research

## References

1. Directorate of Economics and Statistics, Department of Agriculture and Cooperation. Government of India; c2018. p. 127-129.
2. Bernfeld P. Amylases- $\alpha$ and $\beta$. In: Colowick SP, Kaplan NO, eds. Methods in Enzymology. New York: Academic Press. 1955;1:149-155.
3. Bhanuprakash K, Yogeesha HS, Naik LB, Arun MN. Studies on physiological and biochemical changes in relation to seed viability in aged onion seeds. J Horticultural Sci. 2006;1(1):15-18.
4. Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F. A colorimetric method for the determination of sugars. Nature. 1951;168:167.
5. Francisco CK, Barros FN, Marcos G, Milton K. Evaluation of lignin content of soybean seed coat stored in a controlled environment. Rev Bras Sementes. 2008;30(2):220-223.
6. Kakde RB, Chavan AM. Deteriorative changes in oilseeds due to storage fungi and efficacy of botanicals. Curr Biol. 2011;2(1):17-22.
7. Lavanya KS. Investigation on seed storability in soybean genotypes (*Glycine max* (L.) Merrill). 2015. M.Sc(Agri) Thesis. University of Agricultural Sciences Dharwad, Karnataka, India.
8. McDonald MB. Seed deterioration: physiology, repair, and assessment. Seed Sci Technol. 1999;27(1):177-237.
9. Miller AND, Houghton. The micro-Kjeldahl determination of nitrogen content of amino acids and proteins. J Biol Chem. 1945;169:373-383.
10. Shaban M. Review on physiological aspects of seed deterioration. Int J Agric Crop Sci. 2013;6(11):627-631.
11. Oliveira MD, Mathew S, Powell AA. The role of split seed coat in determining seed vigour in commercial seed lots of soybean as measured by the electrical conductivity test. Seed Sci Technol. 1984;12:659-668.
12. Priestley DA. Seed Ageing. Ithaca, NY: Comstock Publishing Associates; c1986.
13. Sadasivam S, Manickam A. Biochemical methods for agricultural sciences. 3rd ed. New Delhi: New Age International; 1996:1-21.
14. Scialabbal A, Bellani LM, Aquila A. Effects of ageing on peroxidase activity and localization in radish (*Raphanus sativus* L.) seeds. Eur J Histochem. 2002;46:351-358.
15. Sharma S, Kaur A, Bansal A, Gill BS. Positional effects on soybean seeds composition during storage. J Food Sci. Technol. 2013;50(2):353-359.
16. Stewart RC, Bewley JD. Lipid peroxidation associated with accelerated ageing in soybean axes. Plant Physiol. 1980;65:245-248.

17. Sung JM, Chiu CC. Lipid peroxidation and peroxide scavenging enzymes of naturally aged soybean seed. Plant Sci. 1995;110:45-52.
18. Verma SS, Tomer RP, Verma U. Loss of viability and vigour in Indian mustard seeds stored under ambient conditions. Seed Res. 2003;31(1):98-101.
19. Vijayalaksmi N. Influence of packaging materials and desiccant on storability of kernel and pod of summer groundnut (*Arachis hypogaea*). M.Sc. Thesis. University of Agricultural Sciences Dharwad; c2017.
20. Wilson DD, McDonald MB. The lipid peroxidation model of seed aging. Seed Sci. Technol. 1986;14(2):269-300.