“सोयबीन में बीज जीवनक्षमता और ओज की संबद्धता वाले बीज आवरण अभिलक्षण”

“Seed coat characteristics in relation to seed viability and vigour in soybean”

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“Seed coat characteristics in relation to seed viability and vigour in soybean”

A Thesis

By

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CERTIFICATE

This is to certify that the thesis entitled **“ Seed coat characteristics in relation to seed viability and vigour in soybean ”** submitted to the faculty of the Post Graduate School, Indian Agricultural Research Institute, New Delhi in partial fulfillment of **Master of Science in Seed Science and Technology,** embodies the results of *bonafide* research work carried out by **Mr. C Balachandan Gowda, Roll No. 20733** under my guidance and supervision, and that no part of thesis has been submitted for any other degree or diploma.

The assistance and help availed during the course of investigation as well as source of information have been duly acknowledged by him.

Date:
Place: New Delhi **(S.K. Jain)**

Dedicated to My beloved Parents and sister

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LIST OF ABBREVIATIONS

m	:	meter
g	:	gram
ha	:	hectare
mha	:	million hectare
%	:	per cent
mm	:	milli meter
ml	:	milli litre
mg	:	milligram
µ	:	micro
µl	:	micro litre
ISTA	:	International Seed Testing Association
viz.	:	namely
etc.	:	et cetera
et al.	:	and other workers
/	:	per
RH	:	Relative humidity
°C	:	Degree centigrade
cm	:	centimeter
GAE	:	Gallic acid equivalent
nm	:	nano meter
OD	:	Optical Density

G%	:	Germination percentage
CD	:	Critical difference
BSA	:	Bovine Serum Albumin
GR	:	Glutathione reductase
GSSG	:	Oxidized glutathione
h	:	hour
min	:	minute
EC	:	Electrical conductivity
MBTH	:	3-methyl benzthiazolinone-2-hydrazone
TBA	:	Tetra Butyl Ammonium hydroxide
NADPH	:	β Nicotinamide Adenine Dinucleotide Phosphate
ROS	:	Reactive oxygen species
rpm	:	rotation per minute
t	:	tonne
w/ v	:	weight per volume
ΔA	:	change in absorption
Fig	:	figure
ng	:	nano gram
mt	:	million tones
t-test	:	Student t test

1. INTRODUCTION

Soybean [*Glycine max* (L) Merrill] is a native of eastern Asia, belongs to botanical family *Fabaceae*. The soy is derived from the Japanese word Shoyu, which means soy sauce. It used to be a considered a pulse crop, but due to high oil content, it has been now placed in the category of oil seed crops. It contain 20 percent oil and 38-42 percent high quality protein possessing high level of essential amino acids like lysine (5%), minerals (4%), phospholipids (2 %) and vitamins A and D (Hymowitz and Harlan, 1983). It is also 'treated as manmade meat' because of its rich source of protein and fat. Soybean also has medicinal value and helps in prevention as well as treating chronic diseases like heart ailments, osteoporosis, cancer, kidney ailments and menopausal syndromes. Hence, it is used in manufacturing variety of processed food products.

Soybean is a grain legume considered as the miracle crop of 20th century or 'Golden bean' because of its versatile nutritional qualities. It was introduced to India during 1880's. It is the second largest oilseed crop in India after groundnut. It is being grown in varied agro-climatic conditions. In recent past it has emerged as one of the important commercial crops in many countries.

Globally soybean is grown over an area of 91.4 million ha with a production of 204.0 million tonnes producing 2233 kg per ha. The commercial cultivation of soybean has been widely adopted both in Northern and Southern India. In India, it is grown on an area of 12.03 mha with annual production of 11.8 mt with an average productivity of 1079 kg per ha (Anon 2016). The year 2016 was considered as the "international year of pulses". It is estimated that there are hundreds of soybean varieties, including many local varieties that are not exported or grown worldwide. The genetic diversity of these varieties is an essential component for on-farm soil, especially for small-scale farmers. Farmers can also choose from numerous varieties of pulses to select those that are more resilient to drought, floods or other effects of climate change. Increasing species diversity of cropping systems could translate into not only a more efficient use of resources but also helps in getting higher returns due to increased yield and a low risk of crop failure (Anon 2016).

Quality seed is the prime factor, which decides the crop productivity for any successive seed programme. It is measured by vigour and viability, which play a major role in seedling establishment and ultimately higher crop yield. Maintenance of seed viability has been a matter of great concern to mankind, since the dawn of the agrarian civilization. Modern agriculture demands that each and every seed should readily germinate and produce a vigorous seedling ensuring high yield. Soybean seed germination and vigour potential is low compared to other grain crops. As it is a poor storer, it looses its vigour and viability very quickly. A major cause for low vigour and viability has been identified as seed ageing. Maintenance of seed viability and vigour up to prescribed germination standard (70%) from harvest to planting is one of the major problem, which is associated with the seed production of soybean in tropical and sub-tropical regions *viz.* India; high temperature and humidity make it difficult to maintain its viability during storage. In most tropical countries the unsold carryover commercial soybean seeds are generally stored without refrigeration for a period of 7-8 months before they are sown in the next season. Under such conditions, its vigour as well as viability is lost rapidly, making it difficult for farmers to use their own seed for planting in the next season. Seed is a biological entity and deterioration is unavoidable beyond physiological maturity until next planting or death of seed. Seed deterioration leads to reduction in seed quality, performance and stand establishment (McDonald, 1999).

At physiological maturity the soybean seed reaches its maximum potential for germination and vigour. This potential is short lived and reduces substantially. A number of seed characters such as seed size, percent hard seededness, permeability, oil content etc are reported to be associated with seed quality in soybean and are under genetic control.

The seed coat is the seed’s primary defence against adverse environmental conditions. The seed coat (often referred to as testa) is therefore, the main modulator of interactions between the internal structures of the seed and the external environment. A hard seed coat protects the seed not only from mechanical stress but also from microorganism invasion and from temperature and humidity fluctuations during storage.

Phenolic compounds in the seed coat contribute to seed hardness and inhibition of microorganism growth. Lignins, complex, phenolic polymers found in the cell wall, are believed to contribute to the compressive strength, resistance to degradation by microbial attack, and water permeability to the polysaccharide protein matrix of the cell wall(Francisco *et al.*, 2001).Soybean seed testa consists of an epidermis of one cell layer of highly cutinized palisade cells, a hypodermis of single layer of large cells with thick anticlinal walls having intercellular spaces, and an inner parenchyma composed of 6–8 layers of flattened thin-walled cells (Carlson 1973). Pereira and Andrew (1985) examined the seed coat characteristics of soybean seeds through scanning electron microscopy and reported that the hourglass cells lying in between the internal palisade and parenchyma cell layers become distorted at the seed coat wrinkle site resulting in seed coat damage.

The seed coat is one of the main determinants of seed germination, vigour and longevity potentials. The soybean seed is highly susceptible to field weathering and mechanical damages which adversely affect its longevity. It is also intimately associated with temporal and spatial dispersion of seed germination.

Research gaps

- There is no inter-varietal reports on changes in the defence signals including anti-oxidative enzyme activity during ageing for protecting the seed from loss of viability.
- Hence, seed coat characteristics in relation to seed viability and vigour of soybean genotypes showing contrasting longevity patterns, needs to be addressed to establish this relationship

In order to develop soybean varieties with better seed longevity role of the seed coat need\s to be understood. Therefore, the present study was undertaken to examine the variations in the seed coat properties of soybean varieties, which directly or indirectly affect the physiological properties and longevity of seeds.

Objectives:

1. To study the seed coat structure in soybean genotypes and its constituents in fresh and stored seeds
2. To understand the correlation of seed coat structure with seed viability and vigour.

2. REVIEW OF LITERATURE

In India the production of soybean is impeded by the lack of maintenance of minimum standards of seed germination and vigour from harvest to the immediate growing season. There are overwhelming evidences that seeds go through a series of physiological and biochemical changes before they finally lose their viability, hitherto the critical factors and the mechanism underlying loss of viability remains unknown. Factors like, genetic variability, mechanical factors, physical structure and shape, pre-harvest and maturation factors, storage environment (seed moisture, relative humidity and temperature), chemical constituents and changes in macromolecules of seed, physiological condition of seed and physical soundness (including microorganisms and insects) are the major factors affect the loss of germination and vigour of seed. The central features of transition from viable to non-viable state in dry seeds include damage to membrane systems in all parts of cell and differential stability of different enzymes. Neither the losses in seed quality can be stopped nor can they be reverted during storage. Bhatia (1996) reported large variations in seed deterioration in soybean, owing to field weathering, whereas mechanical damages by Carbonell and Krzyzanowsky (1995) and physio-biochemical parameters (Ellis et al 1982; Kumar et al 2005).

Further, there is need to understand the genotypic variability in terms of viability of seeds during storage. Keeping these constraints in view, the review of literature pertaining to the objectives of the present investigation on soybean has been presented in this chapter. For convenience the chapter is divided into the following sub headings:

2.1 Seed coat characteristics vis-à-vis seed quality

Zhou et al (2010) observed that seed coat protects seeds against deterioration; and makes seed maintain high quality and viability during storage. Shao et al (2007) and Koizumi et al (2008) reported that seed coat plays an important role in the process of water imbibition and prevents seeds from soaking damage or soaking injury during seed germination or food processing. Mechanical injury can occur at any time during harvesting, drying and conditioning of seeds was shown by (Delouche 1974). Delouche (1974) and Rojanasaroj et al (1976) observed that mechanical damage to an individual seed can include formation of cracks or breaks in the seed coat, cracks in cotyledon, injury or breakage of hypocotyls-radical axis and complete breakage of seed to the point where it would no longer be classified as part of pure seed fraction. Paulsen et al (1981) observed that the varieties having bold seed size were found to have greater percentage split and seed coat damage.

2.1.1 Seed coat colour and seed hilum colour

Green et al (1965) and Delouche (1975) reported that seed size and seed coat colour in soybean plays an important role in maintaining and preserving the seed quality. Poor viability and field emergence due to its inherent composition and seed structure have been reported in soyabean (Bhatnagar 1990; Vijay and Dadlani 2003). Resistance to field weathering in black-seeded lines of soybean is because of thicker seed coat than that of yellow-seeded lines reported by Dassou and Kueneman (1984). Black, smaller soybean seed with grey hilum attributed to better seed quality (Sooganna et al 2015).

Due to physiological, pathological or mechanical causes, seed morphology, anatomical features makes soybean more susceptible to damage than other plant species and results in poor quality seeds (Lori et al 2001). Simic et al (2006) reported that the rafts of different events contribute to loss in seed quality including long duration seed storage. There is great variation in the colour and luster of the seed coat and hilum. Bernard and Weiss (1973) reported that three independent genetic loci *ie I, R* and *T* controlling the seed coat pigmentation in soybean. The type of anthocyanin and pro-anthocyanidin synthesized, are determined by *R* and *T* loci by which a specific seed coat colour is determined as follows: black (*R, T*), imperfect black (*R, t*), brown (*r, T*) and buff (*r, t*). Toda et al (2002) reported that the *T* locus encodes a flavonoid 3′-hydroxylase (F3′H) responsible for synthesis of the cyanidin-based anthocyanins and proanthocyanidins. The (*I*, *ii, ik* and *i*) are the four alleles of *I* locus (inhibitor), which determines the spatial distribution of pigments in the epidermal layer of the seed coat. The production and accumulation of pigments over the entire seed coat, inhibited by *I* allele that resulting in uniformly yellow coloured seeds, whereas the *I* allele leads to completely pigmented seeds by allowing the production and accumulation of pigments over the entire seed coat. The remaining two alleles *ii* and *ik* inhibit pigmentation except in hilum and a saddle-shaped region, respectively. *I* allele for a light (non-pigmented) hilum or the *ii* allele for a dark (pigmented) hilum carried by yellow soybean. Storability among different soybean genotypes is the resultant of different genetic constitutions in different genotypes (Hosamani et al 2013). The difference is also due to lack of fungal growth in black seeded soybean and profuse growth of fungi in yellow seeded soybean seeds (Kueneman and Wein 1981). The yellow seeded temperate varieties such as Clark-63, Lee and Bragg have poor storability than the better storer black-seeded land race varieties of tropical and subtropical regions, such as T-49; and Kalitur was reported in separate studies by Singh and Ram (1986).

Some seed characteristics of soybean such as, hard seed coat were found to be beneficial in maintaining seed viability and vigour (Tiwari and Bhatia 1995), smaller seed size (Paschal and Ellis 1978; Tiwari and Joshi 1989), black seed coat (Dassou and Kueneman 1984) and tight attachment of the seed coat to the cotyledons (Kuchlan et al 2010). The effects of different threshing methods on soybean cultivars (Giza-21, Giza-35 and Giza-111) yield, seed quality and its longevity during storage was evaluated by conducting an experiment by (Abady et al 2012). Their results among soybean cultivars in all parameters studied showed significant differences. On seed yield, germination percent, germination after accelerated ageing, seed and seedling vigour; Giza-21 surpassed Giza-35 and Giza-111. They also reported that with increase storage period up to six months seed viability of soybean cultivars decreased gradually. Kueneman and Wein (1987). Hosamani et al (2013) also evaluated black and yellow seeded varieties of soybean and found that black seeded genotypes were better storers than yellow seeded genotypes. The good storability of soybean seeds can be attributed to the combined effect of seed coat colour, seed size and seed coat hardiness (Zahid 2013).

2.1.2 Seed coat percentage

The proportion of the seed coat is of primary significance in providing guard against mechanical injury during harvesting and processing, which cause noteworthy loss in viability of a seed lot. The black-seeded varieties, ‘Kalitur’ and ‘Birsa Soya 1’, had maximum seed coat to seed proportion (Kuchlan et al 2010). Dassou and Kueneman (1984) reported that black-seeded lines of soybean were defiant to field weathering for the reason that of thicker seed coat than that of yellow-seeded lines. Higher fraction of the seed coat was not correlated with higher seed weight.

2.1.3 Mechanical strength of the seed coat

There was a significant reduction in seed coat hardiness with increase in storage period. The reduction in seed coat hardiness was much more under ambient seed storage than controlled storage (Zahid 2013). Some characteristics of soybean seeds such as, hard seed coat were found to be very beneficial in maintaining seed viability and vigour (Tiwari and Bhatia 1995). In soybean seeds mechanical strength of seed coat had positive correlation with seed storability was reported by Kuchlan (2006). The gap between the cotyledon and seed coat was very thin and uniform in good storer varieties *viz* ‘Birsa Soya 1’, ‘Kalitur’, ‘JS 335’, ‘JS 80-21’, and ‘NRC 2’. On the other hand, this gap was wider in poor storer varieties *viz* ‘PK- 472’, ‘PS-1029’, and ‘NRC-7’. The gap was widest in the region adjacent to hilum in these varieties. The gap between the seed coat and cotyledon makes the seed susceptible to cracking, even during the normal course of processing. Larger gap augments its vulnerability, which was the case in the poor storer varieties. Lesser gap was associated with higher mechanical strength of the seed coat was reported by Kuchlan et al (2010).

2.1.4 Thickness and structure of seed coat (thickness of epidermal and hypodermal layer and length of anticlinal cells)

Verma and Gupta (1975) reported that thicker seed coat tissues in small seeded soybean seeds owing to maintenance of viability which is in contrary to bold seeds where seed viability is not better maintained (Smith and Circle 1978). Soybean seed testa consists of an epidermis of one cell layer of highly cutinized palisade cells, a hypodermis of single layer of large cells with thick anticlinal walls having intercellular spaces, and an inner parenchyma composed of 6–8 layers of flattened thin-walled cells (Carlson 1973). Pereira and Andrew (1985) examined the seed coat characteristics of soybean seeds using scanning electron microscopy and reported that the hourglass cells lying in between the internal palisade and parenchyma cell layers become distorted at the seed coat wrinkle site resulting in seed coat damage. These hourglass cells, with a cushioning effect, help in preventing the wrinkle caused by the hydration and dehydration. However, varietal assessment for this character has not yet been reported. Seed coat surface properties, particularly the presence of pits (pores) and deposits also influence the water permeability, fungal invasion, and the seed longevity in soybean (Calero et al 1981; Wolf et al 1981; Hill and West 1982)

Hourglass cells are not present in the hilum region, but there is a thick parenchymatous layer containing vascular tissue as well as remains of placenta tissue attached to the outer epidermis. This structure indicates that the testa epidermis might be the primary barrier to hydration damage and leakage of intracellular constituents during the imbibition was reported by Carlson (1973).

2.2 The physiological parameters and seed quality

Seed deterioration refers to all those processes which contribute to the loss of vigour and viability of the seeds. Since, soybean is considered as a poor storer, its vigour and viability gets depleted upon storage. The depletion of vigour and viability of soybean seeds may be much pronounced when stored in harsh conditions than controlled storage. Good-storer genotypes were characterized by lower electrolyte leakage with smaller seed size and black testa, whereas poor storer registered more electrolyte leakage and were bold seeded with yellow testa. Strong and positive association between field emergence and standard germination; vigour index and seedling emergence index, whereas there was a negative correlation between 100-seed weight and electrical conductivity (Kharb et al 1994).

2.2.1 100-seed weight

Seed size plays an important role in preserving soybean seed quality. Seed size varied among genotypes. 100-seed weight was found to be negatively correlated with seed quality. Smaller seeded genotypes, namely EC-105790, G-2651 and M-253 were better performer (Sooganna et al 2015). Mechanical damage during harvesting, handling and processing, is likely to cause extensive damage to large, long and irregularly shaped seed varieties which is in contrary to small seeds which are unlikely larger seeds, they escape this injury (Roberts 1972). Burris et al (1971&1973) reported that larger soybean seeds produced larger embryos, exhibited higher respiratory rates, and possessed greater field emergence than small seeds. Gupta (1976) reported that physiologically large seeds of Lee soybean were found by to have higher respiratory rates, greater shoot length, and less leakage of sugars than small seeds. Large seeds germinate more rapidly and had greater field emergence than small seeds (Hopper et al 1979). Edwards and Hartwig (197l) reported that small soybean seeds have faster emergence and better root development. A small-seeded variant of Lee had better germination, greater early hypocotyl development, and lower leakage of sugars than the large-seeded type (Gupta 1976). In several studies, genetically small seeds have been shown to have superior viability (Calero et al 1981; Nangju 1979; Singh et al 1978). However, several reports also suggest that there is no relationship between soybean seed size and germination or field emergence (Fontes and Ohlrogge l972; Johnson and Luedders 1974; Johnson and Wax 1978; Singh et al 1972; Smith and Camper I975). Large seeds tend to be more susceptible to mechanical damage than small seeds (Paulsen et al 1981). Viability in soybean is reported to be maintained better in smaller than in larger seed because small seeds tend to have thicker seed coat tissue (Smith and Circle 1978). The possible explanation for the relationship between the small size and good quality is the positive correlation between the seed size and seed coat permeability (Vyas et al 1990).

2.2.2 Seed germination

Germination is a process dependent on multiple variables, including the integrity of seed and its physiological state. Seed ageing is known to damage cellular and sub cellular structures such as membranes, storage bodies and organelles (Abdul –Baki 1980). Krittigamas et al (2001) reported that soybean seeds stored for six months at a temperature of 15°C maintained high germination (95%) and vigour, a cool storage environment maintained at 60% relative humidity. Zamin et al (2010) reported that the water uptake is an important step towards the initiation of biochemical changes that lead to germination completion. Kuchlan et al (2006) found that after 12 months of ambient storage, 18–30% decrease in germination was recorded in different varieties of soybean. Black and yellow seeds from a bulk of F_4 population derived from the cross between black and yellow seeded parents and establish that the germination of yellow seeds declined faster in storage than compare to that of black seeds (Starzinger and West 1982). Delouche (1973) reported that seeds are uniquely equipped to survive as viable regenerative organisms for a long time; however, like any other form of life, they do eventually deteriorate and loose viability. Seed deterioration is an inexorable, irreversible process which varies among seed populations. Mc Donald (1999) reported that seed deterioration leads to reduction in seed quality, performance and seedling stand establishment and ultimately yield. Basavarajappa et al (1991) reported that the intrinsic factors that are believed to be closely associated with the seed deterioration are loss of membrane integrity, alteration of chemical composition, particularly of macromolecules (Wettlaufer and Leopold 1991; Bailey et al 1996; Ravi et al 1998), changes in enzyme activities, depletion of food reserves (Ravi et al 1998) and genetic aberrations (Elder and Osborne 1993). Tian et al (2008) and Lekic (2003); Tatic et al (2009) observed that accelerated aging of seed is an excellent method to determine the vigour changes during seed storage. The seed aging is characterized by the loss of germination, reduced speed of germination and poor seedling development. This would help not only to identify reasons for improving seed storage life but also to provide information that would enable incorporation of trait for better storability in the genetic background of the high yielding varieties (Kapoor et al 2011). Seed deterioration results in decreased rate of germination and percentage of normal seedlings was indicated by Mohammadi et al (2011).

2.2.3 Electrical conductance from seed leachates

Seed impermeability or permeability is determined by the seed coat (Shao et al 2007). Duke et al (1986) reported that testa is extremely important in protecting the solute from injury by rapid hydration and in maintaining seed viability. The evidences indicate that accelerated aging treatments (which progressively lower seed vigour) cause a marked lowering of early respiration of isolated cotyledons, increase in leakage of electrolytes (David et al 1977). Parrish & Leopold (1977); Meyer et al (2007) reported that the soaking damage of seed takes place in the early stage of imbibition. Meyer et al (2007) observed that the tissue hydration occurs in a controlled way and the internal structures of the cell and organelles are not affected due the presence of seed coat.

Gupta and Aneja (2004); Tatic et al (2009) reported that seed aging is generally marked by reduction in vigour. The germinability and vigour of seeds of different sizes showed that the largest seeds and the low density seeds performed worst in the standard germination test. Single seed leachate conductivity levels were highest for large seeds and low density seeds, indicating low vigour. Bulk conductivity tests showed high levels of leakage in large seeds, but did not detect differences between seeds of high and low density (Hov and Gamble 1985). The Automatic Seed Analyzer (ASA- 610) has been used to predict the germinability of soybean seeds based on the current level of the seed steep water of individual seeds (Mc Donald and Wilson 1980; Steere et al 1981).

Preliminary viability was same (100%) among DPX and Sahar cultivars, the vigour parameters like, electrical conductivity, seedling dry weight and seedling normal percentage had no significant difference between DPX and Sahar cultivars but in Sahar, values of all vigour trials except EC were higher than DPX (Khaliliaqdam et al 2012). Similar conclusions were reported by Kalavathi et al (1994); Venkatareddy et al (1992). Soybean seed deterioration at low storage temperature does not seem to be directly related to the loss of the cell membranes integrity reported by Panobianco and Viera (2007). The cell membrane permeability or rupture was a major factor contributing to the loss of germination after ageing (Robert and Sherlie 1985). Chan (1987) reported that due to ageing, the behaviour of membranes can change as a consequence of unregulated chemical reactions that occur when the tissues are dried. The free fatty acids cause membrane lesions at all moisture levels regardless of the presence of protectants (Crowe et al 1989). Ferguson et al (1990) reported that the damage to membranes during ageing is an important characteristic of deteriorated seed. During ageing, due to contraction plasma membrane vesiculates and the loss of surface area can result in lysis (or) liposomes that fail to swell upon subsequent rehydration (Steponkus et al 1990).

2.2.4 Vigour indices

Vigour indices, in general, decreased with increase seed storage and may serve a good marker of seed quality (Sooganna et al 2015). Johnson and Wax (1978) observed that a number of soybean seed vigour tests are available through seed testing laboratories for use by seed producers and farmers. Laboratory tests of vigour are often employed to predict the performance of seeds under field stress. The standard germination test is the most common, but appears to be incapable of detecting differences in field emergence potential. Among the vigour tests which have been used to predict field emergence are the seedling classification test (Woodstock 1973; Mc Donald 1975), and the conductivity test (Mc Donald 1975). The vigour traits like seedling length and seedling dry weight also differed significantly between the varieties, Giza-111 surpassed Giza-35 and Giza-2; and also the seedling dry weight reduced significantly in six months stored seeds (Abady et al 2012). Seedling establishment and emergence rate is reduced with increased seed storage duration (Verma and Tomer 2003), indicated that environmental conditions and seed aging affected emergence traits and vegetative characteristics in wheat (Soltani et al 2009). The most of the deleterious effects of poor seed vigour are rate of germination and early seedling growth in wheat. Therefore, it was concluded that reduced leaf area and seedling dry weight may be due to the indirect and direct effects of seed ageing (Roberts and Ellis 1989). Heydecker (1972) proposed that the reduction in seedling growth and enhanced susceptibility to stress conditions are good indicators of declining seed vigour. During storage, seed undergoes vigour loss at a rate proportional to the prevalent conditions of temperature and relative humidity. Paschal and Ellis (1978); Ndimande et al (1981) reported that decrease in seed vigour due to pre-harvest environmental conditions and incidence of pathogens was reported in soybean. Dey and Basu (1982); Varier and Agrawal (1992); Mathur (1992) reported that the loss in seed vigour could be estimated in terms of reduced seedling growth as well as enhanced susceptibility to stress conditions during germination (Musgrave et al 1980; Mugnisjah and Nakamura 1986; Shanmugavel 1993). Zahid (2013) reported that seed germination and seed vigour decreased with increased period of seed storage. Seed viability implies the possibility of forming a new plant both under favourable and adverse climatic conditions. Milosevic et al (1995) reported that it is believed that high vigorous seed has uniform emergence ability in the field and thus yield stronger plants that provide higher yield. Seed vigour is related to many other components of physiological seed quality, such as viability and germination changes in overall seed quality that occurs during seed development, maturation, harvesting, conditioning and storage. Balesevic et al (2000); Tatic (2007); Mendes and Moeras (2009) reported that seed vigour testing is used as an indicator of the seed storage potential and proves to be a more reliable indicator than germination test. Attaining the highest level of vigour and viability, a non-dormant seed lot should theoretically record 100% germination at physiological maturity. Powell and Mathews (1984) reported that the symptoms of ageing include reduced rates of germination and field emergence, decreased tolerance to sub-optimal conditions and inferior seedling growth, which could be recorded much before the decline in germination itself. David and Leopold (1978) reported loss of vigour, a decline in early respiratory activity, increased electrical conductance and loss of dry weight due to exposure of soybean seeds to accelerated aging (41^0C and 100 % RH) conditions.

Losses in seed quality occur during all processes from maturation in the field to storage which further worsens due to high humidity and temperature (Shatters et al 1994).

2.3 Biochemical parameters

Biochemical phenotyping involved changes in lignin, total phenol content, volatile aldehyde assays and antioxidant enzyme *ie* glutathione reductase (GR). Nelson et al (1950) reported that the principal components of soybean seed coat are cellulosic type of material 49.3%, pentosans 22.6%, lignin 4.5%, ash 5.1% and nitrogen 1.6%. Acetone extracted hulls of soybean contained 64% alpha cellulose, 16% hemicelluloses and 8% lignin which was analysed by Whistler and Saarnio (1957).

2.3.1 Lignin content

Lignins and complex phenolic polymers found in the cell wall are believed to contribute to the compressive strength, resistance to degradation by microbial attack, and water permeability to the polysaccharide protein matrix of the cell wall. Lignin in the seed coat of soybean reportedly imparts resistance to mechanical damage (Alvarez et al 1997). The higher the lignin content in the seed coat, the greater is the expected resistance to mechanical damage. This resistance is a genetic characteristic that varies among soybean cultivars (Carbonell and Kryzanowski 1995). The seed coat lignin content differed appreciably between the cultivars, and did not considerably change after the 12-month storage period. The results of lignin content in the seed coat and the positioning of the cultivars concur with those previously observed by Alvarez et al (1997). The present study of lignin content between good and poor storer showed significant difference but not between fresh and 8 months stored seeds of same genotype. The steadiness of seed coat lignin yet in storage can be explained by the insolubility and intricacy of the lignin polymer. These distinctiveness craft lignin defiant to degradation by most microorganisms (Campbell and Sederoff 1996). Lofty lignin concentration is thought to be allied with disease resistance (Casler 2001). It was reported that lignin is thought to thwart fungal progress by defending polysaccharide substrates from fungal enzymes, or varying wall components to form an mismatched substrate for fungal enzymes Ride (1983), or displaces water, restraining the action of microbes (Jung 1993) and pathogen enzymes (Peltier 2009).

2.3.2 Total phenolic content

Black seeds have more phenols, tannins etc. in their seed coat as compared to yellow seeds. Therefore, presence of such compounds protects the seeds from fungal attack during storage, thus black seeds maintains better viability than yellow seeded genotypes upon storage (Hosamani et al 2013). Phenolics production and composition, including phenol, lignin, and isoflavones could be associated with disease resistance and mechanical damage resistance. Phenolics trait may be used to screen the resistant germplasm in soybean improvement programme (Nacer Bellaloui 2012). Pro-anthocyanidines (*ie* condensed tannins) are the predominant phenolic compounds found in legume seeds, located mainly in the seed coat (hull), play an important role in the defence system of seeds that are exposed to oxidative damage by many environmental factors such as light, oxygen, free radicals and metal ions (Agnieszka et al 2002).

2.3.3 Changes in volatile aldehydes assay

Mc Donald (1999) explained lipid peroxidation model involving free radical mediated oxidation of unsaturated fatty acids is by far the most plausible hypothesis for the mechanism of seed deterioration. The toxic oxygen species such as superoxide, singlet oxygen and hydroxyl radicals are generated either by auto-oxidation or enzymatically by lipoxygenase present in seeds. Various forms of free radicals have been detected in living tissues, each with a capability of causing cellular damage (Larson 1997). The cross linking of macromolecules and alteration of functional proteins in seeds by the endogenously accumulated aldehydes is cited as one of the reasons of seed deterioration under dry storage conditions (Esashi et al 1997). Volatile aldehydes and esterase activity increased with increased period of seed storage, whereas SOD activity consistently declined with increased period of seed storage (Zahid 2013). Mc Donald (1999); Verma et al (2003) emphasized the connection between lipid peroxidation intensity, *ie* peroxidative degradation of fatty acid and changes of their content in seeds during storage, pointing out the length and conditions of storage, as one of the most important factors that determine the degree of seed damage. Thirty-three soybean genotypes, comprising of 14 black-seeded and 19 yellow-seeded, were selected on the basis of their reported storability for the biochemical phenotyping to establish the role of lipid peroxidation and antioxidant enzymes in seed longevity. The study showed clear genotypic variability with respect to storability among different soybean genotypes. Good-storer genotypes with lower electrolyte leakage were characterized by smaller seed size with black testa colour. The level of volatile aldehydes released and lipoxygenase II enzyme activity were higher in the yellow-seeded genotypes than in the black-seeded genotypes. Significantly higher antioxidant enzyme activity was recorded in the black-seeded genotypes than in the yellow-seeded ones, though there was a reduction in hydroperoxide lyase activity during storage in all the genotypes. The viability of black-seeded genotypes after storage for one year was better than the yellow-seeded genotypes (Hosamani et al 2013). Tubic et al (2005) reported that storage conditions and duration are important factors affecting the degree of biochemical changes in seed. An increase in malondialdehyde (MDA) content in relation to control, *ie* the initial state of seed was observed during seed storage, which showed that lipid peroxidation intensity increased. Results confirmed the possibility of determining the level of lipid peroxidation in seed by determination of MDA content. The greater intensity of lipid peroxidation with the difference of 70% in relation to initial state was observed in soybean seed under conventional storage conditions in 12 months. Content of MDA in seed was increased by prolonged storage indicated that lipid peroxidation was more intensive in aged seed. Some changes also occurred in fatty acids content, especially in linoleic acid. Hosamani et al (2013) reported significantly higher antioxidant enzyme activity in the black-seeded genotypes than in the yellow-seeded ones, though there was a reduction in hydroperoxide lyase activity during storage in all the genotypes. Higher level of esterase activity in soybean seed than in safflower appeared to be critical in the release of free fatty acids, which become the source of free radical attack and greater release of aldehydes in the former (Vijay 2005). Robert et al (1979) reported that soybean seeds age rapidly during storage at high temperature and high relative humidity. The axes of such aged seeds contain high levels of malondialdehyde, a product of the peroxidation of unsaturated fatty acids. It is suggested that aging leads to peroxidative changes to lipids and that these could contribute to loss of viability.

2.3.4 Glutathione reductase (GR)

Loss of seed viability has been reported to be associated with a reduction in the activities of antioxidant enzyme *viz* glutathione reductase (GR), which play a significant role in providing protection against highly reactive free radicals both under accelerated ageing and storage conditions (Bailly et al 1996; Kuchlan 2006). Good storers were also characterised by tightly attached, thicker seed coat and a more efficient system of these radical scavenging enzymes (Kuchlan 2006). The enzyme has been reported to involve in a range of metabolic activities, such as in regulation of the level of indole acetic acid (Siegel and Galston 1955), in cell wall formation (De Jong et al 1967), in regulation of membrane permeability (De Jong 1966), in biotic and abiotic stress (Tomiyama and Stahman 1964), in seed dormancy and germination (Major and Roberts 1967). Sung et al (1995) reported that aging is known to reduce seed viability in many crop species. The phenomenon is due in part to the aging-induced lipid peroxidation, which has the potential to damage membranes of the seed tissues. The results indicated that the aging inhibited seed germination and enhanced lipid peroxidation, but with more rapid seed deterioration and a greater extent of lipid peroxidation at the later temperature. Aging also inhibited the activity of peroxidase, catalase, ascorbate peroxidase, superoxide dismutase, glutathione reductase and lipoxygenase. Hosamani et al (2013) reported that good storers showed significant higher antioxidant activity than poor storer genotypes. However, irrespective of the storage pattern, all the genotypes showed reduced enzyme activity during ambient storage. Various enzymatic and non-enzymatic mechanisms play an important role in the elimination (in oxidative stresses) or homeostasis of AOS (for cellular signaling) in plants. Superoxide dismutase which can be mitochondrial (MnSOD), cystolic (Cu/ZnSOD) or chloroplastic (Cu/ZnSOD, FeSOD), dismutates superoxide radicals into H_2O_2 and oxygen (Bowler et al 1992). The ascorbate-glutathione cycle may also take part in H_2O_2 scavenging, which involves ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase. The enzymes of this cycle which are present in the chloroplast, cytoplasm, mitochondria, peroxisomes and apoplast (Mittler 2002) participate in the regeneration of the powerful antioxidants ascorbic acid, reduced glutathione and α-tocopherol. The activity of SOD, CAT and POX were maximum in fresh seeds but decreased in stored seeds (Jain 1997). Kibinza et al (2006) studied sunflower seed deterioration in relation to moisture content during ageing, energy metabolism and active oxygen species scavenging and reported that higher the energy metabolism during ageing, lower was the seed viability. Loss of seed viability is reported to be associated with an accumulation of hydrogen peroxide and then malondialdehyde and the reduction in the activities of antioxidant enzymes *viz* GR (Bailly et al 1996; Kuchlan 2006), which were the main enzymes involved in cell detoxification. However, inconsistent results were reported with respect to the storage patterns and antioxidant activities in the fresh seeds of different genotypes.

3. MATERIALS AND METHODS

The present research experiment entitled **"Soybean seed coat characteristics and seed quality"** was carried out during 2015-2017 both in the laboratory, and at experimental farm of Division of Seed Science and Technology, ICAR-Indian Agriculture Research Institute (IARI), New Delhi.

3.1. Climate and weather

ICAR-Indian Agricultural Research Institute (IARI): New Delhi is situated at $28^{0}35'$N latitude, $77^{0}12'$E longitudes and at an altitude of 228.6 m above mean sea level. It has a semi-arid and sub-tropical climate, characterized with extreme hot summer and cool winter.

3.2. Experimental material

24 soybean genotypes, as experimental material, procured from Division of Seed Science and Technology, and soybean breeder Division of Genetics, ICAR-IARI, New Delhi used from multiplication in the divisional experimental farm during the *kharif* 2015 (list is enclosed as table 1). In order to minimize mechanical injuries, all post-harvest operations including seed threshing and cleaning were conducted manually.

3.3. Reagents and chemicals

All the reagents and chemicals used in the present study were of analytical grade (SRL and Himedia) or above, unless otherwise stated.

3.4. Seed storability

24 genotypes having sufficient quantity of seed were used for storage. About 200 g of untreated fresh soybean seeds from each genotype were packed in muslin cloth bags and stored in Division of Seed Science and Technology, laboratory under ambient storage environment (average 25±2°C and 65±5% RH). At the start of seed storage, germination (%) of all the soybean genotypes was recorded. From the start of storage, seeds were drawn after 8 months and tested for physical, physiological and biochemical parameters. 24 soybean genotypes having contrasting seed longevity were chosen *ie* 12 genotypes each from good (black seeded) and poor (yellow seeded) storers, and used for detailed studies (table 1). Various physical, physiological and

biochemical parameters were studied for its association with seed quality and vigour. (as good and poor storer genotypes were discriminated and identified from previous studies).

Table 1. List of soybean genotypes used as experimental material

SNo.	Genotype
	Good storer
1.	AMSS-34
2.	G-2265
3.	G-2601
4.	G-2603
5.	G-2605
6.	G-2251
7.	G-2253
8.	G-2614
9.	M-1090
10.	M-11913
11.	MACS-1311
12.	TGX (444-422)
	Poor storer
13.	218 (123)
14.	222 (127)
15.	241 (128)
16.	250 (129)
17.	732 (135)
18.	761 (137)
19.	871 (145)
20.	876(146)
21.	888 (150)
22.	PK -472
23.	TAMS-38
24.	EC-13969

3.5. Parameters studied

3.5.1. Physical phenotyping

3.5.1.1. Seed coat colour

Seed coat colour was recorded using RHS colour chart, by visual assessment, at harvest stage, under natural day light.

3.5.1.2. Seed hilum colour

Seed hilum colour was also recorded using RHS colour chart, by visual assessment, at harvest stage, under natural day light.

3.5.1.3 Seed coat percentage

Seed coats from 5g seeds in two replications were removed carefully with the help of forceps after applying a gentle stroke to seeds placed in-between muslin cloth. Seed coat and cotyledons were collected and weighed separately. The total weight of seed coat and cotyledon was considered as the seed weight. The proportion of seed coat was calculated as follows:

$$\text{Seed coat percentage} = \frac{\text{Weight of seed coat} \times 100}{\text{Weight of seed}}$$

3.5.1.4. Mechanical strength of the seed coat

Mechanical strength of the seed coat was measured as the first break point on the graph of seed cracking using the texture analyzer (Stable Micro System) following the method of Kuchlan *et al* (2006). The seed was placed on the stationary plate of the instrument. The plane perpendicular to the plane of the hilum was parallel to the surface of the plate. The force was applied by gradually increasing the force, which was plotted along the Y-axis. The duration of force applied (until the seed is cracked) was plotted along the X-axis. There was a gradual increase in the graph with the increase of force and at a certain point of time there was a sudden drop on the graph which indicated the first break, on the seed coat surface. Further increase in the force along the graph and next drop on the graph was recorded due to breakage and subsequently for the pieces of seed.

3.5.1.5. Thickness and structure of seed coat (thickness of epidermal and hypodermal layer and length of anticlinal cells)

Various steps, as under were followed for studying the seed coat structure and measuring thickness:

Dehydration of samples and block preparation:

Dehydration of samples was done using TBA series

Seed samples:

Fixed in FAA → Wash in 30%/50%/70% Ethanol →55%TBA (2hr)-75%TBA(overnight) → 85% TBA(1hr) → 95% TBA(1hr) → 85% TBA → 95% TBA(1hr) → 100% TBA (overnight) →Paraffin oil:TBA (1:1) (1hr)Paraffin wax + Paraffin oil+TBA in oven →Paraffin wax (overnight) Paraffin wax (blocks).

After that paraffin blocks were prepared. These blocks were subjected to microtome sectioning and drying.

Staining techniques:

Sections → Xylene (2hr or overnight) → Xylene:Alcohol (1:1) (5 min) 95% Alcohol (5min) → 70% Alcohol (5min) → 50% Alcohol (5min) → Safaranin (45 min) → 50% Alcohol (5min) →70% Alcohol(5 min) → 95% Alcohol (5min) → Fast Green (a dip only) → 95% Alcohol (5 min) → Xylene:Alcohol (1:1) (5 min) Pure Xylene → DPX Mount

Thickness of the seed coat was measured using phase contrast microscope (Leica software) which is connected to a computer. Finally, using phase contrast microscope, the thickness of parenchyma cells (epidermis), thickness of palisade parenchyma (hypodermis) and length of anticlinal cells were measured (µm). The stained seed coat sections were viewed using phase contrast microscope which is connected to a computer. Photographs were recorded using the above set up.

3.5.2. Physiological parameters

Seeds of both good and poor storers were subjected to examination of the following physiological parameters.

3.5.2.1. 100-seed weight

Eight replicates of 100-seeds each were taken for determination of seed weight (Singh & Agrawal 1993). 100-seeds were selected randomly and counted manually from the seed sample. The genotypes were categorized into bold (> 10 g) and small (< 10 g) seeded type on the basis of mean 100-seed weight from eight replicates.

3.5.2.2. Seed germination

Seed germination was tested following ISTA rules (Anon 2015) using 400 seeds using between-paper roll towel paper method at 25°C in four replicates of 100-seeds each. Seeds were placed in between two wet blotters 45 cm wide, 30 cm long. The blotters along with the planted seeds were placed vertically in a seed germinator at 25°C. The germinated seeds were evaluated on 08 day from planting and classified into normal seedlings, abnormal seedlings, hard seeds and dead seeds. Seed germination percentage was recorded on the basis of normal seedlings only.

3.5.2.3. Electrical conductance from seed leachates

The electrical conductance from seed leachates was measured following the standard procedure (Anon 2015) with minor modifications. Three replications of 50 seeds each were soaked in 250 ml double distilled water at 20°C for 24 hr. Seed leachates were collected in 250 ml beaker. The electrical conductance from the seed leachates was measured at room temperature with a conductivity bridge (Eutech-Model 215 R) and expressed as µS/cm/g seed.

3.5.2.4. Seedling dry weight

Seedling dry weight was estimated following the standard method (Gupta 1993). Four replicates of 10 normal seedlings were picked up randomly from the germinated seeds. These were subjected to the measurement of seedling growth and seedling dry weight (70°C oven drying for 17 hr). It was expressed as mg/10 seedlings.

3.5.2.5. Seedling length

Ten normal seedlings were picked up randomly from each replicate of the germination test, on the final count day, and length of seedlings was recorded in cm.

3.5.2.6. Vigour indices

The mean values were used for computing the vigour indices adopting the method of Abdul-Baki and Anderson (1973) using the following formula:

Vigour Index I = Germination (%) x Total seedling length (cm)

Vigour Index II = Germination (%) x Seedling dry weight (mg)

3.5.3. Biochemical phenotyping

Biochemical phenotyping involved changes in lignin, total phenol content, volatile aldehyde assays and antioxidant enzyme *ie* glutathione reductase (GR). The results of change in above different biochemical parameters were correlated with seed germination to identify the better predictor of soybean seed quality. The data on biochemical phenotyping, prior to storage served as control values.

3.5.3.1. Lignin content

Lignin content of the seed coat was determined colorimetrically. The samplewas extracted in NaOH solution and the aliquot was adjusted to pH 7.0 and 12.3. The amount of lignin was calculated by the difference between *A 245* (pH 7.0) and *A 350* (pH 12.3), *ie* E 350/sample.

Moisten 100mg of oven dried experimental material in a mortar with water. Add 2ml of NaoH to residue and extract 70-80^{0}C for 12-16hr. Cool, add 0.45ml of 2N HCl and adjust the pH to 7 or 8 with NaoH. Make up the volume to 3ml with water. Centrifuged at 2000g for 5 min. collect the supernatant. To 0.8ml of extract, add 0.8ml of 0.1M sodium phosphate buffer, pH 7.0. To another aliquot of 0.8ml extract, add 0.8ml of 0.1N NaoH pH 12.3. Measure the absorbance at 245 and 350. Derive the lignin concentration from the difference between *A245* and *A350* on pH 7.0 and 12.3, samples diluted with buffer and NaoH, respectively. Express the amount of lignin as E 350/sample.

3.5.3.2. Total phenolic concentration

Total phenolic concentration was measured using the method of using a Folin-Ciocalteu assay and gallic acid standard. Briefly, soybean seed were ground to powder and 0.5 g of seed powder was extracted twice with 10 ml acetone/water (50:50, v/v). Then, 200 µl of seed extract and 1 ml of Folin-Ciocalteu reagent were mixed. Then, 1.0 ml 20% Na_2CO_3 aqueous solution was added, and the final volume was made to 5 ml with distilled water. The mixture was incubated for 90 min at room temperature for colour development. The concentration of phenol was measured by reading the samples absorbance at 765 nm using a Beckman Coulter DU 800 spectrophotometer (Fullerton, California). Standard curve was drawn using 1-50µg gallic acid (curve-1). The data for total phenol content was expressed as mg of gallic acid equivalent weight (GAE) per 100 g of sample (mg GAE/1g).

3.5.3.3. Glutathione reductase

Glutathione reductase activity was determined at 25°C according to Mavis and Stellwagen (1968), following the rate of NADPH oxidation at 340 nm. Enzyme was extracted as per procedures given at 3.7.3.1.

1.5 ml 100 mM potassium phosphate buffer (pH 7.6) prepared with 3.4 mM EDTA; 0.10 ml of 30 Mm oxidized glutathione (GSSG) prepared in deionised water; 0.35 ml 0.8 mM β Nicotinamide Adenine Dinucleotide Phosphate prepared in five ml cold water; 0.30 ml 1.0% (w/v) Bovine Serum Albumin (BSA) prepared in 100 ml 100 mM potassium phosphate buffer (pH 7.6) and 0.65 ml deionised water were mixed in a cuvette by inversion and incubated at 25°C. The thermostatic spectrophotometer was monitored at 340nm until became constant. To this 0.10 ml enzyme extract or 1.0% (w/v) BSA (in blank) was added. The contents were mixed by inversion; and the decrease at 340nm was recorded for approximately five min. TheΔA340 nm/min was calculated using the maximum linear rate for both the test and blank. GR activity was expressed as µmoles NADPH oxidized/min/g seed fresh weight.

3.5.3.4. Changes in volatile aldehydes assay

Production and release of volatile aldehydes were measured following the procedure of Wilson and McDonald (1986) with slight modification. To perform this test, 250 ml conical flasks were lined with three discs of Whatman No. I filter paper and moistened with distilled water. Twenty seeds were placed over the moist blotter in two replications in separate flasks. A small test tube containing five ml 0.2% (W/V) MBTH (3-methylbenzthiazolinone-2-hydrazone) solution was kept inside each flask with seeds. The flasks were sealed with rubber stoppers and incubated at 25°C in the dark for 48 hr. At the end, the flasks were opened and the reagent tubes were taken out. An aliquot of one ml of MBTH trapping solution from 150 ml conical flasks was pipette into test tubes containing 2.5 ml of 0.23% (w/v) $FeCl_3$ solution and incubated at 25°C for five min. To this, 6.5 ml of acetone was added and the absorbance was measured at 635nm wavelength using a spectrophotometer (Beckman DU-640, USA). A standard curve (curve-2) was constructed using different concentration of formaldehyde (0.5-3.5 μg), and the aldehyde released was expressed as μg HCHO/5g seed fresh weight.

3.5.4 Statistical analyses

3.5.4.1 Analyses of variance

The analysis of variance was done using single factor analysis in excel sheet. Statistical significance was tested using the “F” test. Critical difference (CD) was also used to test the difference between any two means.

3.5.4.2. t-test paired two samples for means

The mean data of both fresh and stored seeds were jointly analysed using t-test paired two samples for means in excel sheet to compare whether there is significant difference exists between the values of fresh and stored seeds.

3.5.4.3. Association between traits

The results of various traits *viz.*, physical characteristics, physiological phenotyping and biochemical phenotyping were correlated with seed germination to identify the better predictor of soybean seed longevity. Association between the traits was calculated using the Pearson correlation coefficient at 5% as well as 1% level of significance, respectively.

4. RESULTS

Seed storability

From the 24 soybean genotypes, various physical, physiological and biochemical parameters were studied both at the start of seed storage and in 08 month stored seeds. These parameters are studied for its correlation with seed quality. Following observations were recorded:

4.1. Physical parameters

Details on various physical parameters studied *viz* seed coat colour, hilum colour, mechanical strength of seed coat, seed coat thickness, seed coat to cotyledon ratio are given (tables 2-14; fig-1).

4.1.1. Seed coat colour

Seed coat colour in soybean plays an important role in maintaining and preserving the seed quality.Black-seeded soybean genotypes being land races reported as superior storer as yellow-seeded ones. Good storer soybean genotypes were black, whereas poor storer soybean genotypes were yellowish-orange. Genotypes with black seed performed better than those to yellow seeded ones. Seed coat colour remains unchanged with advanced seed storage of eight months (table 2). Seed coat colour (black), in general, was identified and proved to be a good indicator of seed quality.

4.1.2. Hilum colour

The black-seeded genotypes by white hilum maintain seed quality higher than IMSCS than those yellow-seeded with brown to grey hilum during at eight months of laboratory ambient storage. Good storer genotypes showed hilum colour (white), whereas the poor storer soybean genotypes showed uniformly black or brown colour. Seed hilum colour also remain unchanged during 08 months of seed storage (table 2). Among the 24 genotypes studied, having white hilum, genotypes performed better than those to brown/black hilum genotypes. White hilum was identified as an indicator of good seed quality (storability).

Table 2. Seed coat and hilum colour* in soybean genotypes of contrasting quality

SNo.	Genotype	Seed coat colour		Hilum colour	
		Fresh seed	**Stored seed**	**Fresh seed**	**Stored seed**
	Good storer				
1.	AMSS-34	Black	Black	White	White
2.	G-2265	Black	Black	White	White
3.	G-2601	Black	Black	White	White
4.	G-2603	Black	Black	White	White
5.	G-2605	Black	Black	White	White
6.	G-2251	Black	Black	White	White
7.	G-2253	Black	Black	White	White
8.	G-2614	Black	Black	White	White
9.	M-1090	Black	Black	White	White
10.	M-11913	Black	Black	White	White
11	MACS-1311	Black	Black	White	White
12.	TGX (444-422)	Black	Black	White	White
	Poor storer				
13.	218(123)	Yellow orange	Yellow orange	Black	Black
14.	222(127)	Yellow orange	Yellow orange	Brown	Brown
15.	241(128)	Yellow orange	Yellow orange	Black	Black
16.	250(129)	Yellow orange	Yellow orange	Brown	Brown
17.	732(135)	Yellow orange	Yellow orange	Black	Black
18.	761(137)	Yellow orange	Yellow orange	Black	Black
19.	871(145)	Yellow orange	Yellow orange	Black	Black
20.	876(146)	Yellow orange	Yellow orange	Black	Black
21.	888(150)	Yellow orange	Yellow orange	Brown	Brown
22.	PK-472	Yellow orange	Yellow orange	Brown	Brown
23.	TAMS-38	Yellow orange	Yellow orange	Black	Black
24.	EC-13969	Yellow orange	Yellow orange	Black	Black

*Determination of colour using RHS chart

Where,

*Average of three replicates with 10 seeds in each replicate
Fresh seed: at the start of seed storage
Stored seed: for 8 months under laboratory ambient storage

4.1.3. Mechanical strength of seed coat

Hard seed coat was found to be beneficial in maintaining seed viability and vigour. Seed coat strength was measured by the amount of force (N) sustained by the seed coat, and hence, the seed. The point of sudden drop observed along the graph, as force increased, which was the point of first break of seed coat, measured as the actual strength of the seed coat/seed. There were significant variations in mechanical strength of seed coat among good and poor storers in the freshly harvested seeds (tables 3). However, these variations were non-significant among fresh and 08 months stored seeds in the present study (tables 16, 17 see appendix).

Mechanical strength of seed coat in soybean genotypes ranged between 85 to160 (N) (table 3). The average of mechanical strength in good storers fresh seed (146.91 N) and after eight months storage was (141.62 N), whereas the average of poor storers fresh seed (109.47 N) and after eight months storage was (103.21 N). The genotype (good storer) G-2251had shown maximum mechanical strength of seed coat (160.53 N) followed by G-2601 (160.49 N), whereas G-2603 had shown minimum mechanical strength of seed coat (137.3 N). The genotype (poor storer) 888 (150) showed maximum mechanical strength of seed coat (119.31 N) followed by 222(127) (119.556 N), whereas genotype 241(128) had shown minimum mechanical strength of seed coat (85.01 N).

4.1.4. Seed coat thickness

Thicker seed coat tissues in small seeded soybean seeds, owing to maintenance of viability which is in contrary to bold seeds, where seed viability is not better maintained.

Thickness of the seed coat was measured using phase contrast microscope (Leica software) which was connected to a computer. The thickness of parenchyma + palisade parenchyma and length of anticlinal cells were measured and presented (table 4). Seed coat thickness in soybean genotypes ranged between 193 to 200 μm (table 4). The mean seed coat thickness in good storer genotypes was 200 μm, whereas it was 193 μm in poor storer genotypes. The genotype (good storer) G-2265 showed thickest seed coat (200.40 μm). The genotype (poor storer) 218(123) showed minimum seed coat thickness (194.76 μm). Soybean genotypes with thicker seed coat in general and

G-2265 in particular were proved to be better with respect to seed quality (fig: 1,2 and tables 18,19,20 see appendix).

Table 3.Mechanical strength (MS)* of seed coat among soybean genotypes of contrasting quality

		MS (N)	
SNo.	**Genotype**	**Fresh seed**	**Stored seed**
	Good storer		
1.	AMSS-34	148.972	148.191
2.	G-2265	138.562	138.171
3.	G-2601	160.491	160.370
4.	G-2603	137.739	137.597
5.	G-2605	140.088	140.346
6.	G-2251	160.536	160.476
7.	G-2253	146.120	146.483
8.	G-2614	147.592	147.211
9.	M-1090	149.768	149.071
10.	M-11913	142.562	142.600
11.	MACS-1311	142.806	142.173
12.	TGX (444-422)	147.779	143.388
	Mean	**14 6.918**	**146.673**
	Poor storer		
13.	218(123)	112.329	112.273
14.	222(127)	118.556	118.276
15.	241(128)	103.198	104.010
16.	250(129)	103.154	103.182
17.	732(135)	104.933	104.450
18.	761(137)	109.230	109.271
19.	871(145)	116.691	116.800
20.	876(146)	106.339	106.128
21.	888(150)	119.314	119.351
22.	PK-472	115.587	115.137
23.	TAMS-38	92.450	92.540
24.	EC-13969	111.96	111.966
	Mean	**109.478**	**109.448**
	MSD @ 5%	**16.610**	**14.076**

Where,

*average of five replicates with five seeds in each replicate

MS: mechanical strength of seed coat,

N: Newton,

MSD: minimum significant difference

Fresh seed: at the start of seed storage

Stored seed: for 8 months under laboratory ambient storage

t-test paired two samples for means P= 0.072> 0.05: non-significant

4.1.5. Seed coat to cotyledon ratio

The proportion of the seed coat is of primary significance in providing guard against mechanical injury during harvesting and processing, which cause noteworthy loss in viability of a seed lot. Seed coats were removed carefully from seeds. Seed coat and cotyledons were collected and weighed separately. The total weight of seed coat and cotyledon was considered as the seed weight.

Seed coat to cotyledon ratio in soybean genotypes ranged between 7.17 to 10.01 (%) (table 5).The average seed coat percentages in fresh seed (9.4146%) and eight months stored were (9.4143%), respectively. The poor storer seeds registered an average 7.6059% in fresh seeds and 7.6051% in eight months stored seeds (fig: 3). The genotype (good storer) G-2614 had shown maximum seed coat percentage (10.01%) followed by G-2251 (9.83%), whereas M-11913 had shown least seed coat percentage (8.97%). The genotype (poor storer) 761(137) had shown seed coat percentage(8.244%) followed by genotype 250(129) (8.046), whereas, 241(128) had shown seed coat percentage (7.17%).Black seeded soybean genotypes had shown, in general, more seed coat to cotyledon ratio than those to yellowish orange genotypes. No significant difference was observed between fresh and stored seeds, irrespective of quality of seed (tables 21, 22 see appendix). Black seeded genotypes G-2614 and G-2251 with more seed coat percentage were identified as better performer (table 5).

4.1.6 Seed coat histology

Thicker seed coat tissues in small seeded soybean seeds were owing to better maintenance of viability, in contrary to bold seeds.

The stained seed coat sections were viewed and photographed using phase contrast microscope. Soybean seed coat consisted of an epidermis of one cell layer (cutinized palisade cells), a hypodermis of single layer of large cells with thick anticlinal walls having intercellular spaces, and an inner parenchyma composed of 6–8 layers of flattened thin-walled cells (fig. 1). Photographs showed that the hour-glass cells, were rather more in number, uniform in shape and distribution along the cross section of the seed coat in good storer varieties, viz. AMSS-34, G-2265 (fig. 1) than those to the yellow-seeded poor storer varieties 218(123), 241(128) (fig. 1).

Table 5. Seed coat (%)* in soybean genotypes of contrasting quality

SNo.	Genotype	Seed coat (%) Fresh seed	Seed coat (%) Stored seed
	Good storer		
1.	AMSS-34	9.155	9.154
2.	G-2265	9.291	9.297
3.	G-2601	9.409	9.405
4.	G-2603	9.291	9.293
5.	G-2605	9.167	9.163
6.	G-2251	9.750	9.755
7.	G-2253	9.346	9.345
8.	G-2614	9.944	9.941
9.	M-1090	9.768	9.763
10.	M-11913	8.970	8.976
11.	MACS-1311	9.337	9.335
12.	TGX (444-422)	9.543	9.544
	Mean	**9.4146**	**9.4143**
	Poor storer		
13.	218(123)	7.642	7.644
14.	222(127)	7.330	7.329
15.	241(128)	7.052	7.055
16.	250(129)	7.943	7.944
17.	732(135)	7.451	7.456
18.	761(137)	8.244	8.242
19.	871(145)	7.255	7.256
20.	876(146)	7.855	7.851
21.	888(150)	7.553	7.554
22.	PK-472	7.728	7.724
23.	TAMS-38	7.648	7.643
24.	EC-13969	7.566	7.562
	Mean	**7.605**	**7.605**
	MSD @ 5%	**0.053**	**0.049**

Where,

*Average of five replicates with five gram seeds in each replicate
Fresh seed: at the start of seed storage
Stored seed: for 8 months under laboratory ambient storage
MSD: minimum significant difference
t-test paired two samples for means P= 0.454 < 0.05: non-significant

Table 4. Seed coat thickness in soybean genotypes of contrasting quality

		Seed coat thickness (µm)		
SNo.	**Genotype**	**Epidermis+palisade**	**Anticlinal**	**Total**
	Good storer			
1.	AMSS-34	112.981	87.243	200.224
2.	G-2265	111.477	88.924	200.401
	Mean	**112.229**	**88.08**	**200.312**
	Poor storer			
3.	218(123)	111.686	83.073	194.759
4.	241(128)	110.354	82.158	193.012
	Mean	**111.020**	**82.865**	**193.012**
	MSD @ 5%	**15.148**	**8.168**	**17.455**

Where,
Fresh seed: at the start of seed storage
MSD: minimum significant difference

4.2. Physiological parameters

Physiological parameters studied in detail were electrical conductance from seed leachates, 100-seed weight, seed germination (%), seedling growth (cm), seedling dry weight (mg) and vigour indices I and II, from good and poor storers. Data on the above parameters are presented (tables 6-10).

4.2.1. Electrical conductance from seed leachates (EC)

Seed coat cell membrane is extremely important in protecting the solute from injury by rapid hydration and in maintaining seed viability. EC is inversely correlated with seed quality. Electrical conductance from seed leachates increased with increased storage period. Electrical conductance as of seed leachates in fresh and stored seeds was considerably higher in yellow-seeded poor storer genotypes, which is in contrary to black-seeded good storer ones (fig: 4). There were significant variations registered in EC values among different genotypes as well as among fresh and stored seeds (tables 35, 36 see appendix). Among good storers soybean genotypes EC ranged between 14.42 to 21.50 µS/cm/g with a mean value of 18.00 µS/cm/g in fresh seeds and 24.89 to 33.40 µS/cm/g with a mean value of 29.12 µS/cm/g in stored seeds. Among poor storer genotypes, it ranged between 23.336 to 28.82 µS/cm/g with a mean value of

Table 6. Variation in EC* in soybean genotypes of contrasting quality

		EC (µS/cm/g seed)	
SNo.	**Genotype**	**Fresh seed**	**Stored seed**
	Good storer		
1.	AMSS-34	14.42	24.89
2.	G-2265	16.05	28.49
3.	G-2601	16.77	30.19
4.	G-2603	17.55	27.55
5.	G-2605	17.80	28.88
6.	G-2251	17.30	31.61
7.	G-2253	15.23	24.33
8.	G-2614	19.67	28.35
9.	M-1090	21.21	34.03
10.	M-11913	21.50	33.40
11.	MACS-1311	20.48	30.42
12.	TGX (444-422)	18.00	27.29
	Mean	**18.00**	**29.12**
	Poor storer		
13.	218(123)	25.44	56.15
14.	222(127)	25.29	55.36
15.	241(128)	26.52	53.57
16.	250(129)	27.04	41.93
17.	732(135)	23.34	48.39
18.	761(137)	24.46	60.12
19.	871(145)	28.82	62.43
20.	876-(146)	25.61	50.02
21.	888(150)	27.25	57.02
22.	PK-472	25.54	64.74
23.	TAMS-38	27.95	52.48
24.	EC-13969	25.52	57.88
	Mean	**26.06**	**55.00**
	MSD @ 5%	**2.849**	**7.955**

Where,
*Average of 03 replicates with 50 seeds in 250 ml distilled water in each replicate
EC: electrical conductance from seed leachates and
Fresh seed: at the start of seed storage
Stored seed: for 8 months under laboratory ambient storage
MSD: minimum significant difference
t-test paired two samples for means $P= 1.543*10^{-9} < 0.05$: significant

26.06 µS/cm/g in fresh seeds and 41.93 to 64.74 µS/cm/g with a mean of 55.1 µS/cm/g in stored seeds, respectively. AMSS-34 had registered the least EC (14.42 µS/cm/g) followed by G-2253 (15.23 µS/cm/g). Genotypes AMSS-34, G-2253 performed better and were identified as better genotypes among the 24 genotypes studied (table 6). EC may be used as a good marker for seed quality (storability).

4.2.2. 100-seed weight

Seed size plays an important role in preserving soybean seed quality. Seed size varied among genotypes. 100-seed weight was found to be negatively correlated with seed quality.100-seed weight in general, was comparable between fresh and stored seed in the present study. Good storer genotypes were usually small seeded, whereas larger seeds were poor storer (fig: 5). 100-seed weight varied significantly among genotypes studied (tables 23, 24 see appendix). 100-seed weight ranged between 6.609 g (TGX 444-422) to 11.327 g (TAMS-38) (table 7), respectively. The average of 100-seed weight among good storers fresh seed 6.914 g and eight months stored seed was 6.916 g. Whereas among poor storers fresh seed 8.852 g and after eight months stored seed was 8.812 g. The genotype (good storer) M-11913 registered maximum100-seed weight 7.42 g, whereas AMSS-34 had shown minimum (6.17 g). The genotype (poor storer) TAMS-38registered 100-seed weight (11.33 g), whereas 218(123) had shown 100-seed weight (7.31 g). Among the genotypes, TGX 444-422 and AMSS-34 were identified as better performers.

4.2.3. Seed germination

Seed germination is the only standard and routinely practised to establish the quality of a given seed lot. The seed germination (%) of good and poor soybean genotypes in fresh and stored seeds is presented (table 8). There was a significant variation registered in germination percentage among different genotypes as well as between fresh and stored seeds (tables 25, 26 see appendix).

Germination of freshly harvested seeds ranged between 93.50 to 98.00% and was comparable among good and poor storer genotypes at the start of the seed storage(fig: 6). The germination percentage ,at the start of seed storage, in all the genotypes (both good and poor storers) was above the Indian Minimum Seed Certification Standards (IMSCS) *ie ≥70%.*None of the good storer genotypes recorded germination below the IMSCS of 70% on the other hand, all poor storer genotypes recorded germination below 70% in 08 months of stored seed.

Table 7. Variation in 100-seed weight* in soybean genotypes of contrasting quality

		100-seed weight (g)	
SNo.	**Genotype**	**Fresh seed**	**Stored seed**
	Good storer		
1.	AMSS-34	6.174	6.178
2.	G-2265	6.521	6.526
3.	G-2601	5.686	5.680
4.	G-2603	7.320	7.322
5.	G-2605	6.819	6.813
6.	G-2251	7.951	7.966
7.	G-2253	7.104	7.116
8.	G-2614	7.196	7.195
9.	M-1090	6.764	6.765
10.	M-11913	7.421	7.425
11.	MACS-1311	7.406	7.403
12.	TGX (444-422)	6.609	6.603
	Mean	**6.914**	**6.916**
	Poor storer		
13.	218(123)	7.310	7.313
14.	222(127)	10.551	10.553
15.	241(128)	8.597	8.591
16.	250(129)	10.469	10.450
17.	732(135)	9.215	9.212
18.	761(137)	7.944	7.946
19.	871(145)	8.977	8.973
20.	876(146)	8.046	8.044
21.	888(150)	8.165	8.161
22.	PK-472	7.514	7.512
23.	TAMS-38	11.309	11.327
24.	EC-13969	8.127	8.125
	Mean	**8.852**	**8.851**
	MSD @ 5%	**0.852**	**0.760**

Where,

*Average of 10 replicates with 100 seeds of each replicate

Fresh seed: at the start of seed storage

Stored seed: for 8 months under laboratory ambient storage

MSD: minimum significant difference

t-test paired two samples for means P= 0.977 > 0.05, non-significant

Table 8. Seed germination* in soybean genotypes of contrasting quality

SNo.	Genotype	Germination (%)	
		Fresh seed	Stored seed
	Good storer		
1.	AMSS-34	96.00	86.75
2.	G-2265	97.25	89.25
3.	G-2601	97.00	87.75
4.	G-2603	97.75	86.50
5.	G-2605	96.25	85.50
6.	G-2251	97.50	86.50
7.	G-2253	97.50	85.00
8.	G-2614	97.00	86.50
9.	M-1090	98.00	88.50
10.	M-11913	97.50	87.75
11.	MACS-1311	97.25	88.50
12.	TGX (444-422)	96.25	87.75
	Mean	**97.104**	**87.187**
	Poor storer		
13.	218(123)	95.25	68.50
14.	222(127)	96.00	67.25
15.	241(128)	96.25	64.75
16.	250(129)	95.75	66.75
17.	732(135)	96.00	65.00
18.	761(137)	94.50	65.50
19.	871(145)	95.00	65.75
20.	876(146)	94.00	67.25
21.	888(150)	94.50	64.25
22.	PK-472	96.00	67.50
23.	TAMS-38	94.25	64.50
24.	EC-13969	93.50	64.75
	Mean	**95.083**	**65.979**
	MSD @ 5%	**3.233**	**4.646**

Where,

*Average of four replicates with 100 seeds in each replicate

Fresh seed: at the start of seed storage
Stored seed: for 8 months under laboratory ambient storage
MSD: minimum significant difference
t-test paired two samples for means $P= 1.455*10^{-9}< 0.05$: significant

The mean germination values in good storer genotypes was 97.10%, whereas in poor storer it was 95.08%,which was declined significantly in all genotypes, but the decline was more pronounced in poor storers genotypes. In case of good storer genotypes, the mean germination was 87.18%, whereas in poor storers it was 65.97%. In case of good storers, at the start of seed storage, genotypes M-1090 and G-2603 had registered maximum germination (98% and 97.75%), whereas, in poor storer, genotypes 222(127), 241(128) had recorded germination up to 96% at the start of seed storage, and genotype TAMS-38, EC-1396 had registered minimum germination (64%) during eight months of ambient seed storage. Black seeded, in general, but genotype 2265 among good storers performed better among all the soybean genotypes studied.

4.2.4. Seedling growth

Seedling growth decreased gradually with increased storage period. There were a significant variations in seedling growth among different genotypes as well as among fresh and stored seeds (tables 27, 28 see appendix). Seedling growth ranged between 25.03 to 31.83 cm and 19.98 to 27.14 cm in fresh and stored seeds, respectively (table 9). The mean seedling growth among good storer genotypes were 28.77cm and 25.08 cm, whereas, in poor storer genotypes it were 26.70 and 21.80 cm, in fresh and stored seeds, respectively. The genotype AMSS-34 had registered maximum seedling growth in fresh and stored seeds. Whereas, genotype 732(135) had registered minimum seedling growth (19.98 cm). AMSS-34 performed better among all the soybean genotypes studied. Seedling growth among physiological markers may also be used to identify better seed quality (storability).

4.2.5. Seedling dry weight

The seedling dry weight, is an excellent index of seed vigour in soybean. Seedling growth, in common, decreased with augmented seed storage. However, turn down was added under ambient storage atmosphere. Seedling dry weight registered significant variation among the genotypes in fresh and stored seeds (tables 29, 30 see appendix). Seedling dry weight ranged between 0.542 to 0.704 g in fresh seeds and 0.313 to 0.462 g in stored seeds, respectively among all the genotypes studied. The mean seedling dry weight among good storer genotypes were 0.655 and 0.411 g in fresh and stored seeds, respectively. Whereas, among poor storer genotypes it were

Table 9. Seedling growth and seedling dry weight* in soybean genotypes of contrasting quality

SNo.	Genotype	Seedling growth (cm)		SDW (g)	
		Fresh seed	Stored seed	Fresh seed	Stored seed
	Good storer				
1.	AMSS-34	31.83	26.54	0.627	0.415
2.	G-2265	28.53	24.77	0.615	0.396
3.	G-2601	28.29	25.19	0.694	0.462
4.	G-2603	30.24	27.14	0.704	0.405
5.	G-2605	27.30	23.72	0.633	0.410
6.	G-2251	27.57	23.79	0.647	0.426
7.	G-2253	27.86	23.54	0.697	0.389
8.	G-2614	27.79	23.51	0.680	0.391
9.	M-1090	30.07	26.75	0.619	0.420
10.	M-11913	28.14	25.23	0.623	0.419
11.	MACS-1311	28.64	25.00	0.698	0.389
12.	TGX (444-422)	29.04	25.85	0.619	0.406
	Mean	**28.775**	**25.088**	**0.655**	**0.411**
	Poor storer				
13.	218(123)	26.18	21.96	0.599	0.415
14.	222(127)	26.08	20.10	0.569	0.351
15.	241(128)	27.46	22.43	0.575	0.347
16.	250(129)	26.82	21.77	0.583	0.313
17.	732(135)	25.03	19.98	0.584	0.343
18.	761(137)	27.16	22.14	0.588	0.379
19.	871(145)	28.18	23.28	0.593	0.406
20.	876(146)	26.28	23.25	0.584	0.375
21.	888(150)	26.32	21.20	0.542	0.384
22.	PK-472	27.17	21.19	0.574	0.348
23.	TAMS-38	26.87	22.28	0.594	0.373
24.	EC-13969	26.58	22.10	0.578	0.372
	Mean	**26.701**	**21.807**	**0.580**	**0.367**
	MSD @ 5%	**1.342**	**1.327**	**0.059**	**0.114**

Where,

*Average of three replicates with 10 seedlings in each replicate

SDW: seedling dry weight

Fresh seed: at the start of seed storage

Stored seed: for 8 months under laboratory ambient storage

MSD: minimum significant difference

t-test paired two samples for means of seedling length $P = 4.182*10^{-17} < 0.05$, significant

t-test paired two samples for means of seedling dry weight $P = 1.974*10^{-19} < 0.05$, significant

0.580 and 0.367 g, in fresh and stored seeds, respectively. The genotype G-2603 had recorded maximum (0.704) seedling dry weight in fresh seeds, whereas in case of poor storers, genotype 250(129) had shown least seedling dry weigh (0.313 g) from stored seeds, respectively (table 9). G-2603may be identified as better genotype for seed quality (storability). Seedling dry weight may also be used as another good marker for seed (storability) quality.

4.2.6. Seed vigour indices I and II

Seed vigour indices, in common, decreased with amplified seed storage. Freshly harvested seed showed superior vigour indices than those to stored seed. Seed vigour index-I ranged between 2402.7 to 3055.6 and 1299.1 to 2366.7 in fresh and stored seeds, respectively among all the genotypes under study. The mean seed vigour index-I, among good storer genotypes, were 2794.1and 2188.08, whereas among poor storer genotypes it were 2538.68 and 1438.76 in fresh and stored seeds, respectively (fig: 7). The genotype AMSS-34 had recorded maximum seed vigour index-I in fresh seeds. Whereas, 732(135) had registered least seed vigour index I (1299.1) in 08 months laboratory ambient stored seeds (tables 31, 30 see appendix).

The decline was more in the poor storers than those to good storers. Seed vigour index-II ranged between 51.27 to 68.89 and 20.93 to 40.62 in fresh and stored seeds, respectively. The mean seed vigour index-IIamong good storer genotypes were 63.61and 35.85in fresh and stored seeds, whereas in poor storer genotypes it were55.21 and 24.26 in fresh and stored seeds, respectively(fig: 8). The genotype G-2603 had recorded maximum seed vigour index-II (68.83) in fresh seeds, whereas 250(129) had registered least seed vigour index II (20.93) among poor storers. Both vigour indices independently, may be used as good marker for seed quality (storability)(table 10). The genotype G-2603 may be identified as better performer among the studied all soybean genotypes (tables 33, 34 see appendix).

Table 10. Seedling vigour indices* in soybean genotypes of contrasting quality

		SVI-I		SVI-II	
SNo.	**Genotype**	**Fresh seed**	**Stored seed**	**Fresh seed**	**Stored seed**
	Good storer				
1.	AMSS-34	3055.6	2302.7	60.25	36.04
2.	G-2265	2773.7	2210.6	59.88	35.39
3.	G-2601	2210.6	2209.6	67.36	40.62
4.	G-2603	2955.9	2347.6	68.83	35.10
5.	G-2605	2347.6	2028.8	60.93	35.09
6.	G-2251	2688.0	2058.1	63.14	36.85
7.	G-2253	2716.3	2000.9	68.03	33.04
8.	G-2614	2695.9	2033.9	65.92	33.87
9.	M-1090	2947.0	2366.7	60.69	37.24
10.	M-11913	2743.8	2214.6	60.75	36.83
11.	MACS-1311	2785.2	2231.6	67.89	34.48
12.	TGX (444-422)	2796.0	2268.4	59.63	35.67
	Mean	**2794.1**	**2188.0**	**63.61**	**35.85**
	Poor storer				
13.	218(123)	2493.8	1504.3	57.13	28.44
14.	222(127)	2503.4	1352.6	54.65	23.62
15.	241(128)	2641.9	1452.4	55.43	22.50
16.	250(129)	2567.9	1352.9	55.89	20.93
17.	732(135)	2402.7	1299.1	56.10	22.28
18.	761(137)	2566.4	1450.4	55.59	24.85
19.	871(145)	2677.2	1530.2	56.36	26.78
20.	876(146)	2470.6	1563.6	54.91	25.24
21.	888(150)	2487.6	1360.6	51.27	24.72
22.	PK-472	2607.8	1430.7	55.11	23.5
23.	TAMS-38	2532.4	1437.0	55.98	24.11
24.	EC-13969	2512.0	1430.9	54.11	25.12
	Mean	**2538.68**	**1438.76**	**55.21**	**24.26**
	MSD at 5%	**146.639**	**129.963**	**1.5596**	**5.7015**

Where,

*average of three replicates with 10 seedlings in each replicate

SVI-I and SVI-II – seedling vigour index –I and seedling vigour index -II

Fresh seed: at the start of seed storage

Stored seed: for 8 months under laboratory ambient storage

MSD: minimum significant difference

t-test paired two samples for means of seedling vigour index –I $P= 4.182*10^{-17} < 0.05$: significant

t-test paired two samples for means of seedling vigour index -II $P= 1.974*10^{-19} < 0.05$: significant

4.3. Biochemical parameters

Details on biochemical phenotyping involved changes in lignin, total phenol content, volatile aldehydes assays and antioxidant enzyme *viz*, glutathione reductase, are presented. The results of changes in lignin, total phenol content, volatile aldehydes assays and antioxidant enzymes *viz.*, glutathione reductase were also correlated with seed germination to identify the better predictor of soybean seed quality. The data on biochemical phenotyping, prior to storage served as control; values are presented (table 11-14).

4.3.1. Lignin content

Lignin is a natural complex phenolic polymer, found in the cell wall, are believed to contribute to the compressive strength, essential for mechanical support, water convey, and resistance in vascular plants (Lewis and Yamamoto 1990; Campbell and Sederoff 1996). In eight months stored seeds, there was no change in lignin content (tables 37, 38 see appendix). Lignin values ranged between 0.353 to 0.552 and 0.352 to 0.557 in fresh and stored seeds, respectively. The means of lignin value, irrespective of quality, were at par in fresh with mean of eight months laboratory ambient stored seeds. However, the mean values recorded were, among good storer genotypes were 0.468and 0.467 in fresh and stored seeds, respectively whereas in poor storer genotypes it were 0.360 and 0.357 in fresh and stored seeds, respectively. The genotype G-2253among good storer had recorded maximum lignin value (0.552) in fresh seeds, whereas among poor storers genotype 888(150) had registered least lignins (0.352) in eight months stored seeds. Lignin independently, may be used as good marker for seed quality (storability) (table 11). The genotype G-2253 was identified as better performer.

4.3.2. Total phenol content

Legume seeds, contain proanthocyanidines (*ie* condensed tannins), the principal phenols content located mainly in the seed coat (hull), play an important role in the defence system of seeds that are expose to damage by many environmental factors such as light, oxygen, free radicals etc. Phenol content have been reported to have seed mechanical damage resistance and disease resistance. The phenol content registered decline significantly among all genotypes with storage (tables 39, 40 see appendix). The decline was more in the poor storers than those to good storers.

Table 11. Lignin content* in soybean genotypes of contrasting quality

		Lignin content (OD_{245}-OD_{350})	
SNo.	**Genotype**	**Fresh seed**	**Stored seed**
	Good storer		
1.	AMSS-34	0.498	0.494
2.	G-2265	0.499	0.492
3.	G-2601	0.430	0.431
4.	G-2603	0.425	0.421
5.	G-2605	0.477	0.471
6.	G-2251	0.470	0.479
7.	G-2253	0.552	0.557
8.	G-2614	0.420	0.421
9.	M-1090	0.437	0.438
10.	M-11913	0.440	0.436
11.	MACS-1311	0.492	0.492
12.	TGX (444-422)	0.476	0.474
	Mean	**0.468**	**0.467**
	Poor storer		
13.	218(123)	0.360	0.354
14.	222(127)	0.359	0.355
15.	241(128)	0.362	0.366
16.	250(129)	0.357	0.351
17.	732(135)	0.360	0.357
18.	761(137)	0.356	0.354
19.	871(145)	0.353	0.355
20.	876(146)	0.369	0.361
21.	888(150)	0.354	0.352
22.	PK-472	0.362	0.362
23.	TAMS-38	0.358	0.355
24.	EC-13969	0.369	0.367
	Mean	**0.360**	**0.357**
	MSD @ 5%	**0.050**	**0.024**

Where,
*average of three replicates
Fresh seed: at the start of seed storage
Stored seed: for 8 months under laboratory ambient storage
MSD: minimum significant difference
t-test paired two samples for means P= 0.05057 > 0.05: non-significant

Table 12. Total phenol content* in soybean genotypes of contrasting quality

SNo.	Genotype	Total phenol content (mg/gdw) Fresh seed	Stored seed
	Good storer		
1.	AMSS-34	0.929	0.923
2.	G-2265	1.223	1.219
3.	G-2601	1.990	1.986
4.	G-2603	1.772	1.763
5.	G-2605	1.478	1.461
6.	G-2251	1.676	1.668
7.	G-2253	1.621	1.617
8.	G-2614	1.616	1.612
9.	M-1090	1.631	1.630
10.	M-11913	1.352	1.345
11.	MACS-1311	1.108	1.101
12.	TGX (444-422)	1.350	1.343
	Mean	**1.735**	**1.472**
	Poor storer		
13.	218(123)	0.373	0.367
14.	222(127)	0.362	0.351
15.	241(128)	0.353	0.348
16.	250(129)	0.379	0.369
17.	732(135)	0.377	0.374
18.	761(137)	0.380	0.379
19.	871(145)	0.360	0.350
20.	876(146)	0.398	0.397
21.	888(150)	0.371	0.368
22.	PK-472	0.392	0.387
23.	TAMS-38	0.381	0.375
24.	EC-13969	0.398	0.389
	Mean	**0.595**	**0.371**
	MSD @ 5%	**0.077**	**0.063**

Where,

*Average of three replicates

Fresh seed: at the start of seed storage
Stored seed: for 8 months ambient storage
MSD: minimum significant difference
t-test paired two samples for means P= $3.58604 \times 10^{-8} < 0.05$: significant

Phenols value ranged between 0.353 to 1.990 (mg/g dw) and 0.348 to 1.986 (mg/g dw) in fresh and stored seeds, respectively (fig: 9). The mean phenol values among good storer genotypes were 1.735 and 1.472 (mg/g dw) in fresh and stored seeds, whereas among poor storer genotypes it were 0.595 and 0.371 (mg/g dw) in fresh and stored seeds, respectively. The genotype G-2601, among good storers, had registered maximum phenol content (1.990 mg/g dw) in fresh and stored seeds. Genotype 241(120) had registered least phenol (0.348 mg/g dw) in stored seeds among poor storers. Present results showed that the total phenol independently, may be used as good marker for seed quality (storability) (table 12). The genotype G-2601 had been identified as better performer.

4.3.3. Volatile aldehydes

Peroxidation of membrane phospholipids, rich in unsaturated fatty acids, results in the production of hydroperoxides, which upon hydration are enzymatically metabolized to various aldehydes and ketones. The level of volatile aldehydes released by the poor storer genotypes was superior than good storer genotypes, though it increased in all the genotypes during storage. This clearly indicated the role of lipid peroxidation in seed longevity behaviour (fig: 10). Volatile aldehydes values ranged between 0.476 to 1.047 (µg HCHO/5g) and 0.991 to 3.978 (µg HCHO/5g) in fresh and stored seeds, respectively (tables 43, 44 see appendix). The mean volatile aldehyde values among good storer genotypes were 0.615 and 1.378 (µg HCHO/5g) in fresh seeds, whereas among poor storer genotypes it were 0.860 and 2.975 (µg HCHO/5g) in fresh and stored seeds, respectively. The genotype AMSS-34, among good storer genotypes had recorded minimal volatile aldehydes in fresh, whereas PK-472, among the poor storer genotypes in stored seeds had registered maximal volatile aldehydes (3.978) (µg HCHO/5g). Volatile aldehydes independently, may be used as good marker for seed quality (storability). AMSS-34 soybean genotype, among the studied genotypes proved to be a better genotype for seed quality (table 13).

4.3.4. Glutathione reductase

Glutathione reductase (GR) takes part in the control of endogenous hydrogen peroxide through an oxido-reduction cycle involving glutathione and ascorbate. GR registered significant increase in all genotypes studied. Freshs seeds, among good storers genotypes registered higher GR values than those to poor storers (tables 41, 42 see appendix). So also changes in GR values were more substantial among stored seeds of good storers than those to poor storers (table 14).

Table 13. Volatile aldehyde content* in soybean seeds of contrasting quality

SNo.	Genotype	Volatile aldehyde (µg/5g seed) Fresh seed	 Stored seed
	Good storer		
1.	AMSS-34	0.476	1.551
2.	G-2265	0.571	1.459
3.	G-2601	0.573	1.239
4.	G-2603	0.605	1.672
5.	G-2605	0.704	1.653
6.	G-2251	0.752	1.011
7.	G-2253	0.662	1.532
8.	G-2614	0.709	1.136
9.	M-1090	0.571	1.754
10.	M-11913	0.680	1.032
11.	MACS-1311	0.575	0.991
12.	TGX (444-422)	0.503	1.515
	Mean	**0.615**	**1.378**
	Poor storer		
13.	218(123)	0.699	2.531
14.	222(127)	0.802	2.684
15.	241(128)	0.774	2.282
16.	250(129)	0.903	2.758
17.	732(135)	0.796	2.781
18.	761(137)	1.047	3.147
19.	871(145)	0.939	3.213
20.	876(146)	0.796	3.115
21.	888(150)	0.703	2.672
22.	PK-472	0.967	3.978
23.	TAMS-38	0.955	3.807
24.	EC-13969	0.939	2.739
	Mean	**0.860**	**2.975**
	MSD @ 5%	**0.064**	**0.134**

Where,

*Average of three replicates
Fresh seed: at the start of seed storage
Stored seed: for 8 months ambient storage
MSD: minimum significant difference
t-test paired two samples for means P= $5.803*10^{-9} < 0.05$: significant

Table 14. Glutathione reductase activity* in soybean seeds of contrasting quality

SNo.	Genotype	GR (µmoles NADPH oxidised/min/g seed) Fresh seed	GR (µmoles NADPH oxidised/min/g seed) Stored seed
	Good storer		
1.	AMSS-34	0.403	1.290
2.	G-2265	0.589	1.249
3.	G-2601	0.699	1.133
4.	G-2603	0.554	1.421
5.	G-2605	0.513	0.983
6.	G-2251	0.442	0.921
7.	G-2253	0.596	1.141
8.	G-2614	0.715	1.182
9.	M-1090	0.776	1.333
10.	M-11913	0.578	1.096
11.	MACS-1311	0.694	1.257
12.	TGX (444-422)	0.673	1.215
	Mean	**0.602**	**1.185**
	Poor storer		
13.	218(123)	0.429	0.836
14.	222(127)	0.554	0.823
15.	241(128)	0.438	0.902
16.	250(129)	0.455	0.918
17.	732(135)	0.654	0.831
18.	761(137)	0.445	0.839
19.	871(145)	0.546	0.794
20.	876(146)	0.669	0.870
21.	888(150)	0.734	0.829
22.	PK-472	0.592	0.840
23.	TAMS-38	0.434	0.818
24.	EC-13969	0.463	0.847
	Mean	**0.534**	**0.845**
	MSD @ 5%	**0.048**	**0.047**

Where,
*Average of three replicates
Fresh seed: at the start of seed storage
Stored seed: for 8 months laboratory ambient storage
MSD: minimum significant difference
t-test paired two samples for means $P= 5.929*10^{-11}< 0.05$: significant
GR: glutathione reductase

GR value ranged between 0.403 to 0.776 (µmoles NADPH oxidized/min/g) and 0.794 to 1.421 (µmoles NADPH oxidized/min/g) in fresh and stored seeds, respectively (fig: 11). The mean GR values, among good storer genotypes, were

0.602 and 1.185 (µmoles NADPH oxidized/min/g) in fresh and stored seeds, whereas among poor storer genotypes, it were 0.534 and 0.845 (µmoles NADPH oxidized/min/g) in fresh and stored seeds, respectively. The genotype AMSS-34, recorded the least GR 0.403 (µmoles NADPH oxidized/min/g) among good storer genotypes; was identified as better, among the studied soybean genotypes for seed quality. GR activity may also be used a good marker for seed quality (storability) (table 14).

5.1. Correlation coefficient among different physical, physiological and biochemical parameters in soybean genotypes of contrasting longevity.

The detail on correlation coefficient values among various physical, physiological and biochemical parameters in good and poor storer soybean genotypes is given (table 15). In the present study, seed germination was either +ve or –ve but significantly correlated with all the parameters of seed quality. There were positive correlations registered between seed germination and physiological parameters like seedling vigour index-I (r = 0.941**), vigour index-II (r = 0.839**), seedling dry weight (r = 0.837**), seedling growth (r = 0.788**), and biochemical parameters like, phenol content (r = 0.927**) and GR activity (r = 0.809**) at 1% significance level. Whereas, there were negative correlations registered between seed germination and physiological parameters like electrical conductance from seed leachates (r = (-) 0.932**) and 100-seed weight(r = (-) 0.706**); and biochemical parameter like volatile aldehydes (r = (-) 0.904**) at 1% significance level. Non–significant correlations were also registered between seed germination and physical parameters like seed coat% (r = 0.094^{NS}), seed coat mechanical strength (r = 0.092^{NS}), lignin content (r = 0.0875^{NS}). The results inferred that SVI followed by SV II, SDW, EC, phenol content, GR activity and volatile aldehyde contents either individually or in combinations may be used as better indices of soybean seed quality and vigour (table 15).

Table 15. Correlation coefficient values among various physical, physiological and biochemical characteristics in soybean genotypes

Soybean seed	**GERM%**	**MS**	**SC%**	**HSW**	**EC**	**SG**	**SDW**	**SV I**	**SV II**	**LIG**	**TP**	**VA**	**GR**
GERM%	1	0.092^{NS}	0.094^{NS}	-0.706**	-0.932**	0.788**	0.837**	0.941**	0.839**	0.0875^{NS}	0.927**	-0.904**	0.809**
MS		1	0.090^{NS}	-0.752**	-0.843**	0.710**	0.840**	0.861**	0.770**	0.0820^{NS}	0.910**	-0.877**	0.718**
SC%			1	-0.684**	-0.905**	0.738**	0.821**	0.882**	0.816**	0.0827^{NS}	0.925**	-0.827**	0.774**
HSW				1	0.624**	-0.648**	-0.682**	-0.711**	-0.545**	-0.0641^{NS}	-0.664**	0.659**	-0.637**
EC					1	-0.722**	-0.776**	-0.869**	-0.821**	-0.0900^{NS}	-0.880**	0.888**	-0.744**
SG						1	0.711**	0.949**	0.640**	0.0665^{NS}	0.697**	-0.659**	0.734**
SDW							1	0.809**	0.916**	0.0718^{NS}	0.889**	-0.771**	0.649**
SVI								1	0.775**	0.0807^{NS}	0.851**	-0.820**	0.820**
SVII									1	0.0744^{NS}	0.874**	-0.764**	0.696**
LIG										1	0.773**	-0.805**	0.637**
TP											1	-0.835**	0.751**
VA												1	-0.717**
GR													1

Note: * Significant at 5 %; ** significant at 1 %, NS: non significant, GERM%: germination %, SC%: seed coat to cotyledon, MS: mechanical strength of seed coat, HSW: 100- seed weight, SDW: seedling dry weight, SG: seedling growth, SVI: seedling vigour index-I, SVII: seedling vigour index-II, EC: electrical conductance from seed leachates, LIG: lignin content,TP: total phenols,VA: volatile aldehydes, GR: glutathione reductase activity,

5. DISCUSSION

5.1 Seed storability

Seeds of 24 genotypes, having adequate quantity, collected from *kharif* 2015, were stored under chosen laboratory: ambient storage environment (average 25±2°C and 65±5%RH). Seed germination in soybean lines declined drastically with higher storage period. The mean germination% at the commence of seed storage was 96%, whereas in eight months of stored seeds, it declined to 76%, under ambient storage environments irrespective of contrasting quality. The decline in seed germination was more evident under ambient storage; more in sensitive storage environment. Seed storage atmosphere (both temperature and RH) were the key factors, answerable for seed aging and seed viability (Mladenet al2012). Normal ageing led to decreased seed germination; significant decrease was pragmatic under usual storage conditions (ambient storage). The current conclusion were in agreement with the interpretation of previous researchers' *viz* Singh and Ram (1986), Zahid (2013) and Sooganna (2015).The results further established that, in addition to genotypes, storage situation and period were other noteworthy factors upsetting the germination of soybean seed (Balasevic et al 2005).

5.2. Physical phenotyping

Physical attributes *viz* seed coat (testa) colour, seed hilum colour, mechanical strength of seed coat and seed coat% were establish to sway storability of soybean seeds by influencing the amount of confrontation to mechanical injury.

5.2.1. Seed coat and hilum colour

Seed coat colour in soybean plays an important role in maintaining and preserving the seed quality. Good storer soybean genotypes, were black, whereas poor storer soybean genotypes were yellowish-orange in my study. Among the 24 genotypes studied, white hilum having genotypes performed better than those to brown/black hilum genotypes. White hilum was identified as an indicator of good seed quality (storability).Seed coat pigmentation in soybean is embarrassed by three sovereign heritable loci *ie I, R* and *T* (Bernard and Weiss 1973). The black-seeded genotypes having white hilum maintained seed quality higher than IMSCS (70%) than those yellow-seeded with brown to grey hilum even at eight months of laboratory ambient storage. These results were in accordance with earlier work of Kumar (2005) and Sooganna (2015) in soybean. Similarly, black seed coat associated with resistance to field weathering (Mugnisjah et al 1987), resistance against pathogen (Starzinger and West 1982) and hence better storer. Srikantaradya (1982) concluded that black-seeded soybean genotypes were superior storer than yellow seeded ones. Kueneman and Wein (1981); Singh and Ram (1986) in a separate studies reported that black-seeded land race varieties of tropical (hot and humid) and sub-tropical regions, such as T-49 and Kalitur have improved storability than yellow-seeded temperate varieties. The black-seeded genotypes were, in common, unimproved land races; contain elevated anthocyanin substance, which acts as an anti-oxidant and inhibits oxidation of oil. Oxidation of linolenic acid and linoleic acid (unsaturated fatty acids) has been reported as one of the major reasons for seed deterioration in soybean. The oil substance in black seeded genotypes is also less; may be accountable for healthier seed storability of black seeded genotypes.

5.2.2 Mechanical strength of seed coat

Hard seed coat was found to be very beneficial in maintaining seed viability and vigour.

There were significant variations in mechanical strength of seed coat among good and poor storers in the freshly harvested seeds. However, these variations were non-significant among fresh and stored seeds in the present study. No clear association was reported between the mechanical strength of the seed and their seed quality, in eight months of ambient laboratory stored soybean seeds (Sooganna 2015). On contrary, Zahid (2013) reported a significant reduction in soybean seed coat hardness, which was from 36 months stored seeds. As stated in para 5.2.1 the black-seeded genotypes with more mechanical strength are land races; rich in anthocyanin, an antioxidant and with less oil; which may accounts for better storability of black-seeded genotypes with greater mechanical strength.

5.2.3 Seed coat percentage

The proportion of the seed coat is of primary significance in providing guard against mechanical injury during harvesting and processing, which cause noteworthy loss in viability of a seed lot. Kuchalan (2006) reported that good storer soybean genotypes are having more seed coat to cotyledon than poor (yellow-orange seeded) storers.

Black-seeded soybean genotypes showed higher seed coat (%) than those to yellow-orange genotypes. Further higher proportion of seed coat was not correlated with higher seed weight. Higher proportion of seed coat may probably has better shielding function to embryonic axis.

5.2.4 Seed coat histology and seed coat thickness

Thicker seed coat tissues in small seeded soybean seeds, owing to maintenance of viability, is in contrary to bold seeds. Soybean genotypes with thicker seed coat were proved to be better with respect to seed quality (viability and vigour). Figures (1&2) depicted that the hour-glass cells, were rather more in number, uniform in shape and distribution along the cross section of the seed coat in good storer genotypes than compared to the yellow-orange seeded poor storers. Menezes et al (2009) reported that transverse sections of the testa in soybean cultivars M-Soy 8400 and M-Soy 8411 verified three observable cell layers: palisade cell layers, an hourglass cell layer, and spongy parenchyma cells.

Carlson (1973); Carlson and Lersten (1987) reported that soybean seed testa includes an epidermis of single cell layer of very much cutinized palisade cells, a hypodermis of single layer of huge cells with fat anticlinal walls having intercellular spaces, plus an inside parenchyma composed of 6–8 layers of compressed thin-walled cells. Pereira and Andrew (1985) reported that the hourglass cells lying and connecting the inner palisade and parenchyma cell layers turn into deformed at the seed coat crinkle site ensuing in seed coat damage. These hourglass cells, with a cushioning effect, facilitate in preventing the wrinkle caused by the hydration and dehydration. Seed coat facade properties, chiefly the existence of pits (pores) and deposits also sway the water permeability, fungal invasion, and the seed longevity in soybean (Calero et al1981; Wolf et al1981; Hill and West 1982).

5.3 Physiological phenotyping

Physiological parameters studied in detail were electrical conductance from seed leachates, 100-seed weight, seed germination (%), seedling growth (cm), seedling dry weight (mg) and vigour indices I and II, from good and poor storers.

5.3.1 Electrical conductance from seed leachates

Seed coat is extremely important in protecting the solute from injury by rapid and slow hydration and in maintaining seed viability. EC is inversely correlated with seed quality. Electrical conductance from seed leachates increased with storage. Electrical conductance as of seed leachates in fresh and stored seeds was considerably higher in yellow-orange seeded, poor storer genotypes, which is in contrary to black-seeded good storer ones. There were significant variations registered in EC values among different genotypes as well as among fresh and stored seeds.

The black-seeded genotypes were well-off in anthocyanin substance, behave as an anti-oxidant and prevent oxidation of oil. Oxidation of linolenic acid and linoleic acid is one of the chief reasons for seed worsening in soybean. The lipid peroxidation, is initiated by the oxygen around unsaturated fatty acids, originate in the phospholipids of cellular membranes. Elevated oil content in yellow-orange seeded genotypes led to more degradation of bio-membranes, thereby higher EC values liable for poor storer. During ageing, ROS build up engender changes in structural and functional properties of membrane of lipids, which amplify membrane permeability (Simon 1974). Soybean with black seed coats, in addition imbibe more water gradually than non-pigmented ones and show lower imbibitional injury (Tully et al 1981). An enhance in electrolyte seepage from seeds was connected with dwindling germination, the change was being more prominent in large seeds (Vyas et al 1990). A significant linear correlation between electrolyte leakage from imbibed seed and germinability had been reported in soybean by several workers (Ferguson et al1990; Hampton et al 1992). Loeffler et al (1988) reported that physical damage to seed coat and seed size unfavourably affects the conductivity test results. From the current study, it was evident that electrical conductance from seed leachates be considered as a good sign for screening soybean genotypes for seed quality and vigour (storability).

5.3.2100-seed weight

Seed size plays an important role in preserving soybean seed quality. Seed size varied among genotypes. 100-seed weight was found to be negatively correlated with seed quality. In the current study, good storer genotypes were usually small seeded, whereas large seeded were poor storer. This was also in conformity of the prior information of Singh (1976); and Srikantaradya (1982) in soybean that varieties with larger seed size normally demonstrate poor storability. Improved storability of small seeds may be accredited to it unconstructive correlation to mechanical damage and seed coat permeability (Paulsen et al 1981; Tekrony et al 1984; Mugnisjah et al 1987). Analogous remarks of better storability of small seeded genotypes in soybean was reported by Vanangamudi 1988; Lal 1996; Kuchlan 2006; Jain 2011; Zahid 2013; Sooganna 2015.

5.3.3 Seed germination

Seed germination is a process dependent on multiple variables, including the integrity of seed and its physiological state. The seed germination (%) of good and poor soybean genotypes both in fresh and stored seeds registered significant variations among different genotypes. Kuchlan et al (2006) found 18–30% decrease in germination in different varieties of soybean in12 months ambient stored seeds. Black and yellow seeds from a bulk of F_4 population derived from the cross between black and yellow seeded parents and establish that the germination of yellow seeds declined faster in storage than compare to that of black seeds (Starzinger and West 1982). Seed deterioration results in decreased rate of germination and percentage of normal seedlings in soybean (Mohammadi et al 2011).

5.3.4 Seedling growth

Seedling growth, in common, decreased with augmented seed storage. However, turn down was added under ambient storage atmosphere. There were significant variations in seedling growth among different genotypes as well fresh and stored seeds. Newly harvested seed were reported to have superior seedling development than those to stored seed in soybean [Zahid (2013); Sooganna (2015)].Such turn down in the seedling length is an outcome of decreased weight in mobilized reserve (Mohammadi2011). Black-seeded, good storer, soybean genotypes registered a lesser turn down in seedling development value in stored seeds and were acknowledged as good performer.

5.3.5 Seedling dry weight

Seedling dry weight registered significant variations among the genotypes as well fresh and stored seeds. The seedling dry weight, an excellent index of seed vigour in soybean (Edje and Burris1971) decreased with higher storage. There were noteworthy differences recorded between genotypes and storage environment. Higher seedling dry weight was pragmatic in freshly harvested seeds, which are in contrary to stored seeds. Mean seedling dry weight at the start of seed storage was higher than those to eight months stored seeds. Such decline in the seedling dry weight may be an outcome of decreased weight in mobilized reserve. The current study was in agreement to the earlier statement of Abady et al (2012) in soybean, where they reported that the vigour traits, akin to seedling length and seedling dry weight differed considerably between the varieties; Giza-111 surpassed Giza-35 and Giza-2; the seedling dry weight declined significantly in six months stored seeds.

5.3.6 Seedling vigour indices

Seed vigour indices, in common, decreased with amplified seed storage. Freshly harvested seed showed superior vigour indices than those to stored seed. The decline was more in the poor storers than those to good storers.

My work was in the line of seedling vigour calculated as seedling length and seedling dry weight decreases during storage eunder normal environment (Dey andBasu1982; Agrawal and Kharlukhi 1985). Freshly harvested seed showed superior vigour indices than those to stored seed (Hosamani 2013). However, turn down was more under ambient storage environment. Such reduction in the seedling development and seedling dry weight may be an outcome of decreased weight in mobilized reserve. The results were in harmony with prior work of Zahid (2013) and Sooganna (2015) in soybean.

5.4. Biochemical phenotyping

Details on biochemical phenotyping involved changes in lignin, total phenol contents, volatile aldehydes assays and antioxidant enzyme *viz* glutathione reductase.

5.4.1Lignin content

Lignin is a natural complex phenolic polymer, found in the cell wall, are believed to contribute to the compressive strength, essential for mechanical support, water convey, and resistance in vascular plants (Lewis and Yamamoto 1990; Campbell and Sederoff 1996). Lignin in the seed coat of soybean apparently imparts resistance to mechanical damage (Alvarez et al 1997). The higher the lignin content in the seed coat, the better is the expected resistance to mechanical damage. This resistance is a genetic attribute that varies amongst soybean cultivars (Carbonell and Kryzanowski 1995). In eight months stored seeds, there was no significant change observed in lignin content. In my present study of lignin content between good and poor storer were significantly different. In soybean, seed coat lignin also play an important role in physical and physiological seed traits (Alvarez et al 1997). The soybean seed coat is awfully thin and squat in lignin content, and provides little protection to the weak radicle that lies in a susceptible location straight beneath the seed coat (Agrawal and Menon 1974; Gupta et al 1973, França Neto and Henning 1984).The steadiness of seed coat lignin yet in storage can be explained by the insolubility and intricacy of the lignin polymer.

5.4.2 Total phenol content

Legume seeds, contain proanthocyanidines (*ie* condensed tannins), the principal phenols content located mainly in the seed coat (hull), play an important role in the defence system of seeds that are exposed to damage by many environmental factors such as light, oxygen, free radicals etc. The phenol content registered decline significantly among all genotypes with storage. The decline was more in the poor storers than those to good storers. Hagermanet al (1998) reported that tannins were 15–30 times more efficient in the quenching of peroxylradicals than simple phenolics. Phenolics, including phenolic acid, lignin and isoflavones, have been reported to have noteworthy role in seed mechanical damage resistance and disease resistance. Phenolic acids and flavonoids have been well thought out as great antioxidants and proved to be more efficient than vitamin C, E and carotenoids. Phenolic traits may be used to screen the resistant germplasm in soybean upgrading programme (Nacer Bellaloui 2012).

5.4.3 Volatile aldehydes

Peroxidation of membrane phospholipids, rich in unsaturated fatty acids, results in the production of hydroperoxides, which upon hydration are enzymatically metabolized to various aldehydes and ketones. In the present study, a sharp increase in the release of volatile aldehydes, concomitant with the reduction in germination was pragmatic in poor storer than good storer. The results was in agreement to that of Kumar (2005), who reported elevated production of volatile aldehydes from bold and yellow-seeded poor storer soybean varieties than the small black-seeded good storer varieties. This clearly indicated the role of lipid peroxidation in seed longevity behaviour. There was an increase in volatile aldehydes content with increased period of seed storage. Accumulation of volatile aldehydes during seed ageing, which are released upon hydration, is a marker of lipid peroxidation (Zhang et al 1993). The level of volatile aldehydes released by the poor storer genotypes was superior to good storer genotypes, though it increased in all the genotypes throughout ageing. This clearly indicated the role of lipid peroxidation in seed longevity behaviour. The cross linking of macromolecules and modification of functional proteins in seeds by the endogenously accumulated aldehydes are cited as the reasons of seed deterioration (Esashi et al1997; Vijjay and Dadlani 2003). Between the advance breeding lines, three genotypes, namelyBragg, DS-12-13 and SL-525 registered minor content of volatilealdehydes in eight months stored seeds, pinpointing of to be better storer.

5.4.4 Glutathione reductase (GR)

Glutathione reductase (GR) takes part in the control of endogenous hydrogen peroxide through an oxido-reduction cycle involving glutathione and ascorbate. In my study, freshs seeds, among good storers registered higher GR values than those to poor storers. So also changes in GR values were more substantial among stored seeds of good storers than those to poor storers. Lipid peroxidation is an indirect indicator of the level of seed deterioration, particularly in oil rich soybean seeds (Wilson and McDonald1986a). Lipid peroxidation is known to be a major cause of loss in seed viability especially in soybean (Sung 1996), peanut (Sung and Jeng 1994) and sunflower (Baily et al1996).Wilson and McDonald (1986b) and McDonald (1999) suggested that seed lipids are subjected to continuous oxidation resulting in formation of hydroperoxides, oxygenated fatty acids and more reactive free radicals, which cannot be quenched and therefore accumulate in dry seeds. The mechanism of lipid peroxidation, which is commonly initiated by the oxygen around unsaturated fatty acids, found in the phospholipids of cellular membranes, results in release and a chain of free radical mediated reactions inside the biological systems causing profound damage to cellular membrane as well as other macro-molecules (Yaklich 1985; Begnami and Cortlazzo 1996; Thapliyal and Connor 1997). A significant variation was noted in the level of lipid peroxidation among different soybean genotypes. The enzymatic and non-enzymatic antioxidative systems play a crucial role in controlling seed deterioration. Keeping this in view, the antioxidative mechanism provided by glutathione reductase (GR) was studied in seeds of 24 selected soybean genotypes of contrasting longevity from eight months laboratory ambient storedseeds. The ascorbate-glutathione cycle may also take part in H_2O_2 scavenging, which involves ascorbate peroxidase and glutathione reductase. The enzymes of this cycle which are present in the chloroplast, the cytoplasm, mitochondria, peroxisomes and apoplast, participate in the regeneration of the powerful antioxidants ascorbic acid and reduced glutathione (Mittler 2002). Loss of seed viability has been reported to be associated with a reduction in the activities of antioxidant enzymes *viz* GR which play a significant role in providing protection against highly reactive free radicals both under accelerated ageing and storage conditions (Bailly et al 1996; Kuchlan 2006; Sooganna 2015). From the present study, it was evident that antioxidant enzyme activity was higher in good storer than those to poor storer genotypes. Loss of viability was associated with the decrease in antioxidant activity under laboratory ambient storage environment.

5.5 Correlation-coefficient(s)

The details on correlation coefficient values among various physical, physiological and biochemical parameters in good and poor storer soybean genotypes is given (table 15). Vieira et al (1999) reported significant correlations among standard germination, electrical conductivity, and seedling field emergence. In the present study, seed germination was either +ve or –ve but significantly correlated with all the traits of seed quality. There were positive correlations registered between seed germination and physiological parameters like, seedling vigour index-I (r = 0.941**), vigour index-II (r = 0.839**), seedling dry weight (r = 0.837**), seedling growth (r = 0.788**); and biochemical parameters like, phenol content (r = 0.927**) and GR activity (r = 0.809**) at 1% significance level. There were negative correlations registered between seed germination and physiological parameters like, electrical conductance from seed leachates (r = (-) 0.932**) and 100-seed weight(r = (-) 0.706**) and biochemical parameter like volatile aldehyde (r = (-) 0.904**) at 1% significance level. However, non–significant correlations were observed between germination and physical parameters like, seed coat % (r = 0.094^{NS}), seed coat mechanical strength (r = 0.092^{NS}) and lignin content (r = 0.0875^{NS}). The results inferred that SVI followed by SV II, SDW, EC, phenol contents, GR activity and volatile aldehyde contents either individually or in combinations may be used as better indices of soybean seed quality and vigour (table 15). Kumar (2005), Zahid (2013), Sooganna (2015) in soybean, had also reported that SVI followed by seedling growth as better indicator of seed vigour in soybean.

Based on above explained observations, in my present study, ***following conclusions were drawn***:

a) Black-seeded having white hilum, thick seed coat and higher seed coat (%) with more and uniform hours glass cells attributed to better seed quality in soybean.
b) Total phenol contents, volatile aldehyde assays and efficiency of antioxidant system like GR, among biochemical phenotyping were identified as good indices of seed coat and in turn seed quality (vigour).
c) Based on studied, various physical, physiological and biochemical parameters, AMSS-34 and G-2265 were identified as better genotypes and may be exploited in soybean crop improvement programme.
d) Based on correlation coefficient values, SVI, EC, total phenol contents and volatile aldehyde assays from soybean seed coat were identified as better indices of seed quality (seed viability and vigour) in studied soybean genotypes. These makers either individually or in combination with black smaller seed, having white hilum may be used as better indices of seed coat characteristics and in turn seed viability and vigour (quality).

Black, smaller seed with white hilum attributed to better seed quality. Based on correlation coefficient values, any of the SV I, SV II, SDW, phenol content and/or EC either individually or in combination may be used as better indices of seed vigour. Volatile aldehyde contents, phenol contents and efficiency of antioxidant system, like GR may also be used as good indices of seed vigour or quality. The good storability of soybean seeds can be attributed to the combined effect of seed coat colour, seed size, seed coat permeability; lower electrical conductance from seed leachates, efficiency of antioxidant system in the seed to counter free radical accumulation.

5.7. Future line of work

Following need to be addressed:

- Histo-chemical characterization of more genotypes and their correlation with seed longevity.
- Alike, MRI tests in human beings, some magneto-resonance image analysis for detailed analysis of seed/seed histology may be developed.
- Molecular characterization of good and poor storers and identification of QTL need to be addressed.

6. SUMMARY AND CONCLUSION

Soybean [*Glycine max* (L) Merrill] genotypes were investigated for phenotyping the seed coat characteristics and its association with seed quality. Based on the previous work, 24 soybean genotypes, having contrasting seed longevity *ie* 12 genotypes each from good (black-seeded) and poor (yellow-orange seeded) storers, were used for detailed study. Fresh seeds were obtained from one cycle of multiplication, *kharif* 2015, at divisional experimental farm. These soybean genotypes, having sufficient quantity of seeds (approx 200g each), were stored for eight months under laboratory ambient environment (average 25 ± 2^0C and 65±5% RH). Various physical, physiological and biochemical parameters were studied to establish its association between seed coat characteristics and seed quality.

The following were the major findings:

1. There weregenotypic variations in seed coat characteristics and soybean seed quality.
2. Black-seeded with white hilum soybean genotypes were found to be better performer.
3. Seed coat and hilum colour remain unchanged with seedstorage.
4. No clear association could be establishedbetween the mechanical strength of the seed and their seed quality from the eight months laboratory ambient stored seeds, in the present study.
5. Black-seeded genotypes with thicker seed coat were proved to be better performer.
6. Black-seeded genotypes with more seed coat (%) were identified as better performer.
7. Transverse sections of the seed coat of soybean seeds depicted three observable cell layers: an outer epidermis (palisade cell layer), an hourglass cell layer and inner spongy parenchyma cells.
8. The hour-glass cells were, rather more in number, uniform in shape and distribution along the cross section of the seed coat in good storers(black-seeded) than those to poor storers (yellow-seeded) genotypes.

9. Seed size varied among genotypes. 100-seed weight was found to be negatively correlated with seed quality. Smaller seeded genotypes, namely AMSS-34, G-2265 and G-2601were found to be better performer.
10. Seed germination decreased in stored seeds among all the genotypes studied.
11. Electrical conductance from seed leachates, irrespective of genotypes, increased in stored seeds; increase was over two folds in poor storer (yellow-seeded) genotypes even from eight months stored seeds.
12. Genotypes AMSS-34, G-2253 and G- 2265 recorded lower EC values after storage, hence identified better performers.
13. Seedling growth and seedling dry weight, in general, decreased with seed storage. Any of these two parameters, either individually or in combination, may be used as good marker of seed vigour and/or seed quality. Vigour indices, in general, decreased with seed storage and may also serve a good marker of seed quality.
14. No clear association was registered between the lignin content and seed quality in the present study.
15. Total phenol contentsfrom seed coat proved to be a good marker linked with seed storability.
16. The volatile aldehyde assays registered higher values in poor storer (yellow-seeded) than those to good storer (black-seeded) genotypes. G-2601 and G-2251 genotypes were found to be better performer.
17. Significantly higher antioxidant enzyme activity GR was recorded in good storer (black-seeded) genotypes. This may also be used as good marker for seed quality (storability).
18. Based on GR enzyme assays, AMSS-34, G-2603 and G-2256 were identified as better genotypes from this present study.
19. Based on correlation coefficient values among physical [MS($r = 0.092^{NS}$), SC% ($r = 0.094^{NS}$)];physiological [100-seed weight($r = (-)0.706$), EC{$r = (-)0.932$}, SG($r = 0.788$), SDW($r = 0.837$), SV I($r = 0.941$), SV II($r = 0.839$);and biochemical [lignin ($r = 0.0875^{NS}$), [phenols ($r = 0.927$), volatile aldehydes {r =

(-)0.904)}, GR activity (r = 0.809)], parameters namely, SV I, SV II, total phenol content, number and distribution of hour glass cell, GR and volatile aldehyde assays from seed were identified as better idices of seed quality (ie seed viability and vigour) in studied soybean genotypes.

Based on above explained observations, in my present study, ***following conclusions were drawn***:

a) Black-seeded having white hilum, thick seed coat and higher seed coat (%) with more and uniform hours glass cells attributed to better seed quality in soybean.

b) Based on correlation coefficient values, SVI, EC, total phenol contents and volatile aldehyde assays from soybean seed coat were identified as better indices of seed quality (seed viability and vigour) in studied soybean genotypes. These makers either individually or in combination with black smaller seed, having white hilum may be used as better indices of seed coat characteristics and in turn seed viability and vigour (quality).

c) Based on studied, various physical, physiological and biochemical parameters, AMSS-34 and G-2265were identified as better genotypes and may be exploited in soybean crop improvement programme.

“Seed coat characteristics in relation to seedviability and vigour in soybean”

ABSTRACT

Soybean [*Glycine max* (L) Merrill], among oilseed crops, is the cheapest source of high quality protein, in eradicating malnourishment and maintaining soil fertility. One of the major constraints in soybean cultivation is the non-availability of good quality seed and post-harvest maintenance of prescribed level of seed germination and vigour until immediate planting season. Hence, the present study, whereseeds of 24soybean genotypes,having contrasting seed longevity *ie* 12 genotypes each from good (black-seeded) and poor (yellow-orange seeded) storers, were used for detailed study.Fresh seeds were obtained from one cycle of multiplication, *kharif* 2015, at divisional experimental farm. These soybean genotypes, having sufficient quantity of seeds (approx 200g each), were stored for eight months under laboratory ambient environment (average 25 ± 2^0C and 65±5% RH). Various physical, physiological and biochemical parameters were studied to establish its association between seed coat characteristics and seed quality.

The results registered a genotypic variability with respect to seed viability and vigour.Black, small-seeded with white hilum soybean genotypes were found to be better storer. Seed germination, seedling growth, seedling dry weight, vigour indices, and total phenols, in general, decreased with seed storage. 100-seed weight, EC and volatile aldehyde contents registered higher values in poor storer than those to good storer genotypes. Parameters like seed coat(%), lignin content and mechanical strength remain unchanged during eight months laboratory ambient storage.There were either a positive or negative but a strong correlation, observed between seed viability and various investigated seed vigour parameters *viz* SVI followed by EC, SVII, seedling dry weight, glutathione reductase, seedling growth and one hundred seed weight, phenol and volatile aldehyde. Investigated all seed coat and/or seed vigour parameters except 100-seed weight, electrical conductance from seed leachates and volatile aldehyde were found to be positively correlated with seed quality ie seed viability and vigour.

Black-seeded having white hilum, thick seed coat and higher seed coat (%) with more and uniform hours glass cells attributed to better seed quality. Based on

correlation coefficient values,more and uniform hours glass cells, EC, SVI, total phenols and volatile aldehydes from soybean seed were identified as better indices of seed viability and vigour in studied genotypes. These indices either individually or in combination may be used as indicator of better seed coat characteristics and in turn seed quality (vigour). Based on studied, various physical, physiological and biochemical parameters in present study, AMSS-34 and G-2265 were identified as better genotypes, which may be exploited in soybean crop improvement programme.

सोयबीन में बीज जीवनक्षमता और ओज की संबद्धता वाले बीज आवरण अभिलक्षण

सारांश

तिलहन फसलों में सोयबीन [*Glycine max* (L) Merrill] उच्च गुणवत्ता प्रोटीन का एक सबसे सस्ता स्रोत है, जो कुपोषण दूर करता है और मृदा उर्वरता बनाए रखता है। सोयबीन की खेती में प्रमुख बाधाएँ उत्तम गुणवत्ता बीज की कटाई उपरांत से अगली रोपाई ऋतु तक अनुशंसित स्तर के बीज अंकुरण एवं ओज के साथ उपलब्ध न होना है। अत: वर्तमान शोधकार्य में विस्तृत अध्ययन के लिए ऐसे 24 सोयाबीन जीनप्ररूपों का उपयोग किया गया है जिनमें बीज जीविता में अंतर है, अर्थात अच्छे भंडारकों (काले-बीज) और खराब भंडारकों (पीले-नारंगी बीज), प्रत्येक के 12-12 जीनप्ररूपों का उपयोग किया गया है। संभाग के प्रायोगिक प्रक्षेत्र से खरीफ 2015 से, बहुगणन के पहले चक्र वाले ताज़े बीज प्राप्त किए गए। इन सोयाबीन जीनप्ररूपों में पर्याप्त मात्रा में बीज (प्रत्येक में लगभग 200 ग्राम) मौजूद थे, उन्हें प्रयोगशाला परिवेश वातावरण (औसत तापमान 25±2^{0}C और आपेक्षिक आर्द्रता 65±5%) के अंतर्गत आठ माह के लिए भंडारित किया गया। इनके बीज आवरण अभिलक्षणों और बीज की गुणवत्ता के संबंध को साबित करने के लिए विभिन्न भौतिक, कार्यिकीय और जैवरासायनिक प्राचलों का अध्ययन किया गया।

परिणामों से प्राप्त हुआ कि बीज जीवनक्षमता और ओज के संदर्भ में जीनप्ररूपों में विविधता मौजूद है। सफेद नाभिका युक्त काले, छोटे-बीज वाले सोयबीन जीनप्ररूप बेहतर भंडारक पाए गए। सामान्य तौर पर बीज के भंडारण के साथ-साथ बीज अंकुरण, बीज विकास, पौध शुष्क भार, फिनॉल, और ओज सूचकांकों में कमी आई। उत्तम भंडारक जीनप्ररूपों की तुलना में खराब भंडारक वाले जीनप्ररूपों में EC और वाष्पशील एल्डिहाइड मात्राओं के अधिक मान दर्ज किए गए। आठ माह के प्रयोगशाला परिवेश भंडारण के दौरान, बीज आवरण (%), लिगनिन मात्रा और यांत्रिकी दृढ़ता में कोई बदलाव नहीं आया। बीज जीवनक्षमता और जाँचे गए विभिन्न बीज ओज प्राचलों, जैसे SVI, EC, SVII, पौध शुष्क-भार, ग्लूटेथायोनरिडक्टेज़, पौध विकास, फिनॉल,वाष्पशील एल्डिहाइड,और 100-बीज भार के साथ सुदृढ़ सहसंबंध, धनात्मक या ऋणात्मक पाए गए। 100-बीज भार, EC (बीज निक्षालक की विद्युत चालकता) और वाष्पशील एल्डिहाइड मात्राओं को छोड़कर जाँचे गए अन्य सभी बीज आवरण और/या बीज ओज प्राचलों का बीज गुणवत्ता, जो कि बीज जीवनक्षमता और ओज है, के साथ धनात्मक सहसंबंध पाया गया।

सफेद नाभिका युक्त कालेबीज, मोटे बीज आवरण, एवं अधिक और समरूप युक्त कोशिका ने बीज गुणवत्ताके बेहतर बनाने में योगदान दिया। अध्ययनाधीन जीनप्ररूपों में,सहसंबंध गुणांक मानों के आधार पर बीज जीवनक्षमता और ओज के बेहतर सूचकांकों के रूप में बीज आवरण में सफेद नाभिका

युक्त काले, छोटे-बीज, अधिक और समरूप युक्त कोशिका, SVI, EC, बीज आवरण के वाष्पशील एल्डिहाइड और फिनॉलकी पहचान की गई। इन सूचकांकों का व्यक्तिगत, या संयुक्त रूप से उपयोग बेहतर बीज आवरण अभिलक्षणों, और परिणामस्वरूप बीज गुणवत्ता (ओज) के सूचक के रूप में किया जा सकता है। विभिन्न भौतिकी, कार्यिकी और जैवरासायनिक प्राचलों के आधार पर वर्तमान शोधकार्य में अध्ययनाधीन बेहतर जीनप्ररूपों के रूप में AMSS-34 और G-2265 की पहचान की गई, जिनका उपयोग सोयबीन फसल सुधार कार्यक्रम में किया जा सकता है।

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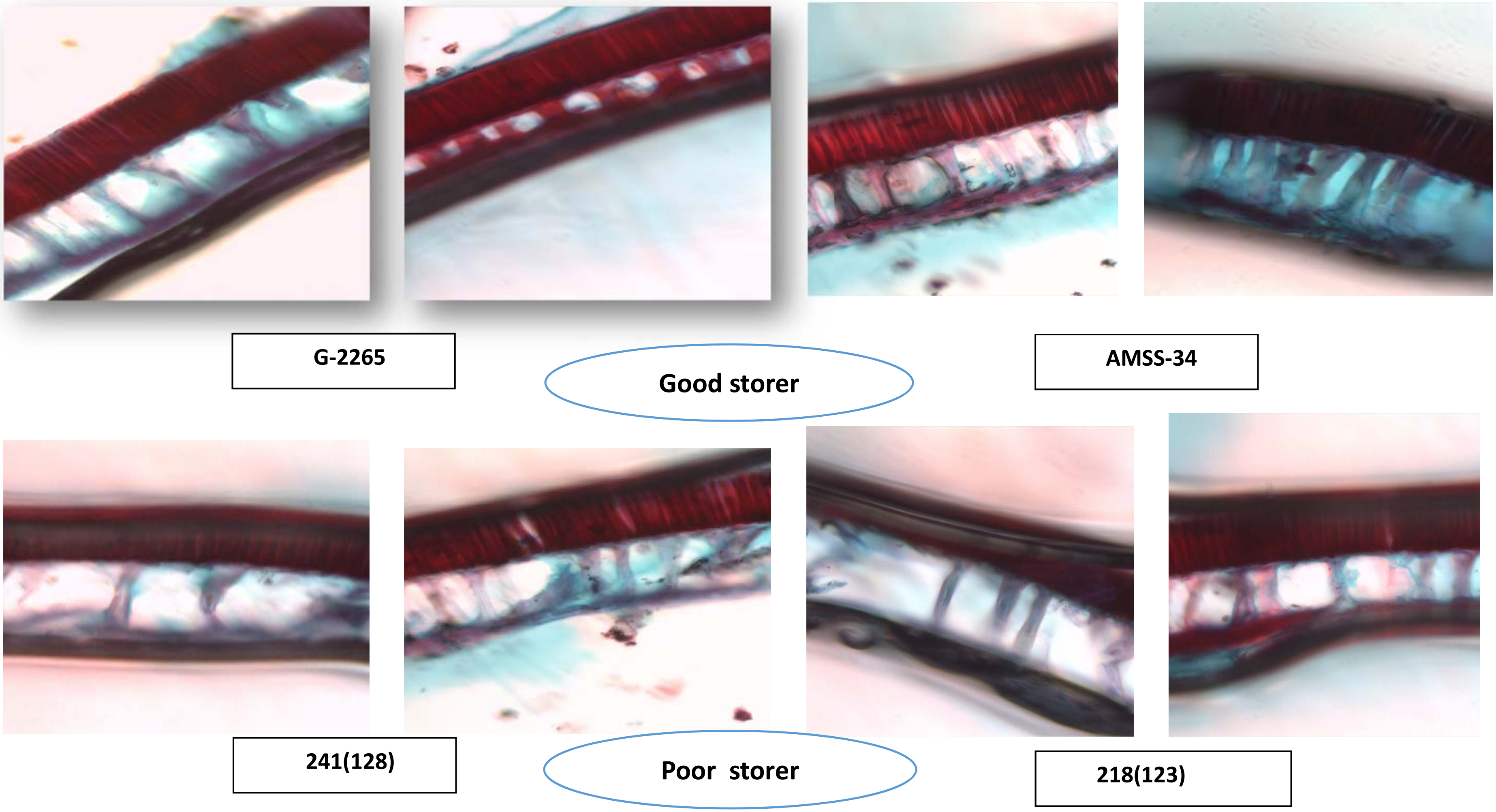


Fig:1 Soybean seed coat cross section view using phase contrast microscope

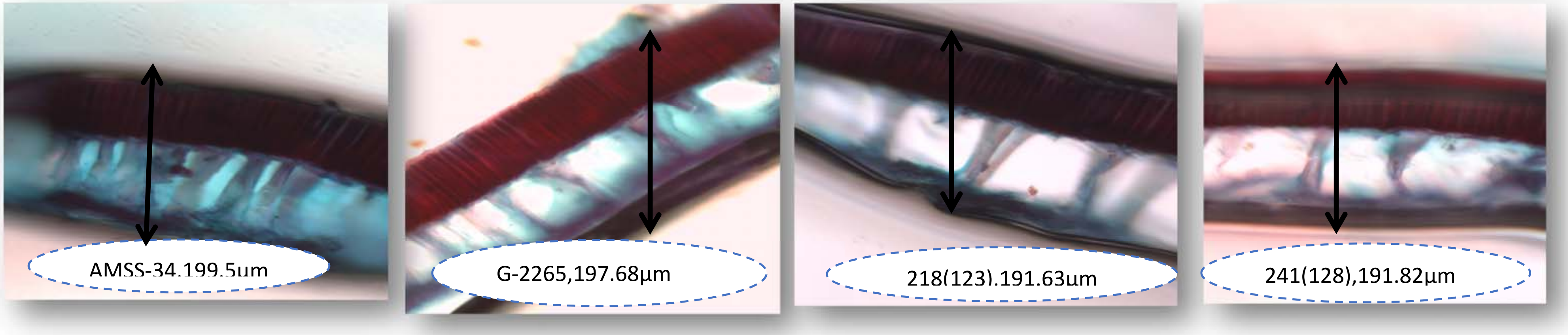


Fig:2 Soybean seed coat thickness using phase contrast microscope

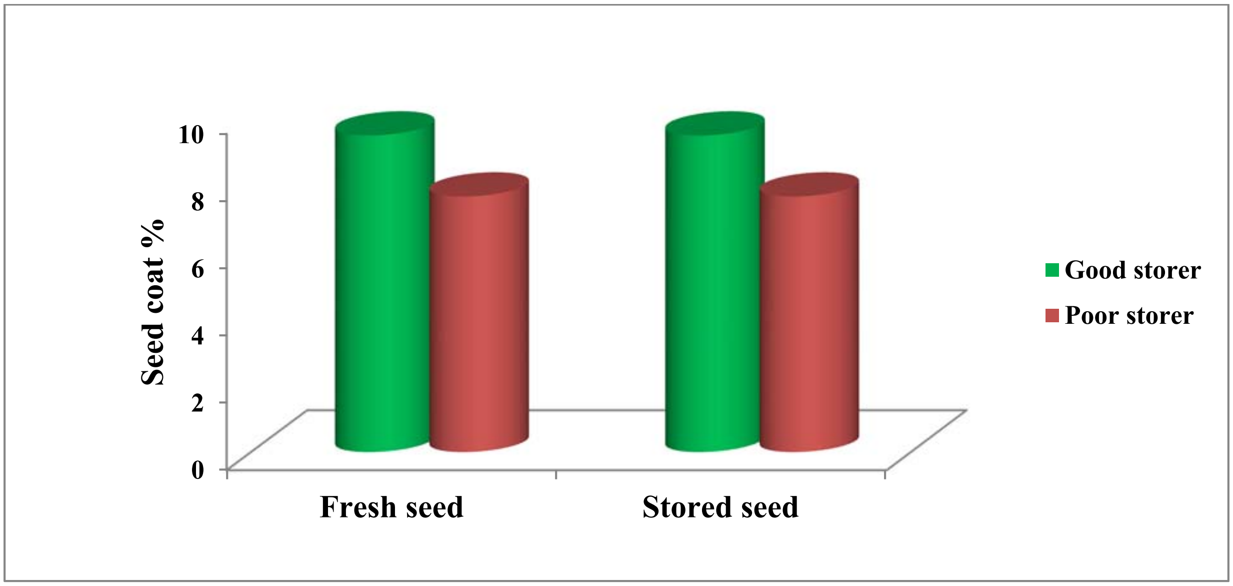


Fig:3 Seed coat percentage from fresh and 08 months ambient stored soybean seeds

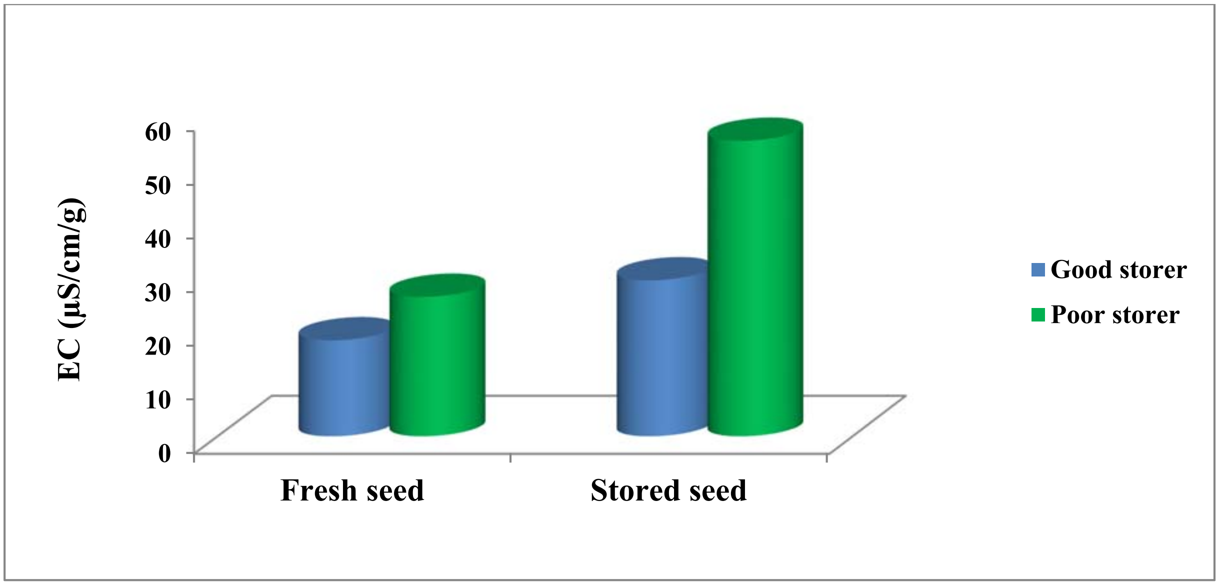


Fig:4 EC from fresh and 08 months ambient stored soybean seeds

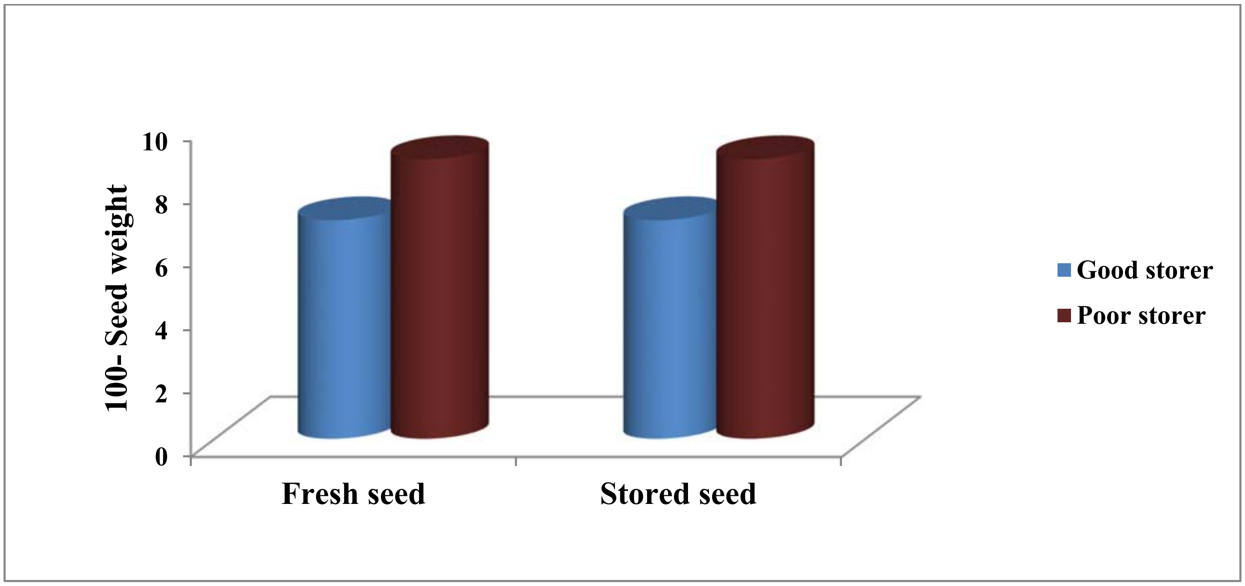


Fig:5 100-seed weight from fresh and 08 months ambient stored soybean seeds

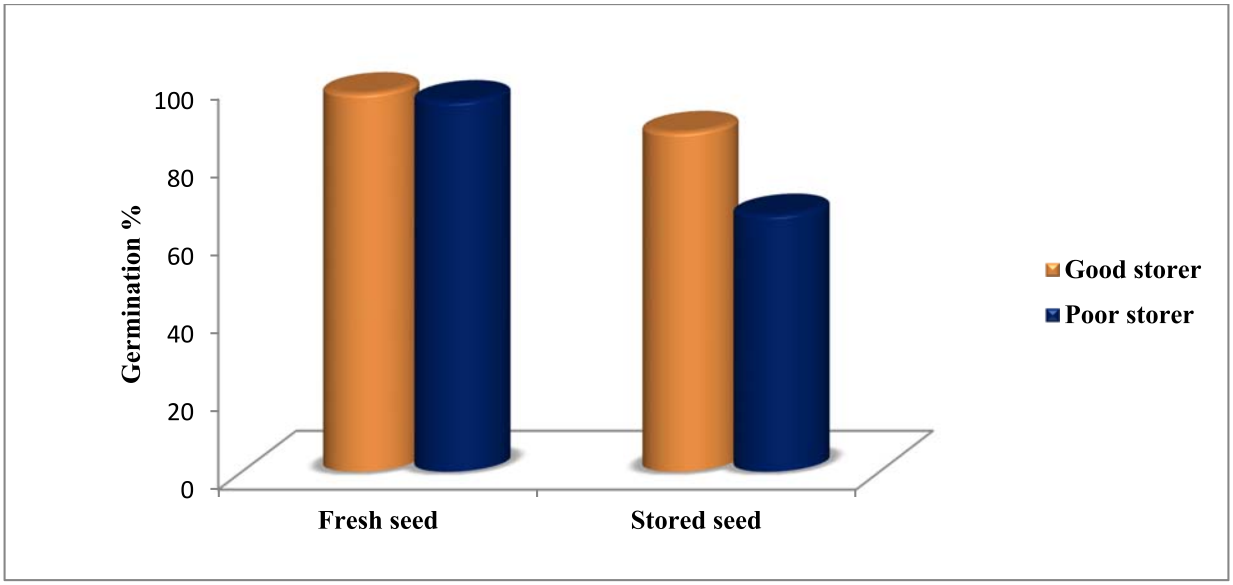


Fig:6 Germination percentage from fresh and 08 months ambient stored soybean seeds

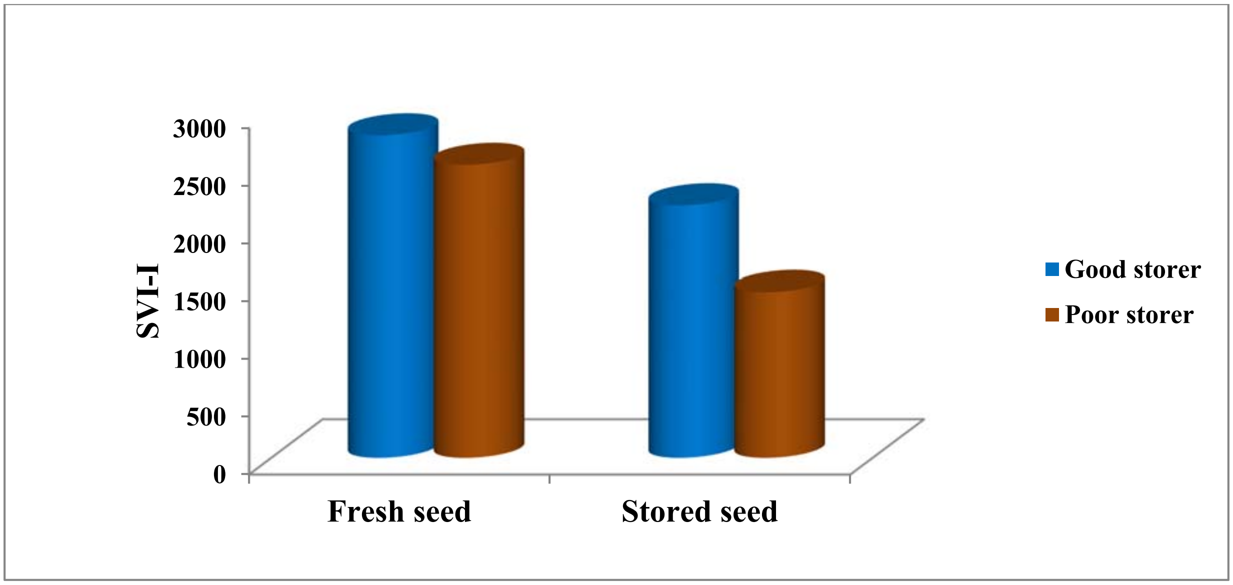


Fig:7 SV-I from fresh and 08 months ambient stored soybean seeds

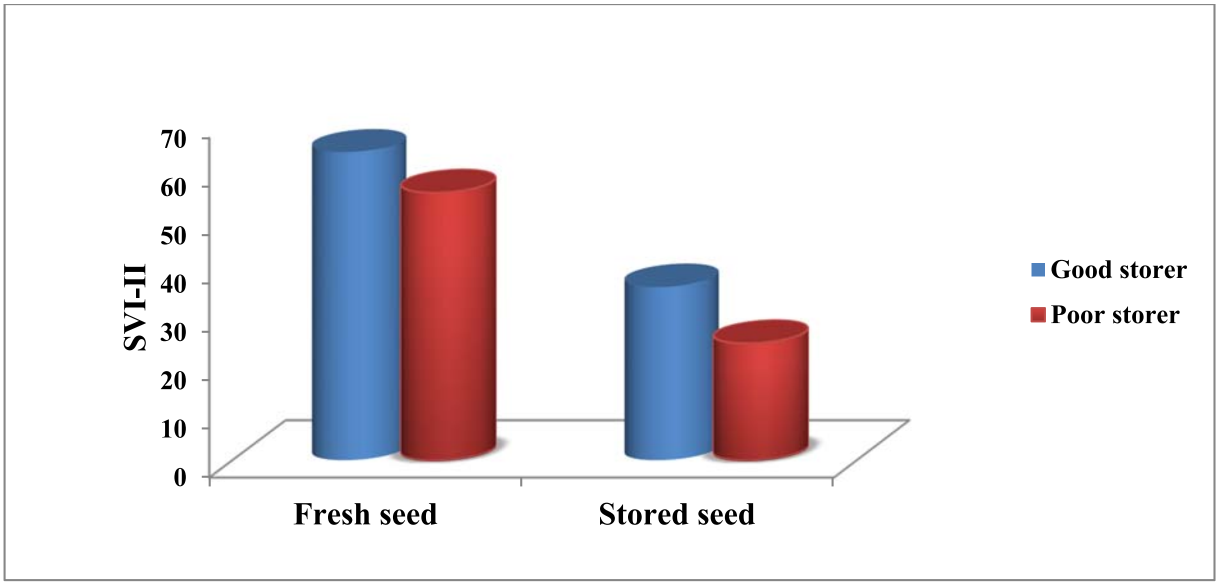


Fig:8 SV-II from fresh and 08 months ambient stored soybean seeds

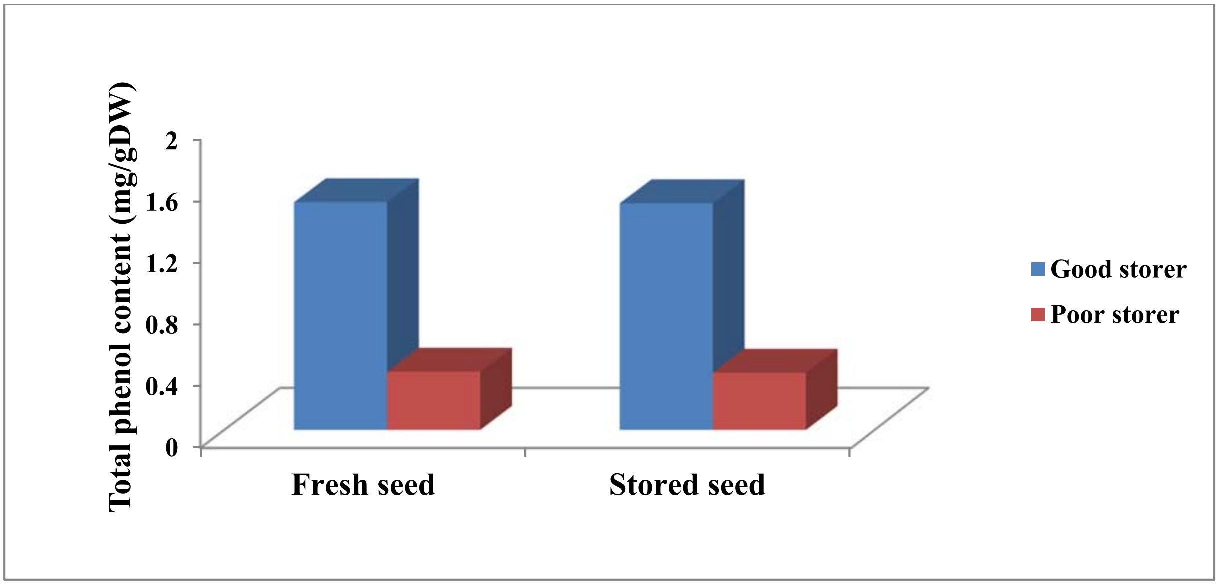


Fig:9 Total phenol content from fresh and 08 months ambient stored soybean seeds

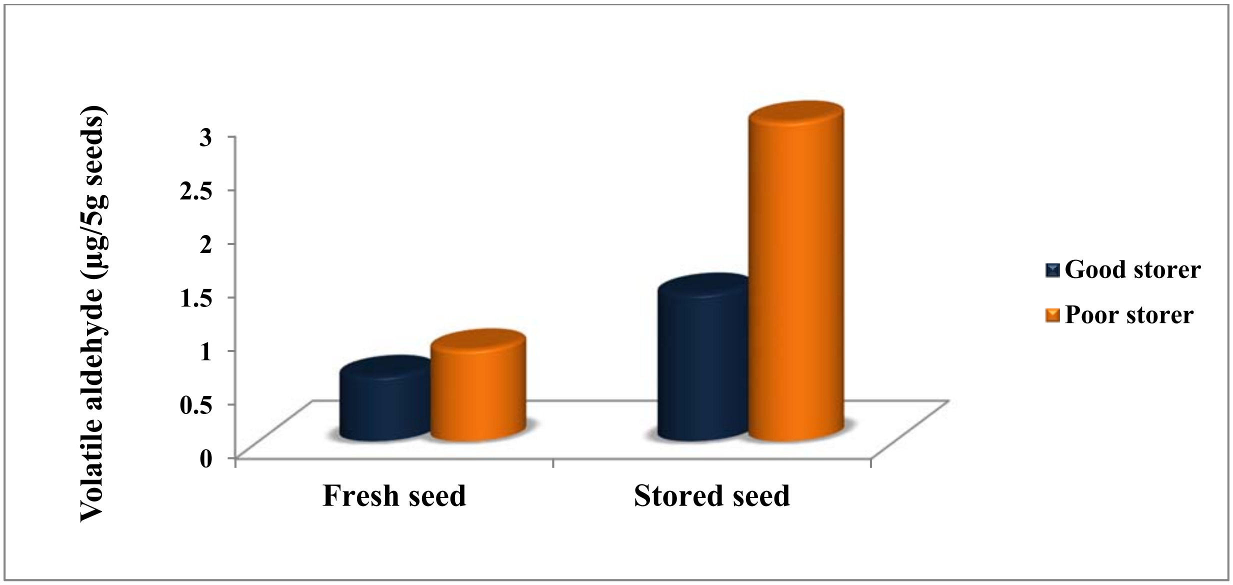


Fig:10 Volatile aldehyde content

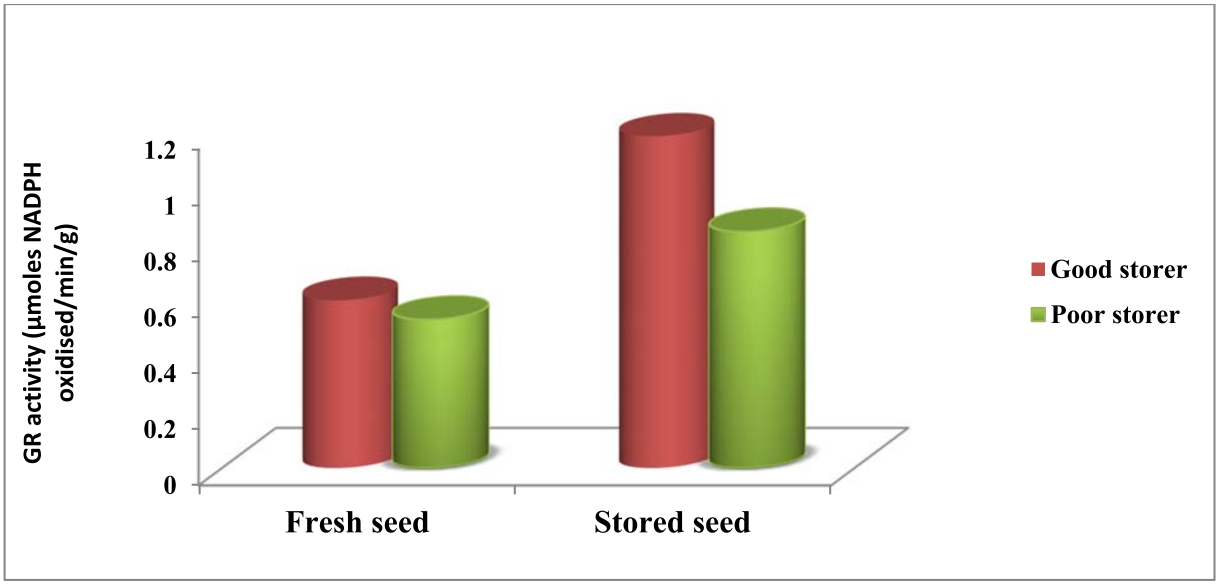


Fig:11 GR content from fresh and 08 months ambient stored soybean seeds

APPENDIX I

Table 16. ANOVA for mechanical strength (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	43.32859	23	1.883852	2789.084*	7.01692E-36	1.993239135
Within group	0.016211	24	0.000675			
Total	43.3448	47				

Where,SS :Sum of square, DF: degree of freedom, MS: mean squares, F: F values,
*: significance @ 5%

Table 17. ANOVA for mechanical strength (08 months ambient stored soybean seeds)

Source of variation	SS	DF	MS	F	P-value	F
Between group	43.32645885	23	1.883759	87922.56*	7.34E-54	1.993239135
Within group	0.000514205	24	2.14E-05			
Total	43.32697306	47				

Where,SS :Sum of square, DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 18. ANOVA for Seed coat epidermis+ hypodermis (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	13.92434	3	4.641445	0.086155	0.966291	3.490295
Within group	646.4821	12	53.8735			
Total	660.4064	15				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 19. ANOVA for Seed coat anticlinal layer (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	114.9155	3	38.30517	2.445317	0.114273	3.490295
Within group	187.9764	12	15.6647			
Total	302.8919	15				

Where,SS :Sum of square, DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

APPENDIX III

Table 20. ANOVA for Seed coat thickness (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	171.4146	3	57.13819	0.798765	0.518023	3.490295
Within group	858.3984	12	71.5332			
Total	1029.813	15				

Where,SS :Sum of square, DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 21. ANOVA for Seed coat to cotyledon ratio (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	43.32859	23	1.883852	*2789.084	7.01692E-36	1.993239135
Within group	0.016211	24	0.000675			
Total	43.3448	47				

Where,SS :Sum of square, DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

APPENDIX IV

Table 22. ANOVA for Seed coat to cotyledon ratio (08 months ambient stored seeds)

Source of variation	SS	DF	MS	F	P-value	F
Between group	43.32645885	23	1.883759	*87922.56	7.34E-54	1.993239135
Within group	0.000514205	24	2.14E-05			
Total	43.32697306	47				

Where,SS :Sum of square, DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 23. ANOVA for 100-seed weight (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	270.6397	23	11.76695	*69.01751	3.1E-58	1.619655
Within group	20.45906	120	0.170492			
Total	291.0988	143				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

APPENDIX V

Table 24. ANOVA for 100-seed weight (08 months ambient stored soybean seeds)

Source of variation	SS	DF	MS	F	P-value	F
Between group	270.7382	23	11.77123	*86.6639	9.98E-64	1.619655
Within group	16.29914	120	0.135826			
Total	287.0373	143				

Where,SS :Sum of square, DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 25. ANOVA for germination (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	153.4063	23	6.669837	*2.716991575	0.000671279	1.680281
Within group	176.75	72	2.454861			
Total	330.1563	95				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

APPENDIX VI

Table 26. ANOVA for germination (08 months ambient stored soybean seeds)

Source of variation	SS	DF	MS	F	P-value	F
Between group	10954.33	23	476.2754	*93.95021	1.23E-44	1.680281
Within group	365	72	5.069444			
Total	11319.33	95				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 27. ANOVA for Seedling length (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	104.4155	23	4.539802899	*10.72690009	9.06682E-08	1.993239
Within group	10.1572	24	0.423216667			
Total	114.5727	47				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 28. ANOVA for Seedling length (08 months ambient stored soybean seeds)

Source of variation	SS	DF	MS	F	P-value	F
Between group	189.8378	23	8.253817	*19.96494	1.3E-10	1.993239
Within group	9.921972	24	0.413416			
Total	199.7598	47				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 29. ANOVA for Seedling dry weight (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	0.201199	23	0.008748	*10.66196718	4.05111E-15	1.680281
Within group	0.059073	72	0.00082			
Total	0.260272	95				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 30. ANOVA for Seedling dry weight (08 months ambient stored soybean seeds)

Source of variation	SS	DF	MS	F	P-value	F
Between group	0.100048	23	0.00435	1.425548	0.129399	1.680281
Within group	0.219701	72	0.003051			
Total	0.31975	95				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance @ 5%

Table 31. ANOVA for Seedling vigour index-I (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	1271493	23	55282.31	*10.95112	7.35632E-08	1.993239135
Within group	121154.4	24	5048.098			
Total	1392648	47				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 32. ANOVA for Seedling vigour index-I (08 months ambient stored soybean seeds)

Source of variation	SS	DF	MS	F	P-value	F
Between group	7222348	23	314015.1	*79.19272	1.94E-17	1.993239
Within group	95164.85	24	3965.202			
Total	7317513	47				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 33. ANOVA for Seedling vigour index-II (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	1194.184	23	51.92105	*9.092657	4.69E-07	1.993239135
Within group	137.0452	24	5.710218			
Total	1331.229	47				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 34. ANOVA for Seedling vigour index-II (08 months ambient stored soybean seeds)

Source of variation	SS	DF	MS	F	P-value	F
Between group	1785.58	23	77.6339	*10.17269	1.54E-07	1.993239
Within group	183.1584	24	7.6316			
Total	1968.738	47				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 35. ANOVA for electrical conductance of seed leachate (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	1421.774	23	61.81628	*32.43544	7.14E-22	1.756759
Within group	91.4796	48	1.905825			
Total	1513.254	71				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

APPENDIX XI

Table 36. ANOVA for electrical conductance of seed leachate (08 months ambient stored soybean seeds)

Source of variation	SS	DF	MS	F	P-value	F
Between group	13672.99	23	594.4777	*40.01058	6.86E-24	1.756759
Within group	713.1847	48	14.85802			
Total	14386.17	71				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance @ 5%

Table 37. ANOVA for lignins (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	0.174944	23	0.007606	*12.7686	1.52E-08	1.993239135
Within group	0.014297	24	0.000596			
Total	0.189241	47				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

APPENDIX XII

Table 38. ANOVA for lignins (08 months ambient stored soybean seeds)

Source of variation	SS	DF	MS	F	P-value	F
Between group	0.18058	23	0.007851	*56.72225	9.52E-16	1.993239
Within group	0.003322	24	0.000138			
Total	0.183902	47				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 39. ANOVA for phenols (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	16.54895	23	0.71952	*2111.835	1.97E-34	1.993239
Within group	0.008177	24	0.000341			
Total	16.55713	47				

Where,SS :Sum of square, DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 40. ANOVA for phenols (08 months ambient stored soybean seeds)

Source of variation	SS	DF	MS	F	P-value	F
Between group	16.53074	23	0.718728	*753.1696	4.57E-29	1.993239
Within group	0.022903	24	0.000954			
Total	16.55364	47				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 41. ANOVA for glutathione reductase (GR) (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	0.886192	23	0.03853	*70.84775	1.6E-29	1.756759381
Within group	0.026105	48	0.000544			
Total	0.912297	71				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

APPENDIX XIV

Table 42. ANOVA for glutathione reductase(GR) (08 months ambient stored soybean seeds)

Source of variation	SS	DF	MS	F	P-value	F
Between group	2.782263	23	0.120968	*233.1959	1.21E-41	1.756759
Within group	0.0249	48	0.000519			
Total	2.807163	71				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 43. ANOVA for volatile aldehydes (fresh soybean seeds at the start of storage)

Source of variation	SS	DF	MS	F	P-value	F
Between group	1.160841	23	0.050471	*52.27821	2.45E-15	1.993239135
Within group	0.023171	24	0.000965			
Total	1.184011	47				

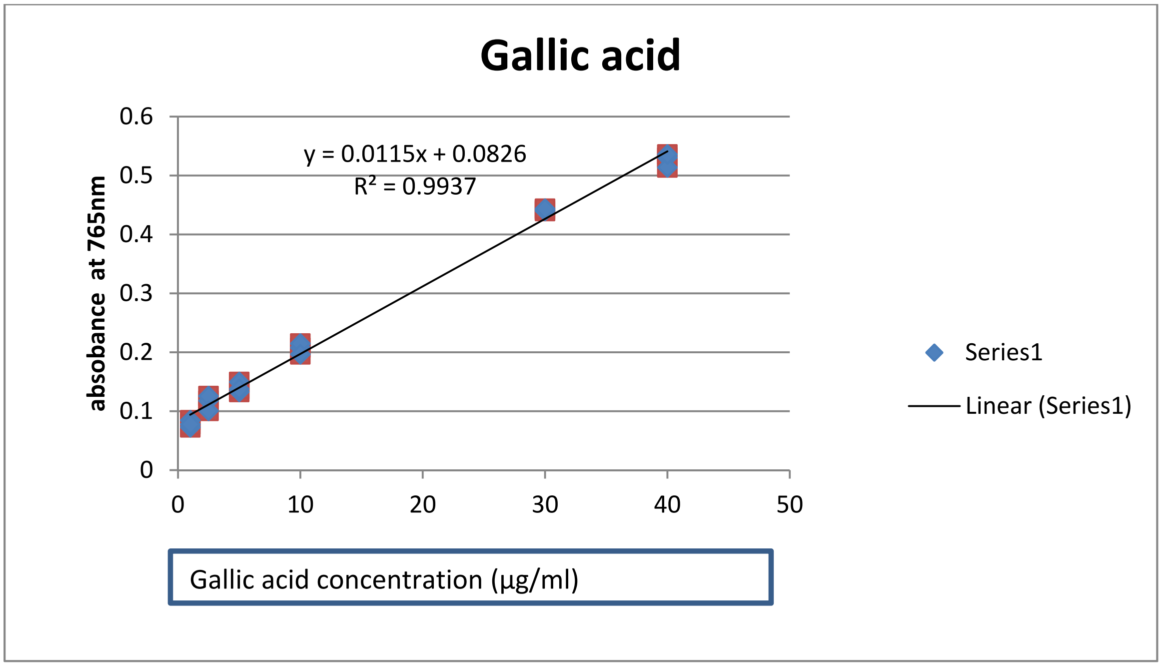
Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Table 44. ANOVA for volatile aldehydes (08 months ambient stored soybean seeds)

Source of variation	SS	DF	MS	F	P-value	F
Between group	37.93477	23	1.649338	*352.8058	3.95E-25	1.993239
Within group	0.112198	24	0.004675			
Total	38.04696	47				

Where, SS :Sum of square,DF: degree of freedom, MS: mean squares, F: F values,
*: significance@ 5%

Curve:1 Phenol standard curve



Curve:2 Volatile aldehyde standard curve