

## Standardization of Controlled Deterioration Test to Assess Seed Vigour in Soybean [*Glycine max* (L.) Merrill.]

C. R. PALLAVI<sup>1</sup>, N. NETHRA<sup>2</sup>, PARASHIVAMURTHY<sup>3</sup>, R. SIDDARAJU<sup>4</sup> AND S. N. NAGESHA<sup>5</sup>

<sup>1,3&4</sup>Department of Seed Science and Technology, <sup>2</sup>Seed Technology Research Unit, AICRP on Seed (Crops),

<sup>5</sup>Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bengaluru - 560 065  
e-Mail : pallavi13697@gmail.com

### AUTHORS CONTRIBUTION

C. R. PALLAVI :

Conduct of experiments, tabulation of the data and drafted the manuscript

N. NETHRA :

Conceptualization, design, supervision and reviewing

PARASHIVAMURTHY;

R. SIDDARAJU &

N. NAGESHA :

Guidence, critical feedback, laboratory support and manuscript corrections

### Corresponding Author :

C. R. PALLAVI

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### ABSTRACT

Soybean [*Glycine max* (L.) Merill] is a vital legume crop, globally contributing significantly to food, feed and industrial sectors. Seeds are rich in protein (40%) and oil content (20%) leads to quick decline of seed viability during storage. Being rich in oil, like other oilseeds, soybean is also highly prone to seed deterioration. Seed aging (seed deterioration) represents an irreversible and gradual decline in seed vigour, ultimately leads to loss of seed viability. This seed deterioration process can be assessed through adoption of Controlled deterioration test (CDT), wherein the seeds are subjected to high humidity and temperatures around 40-45°C and 100 per cent relative humidity. Hence, the present study is focused on standardising the controlled deterioration test (CDT) to asses the seed vigour soybean genotypes (five black and five white). Seed germination in EC993850 (88%) followed by IC501847 (87%) among black genotypes and EC993703 (85%) followed by EC993937 (83%) among white genotypes, showing the higher seed quality parameters among all the genotypes subjected for extreme conditions of CDT. Seeds artificially aged with controlled deterioration (CD) with seed moisture content of 25% per cent for 24 h at 45 °C was found to be effective in assessing the vigour accurately. This study provides valuable insights into the impact of artificial aging on seed quality of different soybean genotypes and informs strategies for seed storage practices.

**Keywords :** Soybean, Seed deterioration, Seed vigour, Seed viability, Controlled Deterioration Test (CDT), Artificial ageing

SOYBEAN [*Glycine max* (L.) Merrill] is a prominent legume crop belongs to family *Leguminaceae* or *Fabaceae*, which is native to Eastern Asia. In India, soybean is popularly used as pulse and oilseed crop, constitute the second largest agricultural produce, next to food grains and these are the important source of our national economy. In India, the area under this crop is 12.09 million hectares with annual production of 11.215 million tonnes and productivity of 1126 kg per hectare (Anonymous, 2020). Major soybean growing states in India are

Madhya Pradesh (the so called 'soy state'), Maharashtra, Gujarat, Rajasthan, Karnataka and Andhra Pradesh. Maharashtra ranks 2<sup>nd</sup> in terms of production of soybean after Madhya Pradesh.

Quick decline of seed vigour during storage is one of the limiting factors for soybean seeds (Purwanti, 2004). Being rich in oil, like other oilseeds, soybean is highly prone to seed deterioration if improperly stored (Chandel *et al.*, 2015). Seed longevity is the maximum period until which a seed

posses its viability and cangerminate. Technically it is defined as the period from seed shedding to death (Roberts,1981). Seed longevity differs between species based on the genetics and storage environmental conditions. The difference in seed longevity within species was reported in rice (Miura *et al.*, 2002 and Sasaki *et al.*, 2005), wheat (Landjeva *et al.*, 2010), arabidopsis (Bentsink *et al.*, 2000 and Clerkx *et al.*, 2004) and lettuce (Schwember and Bradford, 2010). These results show that seed longevity is not a dominant trait and is highly influenced by environmental conditions and plant species. Environmental conditions that influence seedlongevity are storage environment relative humidity, seed moisture content, oxygen pressure and temperature of storage conditions. (Walters, 1998 and Groot *et al.*, 2012).

Seed ageing is an irreversible and inexorable process of a progressive decrease in vigourultimately leading to the loss of seed viability (Galland *et al.*, 2011 and Stewart & Bewley, 1980). The rate of seed ageing depends upon the genotype/ species and the conditions prevailing during storage like seed moisture content, temperature, humidity, seed composition and packaging materials (Roberts, 1973). Assessing deterioration accurately is not possible in standard germination test, vigour tests are of more useful in testing seed vigour. Among different vigour tests, Controlled deterioration test (CDT) is one of the fastest test that may be adopted for soybean.

The principle of CDT is similar with the accelerated aging test (AAT) method, in terms of exposing the seeds to high-temperature conditions, but difference in the moisture content, which is constant over a period of deterioration followed by a standard germination test. It has been reported that high moisture content and high temperature accelerate seed deterioration (Ellis & Hong, 1991 and Goel *et al.*, 2003) based on which seeds are accelerated to artificially age by exposing them to high humidity and temperature of about 40-45p C and 100 per cent RH (Delouche & Baskin, 1973). Seeds with high germination after ageing are considered high-vigour seeds (Powell & Matthews, 1981). The degree of

correlation on viability after CDT treatment may predict optimum seed shelf life.

Artificial ageing is shown to induce many deteriorative changes to seeds during storage like genetic damage, protein degradation, enzyme in activation and loss of membrane integrity (Gall and *et al.*, 2011; Bailly *et al.*, 1996; Bailly, 2004; Ratajczak & Pukacka, 2005; Ratajczak *et al.*, 2015 and Wang *et al.*, 2011) which ultimately lead to reduced germination (Walters, 1998) and loss of viability. Though there are numerous studies on physiology, ROS and its mechanisms of membrane degradation during ageing, there is less knowledge about the changes in seed quality under different conditions of artificially aging the seeds. With this background, an attempt was made to standardise a controlled deterioration test to study the changes in seed viability during artificial ageing among different genotypes of soybean (*Glycine max* (L.) Merrill.) was undertaken at the Seed Technology Research Center, AICRP on Seeds (Crops), Gandhi Krishi Vignan Kendra, University of Agricultural Sciences, Bangalore.

#### MATERIAL AND METHODS

Soybean seeds (Black and White genotypes) collected from All India Coordinated Research Project (AICRP) on Soybean, Zonal Agricultural Research Station, University of Agricultural Sciences, Gandhi Krishi Vignan Kendra, Banagalore were multiplied in the fields of Seed Technology Research Center, AICRP on Seeds (Crops), Gandhi Krishi Vignan Kendra, University of Agricultural Sciences, Bengaluru, which is situated at an altitude of 13p .15'.N and longitude of 77p .32'.E. during 2022-2023.

The lab experiments regarding seed quality testing and Controlled Deterioration Test for Artificial ageing of seeds were conducted at Seed Technology Research Center, AICRP on Seeds (Crops) & Department of Seed Science & Technology, Gandhi Krishi Vignan Kendra, University of Agricultural Sciences, Bengaluru was done at Seed Technology Research Center, AICRP on Seeds (Crops), Gandhi Krishi Vignan Kendra, University of Agricultural Sciences, Bangalore.

**List of Genotypes****TABLE 1**  
**List of Soybean genotypes**

Genotype ID	Genotype colour
EC993695	Off white
EC993937	Off white
EC993987	Off white
EC892808	Off white
PI561348	Off white
EC993752	Off white
EC994027	Off white
EC994017	Off white
EC993703	Off white
EC993922	Off white
EC1037888	Off white
EC993943	Off white
MACS132	Off white
EC1037868	Off white
J202(GOODS2)	Black
IC39088	Black
EC993608	Black
EC993850	Black
IC567498	Black
IC316142	Black
IC501847	Black
EC1037863	Black
EC993431	Black
IC501927	Black

**Screening of Genotypes**

Among the 24 genotypes put for multiplication, based on evaluating the field performance and seed germination test (Table 2), five best white genotypes and five best black genotypes were selected (Plate 1a and 1b respectively). These 10 genotypes were used in the further on going experiments of CDT.

**TABLE 2**  
**Screening of best Soybean genotypes based on seed germination percentage**

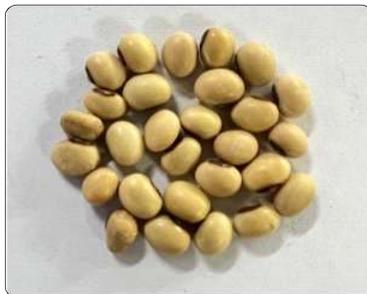
Genotype ID	Lab Germination percentage	Field germination percentage
EC993695	80	80
EC993937	85	85
EC993987	85	85
EC892808	84	83
PI561348	77	75
EC993752	82	82
EC994027	79	78
EC994017	81	79
EC993703	90	90
EC993922	80	80
EC1037888	80	78
EC993943	77	75
MACS132	76	76
EC1037868 (W)	79	79
J202(GOODS2)(B)	82	83
IC39088	82	82
EC993608	82	82
EC993850	89	88
IC567498	88	88
IC316142	80	79
IC501847	77	75
EC1037863	76	73
EC993431	80	78
IC501927	79	78

**Artificial Ageing with Controlled Deterioration Test (CDT)**

Artificial ageing was performed according to ISTA guidelines (Anonymous, 2013). The moisture content of the fresh seed samples was determined by the hot air oven method (Anonymous, 2013) before proceeding with CDT. For CDT, four replicates of 1.0 g seed per genotype were adjusted to desired moisture content using the filter paper method (ISTA, 1995) and then heat sealed in aluminum foil packets and equilibrated at 10°C for 24 hours.



**EC993987**



**EC993937**



**EC993703**



**EC993752**



**EC892808**

Plate 1a : White Soybean Genotypes



**EC993608**



**EC993850**



**J202**



**IC39088**



**IC501847**

Plate 1b : Black Soybean Genotypes



**Determining weight and initial SMC of seeds**



**Increasing Moisture to Desired**



**Sealed Aluminium Packets**



**Stored at 7 °C**



**Ageing chamber set @**



**Germination**

Plate 2 : Protocol of CDT

Then seed samples were deteriorated in water bath/hot air oven at the temperature of 45°C and durations 24 and 48 hours (Plate 2). Following the ageing period, seeds were germination tested expressed as the percentage germination.

**Germination (%)**

Seed germination test was performed within one hour after removal of samples from the hot air oven for 100 seeds in 4 replicates by between paper method

**Experiment detail:**

Genotypes	10 (5 black and 5 white)
Experimental design	CRD
Seed moisture for CDT (%)	25 and 30
CDT duration (days)	2 and 4
CDT temperature (°C)	45
Treatment combination	10 x 2 x 2 = 40
Replications	3

at 25 °C for each ageing treatment and control (fresh, non-aged seeds). The first and final count was taken on the 5<sup>th</sup> and 8<sup>th</sup> day for soybean seeds and the seedlings were evaluated as per International Seed Testing Association guidelines (2013) considering the per centage of normal seedlings to be germinated.

**Seedling Vigour Indices**

To calculate the seedling vigour index-I (SVI-I), the seedling length (root and shoot) of 5 randomly selected seedlings in each replicate was measured while taking the final count of seeds in the germination test. The vigour index was calculated by multiplying germination (%) and seedling length (cm). The lot showing higher values was highly vigourous (Anonymous, 2013).

$$\text{Seedling Vigour Index I} = \text{Germination per cent} \times \text{Seedling Length}$$

To calculate the seedling vigour index-II (SVI-II), the seedling dry weight of 5 randomly selected

**TABLE 3**  
**Soybean seed germination under different conditions of CDT**

Genotypes	Control	Germination percentage (%)			
		CDT for 24 h		CDT for 48 h	
		SMC @ 25%	MC @30%	SMC @ 25%	MC @30%
EC993987	85	77	71	74	65
EC993937	86	76	68	74	56
EC993703	90	81	70	79	63
EC993752	82	72	62	70	48
EC892808	85	75	65	73	54
EC993608	82	73	69	71	56
EC993850	89	80	75	78	67
J202	82	72	68	70	58
IC39088	82	72	67	70	53
IC501847	88	79	74	77	66
S.Em±	1.09	0.16	0.27	0.26	0.41
CD(p=0.01)	0.23	0.80	0.49	1.2	0.77
C.V. (%)	2.28	5.25	5.35	3.68	4.16

seedlings in each replicate was measured while taking the final count of seeds in the germination test. The vigour index was calculated by multiplying germination (%) and seedling dry weight. The lot showing higher values was highly vigourous.

*Seedling Vigour Index II = Germination per cent × Seedling Dry Weight*

### Electrical Conductivity

Membrane deterioration was measured by an electrical conductivity (EC) test as per the

modified ISTA method (Anonymous, 2013). Fifty seeds in two replicates in each ageing treatment

along with control were soaked in 250 ml of distilled water for 24 h at 20p°C and the conductivity of the elute was measured using a conductivity meter (Manufacturer: Systronics, Model: 306).

### Seed Moisture Content

The seed moisture content of seeds was measured before and after ageing using a seed moisture meter.

The seed moisture meter method was used for moisture estimation as it is a non-destructive method and as the seeds need to be used for other analyses after moisture estimation.

### RESULTS AND DISCUSSION

In the present investigation Controlled deterioration test (CDT) has been conducted on white and black soybean genotypes to standardise the optimum condition and results found that there was a significant effect on seed germination under different seed moisture content and duration of ageing at 45 °C which is presented in Table 3. All the genotypes showed highest seed germination (>80%) at initial stages of the experiment (Control). After CDT, all the genotypes reduced significantly in its germination and some are below the minimum seed certification standards (70%).

There was a significant difference among all the genotypes on seed germination (%). Among the white genotypes, genotype EC993703 was having highest



Plate 3 : Soybean seed germination at different CDT condition

germination (81%) followed by EC993987 (77%), whereas among black genotype EC993850 was having highest germination (80%) followed by IC501847 (77%) at the end of 24 hr ageing duration having seed moisture content of 25 per cent. Likewise in seeds aged for 24 hr with seed moisture content of 30 per cent, among the white genotypes, genotype EC993987 was having highest germination (71%) followed by EC993703 (70%), whereas among black

genotype EC993850 was having highest germination (75%) followed by IC501847 (70%). During the ageing period of 48 hr with seed moisture content of 30 per cent, all the genotypes showed rapid decline in germination even below the minimum seed certification standards (70%). Whereas at 25% SMC showed the similar trend as observed in 24 hr *i.e.*, highest germination in EC993703 (79%) and lowest germination in EC993752 (70%) (Plate 3).

**TABLE 4**  
**Soybean seedling length and seedling dry weight under different conditions of CDT**

Genotypes	Control	Mean seedling length (cm)				Control	Mean seedling dry weight (mg)			
		CDT for 24 h		CDT for 48 h			CDT for 24 h		CDT for 48 h	
		SMC @ 25%	SMC @30%	SMC @ 25%	SMC @30%		SMC @ 25%	SMC @30%	SMC @ 25%	SMC @30%
EC993987	22.5	17.3	13.0	14.1	12.6	51.4	43.4	33.7	37.5	33.4
EC993937	24.4	19.2	14.9	16.0	14.6	52.4	44.4	34.7	38.5	34.2
EC993703	25.9	21.6	17.3	18.4	16.7	53.2	46.0	36.3	40.1	35.7
EC993752	22.8	17.6	13.3	14.4	12.8	51.3	43.3	33.6	37.4	33.0
EC892808	24.1	18.9	14.6	15.7	14.3	52.3	44.3	34.6	38.4	34.2
EC993608	22.9	17.7	13.4	14.5	13.1	49.1	41.1	31.4	35.2	31.1
EC993850	26.8	21.6	17.3	18.4	16.8	54.5	46.0	36.3	40.1	35.9
J202	21.2	16.0	11.7	12.8	11.4	47.9	39.9	30.2	34.0	29.7
IC39088	24.4	19.2	14.9	16.0	14.6	49.9	41.9	32.2	36.0	31.8
IC501847	24.7	19.5	15.2	16.3	14.9	53.1	45.1	35.4	39.2	35.1
S.Em±	0.36	0.29	0.17	0.26	0.20	0.72	0.62	0.45	0.54	0.42
CD (p=0.01)	1.12	0.86	0.51	0.76	0.66	2.12	1.86	1.35	1.61	1.29
C. V. (%)	2.71	2.66	2.07	2.52	2.19	2.59	2.50	2.32	2.40	2.30

The higher reduction in germination of white genotypes might be due to production of higher amount of free radicals during ageing which tends to enhanced cell damage and caused less metabolic activities and less production of essential enzymes required for germination and less production of antioxidants counteracting the free radicals. These results were found similar to the previous findings of Santos *et al.* (2003).

The results of mean seedling length of different soybean genotypes is presented in Table 4. There was a significant difference in mean seedling length among all the genotypes. Among the white genotypes, genotype EC993703 was having highest mean seedling length at the end of 24 hr ageing duration having seed moisture content of both 25 and 30 per cent (21.6 cm and 17.3 cm respectively) whereas among black genotype EC993850 was having highest germination (21.6 cm and 17.3 Soybean respectively) at the end of 24 hr ageing duration having seed moisture content of 25 and 30 per cent (Plate 4). During the ageing period of 48 hr with seed moisture content of 25 and 30 per cent, all the genotypes showed rapid decline in mean seedling length. This reduction in seedling length over the period of ageing might be due to, seed ageing hinders cell expansion and cell division more strongly which is greatly affecting its shoot and root elongation (Holfmfridur *et al.*, 2009).

The results of mean seedling dry weight of different soybean genotypes is presented in Table 4. There was a significant difference in mean seedling dry weight among all the genotypes. Among the white genotypes, genotype EC993703 showed highest mean seedling dry weight at the end of 24 hr ageing duration having seed moisture content of both 25 and 30 per cent (46 and 36.3 mg respectively) whereas among black genotype EC993850 showed highest mean seedling dry weight (46 and 36 mg respectively) at the end of 24 hr ageing duration having seed moisture content of 25 and 30 per cent. During the ageing period of 48 hr with seed moisture content of 25 and 30 per cent, all the genotypes showed rapid decline in mean seedling length. When the seeds are subjected to ageing treatments, the pathways for synthesis of required enzymes is hindered and incapable to have its specific function to break the food reserves which ultimately hinders root and shoot growth and lessens its dry weight during ageing. Similar results of decreased mean seedling dry weight over ageing period at high moisture content was reported by Maryam *et al.* (2014).

The results of seedling vigour indices of different soybean genotypes are presented in Fig. 1 and 2. There was a significant difference in seedling vigour indices among all the genotypes. Both white and black genotypes showed highest vigour at the end of 24 hr ageing duration having seed moisture content of 25 per cent, because of the higher seed germination and

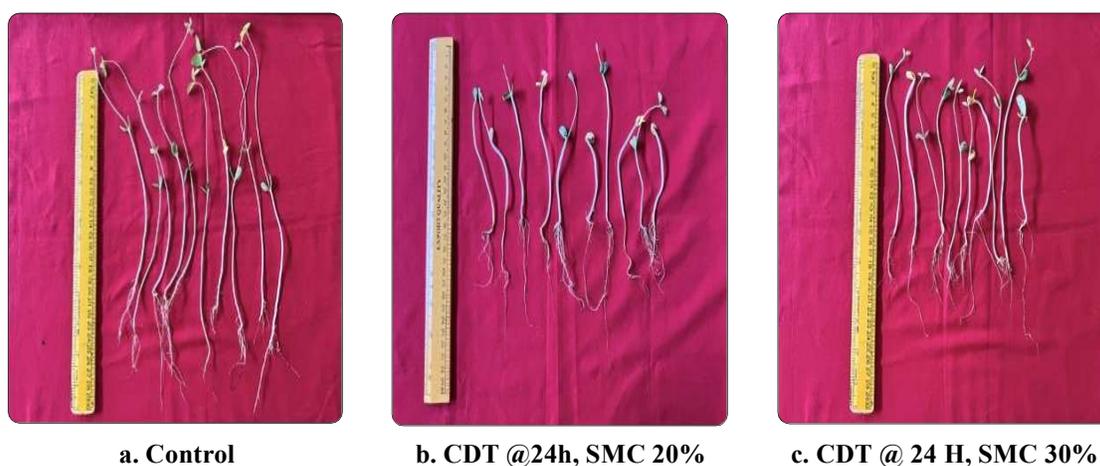


Plate 4 : Soybean seedling length at different CDT conditions

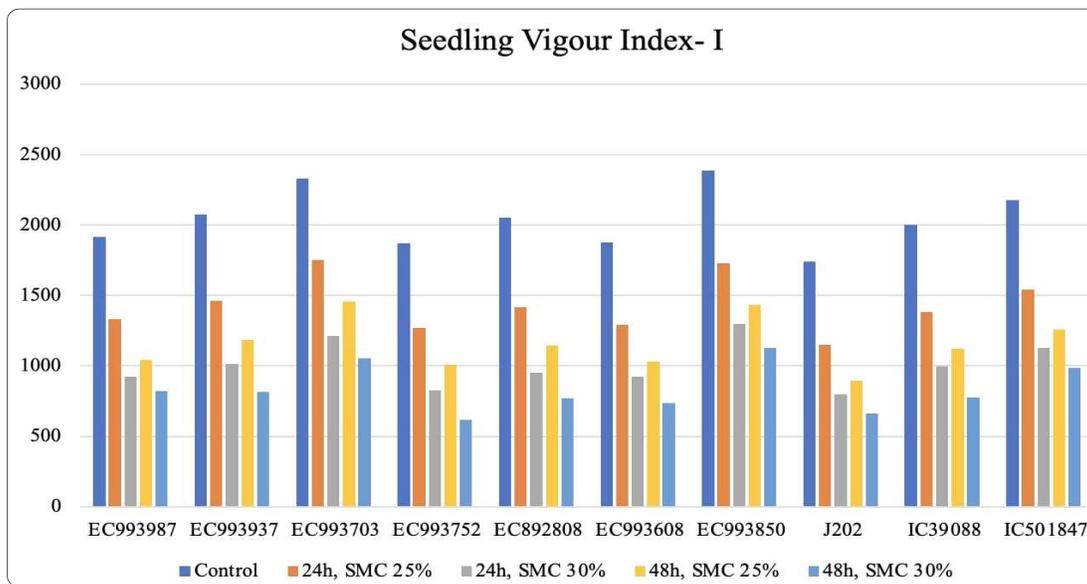


Fig. 1 : Soybean seedling vigour index I at different conditions of CDT

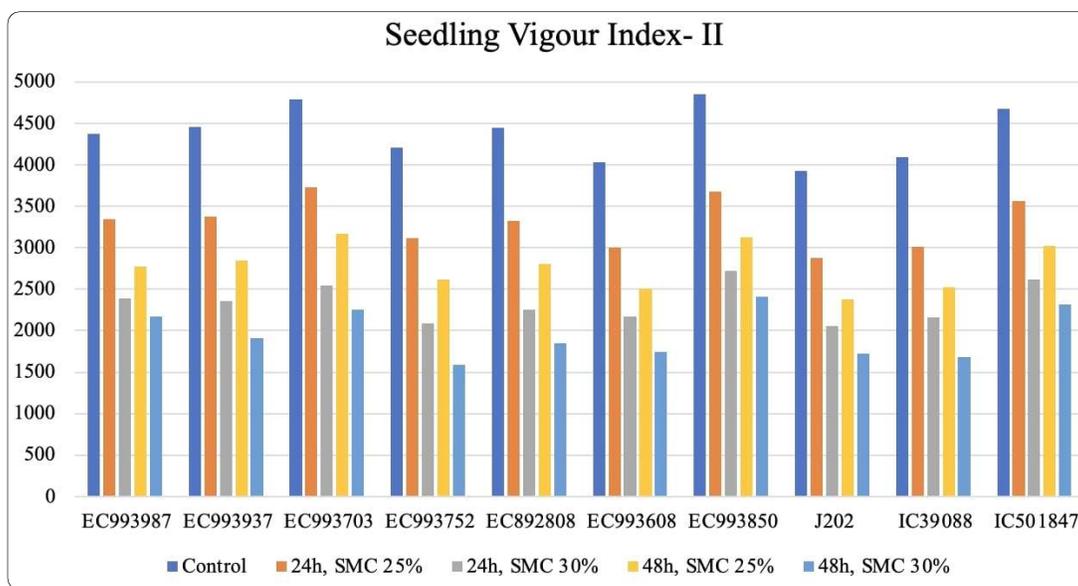


Fig. 2 : Soybean seedling vigour index II at different conditions of CDT

seedling length as well as dry weight at the same condition, since vigour indices are the cumulative effect of these parameters. Among white genotypes, EC993703 (1750 and 3726 respectively) and among black genotypes, EC993850 (1728 and 3680 respectively) recorded highest seedling vigour indices(SVI-I and SVI-II) because of higher germination and seedling length, dry weight. During the ageing period of 48 hr there is decline in vigour indices because of the decline in seed germination

percent and also with seed moisture content of 30 per cent, all the genotypes showed decline in germination even below the minimum seed certification standards (70 %).

The decrease in the seed vigour during storage may be attributed to natural ageing effects, leading to depletion of food reserves due to respiration and metabolic activity of seed and decline in biological activity of the embryo (Gupta and Singh.,1993).

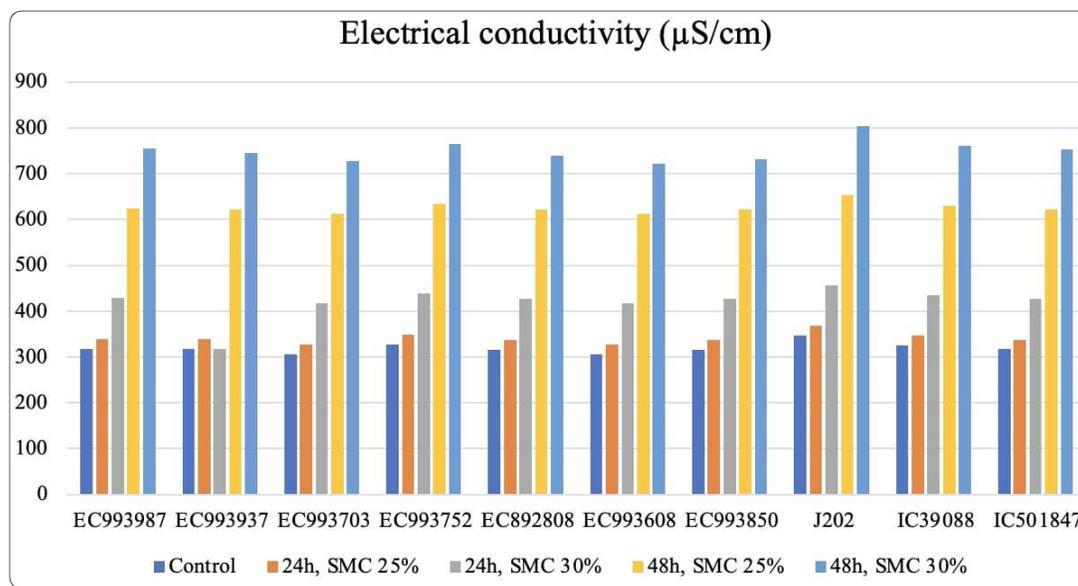


Fig. 3 : Electrical conductivity of Soybean seeds at different conditions of CDT

Studies indicated that oligosaccharide which has been associated in stabilizing membranes decreased during the storage, due to non-enzymatic browning reaction between the carbonyl groups of carbohydrates and amino groups of amino acids and proteins (Crowe *et al.*, 1992).

The results of electrical conductivity (EC) is presented in Fig. There was a significant difference in mean seedling dry weight among all the genotypes. But with the increase in the level of deterioration, electrical conductivity was also found to be increasing irrespective of the genotypes. This rapid increase in EC with the level of seed deterioration might be due to higher rate of leaching of electrolytes, reduction in soluble sugars and potassium content and decrease in dehydrogenase activity in the seeds (Doijode, 1997).

The storage potential of seeds is influenced to a great extent by moisture content, the relative humidity and temperature of the atmosphere surrounding the seed. It has been already documented that the higher the storage temperature and seed moisture content, the shorter was the period of viability (Barton, 1961). Because at higher the storage temperature and seed moisture content, the rate of respiration is also high, which in turn hastens the deterioration process.

In this study, positive trend was observed between seed moisture content, duration of ageing with seed deterioration. As the seed moisture content increased, there is an increase in seed ageing and also with duration of ageing. Among the different regimes of seed moisture content (25% and 30%) and duration (24 and 48 hours) of CDT, seeds with 25% moisture content subjected for 24 hours ageing was standardised to be best for estimating vigour.

This study revealed that, among the CDT conditions there was a noticeable difference observed between duration of CDT *i.e.*, 24 hrs and 48 hrs. CDT with 25 per cent seed moisture content (SMC) 24 hr recorded better results in all tested seed quality parameters, in both white and black soybean genotypes, while other CDT conditions studied showed the quality parameters below the minimum certification standard. Hence Artificial ageing with controlled deterioration test (CDT) of soybean genotypes was found to be more effective with moisture content of 25.0 per cent and duration of 24 hr at 45°C, which can be used as standardised CDT condition in assessing the storability of soybean genotypes. These genotypes artificially aged with CDT were further compared with the naturally aged seeds by testing the seed quality parameters on a monthly basis to know the impact.

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