Seed Ageing and Deterioration during Storage for Germplasm Conservation in Groundnut (*Arachis hypogaea* L.) and its wild relatives

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Doctor of Philosophy

By

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CERTIFICATE

This is to certify that the thesis entitled "Seed Ageing and Deterioration During Storage for Germplasm Conservation in Groundnut (*Arachis hypogaea* L.) and its Wild Relatives" being submitted by Adib Sultana for the award of the degree of Doctor of Philosophy of the Indian Institute of Technology, Kharagpur, is a record of bonafide research work carried out by her under our supervision and guidance. The thesis is, in our opinion, worthy of consideration, for the award of the degree in accordance with the regulations of the Institute. The results embodied in the thesis have not been submitted to any other University or Institute for the award of any degree or diploma.

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To Daddy and Ammi
Aijaz and Rafia Ali
With deep love and heart-felt thanks

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PREFACE

To maintain high viability of seeds stored either for cultivation or germplasm conservation, it is very important to understand the process of seed deterioration due to ageing Groundnut (*Arachis hypogaea* L) is one of the world's principal oilseed crops, and loss of viability during storage is a major problem. This is more so with the post-rainy season (rabi) produce. Such loss of viability leads to poor plant emergence and loss of germplasm in the genebanks. The process of ageing during storage under different environment is poorly known in groundnut and the mechanisms of degradation are not well understood. The present study aims to assess the problem and to determine the extent of seed debilitation in cultivated and wild species of groundnut subjected to different conditions of storage, mainly through a study of physiological and biochemical changes

The subject is introduced in Chapter I and the relevant literature is reviewed in Chapter II The experimental procedures followed are detailed in Chapter III. A comprehensive account of the findings is given in Chapter IV and their significance is discussed in Chapter V. The results are summarized in Chapter VI and references are cited in Chapter VII.

The results from this investigation are expected to stimulate further studies on groundnut and other important oilseed crops. It is hoped that the information obtained on seed deterioration would be useful to groundnut growers and in seed storage for genetic conservation.

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) seeds are valued as rich source of oil and protein. Investigations were carried to determine the extent of loss in seed viability in groundnut during storage under different environments, and to identify important deteriorative processes of ageing that induce cellular debility within seeds. Twenty genotypes of groundnut belonging to four cultivar groups viz , Virginia bunch, Virginia runner, Valencia and Spanish were stored under ambient (22-38°C, 44-80% RH) and medium-term (4°C, 20% RH) conditions, while four genotypes representing these groups were stored under short-term (18°C, 30% RH) and long-term (-20°C) conditions for fifteen months. The results demonstrated that the extent of ageing and consequent deterioration varied considerably with the storage conditions, being acute under ambient condition and much lesser under short- and medium-term storage conditions. Seed deterioration was evident from losses in seed viability and seedling vigor, electrolyte leakage, loss in lipid content and changes in the fatty acid composition. An increase in lipase activity and decrease in peroxidase activity along with increases in acid and peroxide values were recorded. Other biochemical changes due to ageing included a decline in protein content and increase in total soluble sugar. Storage of groundnut in the form of pods has limited advantages over kernel storage, and only under conditions of high temperature and humidity. Groundnut genotypes and cultivar groups showed significant differences in their response to ageing. Wild species of groundnut lost viability more rapidly than the cultivated genotypes when stored under identical conditions. Physiological and biochemical changes due to accelerated ageing were similar to those observed during natural ageing. In both cases the major deteriorative processes appeared to be membrane damage and lipid peroxidation.

Key words: *Arachis hypogaea*, groundnut, germplasm conservation, seed viability, ageing, seed deterioration, enzymatic changes, membrane damage, lipid peroxidation, wild species of groundnut.

Chapter 1

INTRODUCTION

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the principal crops of the world, ranking thirteenth among food crops. Most groundnut is produced in semi-arid regions. Although India ranks first in the world in both area and production, productivity in India (700-800 kg ha^{-1}) is much below the global average of 1000 kg ha^{-1} (FAO, 1992). Poor plant stand and low seedling vigor are important reasons for the low yields. Low seedling vigor is largely due to deterioration of seeds during storage. Between the time of harvest and the next season's planting, seeds undergo the process of ageing, which is a function of time and storage conditions (Priestley, 1986). Seed deterioration is of great concern to groundnut growers who need adequate, good quality seeds and to the seed industry, which must provide fully viable seeds, in order for their own survival in commerce. Seed deterioration as a consequence of ageing is equally important in gene banking, where the primary goal is long term conservation of germplasm.

Groundnut is a crop which is known to have wide genetic diversity. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is the world's largest repository for about 14,000 accessions of groundnut which includes 300 accessions of wild *Arachis* species (Mengesha, 1994), and also provides these basic genetic stocks to the national and international communities. The main source of genetic diversity in groundnut include the primary gene pool, consisting of landraces and cultivated genotypes from the primary centre of origin and diversity in South America and Africa, the cultivars and breeding materials developed in various countries, and the secondary and tertiary genepools consisting of other *Arachis* species (Stalker, 1992). Conservation of this genetic diversity always remains a key issue, and therefore requires an understanding of the different processes of ageing during storage in order to ensure maintenance of viability and genetic integrity of the

samples or collections.

Unfortunately, groundnut seeds are known to suffer loss of viability during storage (Delouche *et al.*, 1973; Nautiyal *et al.*, 1990) as has been observed in several other oilseed crops (Priestley, 1986). However, there is very little information on the deterioration processes during storage and adequate data is not available on several crucial aspects, e.g., genotype, characteristics of seeds including their size, nature of storage material (pods or kernels), the chemical constituents of the seed etc. These variations are yet to be related with the extent of seed deterioration in groundnut.

Groundnut farmers often store their seeds under ambient conditions where the temperature and humidity can be very high, particularly in countries with warmer climate. In India, groundnut is grown in two seasons, and the postrainy season (rabi) harvest often suffers drastic loss of seed viability. In genebanks, the recommended methods include storage of seed germplasm under short-term (18°C, 30% RH), medium-term (4°C, 20% RH) and long-term (-20°C) conditions. Groundnut seeds often have to remain under ambient conditions for varying periods after collection, and before processing and transfer to genebanks.

Seed tissues deteriorate due to ageing and there could be several reasons for such deterioration. The ultimate manifestation of seed deterioration is loss of its ability to germinate, but before that occurs different biochemical and physiological changes at sub-cellular level affect the performance of the seed (Roberts, 1979; Ellis and Roberts, 1980). Among the reasons for seed deterioration during the ageing process, lipid peroxidation mediated membrane damage is considered to be most significant (Koostra and Harrington, 1969). During storage, many polyunsaturated fatty acids found in seeds become highly susceptible to peroxidative degradation, in which not only is the lipid

itself destroyed, but a complex series of reactions generate a variety of potentially toxic products. The consequences of lipid peroxidation for cellular functioning and survival are therefore severe. The peroxidative changes in the phospholipids also affect membrane integrity. Any loss of structural integrity of the cellular membrane has two major consequences. The cell is unable to respond osmotically, failing to maintain proper turgor, while a substantial efflux of seed metabolites possibly stimulates potentially damaging pathogens. In addition to these various individual effects, the age-induced deficiencies interact to induce cellular debility, which is poorly studied in cultivated groundnut and almost unknown in wild species. Even the results of natural ageing do not correspond with the accelerated ageing (Priestly and Leopold, 1979; Pearce and Abdel Samad, 1980).

It can be important to find whether wild species of groundnut can offer resistance to seed deterioration during storage. Such a hope arises from the fact that wild species of cultivated crops have often been useful in donating resistance genes and thereby improving the existing cultivated varieties. The genetics of seed longevity in groundnut so far remains unknown, but there is certainly a need to begin a search for genotypes in which deterioration is minimum.

Maintenance of good quality seeds in groundnut requires a clear understanding of the physiological and biochemical events occurring during storage as a result of seed ageing. The present investigation was therefore undertaken in groundnut to

(a) determine the loss of seed viability in cultivated groundnut (*Arachis hypogaea* L.) and its wild relatives under different storage conditions of germplasm conservation

(b) ascertain the nature and extent of physiological and biochemical changes in seeds due to ageing under different storage conditions.

Chapter 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Deterioration of seed viability is an inevitable and irreversible process of seed ageing and varies from one species to the other depending on the nature of the seed and conditions of storage (Roberts, 1972). Groundnut, an important crop belongs to the leguminous group and is stored both as pod or as seeds after shelling. The information on storability and seed ageing in groundnut appears to be less consistent. In this review, an attempt has been made to organize information on aspects of storage, ageing and consequent seed deterioration in groundnut along with relevant information from other important oilseed crops.

Seed viability and seedling vigor:

Seed ageing and loss of viability are matters of concern in conservation of genetic resources, particularly in the tropical and subtropical regions where high temperature and relative humidity tend to deteriorate stored seeds. Under such conditions various pre- harvest (Austin, 1972) and post-harvest factors (Madhusudhan Rao *et al.*, 1975) contribute towards loss of seed viability during storage, more so under ambient conditions. Nautiyal *et al.* (1990) reported that about 20 to 30 percent groundnut seeds either did not germinate or failed to establish a healthy crop because of loss of viability during storage. They observed a loss of 40 percent viability within 6 months of storage under ambient condition. Ramamoorthy and Karivaratharaju (1989) also observed a decline in the germination of groundnut (Cv. Pollachi 2) seeds to 55 percent when kernels were stored for 12 months under ambient condition with a mean temperature of 33.4°C and a relative humidity (RH) of 73 percent. Norden (1981) and Ketring (1992) also reported that under ambient condition there could be considerable loss of viability in groundnut seeds. Sardar and Islam (1981) observed that groundnut seeds could not be stored satisfactorily even

for a month under ordinary storage conditions, and the progress of seed deterioration became rapid with an increase in the relative humidity of the storage environment. They found that reduction of moisture in the storage atmosphere could cause loss of moisture content of the seeds and thereby improve the seed longevity.

Nautiyal *et al.* (1991), while working with 4 cultivars of Spanish groundnut, reported that moisture stress at pod initiation and pod development stages was responsible for reduction in germination and seedling vigor. Cox *et al.* (1976) also reported that drought during pod development phase could lead to severe loss of viability in groundnut. Zade *et al.* (1987) observed that the moisture level of the pod at harvest and temperature during drying could considerably affect the storability of groundnut. They emphasized the need of low initial pod moisture content and drying under shade for increased seed viability. In fact, high temperature and faster rate of moisture loss during drying could be responsible for seed damage including membrane injury as observed in a number of oil-yielding crops (Herter and Burris, 1989; Seyedin *et al.*, 1984). It has been suggested (Nautiyal and Zala, 1991) that drying temperature of groundnut before storage should not exceed 38°C.

Information on the comparative benefit of preserving shelled or in-shell groundnut seems to be limited. In-shell groundnut requires much greater storage volume and often suffers considerable kernel damage during shelling and the contents of the shell are uncertain. Groundnut as kernel was considered to be a poor storer (Delouche *et al.*, 1973). The experiments of Navarro *et al.* (1989) showed significant improvement in germination when in-shell seeds were used for storage. Hsieh (1981) considered that groundnut seeds should be stored in the form of pods at low temperature in order to maintain a high degree of viability.

High seed moisture and relative humidity besides temperature were the factors considered detrimental to the viability of stored groundnut seeds, because equilibrium moisture content of seeds at a given temperature increased with relative humidity. Ketring (1971), while working with Spanish type groundnut observed that RH was more important in deciding seed viability than temperature during storage. He observed that high RH induced various biochemical changes during storage, which lowered the quality of groundnut seeds. Bass (1973) reported that the loss of viability of groundnut was more rapid at 21°C and 70 percent RH than at 35°C and 50 percent RH. The group working at National Seed Storage Laboratory, USA, considered groundnut to be relatively more responsive to changes in moisture content than other seeds. Norden (1981) observed that seeds with 8-11 percent moisture content deteriorated more rapidly than the seeds with 2-6 percent moisture content. Aung (1991) observed that under similar RH conditions seeds of lower quality deteriorated more rapidly than those of higher quality. Bennett-Lartey (1991) reported from his experiments on soybean, groundnut and pea that at the same relative humidity and temperature conditions, seeds rich in lipid are slower in absorbing moisture with groundnut absorbing least among the three crops.

It has been shown in orthodox seeds that at a given temperature, a logarithmic relationship exists between seed moisture content and longevity (Ellis and Roberts, 1980a,b; Ellis *et al.*, 1986). In soybean it was observed that such relationship continued. In groundnut, discontinuity in such relationship was observed at 2 percent moisture level (Ellis *et al.*, 1990) which was termed "critical". This could be a very low value, and in practice, attainment of such moisture would not be easy. Ultra-dry storage of seeds for conservation, at less than 5 percent moisture content could be of some advantage (IBPGR, 1985) provided there is no alteration in the biochemical profile.

Navarro *et al.* (1989) found that in order to maintain 90 percent germination level in groundnut, the calculated moisture content (termed as critical) was 8 percent at 15°C. To maintain the same germination level at 26°C, a moisture content of 7.1 percent was required. Sejeda Begum and Nasima Akhter (1988) reported that seeds with 10 percent initial moisture content could completely lose their viability after 14 months of storage at ambient temperature. During this period the seeds showed an increase of 13 percent moisture content. When the storage temperature was lowered to 10°C, there was an improvement in viability although, the increase in seed moisture content was very slow. These results suggest that initial moisture content of the seed, its increase during storage, and storage temperature, played important role in groundnut seed viability.

Varietal differences have been identified in soybean (Wein and Kueneman, 1981; Minor and Pascal, 1982) for resistance to deterioration during storage. Kueneman (1983) identified a few soybean varieties for superior seed longevity and suggested that the influence of maternal plant played a major role in seed longevity during storage. In groundnut it was observed by Nordén (1981) and Zade *et al.* (1987) that Spanish genotypes deteriorated more rapidly than the Virginia genotypes. Ketring (1992) in his attempt to determine genetic influence in groundnut observed differences in seed vitality and field emergence between the cultivars, germplasm and breeding lines that were used. From these differences in response to ambient storage condition, he considered that there is genetic potential to improve longevity of seeds during storage. The genetic basis of susceptibility to ageing has possibly been better investigated in corn. Earlier findings (Lindstrom, 1942; Haber, 1950) indicated long storage life to be dominant character, although the possibility of non-cytoplasmic maternal influence was also considered. Rao and Fleming (1979) observed marked influence of cytoplasmic factors with respect to seed storability. More recently, Scott (1981)

investigated on the genetic basis of susceptibility in corn and used artificial ageing procedures to select for seeds with a strong resistance to ageing. He observed significant reduction in sensitivity to accelerated ageing after three selection cycles. Whether such recurrent selection could promote longevity under normal condition of storage remained undetermined, but the experimental results certainly raised a hope that genetic improvement in corn is feasible.

The consequences of seed ageing and/or storage deterioration is most conspicuously manifested through changes in seed viability. There remains the possibility that even after seed germination the seedlings may not maintain normal vigor and/or succumb during the growth period. Heydecker (1972) explained the nature and characteristics of seedling vigor and emphasized that seedling vigor is ultimately the most relevant expression of the seed quality. Seedling vigor was tested in various ways in different crops. In groundnut, seedling vigor has been evaluated from the growth of the shoot, hypocotyl and root or from the dry weight (Nautiyal *et al.*, 1988; Subbaraman and Selvaraj, 1989; Chakraborty *et al.*, 1991). In soybean, seedling vigor was measured from the embryonic axis length of the germinated seedlings which declined with loss of viability (Priestley and Leopold, 1983). Ferguson (1990) established from similar tests that seedling vigor could decline considerably even without any change in the germination. In corn and sesamum, seedling vigor was determined usually from the measurements of shoot and root lengths (Woodstock and Grabe, 1967; Saxena *et al.*, 1985).

Membrane integrity:

Ageing of seeds during storage affects the membrane integrity (Parrish and Leopold, 1978) which has been frequently assessed from the measurement of electrolyte leakage. The conductivity measurements of seed-steep water is an accepted method to determine the extent of electrolyte efflux of the seed

into the imbibition medium (Pandey, 1992). Information on the extent of loss of membrane integrity and subsequent deterioration under various storage conditions, in general, is indirectly available from the measurement of the concentration of seed leachate. High electrical conductivity values of the leachates were reported in aged seeds of groundnut by several researchers (Nautiyal *et al.*, 1988; Parmeswaran *et al.*, 1988; Huang and Fu, 1991; Chakraborty *et al.*, 1991). In other oil-yielding crops also e.g., soybean, mustard, corn (Dey and Mukherjee 1986; 1988), and sunflower (Halder and Gupta, 1982) a rapid increase in electrolyte leakage was observed from the stored seeds. In soybean, accelerated ageing showed a linear relationship with solute leakage, and loss of seedling vigor (Schoettle and Leopold, 1984). Similar observation was also observed in sesamum (Saxena *et al.*, 1985) and groundnut (Pearce and Abdel Samad, 1980). In groundnut, the extent of leakage remained indifferent to the presence or absence of testa around the seed (Abdel Samad and Pearce, 1978).

There are other indirect evidences of deterioration of membrane integrity in groundnut seeds during storage. Groundnut seeds either treated with glutathione, ascorbate, calcium, polyamines or osmoconditioned with polyethylene glycol (PEG) showed a decline in the permeability of the membranes (Chen and Fu, 1986; Fu *et al.*, 1988; Huang and Fu, 1991).

From the ultrastructure studies in aged groundnut seeds, Fu *et al.* (1986) observed contracted plasmalemma of the radicle cells and more or less disintegrated mitochondria. Further, seeds which were viable but expressed very low vigor also showed damaged mitochondria. All these events were considered to be due to membrane damage.

Changes in lipids:

A decrease in total lipids has been noticed in groundnut during ageing

under prolonged storage (Nautiyal *et al.*, 1988; Subbaraman and Selvaraj, 1989). Such decline was also observed in sunflower (Balamurugan *et al.*, 1989). However, accelerated ageing treatment in soybean seeds showed a slight increase in total lipid content, although the seeds showed loss of viability (Priestley and Leopold, 1979). The polar lipid contents (phospholipid and glycolipid) of groundnut seeds during accelerated ageing could decline to almost 90 percent that affected seed viability to a considerable extent (Pearce and Abdel Samad, 1980). Chakraborty *et al.* (1991) also observed a decline in the phospholipid content of groundnut seeds during natural ageing along with a decrease in seed viability. In soybean, in contrast to storage lipid, phospholipid content decreased during accelerated ageing (Priestley and Leopold, 1979). Paulsen *et al.* (1981) also observed 50 percent loss in lipid phosphorus in aged soybean along with a decrease in the germinability of seeds. In the microsomal fraction extracted from embryonic axes of naturally-aged soybean seeds, Senaratna *et al.* (1988) observed about 50 percent reduction in the phospholipid content. In some other oilseed crops also e.g., corn, mustard (Basvarajappa *et al.*, 1991; Dey and Mukherjee, 1988) and sunflower (Halder *et al.*, 1983) a decline in phospholipid content was observed after accelerated ageing. The loss in membrane lipid severely affected seed viability.

The decline in the extractable phospholipid in soybean seeds with "age" was considered to be due to loss of phosphatidyl choline and phosphatidyl ethanolamine (Priestley and Leopold, 1979). Further work in soybean (Simpson and Nakamura, 1989) suggested that there could be loss of phosphatidyl glycerol and phosphatidic acid in addition to phosphatidyl choline and phosphatidyl ethanolamine. In groundnut, Soliya and Chakraborty (1991) observed loss of phosphatidic acid, phosphatidyl choline and phosphatidyl ethanolamine. In addition to phospholipids, other class of membrane lipid component has also been studied with respect to seed ageing. These include

sterols and sterol derivatives, which can remain in small amounts in seeds, and found to be influencing the function of the membrane (Mudd, 1980). In sunflower, Bhattacharyya and Gupta (1983) reported an increase in free sterols and steryl glycerides in the aged seeds.

The chemical changes in lipids with seed deterioration usually involve breakage of the ester linkage between the acyl chain and glycerol backbone (McKersie *et al.*, 1988), or attack of the unsaturated bonds of fatty acid chain (Chan, 1987). During ageing certain changes can also happen in the physical properties of lipid (Vertucci, 1992) such as a decrease in the energy associated with the lipid melting. The different explanations of decreased lipid levels and their constituents in aged seeds have been mostly directed towards the effects of lipid peroxidation or degradation by lipolytic enzymes.

Lipid peroxidation:

Koostra and Harrington (1969) were the first to propose the oxidation of membranes as a major mechanism of seed deterioration and since then, considerable research work has been carried out to identify the role of lipid peroxidation in seed ageing, most of has reviewed by Wilson and McDonald (1986). The various approaches included monitoring changes in lipid bond saturation, lipid and phospholipid content, release of free fatty acids and production of lipid peroxides as well as their breakdown products.

The analysis of fatty acid composition by gas chromatography suggested that seeds in dry storage tend to lose polyunsaturation over time (Priestley, 1986). In oilseeds, the common observations have been a decrease in the proportion of polyunsaturated fatty acids like linoleate and linolenate in the stored seeds. In stored groundnut seeds Uematsu and Ishii (1981) recorded a downward shift in the amount of linoleate which was associated with loss of viability. Ferguson *et al.* (1990) observed that the amount of unsaturated fatty

acids in mitochondria, from the excised axes of soybean seeds, declined shortly after storage. They found double bond index of lipids from mitochondria to be 1.60 at the beginning, which became 1.79 after 10 months of storage. This and other data suggested that decreases in mitochondrial respiration during storage might be associated with the peroxidative changes in mitochondrial lipids, and such changes could occur prior to loss in seed vigor. However, in soybean embryonic axes and cotyledons, Priestley and Leopold (1983) observed only small decrease in the proportion of linoleate and linolenate, although there was a greater decline in vigor and viability.

It has been demonstrated in soybean that seed lipids subjected to accelerated ageing at high temperature and high relative humidity resulted in loss of polyunsaturated fatty acids, and such events remained associated with loss in seed viability (Stewart and Bewley, 1980).

It appears that lipid peroxidation in seeds during storage might not be a compulsory event. In soybean, for instance, Priestley and Leopold (1979) did not observe any decline in the levels of unsaturated fatty acids in the seeds and embryonic axes during accelerated ageing. Pearce and Abdel Samad (1980) observed no change in the total fatty acid composition as well as in neutral lipid, glycolipid and phospholipid fractions in the differently aged groundnut seeds. They opined that loss of seed viability might not be due to lipid peroxidation.

In stored seeds, lipid peroxidation has often been studied through analysis of lipid degradation products. For such purpose, a degradation product like malonaldehyde was used as an index of lipid peroxidation in aged seeds. Higher levels of malonaldehyde in aged groundnut seeds was reported by Chakraborty *et al.* (1991). Seeds subjected to accelerated ageing also showed higher levels of malonaldehyde in soybean (Stewart and Bewley, 1980; Dey and

Mukherjee, 1986), mustard (Rudrapal and Basu, 1982; Dey and Mukherjee, 1988) and corn (Dey and Mukherjee, 1988; Basvarajappa *et al.*, 1991). Chen and Fu (1986) reported that during ageing of groundnut seeds, the increment in the level of lipid peroxide correlated with the decreased levels of glutathione, ascorbate, catalase and superoxide dismutase. Subsequently, Huang and Fu (1991) observed positive correlation between seed vigor and the degree of unsaturation of membranal fatty acids in the axes of groundnut seeds.

Enzyme activities:

The major enzymes in lipid degradation in deteriorating oilseeds are lipases and lipoxygenases. It was reported that lipases hydrolyze the ester linkages between fatty acyl chains and glycerol in triglycerides of storage lipids liberating free fatty acids and glycerol (St. Angelo and Ory, 1983). Free fatty acids could be toxic to cells and cause deleterious effects like uncoupling of oxidative phosphorylation in mitochondria (Earnshaw *et al.*, 1970), inhibit hill reaction in chloroplasts (Krogman and Jagendorf, 1959) and denature soluble enzymes (Tortora *et al.*, 1978).

Lipoxygenase has been found to be responsible for oxidation of polyunsaturated fatty acids and formation of hydroperoxides (St. Angelo and Ory, 1983). Hydroperoxides and their degradation products affect important cellular systems by denaturing proteins and DNA (Benson, 1990).

Phospholipases (an important lipid degrading enzyme) also play an important role during seed ageing and seed deterioration. Phospholipase A cleaves the ester bonds of the glycerol backbone liberating free fatty acids and lysophospholipids. Phospholipase D cleaves the polar head group to leave phosphatidic acid and liberate free fatty acids. These free fatty acids and lysophospholipids are the major components responsible for the increased

membrane damage and consequent damage to the seed (van Bilsen and Hoekstra, 1993).

Increases in different enzyme activities during storage and its association with loss of viability were reported in several oilseed crops. In groundnut (Chakraborty *et al.*, 1991), mustard, corn and soybean (Dey and Mukherjee, 1986) lipase activity showed an increase in the stored seeds along with an increase in free fatty acids. Increase in phospholipase A activity was reported in corn seeds subjected to accelerated ageing (Basvarajappa *et al.*, 1991). In stored soybean seed increase in phospholipase D activity was observed by Nakayama *et al.* (1981).

Apart from lipolytic enzymes, loss of ability of the enzymes to scavenge free radicals has also been considered to be important in increasing seed deterioration. Effective removal of free radicals formed during normal metabolism could be very important for the well-being of all cells including those of the stored seeds. Such removal of free radicals has been possible by various enzymes such as superoxide dismutase, catalase and peroxidase (Benson, 1990). Unfortunately, during seed ageing, activities of these scavenging enzymes could considerably decline to defend the damaging effects of free radicals.

In different oilseeds, e.g. mustard, corn, soybean (Dey and Mukherjee, 1986), sesamum (Saxena *et al.*, 1985) and sunflower (Halder and Gupta, 1982), there are reports on the decline of peroxidase activity due to accelerated ageing. Saxena *et al.* (1985) observed decrease in the activities of superoxide dismutase and catalase in sesamum seeds which were subjected to accelerated ageing. In groundnut, Chen and Fu (1986) observed decreased activities of catalase and superoxide dismutase during ageing of seeds while no change was observed in peroxidase activity (Chakraborty *et al.*, 1991).

Acid and peroxide values:

Hydrolysis of the ester linkages in the presence of lipase liberates free fatty acids which may accumulate leading to lowering of the pH of seed extract. By using pH as an indicator of free fatty acid content, several research workers observed an increase in the acid value with increasing periods of storage. Such increases were observed in oilseeds such as groundnut (Subbaraman and Selvaraj, 1989; Chakraborty *et al.*, 1991), sunflower (Balamurugan *et al.*, 1989), corn (Basvarajappa *et al.*, 1991), mustard and soybean (Dey and Mukherjee, 1986). The seeds of these crops when subjected to accelerated ageing also showed an increase in free fatty acid content. In soybean, Senaratna *et al.* (1988) found a ratio of free fatty acid:phospholipid in membrane axes of aged soybean seeds which was almost 12 times higher than in fresh seeds. This changed ratio was considered to be responsible for complete viability. They also observed that 20 percent of the total fatty acids in aged seeds were in the free form compared with only 1-2 percent of free acyl units observed in highly viable seeds.

Peroxide value has also been reported as an indicator of fat oxidation (Gray, 1978). An elevation of peroxide value, which correlates negatively with loss of viability was observed during prolonged storage of groundnut seeds (Mathur *et al.*, 1956; Uematsu and Ishii, 1981). In soybean, it was observed that viability of seeds and seedling vigor were negatively correlated with peroxide value and iodine value irrespective of the conditions of ageing (Mitrowihardjo, 1989).

Free radicals:

The various biochemical damages occurring at all levels of cellular organization when linked together, develop the phenomena of storage deterioration. The free radical has long been recognized (Halliwell, 1982) as one of the important products of ageing which causes considerable damage to

the biological tissues. Damages resulting from the production of free radicals could cause secondary reactions generating toxic intermediates and breakdown products equally damaging as the free radicals themselves (Chan, 1987). A number of ESR studies such as that of Buchvarov and Gantcheff (1984) demonstrated the presence of organic free radicals in ESR spectra observed in seed component of naturally aged soybean. In this crop, the highest activity of free radicals was observed in the embryonic axes, and it was concluded that different seed components could show different sensitivities to oxidation stress, embryonic axes being more susceptible than other parts. In several instances free radical activities in the seeds were not detected because tests might not have done with (Conger and Randolph, 1968) each organ within the seed. ESR spectroscopy and low level chemiluminescence analysis have been mostly used for the detection of free radicals. But, the very unstable and reactive nature of the free radicals is often responsible data in seeds which failed to prove useful in linking free radical production with other biological damage (Benson, 1990). In groundnut, no information is so far available on the production of free radicals in aged seeds.

Protein and Soluble sugar:

Seed ageing was considered to be determined by the rate of protein denaturation (Crocker and Groves, 1915) and possibly requires re-examination in the light of more recent facts. Solubility properties have commonly been found to change over several years or months, indicating that alterations in protein structure certainly occur, an effect that has been observed in corn (Jones *et al.*, 1942), groundnut (Moorjani and Bhatia, 1954) and soybean (Echigo,1965; Saio *et al.*, 1980). It was also observed that decreased solubility of corn endosperm proteins was associated with a decline in viability (Nikolova and Dencheva, 1984). However, most investigators have not attempted to relate such changes in protein levels to loss of germinability. The answer to

the question how proteins in seeds become denatured is also not very clear. Ovcharov and Genkel (1973) claimed that declining levels of protein in the embryo and endosperm fractions of corn were related to loss of viability in long-term storage. They also suggested that the number of electrophoretic bands that could be resolved diminished consequently with ageing. A decline in the protein content with increased duration of storage was also observed in groundnut (Rao *et al.*, 1970) and bambarra groundnut (Sreeramulu, 1983b). In this context, the effects of microbial or fungal proteinase on stored seeds cannot be discounted entirely (Cherry, 1983). During storage, changes in protein structure might arise from proteins of lipid peroxidation and other forms of deterioration and there were several suggestions on the mechanism of protein denaturation during storage (Sutulov, 1965; Stefanov and Dencheva, 1984).

It has also been observed that during storage of seeds the ageing process tended to elevate the levels of soluble sugars (Anderson and Abdul Baki, 1971). Accumulation of total soluble sugars during storage was observed in groundnut seeds (Rao *et al.*, 1970; Nautiyal *et al.*, 1991) and was found to be negatively correlated with viability. The concentration of soluble sugars, in the seed leachates, was also negatively correlated with the viability in groundnut (Parmeswaran *et al.*, 1988; Suneja and Nagaraj, 1989; Nautiyal *et al.*, 1988), sunflower (Halder and Gupta, 1982) and sesamum (Saxena *et al.*, 1985).

Accelerated ageing:

The accelerated ageing technique has proved useful in understanding seed deterioration due to ageing. Most of the methods of accelerated ageing are based on the precepts of Roberts (1973) that seeds in presence of high moisture and high temperature during storage gradually deteriorate and eventually lose viability. Accelerated ageing commonly aimed to simulate natural ageing has been considered a true time lapse process (Delouche, 1965). The principal

process of ageing during storage is similar to natural ageing except that the rate is different (Likatchev *et al.*, 1984). With this technique, it might be possible to eliminate variables characteristic of long-term storage and natural ageing (Chen, 1970), and to examine in more uniform sublots.

Delouche and Baskin (1973) considered that successful accelerated ageing should require exposure of seeds to 100 percent RH at 40-45°C temperatures for 2-8 days. In some cases ageing regime of 30°C and 75 percent RH for 6-24 weeks proved equally useful. This technique has been used in several oilseeds including soybean, corn, sesamum, mustard and groundnut. In soybean, the technique involved exposing the seeds to 40°C and 100 percent RH (Buchvarov and Gantcheff, 1984). It was observed that viability of soybean seeds declined sharply even after 4 days of accelerated ageing (Priestley and Leopold, 1979). Under similar conditions of ageing Parrish and Leopold (1978) observed total loss of viability in 7 days. In corn, the temperature used was mostly 40-42°C, while the relative humidity maintained was 100 percent (Scott, 1981; Basvarajappa *et al.*, 1991). In sesamum, total loss of viability was observed (Saxena *et al.*, 1985) after 8 days of accelerated ageing at 45°C and 100 percent RH. In mustard, accelerated ageing for 15 days at 40° and 100 percent RH showed a survival of 36 percent (Dey and Mukherjee, 1988). In groundnut, accelerated ageing was done by storing the seeds at 38°C and 90 percent RH for 28 days during which the viability declined to 15 percent (Pearce and Abdel Samad, 1980). Singh and Khatra (1984) observed that accelerated ageing for 5 days at 40°C and 100 percent RH could lower groundnut seed viability to 35 percent. An increase in temperature to 42°C, and a reduction in RH to 76 percent (Ramamoorthy and Basu, 1984) could increase seed viability in groundnut suggesting that increase in humidity could be more damaging than increase in temperature during accelerated ageing. It would thus appear that accelerated ageing as a technique to mimic natural ageing showed somewhat variable results in different situations.

Chapter 3

MATERIALS AND METHODS

MATERIALS AND METHODS

The germplasm accessions of cultivated groundnut (*Arachis hypogaea* L.) and its wild species used in the present investigation are listed in Tables 1 and 2. The seed and pod samples of these genotypes were obtained from the Genetic Resources Division, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. In order to encompass the range of genetic variation, four cultivar groups representing two subspecies and three botanical varieties were used (Krapovickas, 1968; Gregory and Gregory, 1976). The Virginia type included two subdivisions (Krapovickas, 1968). The groups and their botanical description is given below:

I	Virginia bunch	-	*Arachis hypogaea* subsps. *hypogaea* var. *hypogaea* (type Virginia)
II	Virginia runner	-	*Arachis hypogaea* subsps. *hypogaea* var *hypogaea* (type Virginia)
III	Valencia	-	*Arachis hypogaea* subsps. *fastigiata* var. *fastigiata* (type Valencia)
IV	Spanish	-	*Arachis hypogaea* subsps. *fastigiata* var. *vulgaris* (type Spanish)

Five genotypes from each group were selected to represent variations in shell thickness and seed size, characteristics that could influence seed viability in storage. One of the genotypes, which represented a released cultivar was used as 'check'. Pod and seed characteristics of all the genotypes are given in Table 4.

To produce sufficient amount of seed materials for storage experiments, and to achieve uniform pre- and post-harvest conditions, all the genotypes were grown during the post-rainy season of 1990/91 (November-April) at the

ICRISAT Asia Center farm, located at 17°N, 78°E near Hyderabad. The soil of the experimental plot was a typical alfisol. Planting was done in a randomized block design in three replications. At maturity the crop was harvested, cleaned, and dried in shade for a week. Healthy pods and kernels were selected for storing as pods and seeds. The pod and seed samples were kept in different containers under different storage conditions as shown in Table 3.

All the 20 genotypes of the cultivated types were stored under ambient and medium-term conditions representing storage conditions prevailing with growers (ambient) and conditions generally maintained in genebanks (medium-term). Only four genotypes (one of each group) were stored under short-term and long-term storage conditions. For wild species, only pods were stored under ambient and medium-term conditions. Seven wild species were included in the experiment.

Before transferring seeds and pods into different storage conditions, the seed viability, seedling vigor, seed leachate, oil content, protein content, total soluble sugar content, fatty acid composition, and enzyme activity of all the genotypes were determined (initial). After storage, seed samples were drawn at 3-month intervals over a period of 15 months and analyzed. Both at the initial stage and at the end of the experiment, the seeds were also analyzed for lipid fractionation, acid value, peroxide value, and protein profile.

Table 1. Sources and identity of the genotypes of cultivated groundnut (*Arachis hypogaea* L.).

Accession Number*	Other identity	Subspecies	Cultivar group	Origin
ICG 4906	AH erect	*hypogaea*	Virginia Bunch	Sri Lanka
ICG 2742	Gunajato 2	"	"	Sri Lanka
ICG 5067	48-45	"	"	Zimbabwe
ICG 2484	AH 7307	"	"	China
ICGS 76	ICGV 87141	"	"	India (ICRISAT)
ICG 4344	No. 89	"	Virginia Runner	Senegal
ICG 4342	NC 15	"	"	USA
ICG 4236	AH 7641	"	"	India
ICG 4479	NC 5	"	"	USA
ICG 156	M 13	"	"	India
ICG 10633	M 64-72	*fastigiata*	Valencia	Bolivia
ICG 10035	SPZ 480 Purple	"	"	Peru
ICG 3041	Mantredi 112	"	"	Argentina
ICG 10766	TGR 1387	"	"	Zimbabwe
ICG 2738	Gangapuri	"	"	India
ICG 2387	Pant.G.S. 29	"	Spanish	India
ICG 2959	X.14-4-8-19-B	"	"	India
ICG 2988	AH 7194	"	"	Australia
ICG 3209	S 7-2-19	"	"	Tanzania
ICGS 44	ICGV 87128	"	"	India (ICRISAT)

* All accessions were drawn from genebank at ICRISAT Asia Centre

Table 2. Details and taxonomic affinities of *Arachis* wild species.

Accession No. (ICG)	Other identity	Species	Section	Series	Origin
13242	KSSc 38900	*A. duranensis*	Arachis	Annuae	Argentina
8211	GKBSPSc 30083	*A. batizocoi*	Arachis	Annuae	Bolivia
12165	KSSc 36015-or G 3-2	*A. cardenasii*	Arachis	Perennes	Bolivia
13240	GK 10585	*A. paraguariensis*	Erectoides	Tetrafoliate	Bolivia
8129	GKP 10002	*A. appressipila*	Erectoides	Procumbensae	Brazil
8131	GK 12922	*A. triseminalis*	Triseminala	Procumbensae	Brazil
8197	GKBSPSc 30062	*A. monticola*	Arachis	Amphidiploides	Argentina

Table 3. Details of storage conditions.

Storage condition	Temperature during storage	Relative humidity during storage	Container used for storage
Long-term	-20°C	--	Aluminum foil packets
Medium-term	4°C	20%	Plastic containers
Short-term	18°C	35%	Plastic containers
Ambient[1]	22-35°C	44-80%	Gunny bags

[1]Temperature and humidity were continuously recorded in the room where pods and seeds were stored under ambient condition.

Table 4. Pod and seed characteristics of cultivated *Arachis hypogaea* genotypes.

Cultivar group	Accession No (ICRISAT)	Pod thickness (mm)	100-seed mass (g)
Virginia bunch	ICG 4906	0 95	20
	ICG 2742	0 81	86
	ICG 5067	0 40	56
	ICG 2484	1 88	60
	ICGS 76*	1 02	78
Virginia runner	ICG 4344	0 95	41
	ICG 4342	0 81	89
	ICG 4236	0 26	43
	ICG 4479	1 15	85
	ICG 156*	1 23	63
Valencia	ICG 10633	0 78	35
	ICG 10035	1 08	82
	ICG 3041	0 40	55
	ICG 10766	1 56	60
	ICG 2738*	1 04	48
Spanish	ICG 2387	0 26	35
	ICG 2959	0 46	66
	ICG 2988	0 18	41
	ICG 3209	0 90	60
	ICGS 44*	0 83	58

* check

Accelerated ageing:

To determine biochemical and physiological changes during seed deterioration, the method of Matthews (1980) was adopted to accelerate the process of ageing. This not only permitted to simulate a given storage condition and study the consequent seed deterioration, but also circumvented the need for experimental analysis that would otherwise extend over many years of storage. Seeds were kept under high temperature and high moisture conditions for a period of 20 days. A weighed sample of known moisture content, determined by the method of ISTA (1985) was used. The moisture content of the groundnut seeds was raised by placing the seeds on moist filter paper and allowed to imbibe to the required level of 13.5 percent. The attainment of this moisture level was checked by frequent weighing. The partially imbibed seeds were held in a sealed container overnight at 5°C to ensure an even distribution of moisture and then the sample was sealed in laminated aluminum foil packets and kept in an oven at 40°C. Samples of seeds were withdrawn at 4-day intervals over a period of 20 days for viability tests and analyses of biochemical changes. The experiment had 3 replications.

Seed viability:

Seed viability was measured through germination count as per the rules of the International Seed Testing Association (ISTA, 1985). Initially the seeds were dressed with the fungicide Thiram (Tetra methylthioperoxy dicarbonic diamide) to prevent fungal contamination. The treated seeds were plated in germination boxes containing 1% agar. Each replication represented a sample of 50 seeds. In seeds showing dormancy (genotypes belonging to subspecies *hypogaea* and wild *Arachis* species), 20 ppm ethrel was sprayed to initiate the germination process. The germination boxes were kept in germination chambers maintained at of 25°C and 80% RH. Germination counts were made after an interval of ten days and all germinated seeds were considered viable.

Seedling vigor:

To assess seedling vigor, five seedlings from each sample were used for the measurement of root, shoot, and hypocotyl lengths as per ISTA (1985) method. These seedlings were dried in an oven at 80°C for 24 h following the technique of Copeland and Mcdonald (1985). The dried samples were cooled in a desiccator for 2-4 h and weighed for their dry mass.

Seed moisture content:

Moisture content was determined using the low constant oven method (ISTA, 1985). The groundnut seeds were powdered using Krup's blender, and 5 g of the meal was placed in a preweighed metallic container with lid. This was then weighed and kept in an oven maintained at a temperature of 103±2°C for 17±1 h. After drying, the containers were left to cool in a desiccator for 30-45 min and then weighed. Moisture content was calculated using the following formula:

$$\text{Moisture content \%} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

where M_1 = weight of container, M_2 = weight of container + contents before drying, M_3 = weight of container + contents after drying

Electrolyte leakage:

Electrical conductivity of the seed leachate was measured by using a YSI Model 32 conductivity meter. Five seeds were soaked in 15 mL deionized water at room temperature for 24 h since our preliminary studies had shown that beyond 24 h imbibition there was no further increase in electrical conductivity. The seed leachate was collected after 24 h imbibition and the electrical conductivity was measured. The ionic concentration was expressed as mmho/cm. The experiment was replicated thrice.

Oil estimation:

The oil content of groundnut seeds was determined by the Nuclear Magnetic Resonance Spectrometry (NMR) method as described by Jambunathan *et al.* (1985). The experiments were carried out using Newport Analyzer Mark III (Newport Instruments Limited, Newport, UK.). A steady field value of 635 X 10^{-4} T and a radio frequency of 2.7 MHz was used for all analyses. The integration period was kept at 32S, at a gatewidth of 1.5 X 10^{-4} T and a radio frequency (RF) value of 100 μa. The amplitude frequency gain, although variable was usually 300. The samples in NMR tubes (Nessler glass tubes) were filled to an etched mark for which about 18 g of groundnut seeds and 22 g of oil were required (varying levels of reference oil in NMR tubes or the weight of the seed in the tube had little influence on the percentage values obtained in the seed sample). The groundnut oil was used as reference oil for calibration of the instrument. The reference oil was extracted in bulk by the Soxhlet method. A weighed quantity (22 g) of oil was used for NMR reading. Seed samples were loaded in NMR tubes (up to the etched mark), weighed and then dried in an oven at 110°C for 16 h. The tubes were closed with stopper and allowed to cool at room temperature. The weight of the dried sample was recorded after NMR readings were obtained. Oil % was obtained using the following formula:

$$\text{Oil \%} = \frac{\text{Weight of oil}}{\text{NMR reading of oil}} \times \frac{\text{NMR reading of sample}}{\text{Dry weight of sample}} \times 100$$

Fatty acid composition:

The fatty acid methyl esters of triglycerides were prepared according to Hovis (1979). Seeds were ground and approximately 300 mg of ground meal was weighed into a 50 mL glass culture tube. To this 15 mL of petroleum ether was added and shaken on a tube rotator for 30 min. The contents were

centrifuged at 4000 rpm for 5 min and 5 mL supernatant was taken in a small culture tube and the solvent was evaporated under a stream of nitrogen gas. The content was dissolved in 1.3 mL of 0.5 N NaOH in methanol and heated in a boiling water bath for 5 min. After cooling, 2 mL of BF_3 (boron trifluoride) in methanol was added. This was heated for 5 min in a boiling water bath. The tubes were then cooled and 2 mL of saturated NaCl solution was added followed by shaking on a tube rotator for 10 min. This process was repeated with the addition of 2 mL of petroleum ether. The tubes were centrifuged at 4000 rpm for 5 min. The supernatant petroleum ether layer from the tubes was transferred to a sample vial.

Fatty acid methyl esters were analyzed according (Mercer, 1990) using Gas Chromatograph (Shimadzu GC-9A equipped with temperature programmable oven and flame ionization detector). Fatty acid methyl esters (detailed above) were separated on a glass column (2.1 m x 3 mm), packed with 10% Alltech CS-10 on Chromosorb W-AW (80-100 mesh). The carrier gas (helium) flow was adjusted to 50 mL/min (primary pressure 6 kg/cm^2) and after ignition of the flame ionization detector, the hydrogen gas flow was maintained at 0.6 kg/cm^2, and air at 0.5 kg/cm^2 while the injection port and flame ionization detector temperatures were maintained at 260°C. The column temperature was programmed to hold the column at 190°C for 4 min initially, followed by a step up of 10°C/min to reach a final temperature of 250°C, which was maintained for 2 min. Then 1 µL of the sample from the vials containing fatty acids in methyl esters was injected into the gas chromatograph. Peaks were identified by matching their retention times to the reference standard mixture of fatty acids (Nucheck 21A, peanut fatty acid composition). The order of elution was: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), arachidic (20:0), eicosenoic (20:1), behenic (22:0), and lignoceric (24:0). Fatty acid methyl esters were quantified using the area normalization method.

Lipid separation:

Total lipids were extracted by grinding 30 g of seeds in 100 mL propanol with a pestle and mortar. The homogenate was filtered with filter paper and the filter residue was reground with 100 mL chloroform-methanol (2:1). The homogenate was filtered and the filter residue washed with 50 mL chloroform-methanol. All these filtrates were concentrated in vacuum to obtain the lipid.

The lipid was separated into neutral lipids, phospholipid, and glycolipid fractions by means of column chromatography. A slurry of 25 g silica gel in 75 mL chloroform-methanol (2:1) was prepared and poured into the chromatography tube. The stop cock was kept open and the tube tapped gently to dislodge all air bubbles and aid in settling of the column, the height of which was 40 cm. The solvent level was dropped to the top of the silica gel with care to prevent air bubbles entering the column. 5 g of lipid dissolved in 100 mL chloroform was carefully added in the column to ensure that no quantitative loss occurs during transfer. Elution of the column was carried out at a flow rate of 3 mL/min with the following solvents in sequence (a) chloroform to obtain neutral lipid (a relatively large amount of chloroform was used to remove all neutral lipid), (b) chloroform-acetone (1:1) and acetone to obtain glycolipids, (c) chloroform-methanol (1:1) and methanol to obtain phospholipids. The completion of elution in each step was confirmed by microslide thin layer chromatography (TLC). For TLC a uniform slurry of 50 g of silica gel G in 120 mL chloroform was prepared and poured into a 250 mL beaker. Two slides were dipped in the slurry and the excess removed. The slides were separated, placed on a glass plate (coated side facing up) and initially dried for 1-2 h in air and then dried overnight in a oven at 110°C. For checking the fractions, 1-2 µL of the filtrate was used to develop spots which were compared with different checks e.g. oil extracted by hexane for neutral lipids, digalactosyl diglycerol for glycolipids and soyalecithin for phospholipids.

The spotted slides were allowed to separate in chloroform-methanol- water solvent and then stained. For neutral and phospholipids the slides were developed in iodine vapor for a few minutes, and for glycolipids the slides were sprayed with resorcinol reagent (10 mL of 2 g resorcinol dissolved in 100 mL water and 80 mL of conc. HCl containing 0.5 mL of 1M copper sulfate) and allowed to be at 120°C for 5-10 min (Kates, 1972).

The lipid fractions obtained through column chromatography were evaporated to dryness using a flash evaporator and the quantity determined by weighing. From these fatty acid methyl esters were prepared for analysis.

Acid value:

Acid value in seed provides a measure of concentration of free fatty acids in the oil and was determined following the method prescribed by the American Oil Chemists Society (AOCS, 1981). About 8 g of groundnut oil was weighed into an Erlenmeyer flask and dissolved in 50 mL of neutralized alcohol (iso propyl alcohol is neutralized to a faint color with 0.1 N sodium hydroxide). To the above mixture phenolphthalein indicator was added followed by alkali titration using 0.025 N sodium hydroxide with intermittent shaking till the mixture turned pink. Acid value was calculated as follows:

$$\text{Acid value} = \frac{\text{mL alkali} \times 56.1 \times \text{N}}{\text{Weight of the sample}}$$

where N is the normality of alkali (0.025) and 56.1 is the conversion factor

Peroxide value:

The peroxide value indicates oxidation of the substances during storage and this was measured using the potassium iodide test (AOCS, 1981). The expression was in terms of milliequivalents of peroxide per 1000 grams of

sample, that oxidize potassium iodide under test conditions. For this determination, 5 g oil was weighed into an Erlenmeyer flask and after adding 30 mL acetic acid-chloroform solution, the content was swirled and 0.5 mL saturated potassium iodide was added. After allowing the solution to stand for 1 min at room temperature, 30 mL of distilled water was added. This solution was titrated with 0.01 N sodium thiosulfate until the yellow color of the solution disappeared, following which 0.5 mL of starch indicator (1% soluble starch in distilled water) was added and the solution was again titrated till the blue color disappeared. Peroxide value in milliequivalents of 1000 g of sample was calculated using the following formula.

$$\text{Peroxide value} = \frac{(S-B)\,(N)\,(1000)}{\text{Weight of the sample}}$$

where N = normality of sodium thiosulfate solution; B = titration of blank; S = titration of sample.

Peroxidase activity:

Peroxidase enzyme activity was estimated from 4-day old seedlings (which provided maximum activity) using the Shimadzu UV-VIS spectrophotometer UV-160. From these seedlings 1 g fresh weight of the root tips (2-3 cm long) was homogenized in a glass hand homogenizer using 5 mL of cold 0.1 M potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at 10,000 rpm and the supernatant was used to measure the enzyme activity. In a cuvette 0.1 mL of the supernatant was taken and added with 3 mL phosphate buffer 0.1 M (pH 7.0), and 0.05 mL guaiacol (20.1 mM). The cuvette was placed in the spectrophotometer using enzyme kinetics mode and the reading was adjusted to zero with phosphate buffer blank. This was followed by addition of 0.3 mL H_2O_2 (30%) in the cuvette which on reaction recorded a peak on graph paper to indicate the enzyme activity. Later the

activity was calculated and expressed as max. O.D./fresh weight.

Lipase activity:

For determination of lipase activity titrable acidity procedure (Luddy *et al.*, 1964) was followed in which the reaction mixture pH was kept constant against acid production by the addition of a suitable base. The root tips were collected from 4-day old seedlings, washed and surface dried. From this 1 g of root tips (2-3 cm long) were homogenized in glass hand homogenizer using 5 mL of 1 M Tris buffer (pH 8.0). The homogenate was centrifuged at 10,000 rpm and the supernatant was used to measure the enzyme activity. The reaction mixture consisted of 50 µL (25 mg) triolein, 1 mL of 1 M Tris buffer (pH 8.0), 0.25 mL of (0.05%) sodium deoxycholate, 0.1 mL of (2.2%) calcium chloride. These contents were warmed for 1 min in a water bath maintained at 40°C and 1 mL of crude enzyme extract was added to the flask and subjected to vigorous shaking in the water bath. The reaction was stopped after 3 min by adding 1 mL ethanol. The contents were titrated (with phenolphthalein indicator) against 0.01 N NaOH till the solution turned pink; the enzyme activity is directly proportional to the amount of NaOH used. It was expressed as µ eq. of free fatty acid released per 3 min of assay.

Total soluble sugars:

The total soluble sugars in the seeds were determined according to the method described by Dubois *et al.* (1956). The groundnut meal was initially defatted using n-hexane and 50 mg of the defatted meal was weighed and taken into a boiling-tube, to which 25 mL of hot 80% ethanol was added and shaken on a vortex mixer. The mixture was allowed to settle for 10 to 15 min and filtered into a beaker using Whatman filter paper No. 41. For complete extraction of sugars, the sample was extracted thrice without any change in the protocol and the total extractions were evaporated on a sand bath until

removal of the ethanol. The contents were dissolved in distilled water and made up to 100 mL in a volumetric flask and 0.5 mL of the above solution was pipetted into a test tube and the volume was made up to 1 mL with water. To this 1 mL of phenol and 5 mL of 96% sulphuric acid were added and the content was shaken vigorously on a vortex mixer. The tubes were cooled in a water bath and spectrophotometric reading was taken at 490 nm against a sample blank.

For comparison, standard solution of glucose (100 µg glucose/mL distilled water) was prepared, and 0.1 mL to 0.5 mL of this standard solution was pipetted into test tube and volume made up to 1 mL with water. To these tubes, 1 mL of phenol and 5 mL of 96% sulphuric acid were added and the content was shaken vigorously on a vortex mixer before cooling in a water bath. The spectrophotometric absorbance was read at 490 nm against the reagent blank. The percentage of total sugars was calculated using the formula given below

$$\% \text{ Total soluble sugars} = \frac{\text{Conc. of std. (µg)}}{\text{Absorbance of standard}} \times \text{Absorbance for 1 mL extract} \times \frac{1 \text{ (Conversion of g)}}{1{,}000{,}000} \times \frac{100 \text{ ml (vol. made up)}}{0.5 \text{ ml (sample vol.)}} \times \frac{100 \text{ (percentage)}}{0.05 \text{ g (Sample wt.)}}$$

Protein determination:

Protein content was determined using a Technicon Auto Analyzer (Technicon Corp., New York, USA) following the method described by Singh and Jambunathan (1980). About 60 mg groundnut whole meal sample was weighed and transferred into a Technicon digestion tube (75 mL). In this tube 3 mL of acid mixture of orthophosphoric acid and sulphuric acid (5:100) and

1 Kjel (1.5 g K_2SO_4 and 7.5 mg Se) tablet which acted as a catalyst were added. For digestion, the tube was heated in a block digester maintained at 375°C for 90 min. The digest was cooled and dissolved in water, and volume was made up to 75 mL, and thoroughly mixed. A set of 40 tubes were used for this purpose. A sample of about 5 mL solution from each tube was transferred into a Technicon sample cup for analysis.

Towards calibration, different solutions namely (a) alkaline sodium potassium tartarate (75 g NaOH + 50 g $C_4H_4NaKO_6$ in 1 L water), (b) alkaline phenol (138 mL phenol (88%) + 500 mL 5 N NaOH made to 1 L, with distilled water), (c) 5% NaOCl and (d) wash (Brij) solutions were run through their respective tubings for at least 15 min. Following this, run was given with nitrogen standards (ammonium sulphate) in the sampler tube. Three nitrogen standards were used to obtain standard slopes with which the experimental samples were subsequently compared. After running the samples, sample heights were recorded and the percentage of nitrogen was calculated using the formula

$$N\% = \frac{\text{Sample peak heights x 75 x 100}}{\text{1.8 (slope) x 1000 x sample weight (mg)}}$$

where 75 is the made up volume and 1.8 is the slope (net division on the chart paper for 1 ppm). Protein was calculated by multiplying N% with 5.46 (conversion factor for groundnut).

Statistical analysis:

The experimental data was subjected to statistical analysis. For analysis of variance, factorial randomized block design was performed using the "Genstat" program in the VAX 11/781 computer. The standard errors of the variables in the different experiments are given in appendix.

Chapter 4

RESULTS

RESULTS

4.1 Seed deterioration consequent to ageing in cultivated groundnut

4.1.1 Response of twenty genotypes under ambient and medium-term storage conditions

Experiments were conducted to investigate the effects of seed ageing on different genotypes of groundnut during storage towards germplasm conservation. Both pod and seed samples of 20 cultivated genotypes, five each from 4 different cultivar groups viz., Virginia bunch, Virginia runner, Valencia and Spanish were stored under two different conditions of storage for 15 months. Of these the medium-term storage (4°C, 20% RH) represented the procedure prevailing in the genebanks (IBPGR, 1976), while ambient storage (22-38°C, 44-80% RH) represented a condition under which groundnut growers usually store their seeds. Seed samples in replications were tested at 3 month intervals for germination, seedling vigor, electrolyte leakage, oil, protein and total soluble sugar contents, as well as for fatty acid composition.

Seed viability:

When groundnut seeds were kept under ambient condition there was a decline in their viability after 15 months of storage as may be observed from Table 5. There was considerable difference between the genotypes in their storability. The genotype ICG 10035 showed complete loss of viability following storage while the viability of the genotype ICG 4906 was as high as 67.3 percent. However, a comparison of the mean viability of the genotypes belonging to four cultivar groups showed little difference between the groups Virginia bunch and Virginia runner, while the genotypes belonging to the groups Valencia and Spanish showed on an average lower viability than those genotypes belonging to Virginia bunch or Virginia runner group and the differences were significant. Between Valencia and Spanish groups, the

average loss of viability was significantly more in the Valencia group than the other.

The rate of decline in viability differed with the cultivar groups as shown in Fig. 1a. The genotypes belonging to Valencia and Spanish groups lost their viability more rapidly than the genotypes belonging to Virginia bunch and Virginia runner groups. However, the rate of decline of all the genotypes was more rapid during the later period (9-15 months) than observable during the earlier months as seen in Fig. 1a.

The viability of the seeds stored as unshelled pods in general was about 5% higher than the seeds which were stored as kernels. As regards genotypic differences, the results with storage of pods were not very dissimilar to that observed with the stored kernels as seen in Fig. 2a. The viability of pods of the genotypes belonging to Virginia bunch and Virginia runner was significantly higher than the Valencia and Spanish genotypes.

The size of the seeds and thickness of the pods exerted influence on the seed viability. The small-seeded genotypes ICG 4906, ICG 10063 and ICG 2387 belonging to the Virginia bunch, Valencia and Spanish groups respectively, showed significantly higher viability as compared to the large-seeded genotypes ICG 2742, ICG 10035 and ICG 2959 in the corresponding groups (Fig. 2b). The seed size related viability was observed in both the cases whether the groundnut was stored as seed or pod. In both the cases the small-seeded genotypes showed better viability than the large-seeded ones. However, the genotypes belonging to Spanish group did not show any significant differences in viability when the seeds were small or large in size during storage of pods.

It was observed that in some of the genotypes the thickness of the pod influenced the seed viability while in others this character failed to show any influence as seen from Fig. 2c. In the Virginia bunch group the genotype ICG

5067 showed highest viability with thin-shelled pod, while in the same group the genotype ICG 2484 with thick-shelled pods showed significant decline in viability. In the Spanish group, the genotype ICG 2988 with thin-shelled pod showed significantly higher viability than the genotype ICG 3209 which had thick-shelled pods. None of the genotypes belonging to Virginia runner group showed any significant differences in viability because of the differences in the shell thickness of their pod. In the Valencia group it was observed that ICG 3041 with thin-shelled pod showed a significantly lower viability compared to ICG 10766 with thick-shelled pod.

The results on storage of groundnut seeds under medium-term conditions showed a distinct slow down in the process of seed ageing which was evident from negligible loss in the seed viability as observed from Table 6 and Fig. 1b. The seeds deteriorated very slowly with the time of storage but in most of the genotypes without any perceptible difference. However, a few genotypes, namely, ICG 10035, ICG 3041, and ICG 3209 belonging to the Valencia and Spanish groups lost viability to a considerable degree, particularly the genotype ICG 10035 which lost 40% viability irrespective of whether the seeds were stored as pod or kernel. The reduction in viability was much less in the genotypes ICG 3041 and ICG 3209 showing only 4% viability loss during 15 months of storage. The rate of decline in viability, once extrapolated showed that the permissible limit of viability for germplasm rejuvenation (85%) would be reached in about 4 years for the genotypes ICG 3041 and ICG 3209. Under medium-term storage condition significant differences in the viability in terms of seed size were observed. Small-seeded genotypes showed significantly higher viability than large seeded genotypes. No significant differences were observed in terms of viability between the stored kernels or pods and pods with different shell thickness, when these were stored under medium-term condition.

Table 5. Viability* (%) of different cultivated genotypes of groundnut following storage of seeds or pods for different durations under ambient condition.

Cultivar group/ genotype**	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. hypogaea var. hypogaea (Virginia bunch)											
ICG 4906	98.7	94.7	91.3	86.7	76.0	67.3	97.3	96.0	93.3	88.0	68.0
ICG 2742	100.0	94.7	94.7	86.0	75.3	56.0	98.7	98.0	96.7	79.3	57.3
ICG 5067	98.7	92.0	91.3	81.3	78.0	64.6	94.7	92.0	88.0	82.7	70.0
ICG 2484	97.3	92.7	87.3	86.7	68.0	58.0	96.7	96.7	96.7	78.7	64.6
ICGS 76	100.0	96.7	92.0	84.0	68.7	60.0	99.3	96.7	92.7	86.7	61.3
Mean	98.9	94.1	91.3	84.9	73.2	61.2	97.3	95.8	93.4	83.0	64.2
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	98.7	97.3	95.3	85.3	72.7	60.0	98.7	97.3	94.7	77.3	64.6
ICG 4342	98.0	93.3	85.3	82.7	68.7	58.0	93.3	92.7	88.7	76.7	65.3
ICG 4236	98.7	98.0	94.7	90.7	74.7	62.0	98.7	97.3	92.0	84.7	63.3
ICG 4479	97.3	92.7	89.3	83.3	68.0	54.7	97.3	91.3	91.3	82.0	61.3
ICG 156	97.3	94.0	91.3	86.0	72.0	60.0	94.7	93.3	93.3	88.0	70.0
Mean	98.0	95.0	91.2	85.6	71.2	58.9	96.5	94.4	92.0	81.7	64.9
Arachis hypogaea ssp. fastigiata var. fastigiata (Valencia)											
ICG 10063	100.0	94.0	93.3	90.0	72.7	60.0	96.0	96.0	94.0	76.7	64.6
ICG 10035	95.3	83.3	75.3	70.0	38.7	00.0	88.0	75.3	68.7	46.7	10.3
ICG 3041	100.0	90.0	82.0	76.0	46.7	30.0	90.0	80.0	76.0	66.0	40.0
ICG 10766	97.3	90.0	90.0	82.7	70.7	55.3	94.0	94.0	86.0	72.7	60.0
ICG 2738	100.0	96.0	92.0	88.0	78.7	58.2	96.0	92.7	92.0	80.7	60.7
Mean	98.5	90.6	87.3	82.1	61.4	42.2	92.8	88.2	83.7	68.5	49.2
Arachis hypogaea ssp. fastigiata var. vulgaris (Spanish)											
ICG 2387	99.3	94.7	92.7	88.0	70.7	53.3	96.7	96.7	83.3	73.3	58.0
ICG 2959	98.0	90.0	88.0	76.7	66.0	49.3	92.0	90.7	85.3	69.3	58.6
ICG 2988	98.0	93.3	93.3	84.0	64.7	53.3	96.0	95.3	93.3	70.7	60.6
ICG 3209	97.3	87.3	81.3	80.0	56.7	42.7	88.0	87.3	85.3	68.0	49.3
ICGS 44	98.7	92.7	91.3	89.3	66.7	56.7	94.7	91.3	90.7	72.7	58.7
Mean	98.2	91.6	89.3	83.6	64.9	51.0	93.4	92.2	87.6	70.8	57.0
S.E.	(S) ±0.441,		(G) ±0.881,		(M) ±0.279;		CV (%) 6.0				

S=Storage, G=Genotype, M=Material; * determined by germination test; ** indicated by accession number

Table 6. Viability* (%) of different cultivated genotypes of groundnut following storage of seeds or pods for different durations under medium-term condition.

Cultivar group/ genotype**	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	98.7	98.0	98.0	97.3	97.3	97.3	98.7	98.0	97.3	97.3	97.3
ICG 2742	100.0	99.3	98.7	98.7	98.7	98.0	99.3	98.7	98.7	98.7	98.0
ICG 5067	98.7	98.0	98.0	97.3	97.3	97.3	98.0	97.3	97.3	97.3	97.3
ICG 2484	97.3	96.7	96.7	95.3	95.3	95.3	96.7	96.7	95.3	95.3	95.3
ICGS 76	100.0	99.3	99.3	98.7	98.7	98.7	99.3	99.3	98.7	98.7	98.7
Mean	98.9	98.2	98.1	97.4	97.4	97.3	98.4	98.0	97.4	97.4	97.3
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	98.7	98.0	98.0	97.3	97.3	97.3	98.7	97.3	97.3	97.3	97.3
ICG 4342	98.0	98.0	97.3	97.3	96.7	96.7	97.3	97.3	96.7	96.7	96.7
ICG 4236	98.7	98.0	97.3	97.3	96.7	96.7	98.7	98.0	97.3	96.7	96.7
ICG 4479	97.3	97.3	96.0	96.0	95.3	95.3	97.3	96.7	96.0	95.3	95.3
ICG 156	97.3	97.3	97.3	96.7	96.0	96.0	97.3	97.3	96.7	96.0	96.0
Mean	98.0	97.7	97.2	96.9	96.4	96.4	97.8	97.3	96.8	96.4	96.4
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	100.0	99.3	98.7	98.0	97.3	97.3	99.3	98.7	98.0	98.0	97.3
ICG 10035	95.3	89.3	86.0	73.3	68.0	60.0	92.0	86.7	80.7	71.3	60.0
ICG 3041	100.0	99.3	98.0	97.3	96.0	96.0	99.3	98.0	98.0	96.0	96.0
ICG 10766	97.3	97.3	97.3	96.7	96.0	96.0	97.3	97.3	96.7	96.0	96.0
ICG 2738	100.0	99.3	99.3	99.3	98.7	98.0	98.8	98.3	98.0	98.0	98.0
Mean	98.5	96.9	95.8	92.9	91.2	89.4	97.4	96.0	94.5	91.8	89.4
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	99.3	99.3	98.7	98.7	98.0	98.0	99.3	98.7	98.0	98.0	98.0
ICG 2959	98.0	98.0	97.3	97.3	96.7	95.3	98.0	97.3	96.7	96.7	95.3
ICG 2988	98.0	97.3	97.3	97.3	96.7	96.0	98.0	97.3	97.3	96.7	96.0
ICG 3209	97.3	96.7	95.3	94.7	94.0	93.3	97.3	96.7	96.0	95.3	93.3
ICGS 44	98.7	98.0	98.0	97.3	96.7	96.7	98.0	97.3	97.3	96.7	96.7
Mean	98.2	97.8	97.3	97.0	96.4	95.8	98.1	97.4	97.0	96.6	95.8

S.E. (S) ±0.213, (G) ±0.427, (M) ±0.135; CV (%) 2.4

S=Storage, G=Genotype, M=Material; * determined by germination test; ** indicated by accession number

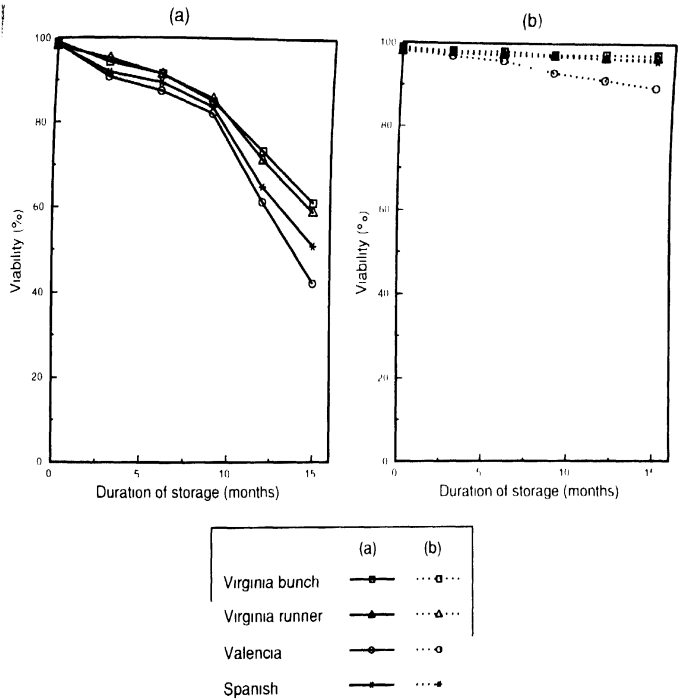

Figure 1. Mean seed viability of cultivated groups of groundnut in relation to time of storage under ambient (a) and medium-term (b) conditions.

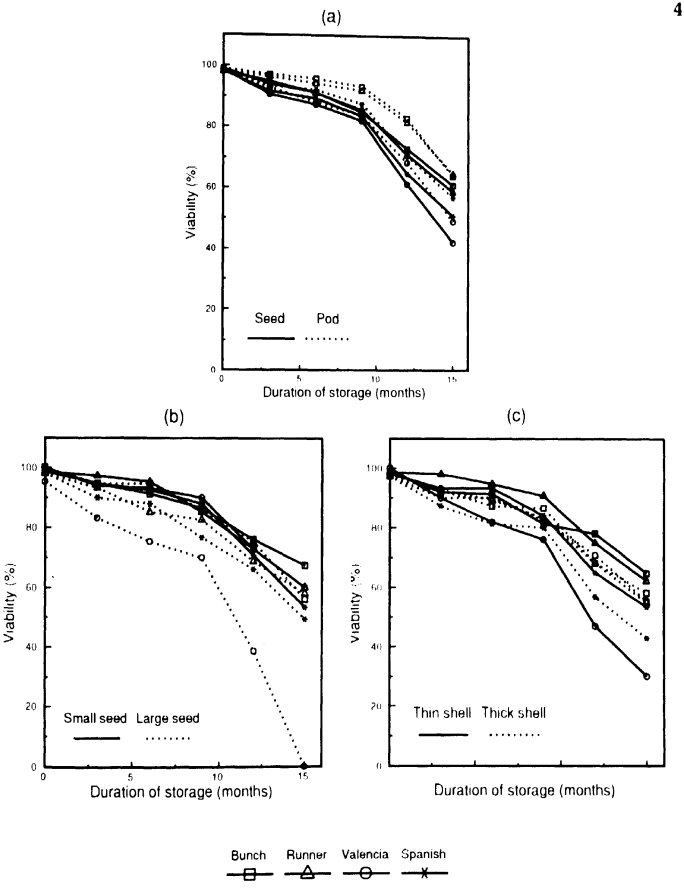

Figure 2. Mean seed viability of genotypes belonging to 4 cultivar groups of groundnut following storage under ambient condition in relation to differences in (a) seed/pod, (b) small/large seed size, (c) thin/thick shell of pods.

Seedling vigor:

A decline in the seedling vigor was observed with the time of storage which was noticeable from the reduction in shoot, hypocotyl and root lengths as well as dry weight of seedlings as seen in Tables 7, 8, 9, and 10. The rate of decline was almost linear observable from Fig. 3, and 4. It was observed that the reduction in shoot and root length was more among the genotypes belonging to Valencia as compared to Virginia bunch, Virginia runner and Spanish. As regards seedling vigor, no significant difference was observed between the groundnut stored as kernels or as pods. The small-seeded genotypes showed larger shoot and hypocotyl lengths than the large-seeded genotypes. Such increased shoot and hypocotyl lengths could be observed in the genotypes ICG 4906, ICG 4344, ICG 10633, and ICG 2387. However, a comparison between small- and large-seeded genotypes as regards root length and dry weight showed no significant differences in most of the cases.

The decline in seedling growth due to storage of seeds under ambient condition was more clear when the dry weight of the seedlings were compared (Table 10). In all the genotypes, a decline in the dry weight with the time of storage was observed. Although considerable genotypic differences were observed, as a group, the reduction was minimum in Virginia runner followed by Virginia bunch, Spanish, and Valencia.

Under medium-term storage conditions it was observed that there was a gradual decrease in seedling vigor with storage time as evident from the reduction in shoot, hypocotyl and root lengths and dry weight of seedlings (Tables 11, 12, 13, and 14). The rate of decline was considerably slow as compared to seedlings obtained following ambient storage as seen in Fig. 3, and 4. There were genotypic differences and the average loss in seedling vigor was highest among the genotypes belonging to Valencia group as compared to the genotypes belonging to other three groups. The loss in seedling vigor did

not show any significant differences between the small- and large-seeded types or between seedlings derived from kernels and pods.

In was generally observed that loss of seedling vigor was more conspicuously seen from the data on the shoot growth than the root growth irrespective of the genotypes and the conditions of storage.

Electrolyte leakage:

The damage caused due to ageing of seeds while in storage was evident from the electrolyte leakage, since leakage of electrolytes from seeds indicates possible membrane disruption. It was seen that under ambient condition of storage, the electrolyte leakage was considerably more as compared to that observed in the seeds stored under the medium-term storage conditions. Under ambient condition it was observed that the electrical conductivity of the seed leachate was as high as 1.180 mmho/cm in case of genotype ICG 10035 as compared to ICG 4906 where the seed leachate showed a conductivity of 0.249 mmho/cm as shown in Table 15. In general, genotypes belonging to Valencia and Spanish types showed greater loss of electrolytes and consequent damage as compared to the genotypes belonging to Virginia bunch and Virginia runner groups. As regards the rate of such deterioration it was observed that the amount of electrolyte leakage, showed considerable increase after 6 months of storage which continued up to 15 months (Fig. 5). A lower amount of electrolyte leakage was estimated from groundnut seeds which were stored as pods as compared to seeds which were stored without shell (kernel).

When the seeds were stored under medium-term conditions the increase in electrolyte leakage over time was very slow except in Valencia group where the electrolyte leakage of ICG 10035 genotype showed a definite increase (Table 16). The mean value of electrolyte leakage of the Valencia group was higher as compared to the other groups as shown in Fig. 5.

Table 7. Shoot length (cm) of seedlings of cultivated genotypes of groundnut following storage as seeds or pods for different durations under ambient condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	6.4	6.0	5.8	5.8	5.0	4.5	6.0	5.8	5.5	5.0	4.5
ICG 2742	4.4	4.2	3.9	3.7	2.8	2.2	4.2	3.8	3.0	2.8	2.4
ICG 5067	6.0	5.5	5.2	5.0	4.6	4.0	5.7	5.4	5.4	5.4	3.8
ICG 2484	5.0	4.5	4.4	4.0	3.2	2.5	4.7	4.5	4.1	3.4	2.5
ICGS 76	5.6	5.5	5.2	4.9	4.7	3.8	5.4	5.0	5.0	4.3	3.8
Mean	5.4	5.1	4.9	4.6	4.1	3.4	5.2	4.9	4.6	4.1	3.4
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	5.9	5.5	5.3	5.1	4.4	3.8	5.5	5.5	5.0	4.5	4.1
ICG 4342	4.4	4.0	3.8	3.3	2.8	2.4	4.0	3.8	2.9	2.8	2.7
ICG 4236	6.6	6.1	5.5	5.3	5.0	4.6	6.2	5.8	5.2	5.0	4.0
ICG 4479	4.5	4.1	3.9	3.5	2.8	2.1	4.0	3.8	3.8	3.4	3.0
ICG 156	4.4	4.3	4.0	3.8	3.0	2.8	4.3	4.1	3.7	3.3	2.5
Mean	5.1	4.8	4.5	4.2	3.6	3.1	4.8	4.6	4.1	3.8	3.2
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	6.9	6.4	5.9	5.3	4.2	4.2	6.6	6.3	5.3	5.0	4.0
ICG 10035	4.0	3.8	3.5	2.9	2.0	0.8	3.8	3.6	2.9	2.0	1.9
ICG 3041	4.6	4.4	4.2	3.9	3.3	2.3	4.4	4.2	4.0	3.2	2.7
ICG 10766	6.6	6.2	5.9	5.4	4.8	3.5	6.1	5.9	5.3	4.9	3.4
ICG 2738	5.4	5.2	5.0	4.7	4.7	3.9	5.1	5.0	5.0	4.4	4.0
Mean	5.5	5.2	4.9	4.4	3.8	2.9	5.2	5.0	4.5	3.9	3.2
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	6.3	6.0	6.0	5.6	5.2	4.2	6.1	5.8	5.7	5.3	4.0
ICG 2959	5.9	5.5	5.2	4.8	4.4	3.8	5.6	5.3	4.8	4.6	4.0
ICG 2988	6.6	6.3	5.7	5.2	4.5	4.2	6.4	5.8	5.3	4.6	4.1
ICG 3209	5.9	5.3	5.0	4.5	3.7	3.2	5.7	5.2	4.5	4.0	3.0
ICGS 44	5.9	5.9	5.6	4.9	4.9	3.2	5.7	5.9	4.7	4.5	3.4
Mean	6.1	5.8	5.5	5.0	4.5	3.7	5.9	5.6	5.0	4.6	3.7
S.E.	(S) ±0.043, (G) ±0.086, (M) ±0.027; CV (%) 10.7										

S=Storage, G=Genotype, M=Material

Table 8. Hypocotyl length (cm) of seedlings of cultivated genotypes of groundnut following storage as seeds or pods for different durations under ambient condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	2.8	2.7	2.5	2.2	2.0	1.8	2.6	2.2	2.0	2.0	1.4
ICG 2742	3.5	3.3	3.0	2.6	2.0	1.8	3.5	3.1	2.8	2.0	2.0
ICG 5067	2.4	2.3	2.1	1.9	1.6	1.4	2.3	2.3	2.2	2.0	1.5
ICG 2484	3.9	3.8	3.4	3.0	2.2	2.0	3.9	3.5	3.0	3.0	2.0
ICGS 76	3.2	3.1	3.0	2.6	2.2	2.0	3.2	3.0	2.8	2.0	2.0
Mean	3.1	3.0	2.8	2.4	2.0	1.8	3.1	2.8	2.5	2.2	1.7
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	3.4	3.3	3.0	2.7	2.4	2.2	3.2	3.0	2.8	2.4	2.2
ICG 4342	3.2	3.0	2.8	2.5	2.3	1.8	3.0	2.8	2.6	2.3	1.8
ICG 4236	3.2	3.0	2.8	2.6	2.2	2.2	3.0	2.8	2.6	2.2	2.2
ICG 4479	3.8	3.4	3.0	2.6	2.3	1.9	3.5	3.0	2.7	2.3	1.9
ICG 156	3.2	3.0	2.9	2.6	2.3	1.9	3.0	2.9	2.8	2.3	1.9
Mean	3.3	3.1	2.9	2.6	2.3	2.0	3.1	2.9	2.7	2.3	2.0
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	4.8	4.8	4.4	3.7	3.5	3.3	4.7	4.3	4.0	3.5	3.3
ICG 10035	5.0	4.6	4.3	4.0	2.5	1.2	4.5	4.3	3.5	2.6	1.2
ICG 3041	3.8	3.7	3.1	3.0	3.0	2.5	3.5	3.2	3.0	3.0	2.3
ICG 10766	5.0	4.9	4.5	3.7	3.8	3.3	4.6	4.4	4.4	3.9	3.5
ICG 2738	4.9	4.9	4.2	3.7	3.7	3.2	4.7	4.3	4.0	3.9	3.5
Mean	4.7	4.5	4.1	3.6	3.3	2.7	4.4	4.1	3.7	3.3	2.8
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	3.7	3.6	3.4	3.0	2.7	2.3	3.7	3.4	3.1	3.0	2.3
ICG 2959	4.4	3.9	3.5	2.9	2.8	2.4	3.9	3.6	3.0	2.8	2.8
ICG 2988	4.1	3.9	3.8	3.4	2.7	2.3	3.7	3.6	3.3	3.0	2.6
ICG 3209	4.5	4.3	3.8	3.3	2.7	2.0	4.3	3.7	3.4	3.0	2.3
ICGS 44	3.0	2.8	2.5	2.4	2.1	2.0	2.7	2.7	2.2	2.0	1.9
Mean	3.9	3.7	3.4	3.0	2.6	2.2	3.6	3.4	3.0	2.7	2.4
S.E.	(S) ±0.035,		(G) ±0.071,			(M) ±0.022;		CV (%) 13.4			

S=Storage, G=Genotype, M=Material

Table 9. Root length (cm) of seedlings of cultivated genotypes of groundnut following storage as seeds or pods for different durations under ambient condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	14.4	13.5	13.0	12.9	11.4	10.3	13.9	13.7	13.4	11.4	10.7
ICG 2742	17.2	16.9	15.4	13.8	12.4	11.0	17.0	16.4	15.3	13.3	11.3
ICG 5067	16.5	15.6	14.5	13.2	12.9	11.7	16.1	15.6	14.8	13.8	11.8
ICG 2484	16.3	15.7	14.5	14.2	13.9	11.9	15.8	15.0	14.4	13.9	11.0
ICGS 76	16.4	15.8	15.1	14.9	13.9	11.5	15.7	14.8	14.1	13.1	11.2
Mean	16.1	15.5	14.5	13.8	12.9	11.2	15.7	15.1	14.4	13.1	11.2
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	18.7	17.9	17.1	16.0	14.9	13.6	17.9	17.1	16.1	14.1	14.0
ICG 4342	14.9	14.5	14.1	13.7	12.0	11.0	14.6	14.2	14.0	12.3	10.7
ICG 4236	17.1	16.8	16.2	15.9	13.0	11.6	16.9	16.5	15.9	13.1	12.0
ICG 4479	17.4	16.8	15.9	15.5	13.4	12.2	16.9	16.8	15.9	13.2	11.6
ICG 156	17.6	17.0	16.7	16.4	13.2	12.1	16.3	16.0	15.6	14.2	13.2
Mean	17.1	16.6	16.0	15.5	13.3	12.1	16.5	16.1	15.5	13.3	12.3
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	20.5	19.1	18.0	17.1	14.4	12.0	19.1	18.4	17.4	14.7	12.5
ICG 10035	15.9	14.9	13.3	12.4	10.9	5.0	15.5	14.1	13.6	11.9	8.5
ICG 3041	16.9	16.5	15.5	13.7	13.2	9.9	16.6	15.8	14.8	13.2	10.9
ICG 10766	17.9	17.4	16.8	15.6	14.7	11.3	17.2	16.8	16.0	14.2	11.6
ICG 2738	18.2	17.6	17.0	16.7	15.8	12.3	17.6	17.4	16.7	15.9	11.9
Mean	17.8	17.1	16.1	15.1	13.8	10.1	17.2	16.5	15.7	13.9	11.1
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	16.5	15.8	15.0	14.6	12.7	10.0	16.0	16.0	14.6	13.4	11.0
ICG 2959	14.9	14.0	13.9	13.3	10.2	8.8	13.9	13.7	13.5	10.7	9.3
ICG 2988	16.0	15.5	15.2	14.6	12.5	10.0	15.6	15.3	15.0	12.6	10.1
ICG 3209	16.2	15.8	15.0	13.9	11.8	9.0	15.9	15.6	14.1	11.9	9.0
ICGS 44	17.4	16.9	16.4	15.6	13.8	11.0	17.1	16.9	15.3	13.9	11.1
Mean	16.2	15.6	15.1	14.4	12.2	9.7	15.7	15.5	14.5	12.5	10.1
S.E.	(S) ±0.081 (G) ±0.162, (M) ±0.051; CV (%) 6.3										

S=Storage, G=Genotype, M=Material

Table 10. Dry weight (g) of seedlings of cultivated genotypes of groundnut following storage as seeds or pods for different durations under ambient condition.

Cultivar group/ genotype	Seeds: Storage duration (months)						Pods: Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	0.91	0.89	0.80	0.76	0.65	0.49	0.86	0.81	0.79	0.67	0.52
ICG 2742	3.12	3.01	2.96	2.67	2.01	1.79	3.09	2.83	2.65	2.01	1.88
ICG 5067	2.72	2.55	2.34	2.20	1.79	1.35	2.48	2.38	2.27	1.92	1.39
ICG 2484	2.93	2.77	2.55	2.38	1.87	1.47	2.80	2.70	2.51	2.02	1.57
ICGS 76	2.52	2.32	2.21	2.19	1.88	1.55	2.26	2.22	2.11	2.07	1.84
Mean	2.44	2.31	2.17	2.04	1.64	1.33	2.30	2.19	2.07	1.74	1.44
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	2.38	2.32	2.25	2.06	1.74	1.56	2.29	2.27	2.06	1.79	1.53
ICG 4342	3.37	3.22	3.09	2.85	1.97	1.76	3.30	3.02	2.81	1.94	1.77
ICG 4236	2.42	2.35	2.16	1.98	1.61	1.42	2.34	2.19	1.95	1.62	1.52
ICG 4479	3.04	2.99	2.70	2.51	2.13	1.75	2.98	2.70	2.57	2.15	1.75
ICG 156	3.44	3.32	3.21	3.10	2.67	2.31	3.34	3.26	3.11	2.73	2.49
Mean	2.93	2.84	2.68	2.50	2.02	1.76	2.85	2.69	2.50	2.05	1.81
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	1.82	1.63	1.57	1.54	1.22	1.01	1.71	1.63	1.43	1.23	1.01
ICG 10035	3.40	3.05	2.84	2.39	1.73	1.35	3.12	3.08	2.30	2.02	1.21
ICG 3041	2.50	2.28	1.76	1.69	1.30	1.01	2.35	2.07	1.81	1.34	1.09
ICG 10766	2.23	2.11	1.80	1.76	1.30	1.01	2.11	2.01	1.85	1.36	1.04
ICG 2738	2.25	2.10	2.01	1.89	1.50	1.02	2.12	2.02	1.81	1.55	1.10
Mean	2.44	2.23	2.00	1.85	1.41	1.08	2.28	2.16	1.84	1.50	1.09
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	2.03	1.86	1.86	1.69	1.24	1.15	1.89	1.82	1.77	1.22	1.07
ICG 2959	2.74	2.59	2.29	2.15	1.49	1.21	2.40	2.32	2.32	1.44	1.23
ICG 2988	2.17	1.89	1.85	1.76	1.23	1.10	1.92	1.87	1.84	1.54	1.36
ICG 3209	2.94	2.53	2.35	2.01	1.40	1.26	2.48	2.44	2.06	1.47	1.29
ICGS 44	3.03	2.76	2.76	2.32	1.79	1.68	2.76	2.62	2.42	1.78	1.61
Mean	2.58	2.33	2.22	1.99	1.43	1.28	2.29	2.21	2.08	1.49	1.31
S.E.	(S) ±0.027, (G) ±0.054, (M) ±0.017; CV (%) 15.1										

S=Storage, G=Genotype, M=Material

Table 11. Shoot length (cm) of seedlings of cultivated genotypes of groundnut following storage as seeds or pods for different durations under medium-term condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	6.4	6.2	6.2	6.1	6.0	6.0	6.3	6.3	6.1	6.1	6.0
ICG 2742	4.4	4.2	4.2	4.0	4.0	4.0	4.2	4.2	4.0	4.0	4.0
ICG 5067	6.0	5.8	5.8	5.7	5.7	5.7	5.9	5.9	5.7	5.7	5.7
ICG 2484	5.0	4.8	4.8	4.7	4.7	4.7	4.9	4.9	4.7	4.7	4.7
ICGS 76	5.6	5.5	5.5	5.5	5.3	5.3	5.6	5.6	5.5	5.5	5.4
Mean	5.4	5.3	5.3	5.2	5.1	5.1	5.3	5.3	5.2	5.2	5.1
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	5.9	5.7	5.7	5.7	5.7	5.6	5.9	5.9	5.9	5.7	5.7
ICG 4342	4.4	4.4	4.4	4.2	4.2	4.0	4.4	4.3	4.3	4.2	4.2
ICG 4236	6.6	6.5	6.5	6.5	6.5	6.4	6.6	6.5	6.5	6.4	6.4
ICG 4479	4.5	4.4	4.4	4.2	4.2	4.0	4.4	4.3	4.3	4.2	4.2
ICG 156	4.4	4.2	4.2	4.0	4.0	4.0	4.4	4.2	4.2	4.0	4.0
Mean	5.1	5.0	5.0	4.9	4.9	4.8	5.1	5.0	5.0	4.9	4.9
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	6.9	6.9	6.9	6.8	6.7	6.7	6.8	6.8	6.8	6.7	6.7
ICG 10035	4.0	4.0	4.0	3.4	3.0	2.6	4.0	3.9	3.4	3.3	3.0
ICG 3041	4.6	4.5	4.5	4.5	4.3	4.3	4.6	4.5	4.5	4.4	4.4
ICG 10766	6.6	6.5	6.5	6.5	6.5	6.4	6.6	6.6	6.5	6.4	6.4
ICG 2738	5.4	5.4	5.4	5.4	5.2	5.2	5.4	5.4	5.4	5.2	5.2
Mean	5.5	5.4	5.4	5.3	5.1	5.0	5.4	5.4	5.3	5.2	5.1
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	6.3	6.3	6.3	6.2	6.2	6.0	6.3	6.3	6.2	6.2	5.8
ICG 2959	5.9	5.9	5.8	5.7	5.7	5.7	5.9	5.8	5.7	5.7	5.3
ICG 2988	6.6	6.5	6.4	6.4	6.4	6.2	6.5	6.5	6.4	6.4	6.0
ICG 3209	5.9	5.9	5.7	5.6	5.6	5.3	5.9	5.6	5.6	5.6	5.2
ICGS 44	5.9	5.9	5.8	5.7	5.7	5.6	5.9	5.8	5.7	5.7	5.3
Mean	6.1	6.1	6.0	5.9	5.9	5.7	6.1	6.0	5.9	5.9	5.5
S.E.	(S) ±0.061,		(G) ±0.122,			(M) ±0.038;		CV (%) 12.4			

S=Storage, G=Genotype, M=Material

Table 12. Hypocotyl length (cm) of seedlings of cultivated genotypes of groundnut following storage as seeds or pods for different durations under medium-term condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	2.8	2.8	2.7	2.7	2.7	2.7	2.8	2.8	2.6	2.6	2.6
ICG 2742	3.5	3.5	3.3	3.3	3.3	3.3	3.5	3.5	3.4	3.4	3.3
ICG 5067	2.4	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.2
ICG 2484	3.9	3.8	3.7	3.7	3.5	3.5	3.9	3.9	3.8	3.8	3.6
ICGS 76	3.2	3.2	3.1	3.1	3.1	3.1	3.2	3.2	3.1	3.1	3.0
Mean	3.1	3.1	3.0	3.0	2.9	2.9	3.1	3.1	3.0	3.0	2.9
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	3.4	3.4	3.3	3.3	3.2	3.2	3.4	3.3	3.2	3.2	3.2
ICG 4342	3.2	3.0	3.0	3.0	3.0	3.0	3.1	3.1	3.0	3.0	3.0
ICG 4236	3.2	3.0	3.0	3.0	2.9	2.9	3.2	3.2	3.1	3.1	3.0
ICG 4479	3.8	3.6	3.6	3.6	3.5	3.5	3.8	3.8	3.7	3.7	3.5
ICG 156	3.2	3.1	3.1	3.1	3.0	3.0	3.2	3.0	3.0	3.0	2.8
Mean	3.3	3.2	3.2	3.2	3.1	3.1	3.3	3.2	3.2	3.2	3.1
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	4.8	4.8	4.8	4.6	4.6	4.5	4.7	4.6	4.6	4.6	4.5
ICG 10035	5.0	4.6	4.6	4.5	4.5	4.0	4.7	4.6	4.6	4.5	4.0
ICG 3041	3.8	3.7	3.7	3.5	3.5	3.5	3.8	3.7	3.7	3.5	3.5
ICG 10766	5.0	4.9	4.9	4.9	4.9	4.8	5.0	4.9	4.9	4.9	4.9
ICG 2738	4.9	4.9	4.9	4.7	4.7	4.7	4.9	4.9	4.9	4.7	4.7
Mean	4.7	4.5	4.5	4.4	4.4	4.3	4.6	4.5	4.5	4.4	4.3
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	3.7	3.7	3.6	3.6	3.4	3.4	3.7	3.7	3.7	3.5	3.4
ICG 2959	4.4	4.3	4.3	4.3	4.2	4.1	4.3	4.3	4.3	4.2	4.1
ICG 2988	4.1	3.9	3.9	3.9	3.9	3.8	4.0	4.0	4.0	3.9	3.8
ICG 3209	4.5	4.3	4.3	4.3	4.2	4.1	4.3	4.3	4.3	4.1	4.1
ICGS 44	3.0	2.9	2.9	2.9	2.8	2.8	3.0	2.9	2.9	2.9	2.8
Mean	3.9	3.8	3.8	3.8	3.7	3.7	3.8	3.8	3.8	3.7	3.7
S.E.	(S) ±0.025,		(G) ±0.050,			(M) ±0.016;	CV (%) 7.6				

S=Storage, G=Genotype, M=Material

Table 13. Root length (cm) of seedlings of cultivated genotypes of groundnut following storage as seeds or pods for different durations under medium-term condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	14.4	14.1	14.1	14.0	14.0	13.6	14.4	14.1	14.0	14.0	13.9
ICG 2742	17.2	17.0	17.0	16.9	16.9	16.5	17.1	17.0	17.0	17.0	16.8
ICG 5067	16.5	16.3	16.3	16.1	16.1	15.9	16.4	16.3	16.2	16.2	16.0
ICG 2484	16.3	16.1	16.1	15.9	15.9	15.4	16.1	16.1	15.9	15.9	15.7
ICGS 76	16.4	16.2	16.2	16.1	16.1	15.9	16.3	16.2	16.1	16.1	15.9
Mean	16.1	15.9	15.9	15.8	15.8	15.4	16.0	15.9	15.8	15.8	15.6
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	18.7	18.5	18.5	18.4	18.4	18.4	18.7	18.5	18.5	18.4	18.4
ICG 4342	14.9	14.7	14.7	14.5	14.5	14.5	14.9	14.7	14.7	14.7	14.5
ICG 4236	17.1	16.9	16.9	16.8	16.8	16.6	17.0	16.9	16.9	16.8	16.6
ICG 4479	17.4	17.1	17.1	17.1	16.8	16.5	17.1	17.1	17.1	16.9	16.5
ICG 156	17.6	17.4	17.4	17.4	17.4	16.8	17.5	17.4	17.4	17.1	16.8
Mean	17.1	16.9	16.9	16.8	16.7	16.5	17.0	16.9	16.9	16.7	16.5
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	20.5	20.3	20.1	20.0	19.5	19.5	20.3	20.1	19.7	19.7	19.5
ICG 10035	15.9	15.6	14.9	14.3	13.9	13.1	15.6	15.5	14.7	14.7	13.9
ICG 3041	16.9	16.8	16.7	16.5	16.2	16.1	16.8	16.7	16.5	16.5	16.2
ICG 10766	17.9	17.8	17.4	17.4	17.4	17.2	17.8	17.6	17.6	17.6	17.4
ICG 2738	18.2	18.1	18.0	18.0	17.8	17.8	18.1	18.0	17.8	17.8	17.8
Mean	17.8	17.7	17.4	17.2	16.9	16.7	17.7	17.5	17.2	17.2	16.9
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	16.5	16.3	16.3	16.3	16.0	15.8	16.5	16.5	16.3	16.0	15.8
ICG 2959	14.9	14.7	14.7	14.7	14.5	14.3	14.8	14.8	14.7	14.5	14.3
ICG 2988	16.0	16.0	16.0	15.8	15.5	15.5	16.0	16.0	15.8	15.8	15.5
ICG 3209	16.2	16.0	16.0	15.9	15.7	15.5	15.9	15.9	15.9	15.9	15.5
ICGS 44	17.4	17.4	17.4	17.1	16.9	16.7	17.2	17.1	16.9	16.9	16.7
Mean											

S.E. (S) ±0.098, (G) ±0.196, (M) ±0.062; CV (%) 6.5

S=Storage, G=Genotype, M=Material

Table 14. Dry weight (g) of seedlings of cultivated genotypes of groundnut following storage as seeds or pods for different durations under medium-term condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	0.91	0.91	0.91	0.91	0.89	0.89	0.91	0.91	0.91	0.89	0.89
ICG 2742	3.12	3.11	3.09	3.09	3.05	3.05	3.10	3.10	3.10	3.06	3.06
ICG 5067	2.72	2.71	2.71	2.71	2.68	2.68	2.71	2.71	2.71	2.69	2.69
ICG 2484	2.93	2.92	2.90	2.90	2.85	2.85	2.93	2.90	2.90	2.85	2.85
ICGS 76	2.52	2.52	2.50	2.50	2.47	2.47	2.52	2.50	2.50	2.48	2.48
Mean	2.44	2.43	2.42	2.42	2.39	2.39	2.43	2.42	2.42	2.39	2.39
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	2.38	2.35	2.35	2.35	2.33	2.33	2.38	2.38	2.36	2.36	2.36
ICG 4342	3.37	3.34	3.34	3.31	3.30	3.30	3.36	3.36	3.32	3.32	3.30
ICG 4236	2.42	2.42	2.42	2.40	2.37	2.37	2.40	2.40	2.40	2.40	2.38
ICG 4479	3.04	3.04	3.04	3.00	2.97	2.97	3.03	3.03	3.00	3.00	2.97
ICG 156	3.44	3.40	3.40	3.40	3.37	3.37	3.41	3.41	3.40	3.40	3.38
Mean	2.93	2.91	2.91	2.89	2.87	2.87	2.92	2.92	2.90	2.90	2.88
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	1.82	1.80	1.80	1.80	1.76	1.76	1.81	1.80	1.80	1.79	1.79
ICG 10035	3.40	3.22	3.22	2.81	2.81	2.62	3.28	3.22	3.22	2.53	2.53
ICG 3041	2.50	2.47	2.47	2.47	2.43	2.42	2.50	2.47	2.47	2.45	2.45
ICG 10766	2.23	2.21	2.21	2.20	2.19	2.18	2.23	2.21	2.21	2.21	2.21
ICG 2738	2.25	2.24	2.24	2.24	2.23	2.23	2.25	2.24	2.24	2.22	2.22
Mean	2.44	2.39	2.39	2.30	2.28	2.24	2.41	2.39	2.39	2.24	2.24
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	2.03	2.03	2.01	2.01	1.97	1.97	2.03	2.01	2.01	1.98	1.98
ICG 2959	2.74	2.72	2.70	2.68	2.67	2.67	2.72	2.71	2.71	2.68	2.68
ICG 2988	2.17	2.17	2.15	2.15	2.11	2.11	2.17	2.15	2.15	2.12	2.12
ICG 3209	2.94	2.94	2.91	2.87	2.87	2.87	2.94	2.90	2.90	2.88	2.88
ICGS 44	3.03	3.01	3.01	2.98	2.98	2.98	3.01	3.01	3.01	2.98	2.98
Mean	2.58	2.57	2.56	2.54	2.52	2.52	2.57	2.56	2.56	2.53	2.53
S.E.	(S) ±0.037, (G) ±0.074, (M) ±0.023; CV (%) 16.0										

S=Storage, G=Genotype, M=Material

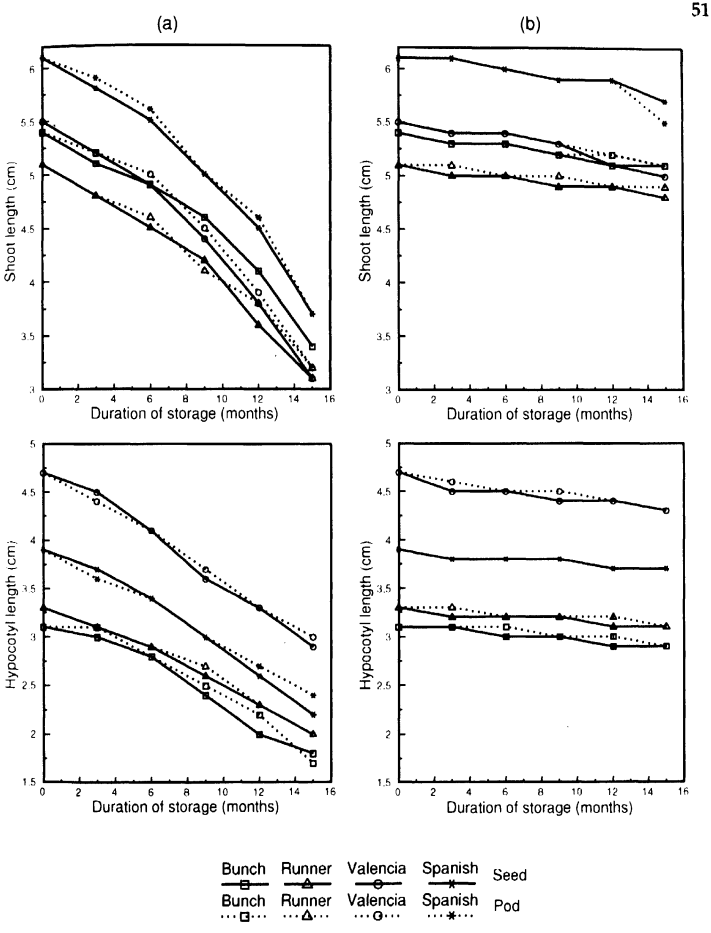

Figure 3. Mean length of shoot and hypocotyl of seedlings of genotypes of 4 cultivar groups of groundnut following storage under (a) ambient and (b) medium-term conditions as seeds and pods.

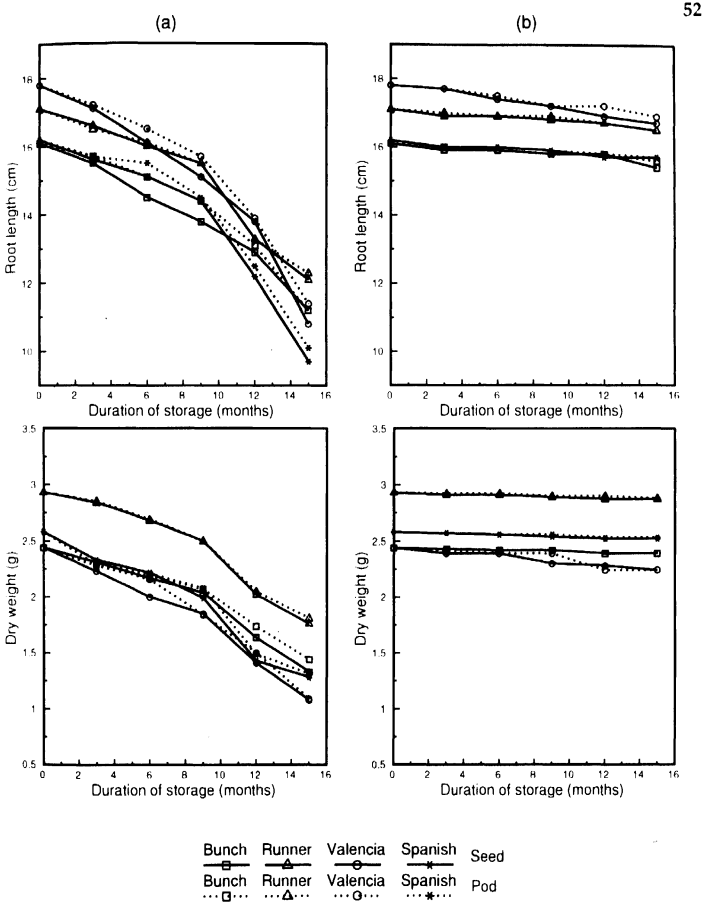

Figure 4. Mean root length and dry weight of seedlings of genotypes of 4 cultivar groups of groundnut following storage under (a) ambient and (b) medium-term conditions as seeds and pods.

Table 15. Electrolyte leakage (mmho/cm) from the seeds of cultivated genotypes of groundnut following storage as seeds or pods for different durations under ambient condition.

Cultivar group/ genotype	Seeds: Storage duration (months)						Pods: Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	0.095	0.101	0.109	0.114	0.201	0.249	0.105	0.105	0.118	0.199	0.237
ICG 2742	0.215	0.227	0.230	0.271	0.408	0.418	0.226	0.245	0.270	0.376	0.408
ICG 5067	0.188	0.199	0.213	0.236	0.459	0.524	0.195	0.215	0.249	0.445	0.522
ICG 2484	0.245	0.257	0.307	0.390	0.495	0.573	0.261	0.305	0.386	0.490	0.551
ICGS 76	0.144	0.172	0.197	0.213	0.462	0.606	0.167	0.192	0.223	0.356	0.562
Mean	0.177	0.191	0.211	0.244	0.405	0.474	0.190	0.212	0.249	0.373	0.456
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	0.104	0.107	0.131	0.193	0.357	0.406	0.107	0.135	0.183	0.351	0.382
ICG 4342	0.162	0.183	0.198	0.288	0.498	0.545	0.176	0.197	0.271	0.464	0.527
ICG 4236	0.104	0.109	0.118	0.244	0.270	0.344	0.108	0.117	0.238	0.261	0.338
ICG 4479	0.148	0.161	0.183	0.301	0.387	0.415	0.157	0.198	0.298	0.376	0.406
ICG 156	0.180	0.201	0.220	0.264	0.473	0.499	0.195	0.226	0.250	0.467	0.492
Mean	0.139	0.152	0.170	0.258	0.397	0.441	0.148	0.174	0.248	0.383	0.429
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	0.122	0.134	0.143	0.188	0.220	0.275	0.133	0.141	0.181	0.219	0.269
ICG 10035	0.402	0.469	0.701	0.890	1.121	1.180	0.490	0.664	0.852	0.973	1.115
ICG 3041	0.256	0.260	0.300	0.353	0.698	0.751	0.261	0.305	0.421	0.620	0.670
ICG 10766	0.181	0.186	0.203	0.272	0.351	0.441	0.184	0.205	0.272	0.329	0.429
ICG 2738	0.097	0.106	0.118	0.134	0.272	0.277	0.101	0.110	0.130	0.270	0.270
Mean	0.211	0.231	0.293	0.367	0.532	0.584	0.233	0.285	0.371	0.482	0.550
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	0.201	0.207	0.237	0.301	0.325	0.424	0.204	0.233	0.299	0.306	0.406
ICG 2959	0.310	0.320	0.361	0.402	0.557	0.697	0.317	0.365	0.404	0.554	0.681
ICG 2988	0.209	0.213	0.251	0.307	0.426	0.445	0.217	0.257	0.310	0.416	0.439
ICG 3209	0.297	0.334	0.407	0.487	0.703	0.842	0.333	0.404	0.477	0.686	0.761
ICGS 44	0.168	0.176	0.199	0.279	0.622	0.647	0.179	0.206	0.274	0.595	0.627
Mean	0.237	0.250	0.291	0.355	0.526	0.611	0.249	0.293	0.352	0.511	0.583

S.E. (S) ±0.0043, (G) ±0.0087, (M) ±0.0027; CV (%) 13.9

S=Storage, G=Genotype, M=Material

Table 16. Electrolyte leakage (mmho/cm) from the seeds of cultivated genotypes of groundnut following storage as seeds or pods for different durations under medium-term condition.

Cultivar group/ genotype	Seeds: Storage duration (months)						Pods: Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	0.095	0.097	0.097	0.099	0.102	0.105	0.097	0.097	0.099	0.101	0.101
ICG 2742	0.215	0.217	0.217	0.222	0.223	0.224	0.217	0.217	0.218	0.220	0.223
ICG 5067	0.188	0.190	0.191	0.194	0.194	0.196	0.188	0.190	0.191	0.191	0.193
ICG 2484	0.245	0.247	0.247	0.249	0.254	0.255	0.245	0.249	0.250	0.252	0.255
ICGS 76	0.144	0.147	0.147	0.150	0.155	0.157	0.145	0.145	0.147	0.147	0.151
Mean	0.177	0.179	0.179	0.182	0.185	0.187	0.178	0.179	0.181	0.182	0.184
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	0.104	0.103	0.107	0.107	0.109	0.112	0.105	0.107	0.107	0.109	0.111
ICG 4342	0.162	0.165	0.167	0.167	0.170	0.170	0.163	0.165	0.165	0.166	0.109
ICG 4236	0.104	0.103	0.105	0.107	0.107	0.105	0.105	0.105	0.107	0.107	0.019
ICG 4479	0.148	0.150	0.150	0.152	0.151	0.152	0.150	0.151	0.151	0.153	0.153
ICG 156	0.180	0.181	0.182	0.183	0.184	0.185	0.181	0.183	0.185	0.185	0.187
Mean	0.139	0.140	0.142	0.143	0.144	0.145	0.140	0.142	0.143	0.144	0.145
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	0.122	0.124	0.126	0.128	0.129	0.132	0.124	0.124	0.126	0.127	0.127
ICG 10035	0.402	0.515	0.644	0.674	0.826	0.930	0.505	0.554	0.671	0.801	0.887
ICG 3041	0.256	0.258	0.260	0.260	0.262	0.264	0.256	0.258	0.258	0.260	0.262
ICG 10766	0.181	0.182	0.186	0.189	0.192	0.192	0.181	0.184	0.185	0.185	0.186
ICG 2738	0.097	0.103	0.107	0.106	0.110	0.117	0.099	0.099	0.101	0.103	0.103
Mean	0.211	0.236	0.266	0.277	0.311	0.338	0.233	0.243	0.268	0.295	0.313
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	0.201	0.203	0.204	0.207	0.207	0.213	0.203	0.203	0.206	0.207	0.209
ICG 2959	0.310	0.312	0.317	0.320	0.325	0.328	0.312	0.317	0.320	0.325	0.328
ICG 2988	0.209	0.213	0.217	0.219	0.219	0.221	0.213	0.217	0.219	0.219	0.221
ICG 3209	0.297	0.299	0.303	0.307	0.309	0.311	0.299	0.303	0.303	0.305	0.305
ICGS 44	0.168	0.170	0.170	0.172	0.174	0.178	0.170	0.170	0.174	0.174	0.173
Mean	0.237	0.239	0.242	0.245	0.247	0.250	0.239	0.242	0.244	0.246	0.247
S.E.	(S) ±0.0032,		(G) ±0.0065,		(M) ±0.0020;		CV (%) 16.9				

S=Storage, G=Genotype, M=Material

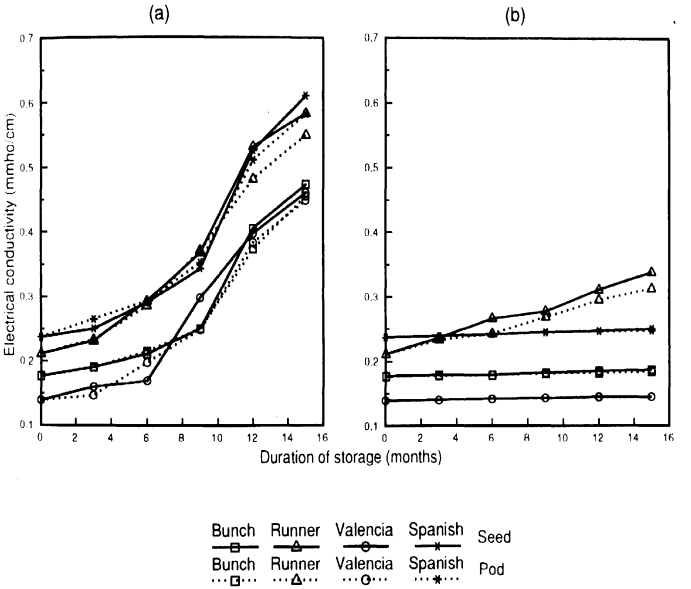

Figure 5. Electrolyte leakage from seeds of cultivated groundnut belonging to 4 cultivar groups (mean) stored as seeds or pods under (a) ambient and (b) medium-term conditions.

Oil content:

A decrease in the oil content of the seeds in almost all the genotypes was observed when the seeds were stored under ambient condition for 15 months as shown in Table 17. There was a progressive decrease in the seed oil content with time and the decline was more conspicuous between the period 9-12 months of storage (Fig. 6). The loss in the seed oil content did not show much differences among the genotypes belonging to different groups. The exception was in the genotype ICG 10035 where the reduction was as high as 3.6 percent. With regards to the loss of oil content there was no significant difference between the small-seeded or large-seeded genotypes. There were also no differences among the groundnut genotypes or groups with respect to storage of kernels or pods.

When the seeds were stored under medium-term storage condition no loss of oil content was observed except in the genotype ICG 10035 as seen from Table 18 and Fig. 6.

Fatty acid composition:

An analysis of the fatty acid composition of groundnut seeds stored under ambient condition showed a change only in the oleic and linoleic acid contents, while palmitic, stearic, arachidic, eicosenoic, behenic, and lignoceric acids remained unchanged as shown in Table 19.

A gradual decrease in linoleic acid content was noticed in the seeds which were stored under ambient conditions (Table 20 and Fig. 7). The extent of such decrease was more in the genotypes belonging to Valencia and Spanish groups compared to genotypes belonging to Virginia bunch and Virginia runner groups. There were no significant differences between small-seeded and large-seeded genotypes as well as between stored kernels and pods in terms of changes in linoleic acid content. There was an increase in the oleic acid content

of the seeds. The changes in both these fatty acids caused an increase in the O/L ratio of the seeds.

When the seeds were stored under medium-term condition a gradual lowering in the linoleic acid content was observed except in ICG 10035 where the decrease was considerably more as seen in Table 22. However, decrease in the linoleic acid content was much slower in the seeds stored under medium-term condition as compared to storage under ambient condition.

Table 17. Oil content (%) of seeds of cultivated genotypes of groundnut following storage as seeds or pods for different durations under ambient condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	45.4	45.2	45.0	45.0	44.0	44.0	45.2	44.7	44.6	44.3	44.0
ICG 2742	45.6	45.2	45.0	45.5	44.0	44.0	45.2	45.0	45.0	44.0	44.0
ICG 5067	48.6	48.2	48.0	47.6	47.0	46.8	48.4	48.2	47.7	47.0	46.7
ICG 2484	44.8	44.4	44.1	44.0	43.0	42.9	44.4	44.3	44.0	43.0	42.8
ICGS 76	45.1	45.0	44.5	44.0	43.5	43.5	45.0	44.6	44.0	43.6	43.5
Mean	45.9	45.6	45.3	45.1	44.3	44.2	45.6	45.3	45.0	44.3	44.2
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	42.3	42.0	42.0	42.0	40.9	40.5	42.0	42.0	42.0	40.9	40.5
ICG 4342	45.9	45.7	45.7	45.2	44.8	44.2	45.7	45.7	45.2	44.6	44.3
ICG 4236	43.7	43.5	43.5	43.5	42.4	42.0	43.5	43.5	43.5	42.5	42.0
ICG 4479	46.1	46.0	45.7	45.7	44.7	44.4	46.0	45.8	45.3	44.9	44.5
ICG 156	46.4	46.2	46.2	46.0	45.1	44.8	46.2	46.2	46.2	45.1	44.7
Mean	44.8	44.6	44.6	45.3	44.8	44.2	44.6	44.6	44.4	43.6	43.2
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	45.9	45.9	45.5	45.0	44.5	44.5	45.8	45.5	45.0	44.5	44.5
ICG 10035	48.6	48.3	47.9	47.8	47.2	45.0	48.3	48.0	47.8	47.0	45.0
ICG 3041	47.2	47.0	46.7	46.1	45.1	44.7	47.0	46.1	46.1	45.7	44.7
ICG 10766	43.7	43.5	43.1	43.0	42.7	42.2	43.5	43.1	43.0	42.6	42.2
ICG 2738	43.0	43.0	42.8	42.3	42.0	41.5	43.0	43.0	42.9	42.3	41.5
Mean	45.6	45.5	45.2	44.8	44.3	43.5	45.5	45.1	44.9	44.4	43.5
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	45.4	45.3	45.0	45.0	43.5	43.5	45.4	45.3	45.0	44.5	43.6
ICG 2959	46.3	46.0	45.7	45.5	44.4	44.1	46.0	45.5	45.0	44.5	44.0
ICG 2988	46.9	46.1	46.0	45.5	45.1	44.8	46.3	46.0	45.4	45.3	44.8
ICG 3209	45.9	45.6	45.2	45.0	44.5	43.5	45.2	45.1	45.1	44.3	43.5
ICGS 44	47.0	46.5	46.5	46.5	45.3	45.1	46.5	46.2	46.0	45.8	45.2
Mean	46.3	45.9	45.6	45.5	44.5	44.2	45.8	45.6	45.3	44.8	44.2

S.E. (S) ±0.047, (G) ±0.095, (M) ±0.030; CV (%) 1.2

S=Storage, G=Genotype, M=Material

Table 18. Oil content (%) of seeds of cultivated genotypes of groundnut following storage as seeds or pods for different durations under medium-term condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	45.4	45.8	45.2	45.5	45.4	45.8	45.3	45.2	45.1	45.8	45.6
ICG 2742	45.6	45.9	45.3	45.6	45.5	45.9	45.7	46.0	45.8	45.3	45.5
ICG 5067	48.6	48.8	48.7	47.9	48.3	48.0	48.9	48.5	48.8	48.9	48.6
ICG 2484	44.8	44.9	45.2	45.3	44.9	45.0	45.0	45.2	45.0	44.1	44.7
ICGS 76	45.1	45.0	45.4	45.0	45.0	44.8	45.2	45.2	45.0	45.0	45.2
Mean	45.9	46.0	45.9	45.8	45.8	45.9	46.0	46.0	45.9	45.8	45.9
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	42.3	42.0	42.2	43.1	43.6	42.2	42.4	42.7	42.5	43.1	42.3
ICG 4342	45.9	45.5	45.4	45.4	44.8	45.9	46.0	45.8	45.5	45.5	45.9
ICG 4236	43.7	44.5	44.5	44.0	43.4	43.6	44.0	43.4	43.9	43.2	43.7
ICG 4479	46.1	46.4	46.2	46.0	45.5	46.1	46.2	46.3	46.2	46.2	45.8
ICG 156	46.4	46.5	46.2	46.3	46.1	46.3	46.0	46.5	46.4	46.2	46.5
Mean	44.8	44.9	44.9	44.9	44.6	45.0	44.9	44.7	44.9	44.8	44.8
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	45.9	45.9	46.0	45.8	45.9	45.9	46.0	46.0	45.9	45.8	45.9
ICG 10035	48.6	48.4	48.2	48.2	47.4	47.0	48.5	48.2	48.0	47.6	47.5
ICG 3041	47.2	47.2	47.0	47.2	47.0	47.0	47.1	47.2	47.0	47.2	47.1
ICG 10766	43.7	43.7	44.0	43.6	43.8	43.8	43.7	44.0	43.8	43.6	43.7
ICG 2738	43.0	42.9	43.0	43.1	43.0	43.0	43.1	43.0	43.0	43.0	43.0
Mean	45.6	45.6	45.6	45.5	45.4	45.3	45.7	45.6	45.5	45.4	45.4
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	45.4	45.5	45.6	45.2	45.9	45.6	45.6	45.9	45.9	45.6	45.5
ICG 2959	46.3	46.7	46.7	46.9	46.3	46.1	46.4	46.1	46.0	46.4	46.4
ICG 2988	46.9	47.1	47.2	47.2	46.9	46.9	47.1	47.5	46.9	46.9	47.0
ICG 3209	45.9	46.4	46.4	46.2	46.0	45.8	46.0	45.7	45.8	45.8	45.8
ICGS 44	47.0	46.0	46.3	46.6	46.8	47.2	47.0	46.7	47.0	46.9	46.9
Mean	46.3	46.3	46.4	46.4	46.3	46.3	46.4	46.3	46.3	46.3	46.3

S.E. (S) ±0.053, (G) ±0.107, (M) ±0.033; CV (%) 1.3

S=Storage, G=Genotype, M=Material

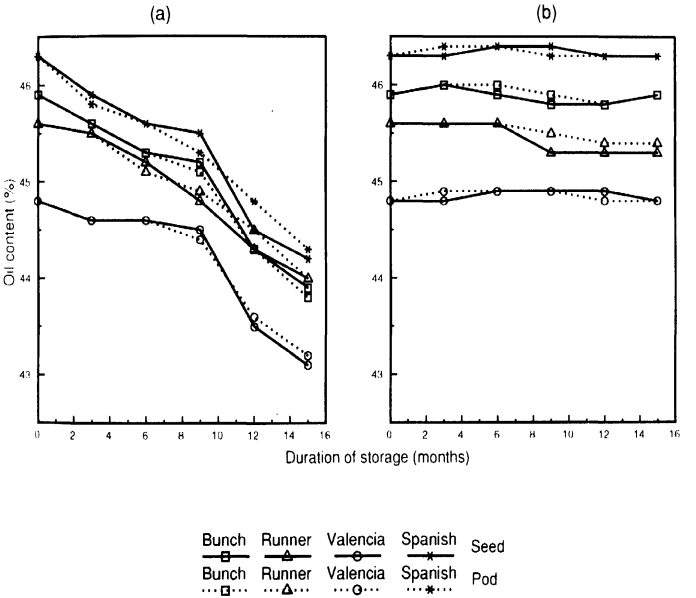

Figure 6. Changes in oil content of seeds of genotypes of 4 cultivar groups (mean) of groundnut following storage as seeds or pods under (a) ambient and (b) medium-term conditions.

Table 19. Fatty acid composition of seeds of cultivated genotypes of groundnut following storage under ambient condition.

Fatty acid/ Genotype		Palmitic	Stearic	Oleic	Linoleic	Arachidic	Eicosenoic	Behenic	Lignoceric	O/L ratio
ICG 4906	F	12.2	3.3	38.3	36.4	1.7	1.8	3.9	1.9	1.05
	A	12.8	3.2	41.4	34.8	1.7	1.4	3.0	1.6	1.19
ICG 2742	F	12.2	3.8	35.7	39.1	1.8	1.5	3.2	1.6	0.91
	A	12.9	3.3	39.1	35.5	1.7	1.0	3.1	1.3	1.10
ICG 5067	F	11.8	3.9	39.0	38.0	1.4	0.8	3.0	1.7	1.03
	A	12.3	3.2	42.1	34.4	1.7	1.1	3.0	1.2	1.23
ICG 2484	F	12.4	3.9	35.8	38.2	1.8	1.7	3.5	1.9	0.94
	A	11.5	3.2	41.8	35.0	1.2	1.0	2.7	1.0	1.20
ICGS 76	F	11.8	2.2	36.1	41.7	1.6	1.4	3.0	1.7	0.87
	A	11.8	3.2	40.9	37.8	1.4	1.6	2.8	1.5	1.08
ICG 4344	F	11.3	2.9	38.1	39.6	1.5	1.7	3.0	1.6	0.96
	A	12.4	3.4	40.0	36.2	1.5	1.5	3.1	1.2	1.10
ICG 4342	F	12.8	3.0	34.9	41.2	1.8	1.6	2.7	1.7	0.85
	A	12.8	3.1	39.6	38.3	1.8	1.8	2.9	1.7	1.03
ICG 4236	F	10.7	4.0	35.8	41.6	1.5	1.4	3.1	1.6	0.86
	A	11.8	3.6	40.0	37.0	1.6	1.6	3.2	1.6	1.08
ICG 4479	F	11.2	3.6	37.5	40.0	1.6	1.7	2.9	1.4	0.94
	A	13.4	3.8	40.1	34.6	1.9	1.8	3.3	1.9	1.16
ICG 156	F	11.2	3.8	38.0	38.3	1.6	1.2	3.5	1.9	0.99
	A	11.6	3.5	40.2	35.0	1.6	1.6	3.2	1.5	1.15
ICG 10063	F	11.3	3.3	41.3	35.4	1.7	1.6	3.8	1.8	1.16
	A	11.4	3.1	44.8	32.2	1.7	1.5	3.2	1.4	1.39
ICG 10035	F	11.3	3.2	44.1	33.9	1.6	1.5	3.6	2.0	1.30
	A	11.0	3.1	48.2	31.3	1.3	1.1	2.8	1.1	1.54
ICG 3041	F	11.5	2.2	41.9	38.4	1.1	1.2	2.4	1.3	1.09
	A	11.9	3.0	46.1	35.1	1.2	1.2	2.7	1.2	1.31
ICG 10766	F	12.2	2.9	42.1	34.8	1.7	1.6	2.5	1.9	1.21
	A	11.3	2.9	47.0	30.6	1.5	1.3	2.8	1.3	1.53
ICG 2738	F	11.0	2.2	49.1	29.2	1.3	1.5	3.7	1.9	1.68
	A	12.2	3.2	50.9	27.0	1.4	1.5	2.5	1.2	1.88
ICG 2387	F	10.6	2.3	43.6	34.7	1.3	1.8	3.4	2.0	1.25
	A	11.7	3.0	45.6	32.5	1.4	1.5	2.7	1.0	1.40
ICG 2959	F	10.4	2.9	48.3	32.2	1.2	1.5	2.0	1.5	1.50
	A	10.0	2.6	49.7	30.3	1.0	1.0	2.3	0.7	1.64
ICG 2988	F	10.8	2.1	44.0	34.5	1.3	1.8	3.5	1.9	1.27
	A	11.6	3.0	46.5	33.0	1.4	1.2	2.5	1.2	1.41
ICG 3209	F	10.6	2.4	45.9	33.6	1.5	1.8	2.9	1.9	1.36
	A	11.3	3.0	47.7	31.4	1.3	1.2	2.5	1.0	1.52
ICGS 44	F	12.2	2.5	47.1	30.8	1.4	1.5	2.9	1.5	1.53
	A	12.1	3.1	49.0	29.8	1.3	1.3	2.8	1.1	1.65

F=Fresh seeds, A=Aged seeds, stored under ambient condition

Table 20. Linoleic acid content (%) of seeds of cultivated genotypes of groundnut following storage as seeds or pods for different durations under ambient condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	35.4	35.2	35.0	34.6	34.6	33.5	35.2	35.2	34.5	34.5	33.7
ICG 2742	33.9	33.5	32.9	32.2	31.7	31.5	33.5	33.5	32.9	32.4	31.3
ICG 5067	38.4	38.2	38.0	37.8	37.6	36.5	38.2	38.0	37.6	37.6	36.3
ICG 2484	34.8	34.5	33.8	33.1	32.2	31.7	34.5	34.0	33.3	33.1	31.3
ICGS 76	29.2	28.9	28.5	28.0	27.8	27.6	28.9	28.9	28.5	28.2	28.2
Mean	34.3	34.0	33.6	33.1	32.7	32.1	34.0	33.9	33.3	33.1	32.1
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	34.7	33.7	33.6	33.2	33.0	32.5	33.7	33.4	32.9	32.9	32.5
ICG 4342	32.2	31.9	31.7	31.5	30.9	30.3	31.7	31.6	31.5	30.9	30.1
ICG 4236	34.5	34.3	34.0	33.8	33.6	33.0	34.5	34.3	34.1	33.4	33.0
ICG 4479	33.6	33.2	33.0	32.5	31.8	31.4	33.5	33.3	32.8	31.8	31.2
ICG 156	30.8	30.8	30.6	30.5	30.0	29.8	30.5	30.4	30.2	29.9	29.9
Mean	33.1	32.7	32.5	32.3	31.8	31.4	32.7	32.6	32.3	31.7	31.3
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	39.6	39.0	38.0	37.6	37.4	37.0	39.0	38.4	38.0	37.2	37.0
ICG 10035	41.2	40.6	39.1	38.5	37.2	36.0	40.6	39.1	38.1	37.9	36.0
ICG 3041	41.6	41.1	40.0	39.5	38.1	37.0	41.0	40.0	39.5	38.4	37.2
ICG 10766	40.0	39.6	39.2	38.5	37.9	37.6	39.8	39.2	38.6	37.8	37.5
ICG 2738	38.3	38.3	37.7	37.0	36.5	36.0	38.2	37.8	37.2	36.8	36.1
Mean	40.1	39.7	38.7	38.2	37.4	36.7	39.7	38.9	38.2	37.6	36.7
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	36.5	36.0	35.8	35.0	34.9	34.9	36.0	35.4	34.9	34.8	34.8
ICG 2959	39.1	38.4	38.1	37.6	37.4	36.5	38.5	38.0	37.5	37.2	36.8
ICG 2988	38.0	37.5	37.0	36.5	36.5	36.0	37.3	36.9	36.0	36.0	36.0
ICG 3209	38.2	37.2	37.2	36.9	35.9	35.0	37.7	37.2	37.2	36.0	35.0
ICGS 44	41.7	41.4	40.7	40.0	39.7	39.7	41.5	40.7	40.1	39.8	39.8
Mean	38.7	38.1	37.7	37.2	36.8	36.4	38.1	37.6	37.1	36.7	36.4

S.E. (S) ±0.048, (G) ±0.061, (M) ±0.030; CV (%) 1.5

S=Storage, G=Genotype, M=Material

Table 21. Linoleic acid content (%) of seeds of cultivated genotypes of groundnut following storage as seeds or pods for different durations under medium-term condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	35.4	35.4	35.4	35.3	35.3	35.2	35.4	35.4	35.3	35.3	35.2
ICG 2742	33.9	33.8	33.8	33.8	33.8	33.7	33.8	33.8	33.8	33.8	33.7
ICG 5067	38.4	38.2	38.2	38.0	38.0	38.0	38.4	38.4	38.2	38.2	38.2
ICG 2484	34.8	34.7	34.7	34.7	34.5	34.5	34.6	34.6	34.6	34.5	34.5
ICGS 76	29.2	29.0	29.0	28.9	28.9	28.9	29.1	29.1	29.1	29.0	29.0
Mean	34.3	34.2	34.2	34.1	34.1	34.0	34.2	34.2	34.2	34.1	34.1
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	34.7	34.5	34.5	34.5	34.4	34.4	34.6	34.6	34.6	34.6	34.5
ICG 4342	32.2	32.1	32.1	32.1	32.1	32.0	32.2	32.2	32.2	32.0	32.0
ICG 4236	34.5	34.4	34.4	34.4	34.3	34.2	34.4	34.4	34.4	34.3	34.3
ICG 4479	33.6	33.6	33.6	33.6	33.4	33.4	33.6	33.5	33.5	33.3	33.3
ICG 156	30.8	30.6	30.6	30.6	30.5	30.5	30.6	30.6	30.6	30.5	30.5
Mean	33.1	33.0	33.0	33.0	32.9	32.9	33.0	33.0	33.0	32.7	32.7
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	39.6	39.5	39.5	39.5	39.4	39.4	39.5	39.5	39.5	39.4	39.4
ICG 10035	41.2	40.8	40.5	40.0	39.8	39.0	40.8	40.5	39.8	39.8	39.0
ICG 3041	41.6	41.4	41.4	41.1	41.1	41.1	41.6	41.6	41.4	41.0	41.0
ICG 10766	40.0	39.8	39.8	39.8	39.7	39.6	40.0	40.0	40.0	39.8	39.8
ICG 2738	38.3	38.2	38.2	38.2	38.1	38.0	38.2	38.2	38.2	38.1	38.0
Mean	40.1	39.9	39.8	39.7	39.6	39.4	40.0	39.9	39.7	39.6	39.4
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	36.5	36.4	36.4	36.4	36.3	36.2	36.4	36.4	36.4	36.3	36.2
ICG 2959	39.1	38.8	38.8	38.5	38.5	38.5	38.8	38.8	38.5	38.5	38.5
ICG 2988	38.0	37.8	37.8	37.8	37.6	37.6	37.8	37.8	37.8	37.6	37.6
ICG 3209	38.2	38.0	38.0	37.7	37.7	37.7	38.0	38.0	37.7	37.7	37.7
ICGS 44	41.7	41.7	41.7	41.7	41.6	41.5	41.6	41.6	41.6	41.5	41.4
Mean	38.7	38.5	38.5	38.4	38.3	38.3	38.5	38.5	38.4	38.3	38.2

S.E. (S) ±0.053, (G) ±0.106, (M) ±0.033; CV (%) 1.6

S=Storage, G=Genotype, M=Material

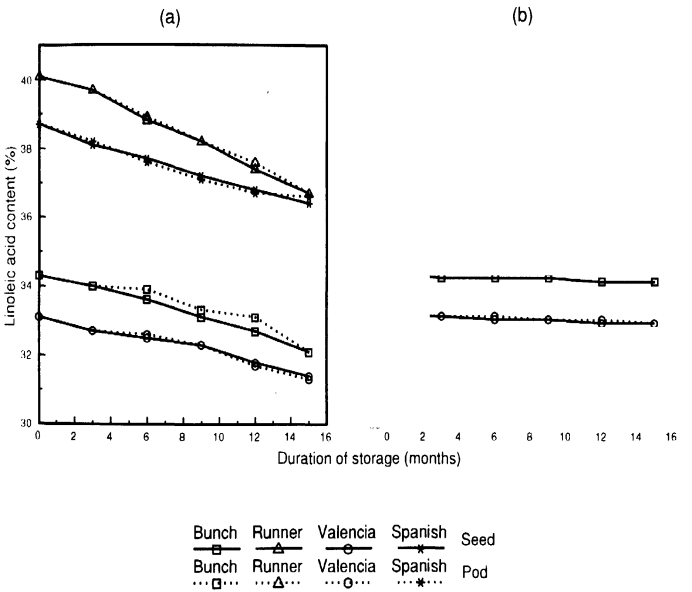

Figure 7. Changes in linoleic acid content of seeds of genotypes of 4 cultivar groups (mean) of groundnut following storage as seeds or pods under (a) ambient and (b) medium-term conditions.

Protein content:

A decrease in the protein content of the seeds was observed in almost all the genotypes stored under ambient conditions for 15 months as shown in Table 22. A progressive decline in the protein content in relation to time was observed and the rate of decline was conspicuously more during the latter period of storage (6-15 months) than the earlier period of storage as seen in Fig. 9. The decline in protein content was almost similar in all the genotypes belonging to different groups except in the genotypes ICG 10035, ICG 3041, and ICG 3209 where the reduction was comparatively more. There were no significant differences between the small-seeded or large-seeded genotypes and stored kernels and pods as regards the extent of decline in protein content. When the seeds were stored under medium-term condition decline of protein content was not observed in any of the genotypes except the genotype ICG 10035 as seen in Table 23 and Fig. 7.

Total soluble sugar content:

An increase in the total soluble sugar content of the seeds was observed in almost all the genotypes stored under ambient conditions for 15 months as shown in Table 24. A progressive increase was observed in the total soluble sugar content as the storage period increased, although the increase was more conspicuous during the period 9-15 months of storage (Fig. 9). The extent of the increase in total soluble sugar content was more among the genotypes belonging to Valencia and Spanish groups compared to the Virginia bunch and Virginia runner genotypes. As regards increase in the soluble sugar content, there was no significant differences between stored kernels or pods as well as between the small- or large-seeded genotypes. The seeds stored under medium-term conditions showed no significant increase in the total soluble sugar content as shown in Table 25 and Fig. 8 except in the genotype ICG 10035, where an increase of 1.1% was observed.

Table 22. Protein content (%) of seeds of cultivated genotypes of groundnut following storage as seeds or pods for different durations under ambient condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	24.7	24.7	24.5	24.0	23.8	23.7	24.6	24.6	24.0	23.8	23.7
ICG 2742	22.4	22.4	22.2	22.0	21.8	21.5	22.4	22.1	22.0	21.8	21.4
ICG 5067	20.2	20.2	20.0	19.8	19.7	19.5	20.2	20.0	19.8	19.7	19.5
ICG 2484	20.8	20.7	20.7	20.5	20.1	20.3	20.8	20.7	20.5	20.3	20.0
ICGS 76	23.7	23.8	23.5	23.2	23.0	23.0	23.7	23.5	23.3	23.0	23.0
Mean	22.3	22.3	22.1	21.9	21.7	21.5	22.4	22.1	21.9	21.7	21.5
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	21.5	21.5	21.2	21.0	20.8	20.8	21.5	21.3	21.1	20.8	20.7
ICG 4342	21.1	21.0	21.0	20.5	20.3	20.3	21.0	21.0	20.6	20.3	20.3
ICG 4236	21.8	21.8	21.5	21.5	21.4	21.0	21.8	21.7	21.3	21.0	21.0
ICG 4479	21.9	21.9	21.7	21.5	21.3	20.9	21.8	21.7	21.5	21.3	20.9
ICG 156	20.6	20.6	20.4	20.2	20.0	19.5	20.6	20.5	20.0	20.2	19.8
Mean	21.3	21.3	21.1	20.9	20.7	20.5	21.3	21.2	20.9	20.6	20.5
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	26.8	26.7	26.7	26.5	26.4	25.8	26.7	26.7	26.5	26.1	25.8
ICG 10035	24.9	24.7	24.6	24.6	24.2	23.2	24.7	24.6	24.5	24.0	23.2
ICG 3041	24.7	24.6	24.5	24.3	24.2	23.5	24.6	24.3	24.3	24.2	23.5
ICG 10766	27.8	27.8	27.6	27.5	27.4	26.8	27.7	27.7	27.5	27.2	26.8
ICG 2738	28.6	28.6	28.3	28.2	28.1	27.8	28.6	28.4	28.2	28.1	27.8
Mean	26.5	26.5	26.3	26.2	26.0	25.4	26.4	26.3	26.2	25.9	25.4
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	23.8	23.8	23.6	23.5	23.1	23.0	23.8	23.6	23.4	23.2	23.0
ICG 2959	25.9	25.8	25.7	25.6	25.2	25.0	25.9	25.7	25.7	25.3	25.0
ICG 2988	23.4	23.4	23.2	23.0	22.7	22.5	23.3	23.2	23.0	22.7	22.5
ICG 3209	26.5	26.5	26.2	26.0	25.5	25.3	26.5	26.2	26.0	25.7	25.3
ICGS 44	24.5	24.4	24.3	24.2	23.6	23.6	24.4	24.2	24.0	23.7	23.6
Mean	24.8	24.8	24.6	24.4	24.0	23.8	24.7	24.5	24.4	24.1	23.8

S.E. (S) ±0.064, (G) ±0.128, (M) ±0.040; CV (%) 3.0

S=Storage, G=Genotype, M=Material

Table 23. Protein content (%) of seeds of cultivated genotypes of groundnut following storage as seeds or pods for different durations under medium-term condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	24.7	24.5	24.8	25.0	24.7	24.7	24.5	24.6	24.7	24.7	24.6
ICG 2742	22.4	22.5	22.4	22.2	22.4	22.5	22.5	22.5	22.4	22.3	22.5
ICG 5067	20.2	20.3	20.0	20.4	20.3	20.1	20.2	20.0	20.2	20.1	20.1
ICG 2484	20.8	21.0	20.8	20.6	20.8	20.8	20.8	21.0	20.9	20.6	20.9
ICGS 76	23.7	23.6	23.8	23.5	23.7	23.6	23.5	23.8	23.6	23.7	23.6
Mean	22.3	22.3	22.3	22.3	22.3	22.3	22.3	22.3	22.3	22.2	22.3
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	21.5	21.6	21.6	21.5	21.4	21.4	21.6	21.5	21.4	21.4	21.4
ICG 4342	21.1	21.2	21.2	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0
ICG 4236	21.8	21.7	21.9	22.0	21.7	21.7	21.9	21.7	21.7	21.7	21.7
ICG 4479	21.9	22.0	21.7	22.0	21.7	22.0	22.0	22.0	21.7	21.7	22.0
ICG 156	20.6	20.5	20.5	20.3	20.8	20.5	20.4	20.5	20.4	20.4	20.5
Mean	21.3	21.4	21.3	21.3	21.3	21.3	21.3	21.3	21.2	21.2	21.3
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	26.8	26.6	26.7	26.6	26.7	26.7	26.7	26.9	26.7	26.7	26.7
ICG 10035	24.9	24.7	24.7	24.6	24.5	24.5	24.8	24.7	24.7	24.5	24.5
ICG 3041	24.7	24.8	24.6	24.6	24.6	24.7	24.6	24.8	24.6	24.5	24.6
ICG 10766	27.8	27.7	27.6	27.6	27.7	27.6	27.6	27.6	27.5	27.6	27.7
ICG 2738	28.6	28.5	28.4	28.4	28.4	28.5	28.6	28.6	28.7	28.5	28.6
Mean	26.5	26.6	26.4	26.4	26.3	26.3	26.4	26.4	26.4	26.3	26.3
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	23.8	24.0	23.7	24.0	23.9	24.0	24.0	23.7	23.8	24.0	23.9
ICG 2959	25.9	25.8	26.0	25.9	25.7	25.7	26.0	25.8	25.7	25.8	25.7
ICG 2988	23.4	23.5	23.3	23.2	23.5	23.5	23.5	23.5	23.4	23.5	23.5
ICG 3209	26.5	26.4	26.6	26.7	26.4	26.4	26.4	26.6	26.6	26.7	26.6
ICGS 44	24.5	24.6	24.6	24.4	24.4	24.7	24.5	24.4	24.4	24.5	24.4
Mean	24.8	24.8	24.7	24.8	24.7	24.8	24.8	24.7	24.7	24.8	24.8

S.E. (S) ±0.042, (G) ±0.084, (M) ±0.026; CV (%) 2.0

S=Storage, G=Genotype, M=Material

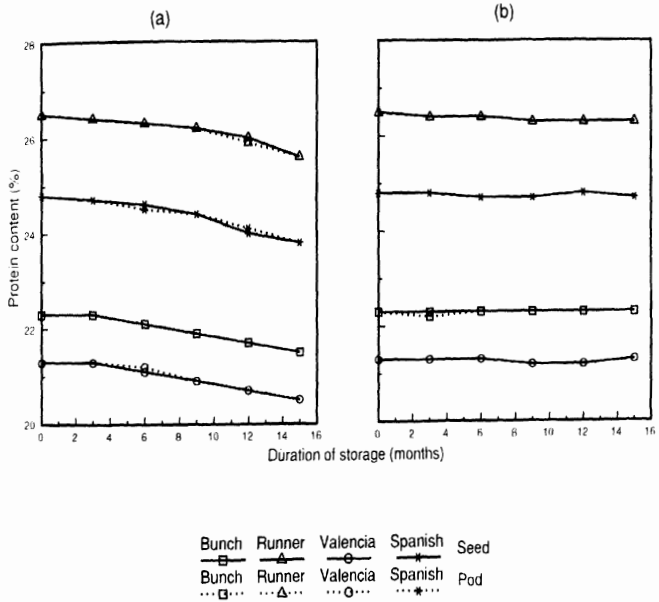

Figure 8. Changes in protein content of seeds of genotypes of 4 cultivar groups (mean) of groundnut following storage of seeds or pods under (a) ambient and (b) medium-term conditions.

Table 24. Total soluble sugar content (%) of seeds of cultivated genotypes of groundnut following storage as seeds or pods for different durations under ambient condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	14.5	14.5	14.8	14.8	15.5	15.8	14.6	14.7	14.9	15.6	15.8
ICG 2742	15.4	15.6	15.7	16.0	16.7	17.4	15.6	15.7	16.0	16.7	17.4
ICG 5067	14.2	14.3	14.4	14.7	15.0	15.9	14.3	14.5	14.7	15.4	15.8
ICG 2484	15.7	16.0	16.0	16.2	17.0	17.5	16.0	16.0	16.5	17.0	17.5
ICGS 76	14.4	14.5	14.7	14.9	15.0	16.0	14.5	14.7	14.9	15.1	16.0
Mean	14.8	14.9	15.1	15.3	15.8	16.5	15.0	15.1	15.4	15.9	16.6
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	15.4	15.6	15.6	16.1	16.4	16.7	15.7	15.7	16.0	16.4	16.7
ICG 4342	15.7	15.9	15.9	16.0	16.7	17.5	15.9	16.0	16.4	17.0	17.5
ICG 4236	15.9	16.1	16.2	16.2	17.1	17.7	16.0	16.2	16.4	16.9	17.5
ICG 4479	16.6	16.7	16.8	17.0	18.1	18.3	16.7	16.9	17.1	17.9	18.2
ICG 156	15.6	15.8	15.8	16.2	16.8	17.0	15.8	16.0	16.2	16.6	17.0
Mean	15.8	16.0	16.0	16.3	17.0	17.4	16.0	16.1	16.4	16.9	17.2
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	11.8	12.1	12.2	12.6	13.0	13.4	11.9	12.3	12.5	12.9	13.4
ICG 10035	12.1	12.4	12.8	13.1	15.9	16.8	12.4	12.9	13.9	14.6	16.8
ICG 3041	11.7	12.0	12.4	12.6	13.2	14.5	11.9	12.3	12.5	13.1	14.5
ICG 10766	11.6	11.7	12.1	12.5	13.0	13.4	11.9	12.0	12.4	12.7	13.4
ICG 2738	11.8	11.9	12.0	12.4	13.0	13.5	11.9	12.1	12.5	13.2	13.5
Mean	11.8	12.0	12.3	12.6	13.6	14.3	11.8	12.1	12.3	12.9	13.5
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	11.0	11.4	11.5	11.8	12.3	12.9	11.3	11.6	11.9	12.5	12.9
ICG 2959	10.5	10.7	11.0	11.2	11.7	12.9	10.7	10.9	11.1	12.6	12.2
ICG 2988	11.8	12.0	12.4	12.5	13.1	13.8	12.0	12.2	12.5	13.3	13.9
ICG 3209	11.7	12.0	12.1	12.5	13.2	14.0	11.9	12.2	12.4	13.0	13.9
ICGS 44	13.3	13.3	13.5	13.7	14.5	14.8	13.5	13.5	13.7	14.2	14.6
Mean	11.6	11.8	12.1	12.3	12.9	13.5	11.8	12.0	12.3	13.1	13.5

S.E. (S) ±0.048, (G) ±0.097, (M) ±0.030; CV (%) 3.7

S=Storage, G=Genotype, M=Material

Table 25. Total soluble sugar content (%) of seeds of cultivated genotypes of groundnut following storage as seeds or pods for different duration under medium-term condition.

Cultivar group/ genotype	Seeds						Pods				
	Storage duration (months)						Storage duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia bunch)											
ICG 4906	14.5	14.5	14.5	14.6	14.6	14.5	14.5	14.6	14.7	14.6	14.5
ICG 2742	15.4	15.4	15.5	15.7	15.7	15.5	15.5	15.4	15.6	15.6	15.5
ICG 5067	14.2	14.2	14.2	14.3	14.3	14.3	14.2	14.2	14.3	14.3	14.3
ICG 2484	15.7	15.7	15.7	15.8	15.7	15.7	15.7	15.7	15.8	15.7	15.7
ICGS 76	14.4	14.4	14.4	14.5	14.5	14.4	14.4	14.5	14.5	14.5	14.4
Mean	14.8	14.8	14.8	14.9	14.9	14.8	14.8	14.8	14.9	14.9	14.8
Arachis hypogaea ssp. *hypogaea* var. *hypogaea* (Virginia runner)											
ICG 4344	15.4	15.5	15.6	15.6	15.7	15.7	15.4	15.4	15.5	15.6	15.6
ICG 4342	15.7	15.7	15.6	15.7	15.8	15.8	15.7	15.8	15.7	15.8	15.9
ICG 4236	15.9	15.9	16.0	15.9	15.9	15.9	15.9	15.9	15.9	15.9	15.9
ICG 4479	16.6	16.6	16.6	16.6	16.7	16.7	16.6	16.7	16.8	16.7	16.6
ICG 156	15.6	15.7	15.6	15.7	15.8	15.6	15.6	15.7	15.6	15.7	15.8
Mean	15.8	15.8	15.9	15.9	15.9	15.9	15.8	15.9	15.9	15.9	15.9
Arachis hypogaea ssp. *fastigiata* var. *fastigiata* (Valencia)											
ICG 10063	11 8	11.8	11.8	11.9	11.9	11.9	11.8	11.8	11.9	11.9	11.9
ICG 10035	12.1	12.2	12.6	13.0	13.0	13.2	12.4	12.8	12.9	13.0	13.3
ICG 3041	11.7	11.8	11.8	11.8	11.9	11.9	11.7	11.8	11.8	11.8	11.9
ICG 10766	11.6	11.6	11.7	11.7	11.7	11.7	11.6	11.6	11.7	11.7	11.7
ICG 2738	11.8	11.8	11.8	11.8	11.9	11.9	11.8	11.8	11.8	11.9	11.9
Mean	11.8	11.8	11.9	12.0	12.0	12.1	11.8	11.9	12.0	12.0	12.1
Arachis hypogaea ssp. *fastigiata* var. *vulgaris* (Spanish)											
ICG 2387	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.1	11.0	11.0	11.0
ICG 2959	10.5	10.5	10.5	10.5	10.6	10.7	10.5	10.5	10.6	10.5	10.5
ICG 2988	11.8	11.9	11.8	11.8	11.8	11.9	11.9	11.8	11.8	12.0	11.9
ICG 3209	11.7	11.8	11.7	11.9	11.7	11.7	11.8	11.9	11.7	11.7	11.8
ICGS 44	13.3	13.3	13.4	13.5	13.4	13.4	13.3	13.3	13.3	13.4	13.4
Mean	11.6	11.6	11.6	11.7	11.7	11.7	11.7	11.7	11.6	11.7	11.7
S.E.	(S) ±0.049, (G) ±0.098, (M) ±0.031; CV (%) 3.9										

S=Storage, G=Genotype, M=Material

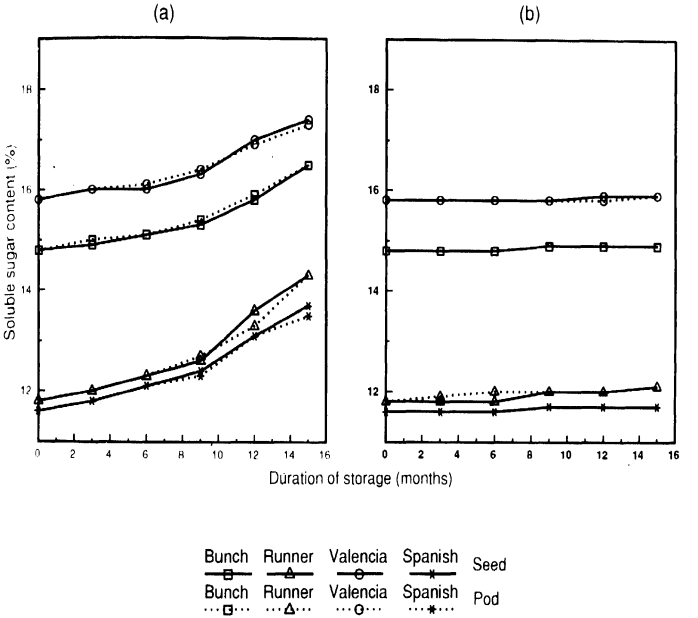

Figure 9. Changes in soluble sugar content of seeds of genotypes of 4 cultivar groups (mean) of groundnut (mean) following storage as seeds or pods under (a) ambient and (b) medium-term conditions.

4.1.2 Changes in groundnut genotypes following storage under different conditions viz., ambient, medium-term, short-term and long-term

In order to have further assessment of the process of groundnut seed ageing during germplasm conservation, a set of experiments were conducted with 4 storage conditions namely (i) ambient (22-38°C, 44-80% RH), (ii) short-term (18°C, 30% RH), (iii) medium-term (4°C, 20% RH) and (iv) long-term (-20°C). The last three storage conditions represent the procedure prevailing in the genebanks (IBPGR, 1976). From each cultivar group, the standard cultivated genotypes which were considered as check in experiment 4.1 were included for this experiment. These were ICGS 76, ICG 156, ICG 2738 and ICGS 44. Following storage, measurements were taken on (1) seed viability (2) seedling vigor, (3) electrolyte leakage, (4) oil, protein, and total soluble sugar contents, (5) fatty acid composition, (6) lipase and peroxidase activities, (7) acid and peroxide values, and (8) fatty acid composition of different lipid fractions including phospholipid and glycolipid contents.

Both kernel and pods were kept under 4 storage conditions for 15 months and different measurements were taken at 3 month intervals; however analyses 6 to 8 (as mentioned above) were done only with the stored seeds and for analyses 7 and 8, the data were recorded only twice i.e. initially before storage and at the completion of storage.

Seed viability:

There was a decline in the viability of seeds stored under ambient, short-term and medium-term conditions, while there was no loss of viability in the seeds stored under long-term storage condition as observed from Table 26. The extent of loss and the rate of decline in the viability of seeds stored under ambient condition was similar to that observed earlier, and the loss of seed viability was much higher when compared to any other storage conditions. It was observed that when the seeds were stored under short-term condition

there was considerable decline in the loss of viability as compared to medium-term condition, but such loss of seed viability was observed to be significantly less as compared to the seeds stored under ambient condition. Such decline was linear as could be observed from Fig. 10. A comparative view on the loss of viability of groundnut seeds under four conditions of storage could be seen in Fig. 10. Genotypes belonging to Valencia and Spanish groups lost comparatively more viability than the genotypes belonging to Virginia bunch and Virginia runner groups under both short-term and medium-term storage. It was observed that the rate of loss in viability was similar for seeds and pods while the extent of loss did not differ between seeds and pods particularly under medium-term and long-term conditions.

Seedling vigor:

When the seeds stored under ambient, short-term and medium-term conditions were germinated, as evident from the measurements of shoot, root and hypocotyl length and dry weight, a decline in the seedling vigor was observed (Tables 27, 28, 29, and 30). There was no loss of seedling vigor in the seeds stored under long-term storage condition. The extent of loss and the rate of decline in the seedling vigor of the seeds stored under ambient and medium-term conditions could be seen in Fig. 11, where it is observable that the loss in seedling vigor was much higher when stored under ambient condition as compared to storage under medium-term condition. It was observed that when the seeds were stored under short-term condition, there was a sharp decline in the shoot, hypocotyl and root length and dry weight as compared to the seedlings obtained from seeds stored under medium-term condition as shown in Fig. 11. However, the seedling vigor was considerably low when compared to seedlings grown from seeds stored under ambient condition and the loss of seedling vigor was linear in relation to the time of storage (Fig. 11). The loss in seedling vigor was more evident from the

reduction in hypocotyl and shoot length as compared to reduction in root length. This was also evident from the decline in dry weight of seedlings from Table 30. In this respect, there were no significant differences between the groundnuts stored as kernels or pods, or between the different genotypes when the seeds were stored under short-term storage condition.

Electrolyte leakage:

There was distinct increase in the electrolyte leakage when the seeds were stored under ambient condition, although such leakage was much less in the seeds stored under three other conditions of storage observable from Table 31. The electrolyte leakage from seeds stored under medium-term and long-term conditions showed no significant changes. Under ambient condition, the extent and the rate of electrolyte leakage from the seeds was observed to be much more during the period 9-15 months of storage, as compared to the amount of leakage recorded during the earlier period i.e. between 3-6 months as could be seen in Fig. 12. The electrolyte leakage of the seeds stored under short-term was significantly lower than that of the seeds stored under ambient condition. As regards electrolyte leakage there was no significant differences between the stored kernels and pods and between the genotypes.

Table 26. Effect of different storage conditions on the viability* (%) of four cultivated genotypes of groundnut stored as seeds or pods for different durations.

Storage condition	Genotypes	Seeds						Pods				
		Initial	Storage duration (months)					Storage duration (months)				
			3	6	9	12	15	3	6	9	12	15
Long-term	ICGS 76	100.0	100.0	99.3	100.0	99.3	100.0	100.0	99.3	100.0	99.3	100.0
	ICG 156	97.3	97.3	96.7	97.3	96.7	97.3	97.3	96.7	97.3	96.7	97.3
	ICG 2738	100.0	100.0	100.0	99.3	100.0	100.0	100.0	100.0	99.3	100.0	100.0
	ICGS 44	98.7	98.7	98.7	98.0	98.0	98.7	98.7	98.7	98.0	98.0	98.7
	Mean	99.0	99.0	98.7	98.7	98.5	99.0	99.0	98.7	98.7	98.5	99.0
Medium-term	ICGS 76	100.0	99.3	98.7	98.7	98.0	98.0	99.3	98.7	98.7	98.0	98.0
	ICG 156	97.3	97.3	96.0	96.7	95.3	95.3	97.3	96.7	96.7	95.3	95.3
	ICG 2738	100.0	99.3	98.7	98.0	98.0	97.3	99.3	98.7	98.0	98.0	97.3
	ICGS 44	98.7	98.0	98.0	97.3	96.7	96.0	98.0	97.3	97.3	96.7	96.0
	Mean	99.0	98.5	98.0	97.7	97.0	96.7	98.5	97.8	97.7	97.0	96.7
Short-term	ICGS 76	100.0	98.0	98.0	96.7	94.7	94.0	98.7	98.7	96.7	94.7	94.0
	ICG 156	97.3	96.0	95.3	94.0	92.7	92.7	96.7	96.0	95.3	93.3	92.7
	ICG 2738	100.0	98.7	96.0	95.3	93.3	91.3	98.7	96.0	95.3	93.3	91.3
	ICGS 44	98.7	97.3	96.0	94.7	92.7	90.7	97.3	96.7	95.3	93.3	90.7
	Mean	99.0	97.5	96.3	95.1	93.3	92.1	97.8	96.8	95.7	93.7	92.1
Ambient	ICGS 76	100.0	97.3	92.7	86.7	70.7	60.0	98.7	94.7	92.7	88.0	64.0
	ICG 156	97.3	96.7	93.7	88.7	74.0	61.3	97.3	96.0	95.3	80.7	68.0
	ICG 2738	100.0	94.0	92.7	88.0	72.7	55.7	97.3	94.0	92.7	74.7	58.3
	ICGS 44	98.7	94.0	92.7	90.7	67.3	55.3	96.0	92.7	91.3	73.3	56.0
	Mean	99.0	95.5	93.0	88.5	71.7	58.0	97.3	94.3	93.0	79.1	60.8
S.E.	(S) ±0.284, (T) ±0.254, (M) ±0.179, (G) ±0.254; CV (%) 3.0											

S=Storage, T=Temperature, M=Material, G=Genotype; * determined by germination test

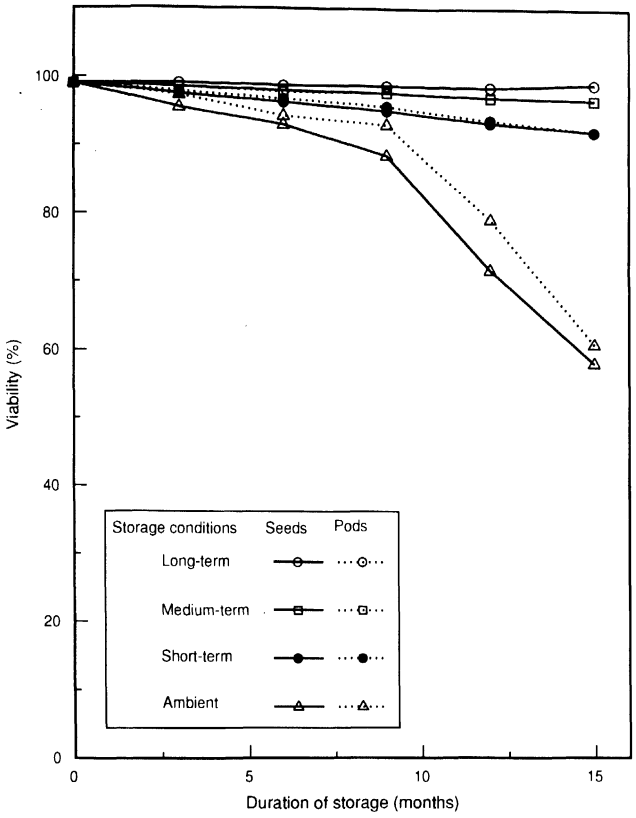

Figure 10. Mean seed viability of genotypes of 4 cultivar groups of groundnut following storage under different conditions

(Note. The extent of loss was almost same irrespective of storage of seed or pod in respect to storage under short-, medium- and long-term conditions and hence the overlap)

Table 27. Shoot length (cm) of seedlings of four cultivated genotypes of groundnut following storage under different conditions and durations as seeds and pods.

Storage condition	Genotypes	Seeds						Pods				
		Initial	Storage duration (months)					Storage duration (months)				
			3	6	9	12	15	3	6	9	12	15
Long-term	ICGS 76	5.6	5.6	5.5	5.5	5.6	5.5	5.6	5.5	5.5	5.6	5.5
	ICG 156	4.4	4.4	4.3	4.3	4.3	4.3	4.4	4.3	4.3	4.3	4.3
	ICG 2738	5.4	5.4	5.4	5.3	5.3	5.3	5.4	5.4	5.3	5.3	5.3
	ICGS 44	5.9	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.7	5.8	5.7
	Mean	5.3	5.3	5.2	5.2	5.2	5.2	5.3	5.2	5.2	5.2	5.2
Medium-term	ICGS 76	5.6	5.5	5.4	5.4	5.3	5.3	5.5	5.5	5.5	5.4	5.4
	ICG 156	4.4	4.4	4.2	4.2	4.0	4.0	4.4	4.4	4.2	4.2	4.2
	ICG 2738	5.4	5.4	5.3	5.3	5.2	5.2	5.4	5.3	5.3	5.2	5.2
	ICGS 44	5.9	5.8	5.7	5.7	5.6	5.6	5.9	5.8	5.7	5.6	5.6
	Mean	5.3	5.2	5.1	5.1	5.0	5.0	5.3	5.2	5.2	5.1	5.1
Short-term	ICGS 76	5.6	5.4	5.4	5.0	4.8	4.8	5.3	5.3	5.0	4.9	4.9
	ICG 156	4.4	4.2	4.2	4.0	4.0	3.9	4.3	4.2	4.1	4.0	3.9
	ICG 2738	5.4	5.3	5.1	5.1	4.9	4.9	5.4	5.3	5.1	5.0	4.9
	ICGS 44	5.9	5.8	5.6	5.4	5.4	5.3	5.8	5.7	5.5	5.4	5.2
	Mean	5.3	5.1	5.0	4.8	4.7	4.7	5.2	5.1	4.9	4.8	4.7
Ambient	ICGS 76	5.6	5.5	5.3	4.9	4.5	3.9	5.4	5.0	4.8	4.4	3.8
	ICG 156	4.4	4.2	4.0	3.8	3.5	2.8	4.3	4.0	3.7	3.0	2.9
	ICG 2738	5.4	5.2	4.9	4.7	4.2	3.9	5.1	5.0	4.7	4.4	4.0
	ICGS 44	5.9	5.8	5.6	5.2	4.9	3.5	5.7	5.2	4.9	4.5	3.6
	Mean	5.3	5.1	4.9	4.6	4.2	3.5	5.1	4.8	4.5	4.0	3.5
S.E.	(S) ±0.066, (T) ±0.059, (M) ±0.042, (G) ±0.059; CV (%) 13.2											

S=Storage, T=Temperature, M=Material, G=Genotype

Table 28. Hypocotyl length (cm) of seedlings of four cultivated genotypes of groundnut following storage under different conditions and durations as seeds and pods.

Storage condition	Genotypes	Seeds						Pods				
			Storage duration (months)					Storage duration (months)				
		Initial	3	6	9	12	15	3	6	9	12	15
Long-term	ICGS 76	3.2	3.2	3.1	3.1	3.2	3.2	3.2	3.1	3.1	3.2	3.2
	ICG 156	3.2	3.2	3.1	3.1	3.2	3.1	3.2	3.1	3.1	3.2	3.1
	ICG 2738	4.9	4.9	4.9	4.8	4.9	4.8	4.9	4.9	4.8	4.9	4.8
	ICGS 44	3.0	3.0	3.0	2.9	3.0	3.0	3.0	3.0	2.9	3.0	3.0
	Mean	3.5	3.5	3.5	3.4	3.5	3.5	3.5	3.5	3.4	3.5	3.5
Medium-term	ICGS 76	3.2	3.2	3.2	3.1	3.1	3.0	3.2	3.2	3.1	3.1	3.0
	ICG 156	3.2	3.2	3.1	3.1	3.0	3.0	3.2	3.0	3.0	3.0	2.9
	ICG 2738	4.9	4.8	4.8	4.7	4.7	4.7	4.9	4.8	4.8	4.7	4.7
	ICGS 44	3.0	3.0	2.9	2.8	2.8	2.7	3.0	2.9	2.9	2.8	2.7
	Mean	3.5	3.5	3.5	3.4	3.4	3.3	3.5	3.4	3.4	3.4	3.3
Short-term	ICGS 76	3.2	3.0	3.0	2.7	2.5	2.5	3.1	3.0	2.8	2.5	2.5
	ICG 156	3.2	3.1	3.1	3.0	2.9	2.7	3.0	3.0	2.9	2.7	2.7
	ICG 2738	4.9	4.7	4.7	4.5	4.5	4.5	4.9	4.7	4.6	4.5	4.5
	ICGS 44	3.0	2.9	2.9	2.7	2.7	2.5	3.0	2.9	2.6	2.6	2.5
	Mean	3.5	3.4	3.4	3.2	3.1	3.0	3.5	3.4	3.2	3.1	3.0
Ambient	ICGS 76	3.2	3.0	2.8	2.6	2.4	1.9	3.2	3.0	2.8	2.5	2.0
	ICG 156	3.2	3.0	2.7	2.6	2.4	1.9	3.0	2.7	2.5	2.3	1.9
	ICG 2738	4.9	4.9	4.5	3.7	3.5	3.2	4.8	4.3	4.0	3.7	3.4
	ICGS 44	3.0	2.9	2.5	2.3	2.1	2.0	2.9	2.7	2.2	2.0	1.9
	Mean	3.5	3.4	3.1	2.8	2.7	2.2	3.4	3.1	2.8	2.6	2.3
S.E.	(S) ±0.032,	(T) ±0.028,		(M) ±0.020,			(G) ±0.028;		CV (%)			9.6

S=Storage, T=Temperature, M=Material, G=Genotype

Table 29. Root length (cm) of seedlings of four cultivated genotypes of groundnut following storage under different conditions and durations as seeds and pods.

Storage condition	Genotypes	Seeds						Pods					
		Initial	Storage duration (months)					Storage duration (months)					
			3	6	9	12	15	3	6	9	12	15	
Long-term	ICGS 76	18.2	18.2	18.0	18.0	18.1	18.2	18.2	18.0	18.0	18.1	18.2	
	ICG 156	17.6	17.6	17.4	17.5	17.4	17.5	17.6	17.4	17.5	17.4	17.5	
	ICG 2738	18.2	18.2	18.0	18.0	18.1	18.2	18.2	18.0	18.0	18.1	18.2	
	ICGS 44	17.4	17.4	17.1	17.3	17.2	17.2	17.4	17.1	17.3	17.2	17.2	
	Mean	17.3	17.3	17.2	17.1	17.3	17.2	17.3	17.2	17.1	17.3	17.2	
Medium-term	ICGS 76	16.4	16.4	16.2	16.1	16.0	15.9	16.4	16.3	16.2	16.0	16.0	
	ICG 156	17.6	17.5	17.4	17.2	17.0	16.8	17.5	17.3	17.2	17.0	16.8	
	ICG 2738	18.2	18.0	18.0	17.8	17.7	17.7	18.1	18.0	17.8	17.7	17.7	
	ICGS 44	17.4	17.3	17.3	17.2	17.0	16.8	17.2	17.1	16.9	16.8	16.7	
	Mean	17.3	17.3	17.2	17.0	16.9	16.8	17.2	17.1	17.0	16.8	16.7	
Short-term	ICGS 76	16.4	16.2	16.0	15.5	15.0	14.8	16.2	16.0	15.6	15.2	15.0	
	ICG 156	17.6	17.4	17.0	16.5	16.2	16.0	17.3	17.0	16.7	16.4	16.0	
	ICG 2738	18.2	18.0	17.7	17.4	17.0	16.3	18.0	17.7	17.7	17.0	16.7	
	ICGS 44	17.4	17.0	16.8	16.4	16.0	15.8	17.1	16.8	16.4	16.2	15.8	
	Mean	17.3	17.2	16.9	16.5	16.1	15.9	17.2	16.9	16.6	16.3	16.0	
Ambient	ICGS 76	16.4	16.0	15.5	15.1	13.5	11.7	15.7	14.9	14.6	13.8	11.9	
	ICG 156	17.6	17.2	16.5	16.0	14.2	12.1	17.3	16.4	15.6	14.8	13.2	
	ICG 2738	18.2	17.6	17.0	16.5	14.8	12.5	17.6	17.0	16.7	15.5	12.4	
	ICGS 44	17.4	16.5	16.0	15.6	13.8	12.0	17.1	16.5	15.3	13.9	12.4	
	Mean	17.3	16.8	16.2	15.8	14.0	12.0	16.9	16.2	15.5	14.5	12.5	

S.E. (S) ±0.063, (T) ±0.056, (M) ±0.040, (G) ±0.056; CV (%) 3.8

S=Storage, T=Temperature, M=Material, G=Genotype

Table 30. Dry weight (g) of seedlings of four cultivated genotypes of groundnut following storage under different conditions and durations as seeds and pods.

Storage condition	Genotypes	Seeds						Pods					
			Storage duration (months)					Storage duration (months					
		Initial	3	6	9	12	15	3	6	9	12	15	
Long-term	ICGS 76	2.52	2.51	2.50	2.50	2.51	2.51	2.51	2.50	2.50	2.51	2.51	
	ICG 156	3.44	3.43	3.43	3.42	3.42	3.43	3.43	3.40	3.42	3.42	3.43	
	ICG 2738	2.25	2.25	2.24	2.24	2.23	2.23	2.25	2.24	2.24	2.23	2.23	
	ICGS 44	3.03	3.02	3.01	3.01	3.01	3.01	3.02	3.01	3.01	3.01	3.01	
	Mean	2.80	2.80	2.78	2.79	2.79	2.80	2.80	2.78	2.79	2.79	2.80	
Medium-term	ICGS 76	2.52	2.51	2.50	2.48	2.47	2.46	2.52	2.50	2.48	2.47	2 47	
	ICG 156	3.44	3.42	3.42	3.40	3.39	3.37	3.42	3.41	3.40	3.39	3.37	
	ICG 2738	2.25	2.25	2.24	2.23	2.23	2.20	2.25	2.24	2.22	2.22	2.20	
	ICGS 44	3.03	3.02	3.01	2.99	2.98	2.96	3.03	3.01	3.00	2.98	2.97	
	Mean	2.80	2.80	2.79	2.77	2.76	2.74	2.80	2.79	2.77	2.76	2.75	
Short-term	ICGS 76	2.52	2.47	2.45	2.31	2.31	2.27	2.50	2.41	2.40	2.32	2.28	
	ICG 156	3.44	3.40	3.21	3.20	3.17	3.13	3.37	3.30	3.20	3.18	3.15	
	ICG 2738	2.25	2.22	2.22	2.13	2.11	2.05	2.22	2.20	2.15	2.10	2.02	
	ICGS 44	3.03	2.97	2.90	2.76	2.61	2.61	2.92	2.90	2.82	2.72	2.61	
	Mean	2.80	2.76	2.69	2.60	2.55	2.51	2.75	2.70	2.64	2.59	2.54	
Ambient	ICGS 76	2.52	2.42	2.20	2.01	1.81	1.65	2.36	2.12	2.01	1.97	1.74	
	ICG 156	3.44	3.30	3.11	3.01	2.77	2.41	3.38	3.26	3.01	2.70	2.49	
	ICG 2738	2.25	2.12	2.01	1.81	1.40	1.02	2.10	2.01	1.81	1.55	1.10	
	ICGS 44	3.03	2.70	2.56	2.22	1.99	1.88	2.76	2.52	2.32	1.98	1.91	
	Mean	2.80	2.62	2.46	2.26	1.98	1.74	2.65	2.47	2.28	2.06	1.83	
S.E.	(S) ±0.036,	(T) ±0.032,		(M) ±0.022,			(G) ±0.032;			CV (%) 13.5			

S=Storage, T=Temperature, M=Material, G=Genotype

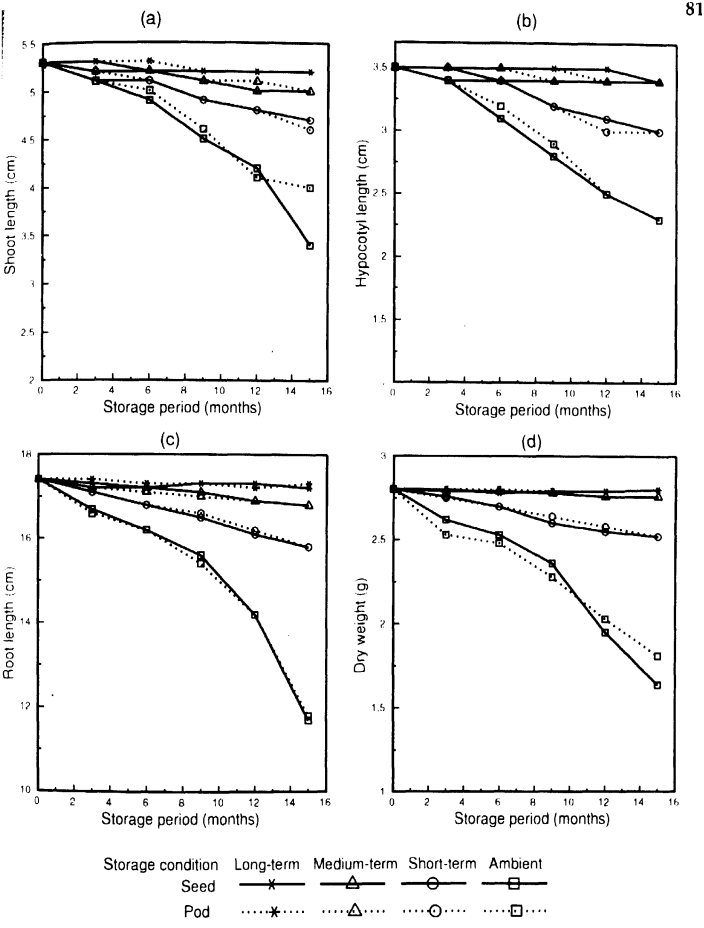

Figure 11. Seedling vigor [mean (a) shoot length (b) hypocotyl length (c) root length and (d) dry weight of seedlings] of genotypes of 4 cultivar groups of groundnut following storage as seed or pod under different conditions.

Table 31. Electrolyte leakage (mmho/cm) from the seeds of four cultivated genotypes of groundnut following storage under different conditions and durations as seeds and pods.

Storage condition	Genotypes	Seeds Initial	Seeds Storage duration (months) 3	6	9	12	15	Pods Storage duration (months) 3	6	9	12	15
Long-term	ICGS 76	0.144	0.143	0.144	0.143	0.144	0.145	0.143	0.144	0.143	0.144	0.145
	ICG 156	0.180	0.180	0.179	0.180	0.181	0.182	0.180	0.179	0.180	0.181	0.182
	ICG 2738	0.097	0.097	0.099	0.101	0.098	0.101	0.097	0.099	0.101	0.098	0.101
	ICGS 44	0.144	0.143	0.144	0.143	0.145	0.146	0.143	0.144	0.143	0.145	0.146
	Mean	0.147	0.147	0.148	0.148	0.148	0.149	0.147	0.148	0.148	0.148	0.149
Medium-term	ICGS 76	0.144	0.147	0.149	0.150	0.154	0.160	0.145	0.149	0.151	0.156	0.156
	ICG 156	0.180	0.181	0.182	0.185	0.186	0.189	0.181	0.181	0.183	0.185	0.188
	ICG 2738	0.097	0.103	0.105	0.106	0.113	0.117	0.099	0.103	0.109	0.109	0.111
	ICGS 44	0.168	0.170	0.172	0.175	0.177	0.180	0.170	0.170	0.172	0.176	0.178
	Mean	0.147	0.150	0.152	0.161	0.168	0.176	0.148	0.149	0.153	0.156	0.158
Short-term	ICGS 76	0.144	0.147	0.157	0.170	0.175	0.199	0.150	0.150	0.162	0.176	0.194
	ICG 156	0.180	0.187	0.193	0.207	0.210	0.237	0.187	0.187	0.205	0.207	0.227
	ICG 2738	0.097	0.099	0.110	0.120	0.126	0.157	0.097	0.102	0.117	0.127	0.145
	ICGS 44	0.168	0.171	0.180	0.195	0.209	0.231	0.171	0.175	0.188	0.197	0.208
	Mean	0.147	0.151	0.160	0.172	0.180	0.206	0.151	0.153	0.168	0.176	0.193
Ambient	ICGS 76	0.144	0.162	0.187	0.203	0.452	0.592	0.169	0.182	0.243	0.396	0.562
	ICG 156	0.180	0.201	0.250	0.294	0.463	0.501	0.205	0.246	0.270	0.487	0.494
	ICG 2738	0.097	0.116	0.138	0.134	0.272	0.279	0.111	0.140	0.150	0.210	0.270
	ICGS 44	0.168	0.206	0.229	0.299	0.522	0.647	0.209	0.236	0.294	0.495	0.627
	Mean	0.147	0.171	0.201	0.237	0.427	0.504	0.173	0.201	0.229	0.397	0.488

S.E. (S) ±0.0026, (T) ±0.0024, (M) ±0.0017, (G) ±0.0024; CV (%) 13.6

S=Storage, T=Temperature, M=Material, G=Genotype

Oil content:

It was observed that the seeds stored under ambient condition showed a decline in the oil content, while there was no such loss in the seeds stored under short-term, medium-term or long-term condition which could be seen in Table 32. The oil content of the seeds stored under ambient condition showed a slow decline both in extent and rate during the earlier period of storage which became much rapid during 9-12 months of storage (Fig. 12). No genotypic difference was observed with respect to decline in the oil content, irrespective of the storage condition.

Fatty acid composition:

Analysis of the fatty acid composition showed that there was a significant decline in the linoleic acid content of the seeds stored under ambient condition, while the decrease was less under short-term and medium-term conditions observed from Table 33. No change was observed in the linoleic acid content of seeds stored under long-term condition. The extent of decline in the linoleic acid content of the seeds stored under ambient condition was similar to that observed earlier i.e. there was a gradual linear decline. The decline in the linoleic acid content of the seeds stored under short-term condition was more than the seeds stored under medium-term condition observed from Fig. 12. There were no significant differences between stored kernels or pods or between genotypes with respect to decrease in linoleic acid content.

Protein content:

The seeds stored under ambient condition showed a significant decline in the protein content as shown in Table 34 and the rate of decline was linear as seen in Fig. 13. There was no significant change in the protein content of the seeds stored under short-term, medium-term and long-term condition.

There was neither any observable genotypic differences, or any difference between stored kernel and pod, as regards the protein content.

Total soluble sugars:

There was a significant increase in the total soluble sugar content of the seeds stored under ambient condition, while a small increase was seen in the seeds stored under short-term condition as shown in Table 35. The seeds stored under medium-term and long-term conditions showed little change in the soluble sugar content. As regards the rate of increase in the total soluble sugar content of the seeds stored under ambient condition, the increase was observed to be slower in the earlier period of storage but more pronounced during 9-15 months of storage as could be seen in Fig. 13. The increase in total soluble sugar content in the seeds stored under short-term condition was significantly less as compared to the seeds stored under ambient condition, while it was more than in the seeds stored under medium-term or long-term condition. There were no significant differences between kernels or pods or between the genotypes in relation to changes in the total soluble sugar content.

Table 32. Oil content (%) of seeds of four cultivated genotypes of groundnut following storage under different conditions and durations as seeds and pods.

Storage condition	Genotypes	Seeds						Pods				
		Storage duration (months)						Storage duration (months)				
		Initial	3	6	9	12	15	3	6	9	12	15
Long-term	ICGS 76	45.1	44.9	45.0	44.9	45.1	45.1	44.9	45.0	44.9	45.2	45.2
	ICG 156	46.4	46.2	46.4	46.2	46.4	46.4	46.2	46.4	46.2	46.4	46.4
	ICG 2738	43.0	42.9	43.0	42.9	42.9	43.0	42.9	43.0	42.9	42.9	43.0
	ICGS 44	47.0	46.9	47.0	46.9	46.9	47.0	46.9	47.0	46.9	46.9	47.0
	Mean	45.2	45.2	45.3	45.2	45.3	45.3	45.2	45.3	45.2	45.3	45.3
Medium-term	ICGS 76	45.1	45.0	45.0	45.0	45.0	45.0	45.1	45.1	45.0	44.9	44.9
	ICG 156	46.4	46.3	46.2	46.3	46.2	46.3	46.3	46.4	46.2	46.2	46.3
	ICG 2738	43.0	42.9	43.0	43.0	43.0	43.0	43.0	43.0	43.0	43.0	43.0
	ICGS 44	47.0	47.0	46.8	46.8	46.8	47.0	46.8	46.8	47.0	46.9	47.0
	Mean	45.2	45.3	45.2	45.2	45.2	45.3	45.3	45.3	45.3	45.2	45.3
Short-term	ICGS 76	45.1	45.0	45.1	45.0	44.9	44.7	45.0	45.0	44.9	45.0	44.7
	ICG 156	46.4	46.3	46.4	46.3	46.2	46.1	46.3	46.3	46.3	46.2	46.1
	ICG 2738	43.0	42.9	43.0	42.9	42.8	42.6	42.9	42.8	42.8	42.8	42.6
	ICGS 44	47.0	46.9	46.9	46.8	46.8	46.7	46.9	46.9	46.9	46.8	46.7
	Mean	45.2	45.2	45.3	45.2	45.1	45.0	45.2	45.2	45.2	45.2	45.0
Ambient	ICGS 76	45.1	45.0	44.7	44.2	43.7	43.5	45.0	44.7	44.1	43.8	43.5
	ICG 156	46.4	46.1	46.1	45.7	45.3	44.8	46.2	46.0	45.7	45.5	44.9
	ICG 2738	43.0	43.0	42.5	42.3	42.0	41.6	43.0	43.0	42.7	42.5	41.5
	ICGS 44	47.0	46.7	46.5	46.0	45.5	45.1	46.5	46.0	45.8	45.5	45.2
	Mean	45.2	45.2	44.9	44.5	44.1	43.7	45.1	44.9	44.5	44.3	43.7

S.E. (S) ±0.043, (T) ±0.038, (M) ±0.027, (G) ±0.038; CV (%) 0.9

S=Storage, T=Temperature, M=Material, G=Genotype

Table 33. Linoleic acid content (%) of seeds of four cultivated genotypes of groundnut following storage under different conditions and durations as seeds and pods.

Storage condition	Genotypes	Seeds						Pods					
		Initial	Storage duration (months)					Storage duration (months)					
			3	6	9	12	15	3	6	9	12	15	
Long-term	ICGS 76	29.2	29.2	29.1	29.2	29.2	29.2	29.2	29.1	29.2	29.2	29.2	
	ICG 156	30.8	30.8	30.7	30.8	30.7	30.7	30.8	30.7	30.8	30.7	30.7	
	ICG 2738	38.3	38.3	38.2	38.3	38.2	38.2	38.3	38.2	38.3	38.2	38.2	
	ICGS 44	41.7	41.6	41.7	41.7	41.6	41.6	41.6	41.7	41.7	41.6	41.6	
	Mean	35.0	34.9	34.9	34.9	34.9	34.9	34.9	34.9	34.9	34.9	34.9	
Medium-term	ICGS 76	29.2	29.0	29.0	29.0	29.0	28.9	29.1	29.1	29.1	29.0	29.0	
	ICG 156	30.8	30.6	30.6	30.6	30.5	30.5	30.6	30.6	30.6	30.5	30.5	
	ICG 2738	38.3	38.2	38.2	38.2	38.1	38.0	38.3	38.2	38.2	38.1	38.0	
	ICGS 44	41.7	41.7	41.6	41.6	41.5	41.3	41.6	41.6	41.5	41.5	41.3	
	Mean	35.0	34.8	34.8	34.8	34.7	34.6	34.8	34.8	34.8	34.7	34.7	
Short-term	ICGS 76	29.2	29.0	29.0	28.8	28.7	28.7	29.1	29.1	28.9	28.7	28.7	
	ICG 156	30.8	30.6	30.4	30.4	30.3	30.3	30.7	30.7	30.5	30.3	30.2	
	ICG 2738	38.3	38.2	38.0	37.8	37.7	37.5	38.2	38.0	37.8	37.7	37.5	
	ICGS 44	41.7	41.5	41.4	41.2	41.0	41.0	41.6	41.5	41.4	41.3	41.0	
	Mean	35.0	34.8	34.7	34.5	34.4	34.3	34.9	34.8	34.6	34.5	34.3	
Ambient	ICGS 76	29.2	28.2	28.5	28.0	27.8	27.6	28.9	28.5	28.5	28.2	28.2	
	ICG 156	30.8	30.8	30.4	30.4	30.0	29.7	30.5	30.4	30.2	29.9	29.9	
	ICG 2738	39.3	39.3	37.7	37.0	36.5	36.0	38.2	37.6	37.0	36.8	36.1	
	ICGS 44	41.7	41.4	40.7	39.7	39.0	38.6	41.5	40.1	39.8	39.5	39.0	
	Mean	35.0	34.8	34.2	33.7	33.3	32.9	34.7	34.1	33.8	33.6	33.3	
S.E.	(S) ±0.046, (T) ±0.041, (M) ±0.029, (G) ±0.041; CV (%) 1.3												

S=Storage, T=Temperature, M=Material, G=Genotype

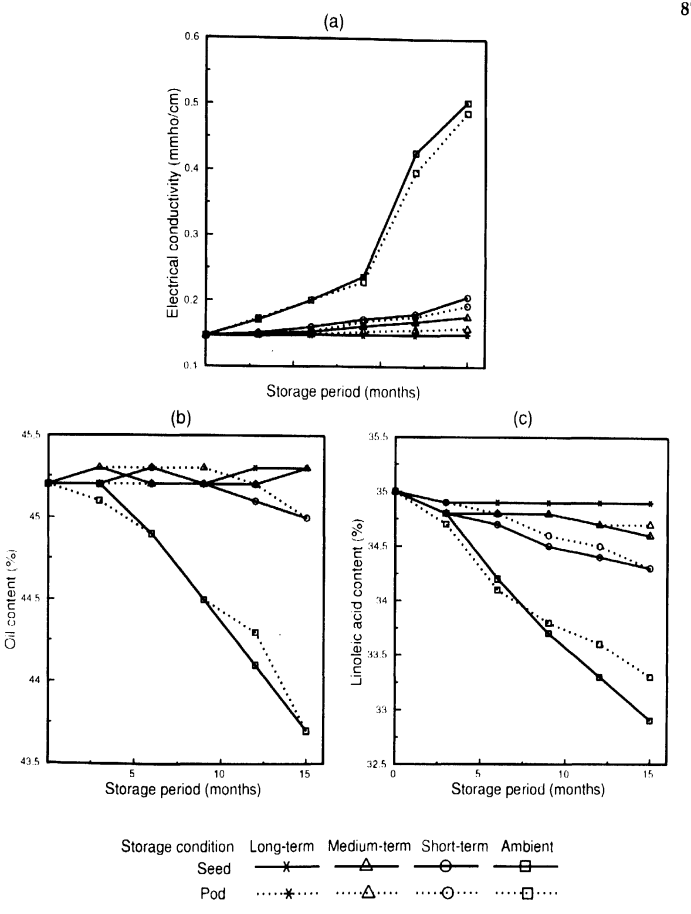

Figure 12. Extent of (a) electrolyte leakage and changes in (b) oil content and (c) linoleic acid content of seeds of genotypes belonging to 4 cultivar groups (mean) of groundnut stored as seeds or pods under different conditions.

Table 34. Protein content (%) of seeds of four cultivated genotypes of groundnut following storage under different conditions and durations as seeds and pods.

Storage condition	Genotypes	Seeds						Pods					
		Initial	Storage duration (months)					Storage duration (months)					
			3	6	9	12	15	3	6	9	12	15	
Long-term	ICGS 76	23.7	23.7	23.6	23.6	23.7	23.7	23.7	23.6	23.6	23.7	23.7	
	ICG 156	20.6	20.5	20.5	20.6	20.6	20.5	20.5	20.5	20.6	20.6	20.5	
	ICG 2738	28.6	28.5	28.5	28.5	28.6	28.6	28.5	28.5	28.5	28.6	28.6	
	ICGS 44	24.5	24.5	24.5	24.5	24.4	24.5	24.5	24.5	24.5	24.4	24.5	
	Mean	24.3	24.3	24.2	24.3	24.3	24.3	24.3	24.2	24.3	24.3	24.3	
Medium-term	ICGS 76	23.7	23.7	23.7	23.6	23.7	23.5	23.7	23.7	23.6	23.7	23.5	
	ICG 156	20.6	20.6	20.6	20.5	20.5	20.4	20.6	20.6	20.5	20.5	20.5	
	ICG 2738	28.6	28.5	28.5	28.6	28.6	28.4	28.6	28.6	28.5	28.5	28.4	
	ICGS 44	24.5	24.4	24.4	24.5	24.4	24.5	24.5	24.4	24.5	24.4	24.4	
	Mean	24.3	24.3	24.3	24.2	24.3	24.2	24.3	24.3	24.2	24.3	24.2	
Short-term	ICGS 76	23.7	23.6	23.5	23.5	23.4	23.4	23.6	23.6	23.5	23.4	23.4	
	ICG 156	20.6	20.5	20.4	20.4	20.4	20.3	20.5	20.5	20.4	20.3	23.3	
	ICG 2738	28.6	28.5	28.4	28.4	28.3	28.3	28.5	28.4	28.4	28.3	28.2	
	ICGS 44	24.5	24.4	24.4	24.3	24.2	24.2	24.5	24.5	24.4	24.3	24.2	
	Mean	24.3	24.2	24.1	24.1	24.0	24.0	24.3	24.2	24.2	24.0	23.9	
Ambient	ICGS 76	23.7	23.5	23.5	23.2	23.0	22.9	23.7	23.5	23.3	23.1	22.8	
	ICG 156	20.6	20.6	20.4	20.4	19.9	19.6	20.6	20.5	20.2	19.8	19.6	
	ICG 2738	28.6	28.4	28.3	28.2	28.0	27.8	28.4	28.4	28.2	28.0	27.7	
	ICGS 44	24.5	24.3	24.3	24.2	23.6	23.4	24.4	24.2	24.1	23.7	23.4	
	Mean	24.3	24.2	24.1	23.9	23.6	23.4	24.2	24.1	23.9	23.6	23.4	

S.E. (S) ±0.047, (T) ±0.042, (M) ±0.030, (G) ±0.042; CV (%) 1.9

S=Storage, T=Temperature, M=Material, G=Genotype

Table 35. Total soluble sugar content (%) of seeds of four cultivated genotypes of groundnut following storage under different conditions and durations as seeds and pods.

Storage condition	Genotypes	Seeds						Pods				
		Storage duration (months)						Storage duration (months)				
		Initial	3	6	9	12	15	3	6	9	12	15
Long-term	ICGS 76	14.4	14.5	14.5	14.4	14.4	14.5	14.5	14.5	14.4	14.4	14.5
	ICG 156	15.6	15.7	15.6	15.6	15.7	15.7	15.7	15.6	15.6	15.7	15.7
	ICG 2738	11.8	11.9	11.9	11.8	11.9	11.9	11.9	11.9	11.8	11.9	11.9
	ICGS 44	13.3	13.4	13.3	13.4	13.3	13.4	13.4	13.3	13.4	13.3	13.4
	Mean	13.8	13.9	13.8	13.8	13.8	13.9	13.9	13.8	13.8	13.8	13.9
Medium-term	ICGS 76	14.4	14.4	14.4	14.5	14.5	14.6	14.4	14.5	14.5	14.5	14.6
	ICG 156	15.6	15.7	15.6	51.7	15.8	15.8	15.7	15.7	15.6	15.7	15.8
	ICG 2738	11.8	11.8	11.9	12.0	12.0	12.0	11.8	11.9	11.9	12.0	12.0
	ICGS 44	13.3	13.4	13.4	13.5	13.5	13.5	13.3	13.3	13.3	13.4	13.4
	Mean	13.8	13.8	13.8	13.9	13.9	13.9	13.8	13.8	13.8	13.9	13.9
Short-term	ICGS 76	14.4	14.5	14.5	14.6	14.8	14.9	14.5	14.5	14.5	14.8	14.9
	ICG 156	15.6	15.7	15.7	15.8	15.8	16.0	15.7	15.8	15.8	15.8	16.0
	ICG 2738	11.8	12.0	12.0	12.1	12.1	12.2	12.0	12.0	12.1	12.1	12.2
	ICGS 44	13.3	13.4	13.4	13.6	13.6	13.7	13.4	13.4	13.6	13.6	13.7
	Mean	13.8	13.9	13.9	14.0	14.1	14.2	13.9	13.9	14.0	14.1	14.2
Ambient	ICGS 76	14.4	14.6	14.7	15.3	16.5	17.0	14.5	14.9	15.5	16.3	16.8
	ICG 156	15.6	15.8	16.0	16.5	17.8	18.0	15.8	16.2	16.7	17.6	17.9
	ICG 2738	11.8	12.0	12.4	12.8	13.5	13.8	11.9	12.5	12.7	13.3	13.9
	ICGS 44	13.3	13.5	13.7	13.9	14.5	14.8	13.4	13.6	14.0	14.6	14.7
	Mean	13.8	13.9	14.2	14.5	15.5	15.9	13.9	14.3	14.6	15.4	15.8
S.E.	(S) ±0.057, (T) ±0.051, (M) ±0.036, (G) ±0.051; CV (%) 4.0											

S=Storage, T=Temperature, M=Material, G=Genotype

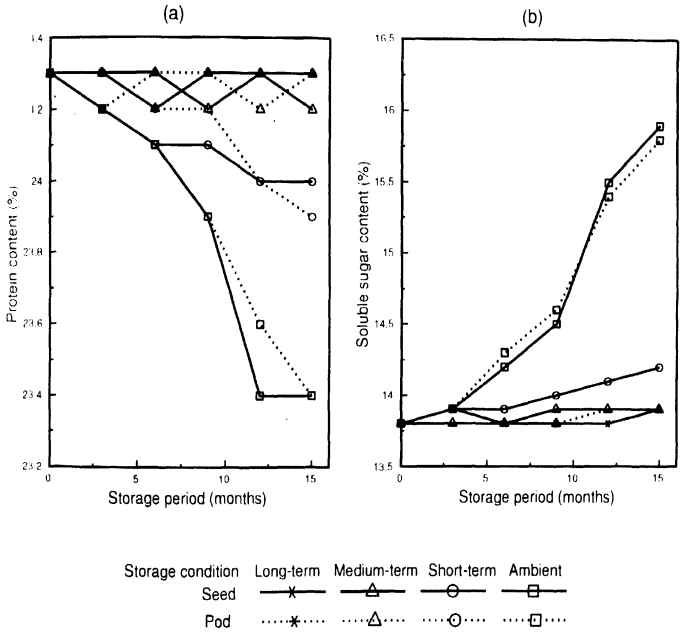

Figure 13. Changes in (a) protein content and (b) soluble sugar content of seeds of genotypes belonging to 4 cultivar groups (mean) of groundnut following storage as seeds or pods under different conditions.

Enzyme activity:

The activity of the enzymes lipase and peroxidase was analyzed in the seeds which were stored under different conditions. It was observed that there was a significant increase in the activity of enzyme lipase during storage of seeds under ambient condition as well as under short-term condition as seen in Table 36. It was also observed that the increase in this enzyme activity was significantly less in the seeds stored under short-term condition when compared to those stored under ambient condition. However, the increase in the enzyme activity noticed in the seeds stored under medium-term or long-term conditions did not differ significantly. Under ambient and short-term storage conditions the rate of increase in lipase activity in the seeds was linear as shown in Fig. 14. The activity of the enzyme peroxidase in the seeds also showed changes when stored under different conditions as could be observed from Table 36. There was almost 55% decrease in the activity of peroxidase in the seeds stored under ambient condition. The rate of decline with time was mostly uniform and linear as seen in Fig. 14. Such decline in the peroxidase activity was also seen in seeds stored under short-term condition, but the amount of reduction was much less compared to the seeds stored under ambient condition. There was no significant decline in the activity of this enzyme among the seeds stored under medium-term and long-term conditions; no significant differences were also observed between the different genotypes.

Acid and Peroxide Values:

The accumulation of free fatty acids and peroxides of fat oxidation were determined from the acid and peroxide value respectively. It was observed that in seeds stored under ambient condition both acid value and peroxide value increased as seen in Table 37 and Fig. 15. Such increase in acid and peroxide values were significantly low in the seeds stored under short-term conditions. There was no increase in these values in seeds stored under

medium-term or long-term condition. There were no genotypic differences observable with respect to free fatty acids or peroxide values.

Content and Fatty acid composition of different lipid fractions:

It was observed that there was a decline in the phospholipid and glycolipid contents of the seeds stored under ambient and short-term conditions seen in Table 38. The changes in the phospholipid and glycolipid contents were significantly less in the seeds stored under short-term condition as compared to the seeds stored under ambient condition (Fig. 16). Genotypic differences were not seen with respect to loss in phospholipid and glycolipid contents. No change was observed in the content of phospholipids and glycolipids in the seeds stored under medium-term and long-term conditions.

Changes in the fatty acid composition were examined in the neutral lipids, phospholipids and glycolipids of the seeds stored under different conditions. The initial fatty acid composition of the three lipid fractions is given in Table 39. It was observed that in the seeds stored under ambient condition, the linoleic acid content of the seeds decreased and the decline was more in phospholipids and glycolipids as compared to neutral lipids seen from Table 40. The changes in linoleic acid content were significantly less in seeds stored under short-term condition as compared to ambient condition observable from Fig. 17. Valencia and Spanish genotypes showed higher amount of such changes compared to Virginia bunch and Virginia runner genotypes. There was almost no change in the linoleic acid content of the seeds stored under medium-term and long-term conditions.

Regression analysis:

Multiple regression analysis of germination percent over various parameters- electrolyte leakage, oil and linoleic acid contents, protein and sugar contents and lipase and peroxidase activities plotted in Figs. 18 and 19

reveal the following relationships.

An inverse relationship was observed between germination percent and parameters like electrolyte leakage, sugar content and lipase activity. A direct relationship was seen between germination percent and the following parameters- oil and linoleic acid contents, protein content and peroxidase activity. High regression coefficients were obtained.

Table 36. Lipase and peroxidase activities of the seeds of cultivated genotypes of groundnut following storage under different conditions and durations.

Storage condition	Genotypes	Lipase activity (μ eq. of free fatty acid released\3 min assay) Storage duration (months) 0*	3	6	9	12	15	Peroxidase activity (max. O.D.\g fresh weight) Storage duration (months) 0*	3	6	9	12	15
Long-	ICGS 76	1.54	1.54	1.54	1.55	1.54	1.54	0.315	0.314	0.315	0.314	0.314	0.315
	ICG 156	1.53	.53	1.54	1.54	1.53	1.54	0.338	0.338	0.337	0.337	0.337	0.337
	ICG 2738	1.57	.57	1.57	1.58	1.58	1.57	0.300	0.299	0.299	0.299	0.300	0.299
	ICGS 44	.48	.48	1.49	1.48	1.48	1.49	0.302	0.302	0.302	0.301	0.301	0.302
	Mean	.53	.53	1.53	1.52	1.52	1.53	0.315	0.313	0.312	0.313	0.312	0.313
Medium-	ICGS 76	.54	.54	1.55	1.54	1.55	1.56	0.315	0.314	0.314	0.314	0.313	0.313
term	ICG 156	.53	.54	1.54	1.53	1.54	1.55	0.338	0.338	0.336	0.335	0.335	0.335
	ICG 2738	.57	1.57	1.57	1.58	1.59	1.59	0.300	0.300	0.299	0.299	0.298	0.297
	ICGS 44	.48	1.48	1.49	1.49	1.50	1.51	0.302	0.299	0.299	0.299	0.298	0.298
	Mean	.53	1.53	1.53	1.53	1.54	1.55	0.315	0.312	0.311	0.311	0.310	0.310
Short-	ICGS 76	1.54	1.55	1.57	1.59	1.66	1.70	0.315	0.310	0.302	0.297	0.291	0.284
term	ICG 156	1.53	1.56	1.59	1.61	1.63	1.70	0.338	0.321	0.315	0.310	0.309	0.303
	ICG 2738	1.57	1.58	1.62	1.65	1.69	1.78	0.300	0.292	0.283	0.275	0.271	0.266
	ICGS 44	1.48	1.51	1.52	1.60	1.68	1.72	0.302	0.291	0.283	0.279	0.271	0.265
	Mean	1.53	1.55	1.57	1.61	1.66	1.72	0.315	0.303	0.295	0.290	0.285	0.285
Ambient	ICGS 76	1.54	1.66	1.79	1.91	2.20	2.40	0.315	0.295	0.283	0.255	0.226	0.206
	ICG 156	1.53	1.64	1.76	1.90	2.24	2.44	0.338	0.316	0.283	0.240	0.235	0.199
	ICG 2738	1.57	1.59	1.69	1.81	2.11	2.41	0.300	0.275	0.266	0.230	0.188	0.201
	ICGS 44	1.48	1.62	1.75	1.86	2.20	2.39	0.302	0.275	0.258	0.227	0.180	0.211
	Mean	1.53	1.62	1.74	1.87	2.18	2.41	0.315	0.289	0.275	0.238	0.218	0.193
		(S) ±0.015, (T) ±0.014, (G)±0.014; CV (%) 6.6						(S) ±0.0022, (T) ±0.0019, (G) ±0.0019; CV (%) 5.3					

S=Storage, T=Temperature, G=Genotype; * value prior to storage

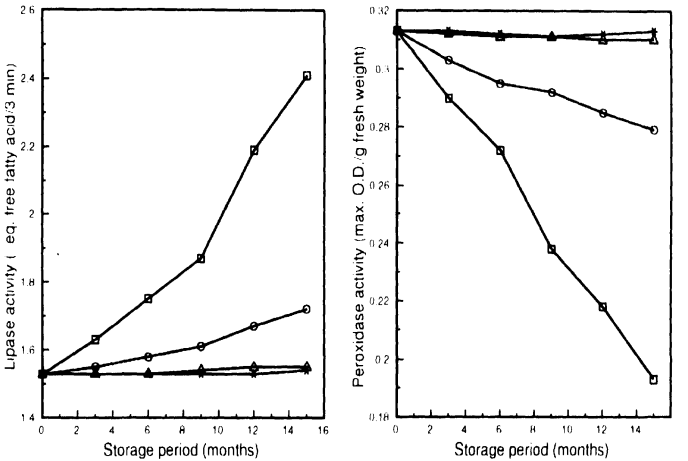

Figure 14. Enzyme (a) lipase and (b) peroxidase activities in the seeds of genotypes belonging to 4 cultivar groups (mean) of groundnut following storage under different conditions .

Table 37. Changes in acid and peroxide values of the seeds of cultivated genotypes of groundnut following storage for fifteen months under different conditions.

	Peroxide value (milliequivalents of peroxide\1000 g of sample)				Acid value (mg KOH per g of sample)			
Genotype Storage condition	ICGS 76	ICG 156	ICG 2738	ICGS 44	ICGS 76	ICG 156	ICG 2738	ICGS 44
Ambient	1.50	1.63	1.10	1.08	7.3	6.2	6.9	7.0
Short-term	0.80	0.75	0.55	0.60	3.8	3.7	3.7	3.8
Medium-term	0.65	0.61	0.41	0.42	3.0	2.8	2.9	2.9
Long-term	0.63	0.60	0.40	0.40	3.0	2.8	2.8	2.9
Initial	0.63	0.59	0.39	0.40	3.0	2.8	2.8	2.8
S.E.	(S) ±0.105, (G)±0.094, S X G ±0.211; CV (%) 1.1;				(S) ±0.012, (G) ±0.010, S X G ±0.024; CV (%) 5.3			

S=Storage, G=Genotype

Table 38. Changes in phospholipid and glycolipid contents of the seeds of cultivated genotypes of groundnut following storage for fifteen months under different conditions.

	Phospholipid content (mg/g dry weight)				Glycolipid content (mg/g dry weight)			
Genotype Storage condition	ICGS 76	ICG 156	ICG 2738	ICGS 44	ICGS 76	ICG 156	ICG 2738	ICGS 44
Ambient	1.78	1.92	1.87	1.97	1.27	1.25	1.23	1.39
Short-term	3.14	3.27	3.27	3.37	2.27	2.24	2.25	2.50
Medium-term	3.50	3.58	3.59	3.75	2.49	2.45	2.52	2.80
Long-term	3.50	3.58	3.59	3.75	2.50	2.47	2.53	2.81
Initial	3.52	3.60	3.59	3.75	2.50	2.47	2.53	2.81
S.E.	(S) ±0.076, (G)±0.068, S X G ±0.153; CV (%) 3.1;				(S) ±0.066, (G) ±0.059, S X G ±0.132; CV (%) 4.6			

S=Storage, G=Genotype

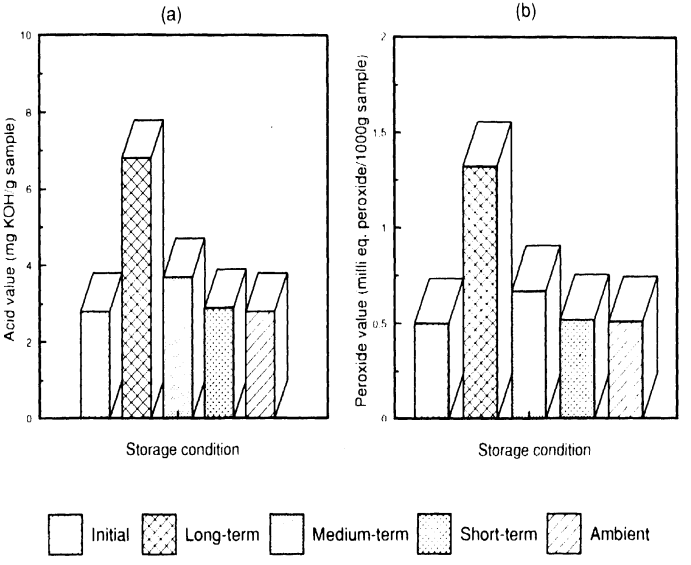

Figure 15. Differences in (a) acid and (b) peroxide values of the seeds belonging to 4 cultivar groups (mean) of groundnut following storage under different conditions.

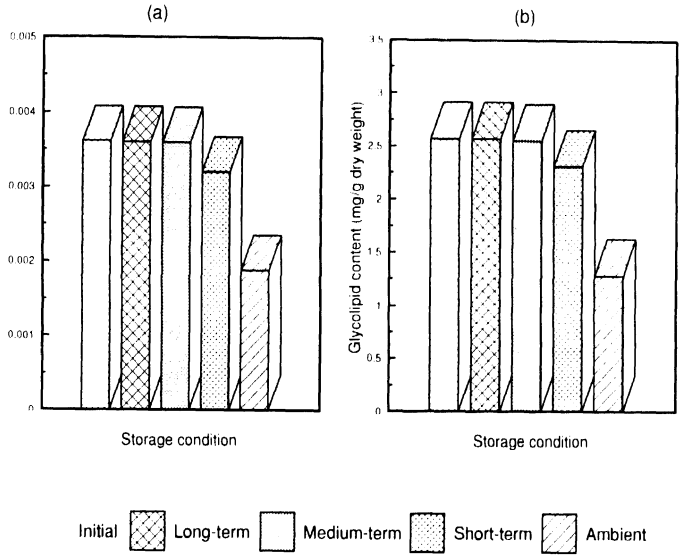

Figure 16. Changes in (a) phospholipid and (b) glycolipid contents in the seeds belonging to 4 cultivar groups (mean) of groundnut following storage under different conditions.

Table 39. Initial fatty acid composition of neutral, phospho- and glycolipids of seeds of four cultivated genotypes of groundnut.

Fatty acid/ Genotype	Palmitic	Stearic	Oleic	Linoleic	Arachidic	Eicosenoic	Behenic	Lignoceric	O/L ratio
				Neutral lipid					
ICGS 76	11.0	2.2	49.1	29.2	1.7	1.6	3.0	1.5	1.68
ICG 156	11.3	2.8	47.1	30.8	1.5	1.5	3.0	1.8	1.53
ICG 2738	12.1	2.7	38.0	38.3	1.6	1.6	3.2	1.5	0.99
ICGS 44	12.1	2.4	36.1	41.7	1.7	1.0	3.4	1.5	0.86
				Phospholipid					
ICGS 76	14.3	4.0	43.3	29.9	1.7	2.0	3.0	1.2	1.44
ICG 156	15.1	4.1	40.0	30.7	2.5	1.9	3.3	1.5	1.30
ICG 2738	15.3	4.0	35.3	35.9	2.5	1.7	3.0	1.5	0.98
ICGS 44	15.0	3.8	34.2	38.1	2.5	1.5	3.1	1.5	0.89
				Glycolipid					
ICGS 76	15.9	5.2	38.7	29.3	2.1	2.3	3.6	2.0	1.32
ICG 156	15.5	5.0	38.9	29.5	2.5	2.0	3.8	2.1	1.31
ICG 2738	13.3	4.0	37.7	36.6	2.0	1.5	3.0	1.5	1.04
ICGS 44	15.1	5.2	32.8	36.7	2.5	2.0	3.5	1.8	0.89

Table 40. Linoleic acid content (%) of the neutral lipid, phospholipid and glycolipid of the seeds of cultivated genotypes of groundnut following storage for fifteen months under different conditions.

	Neutral lipid				Phospholipid				Glycolipid			
Genotype / Storage condition	ICGS 76	ICG 156	ICG 2738	ICGS 44	ICGS 76	ICG 156	ICG 2738	ICGS 44	ICGS 76	ICG 156	ICG 2738	ICGS 44
Ambient	27.8	29.3	36.2	38.3	27.7	28.2	32.8	35.9	27.0	27.4	29.6	30.8
Short-term	28.9	30.5	37.6	40.5	29.6	30.0	35.0	37.5	28.8	29.0	35.1	35.2
Medium-term	29.1	30.7	38.2	41.5	29.7	30.5	35.5	37.8	29.0	29.3	36.0	36.2
Long-term	29.2	30.8	38.3	41.7	29.9	30.7	35.9	38.1	29.2	29.5	36.6	36.7
Initial	29.2	30.8	38.3	41.7	29.9	30.7	35.9	38.1	29.3	29.5	36.6	36.7
	S.E. (S) ±0.060, (G) ±0.054; S X G ±0.121, CV (%) 0.3;				(S) ±0.070, (G) ±0.062; S X G ±0.140, CV (%) 0.7;				(S) ±0.059, (G) ±0.053; S X G ±0.119, CV (%) 0.2;			

S=Storage, G=Genotype

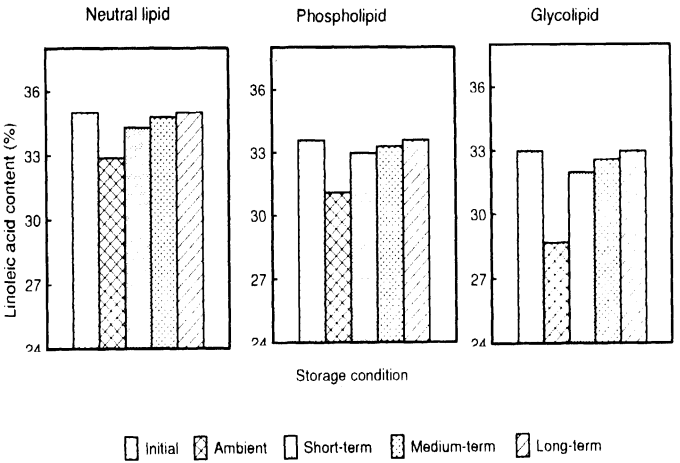

Figure 17. Changes in linoleic acid content in the neutral, phospho- and glycolipids of the seeds of genotypes belonging to 4 cultivar groups (mean) of groundnut following storage under different conditions.

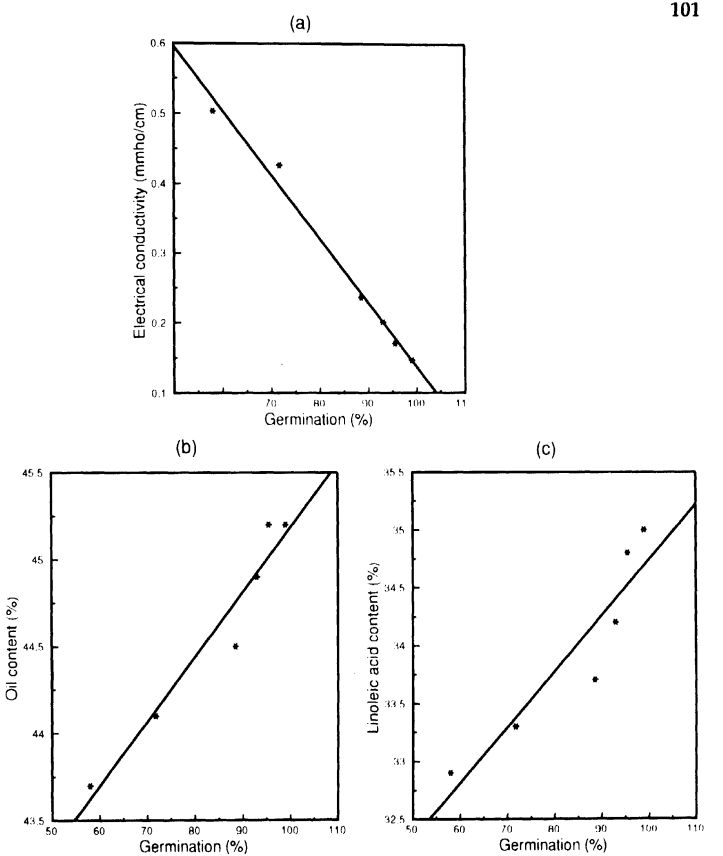

Figure 18. Relationship between germination percent and (a) electrolyte leakage (b) oil content (c) linoleic acid content of the seeds of 4 genotypes (mean) of groundnut stored under ambient condition.

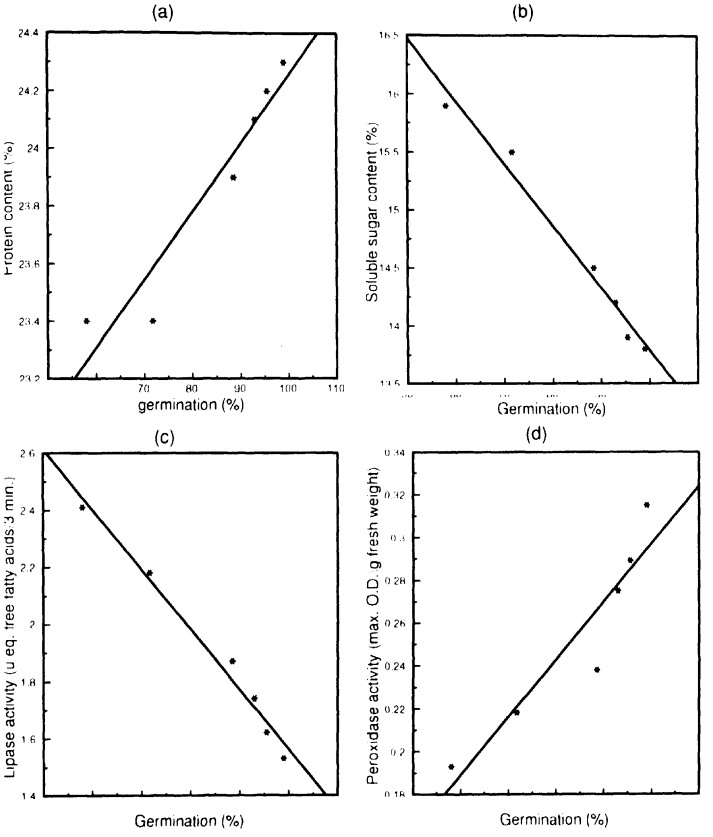

Figure 19. Relationship between germination percent and (a) protein content (b) sugar content (c) lipase activity (d) peroxidase activity of the seeds of 4 genotypes (mean) of groundnut stored under ambient condition.

4.2 Accelerated ageing on groundnut

In order to accelerate the process of ageing and simulate events expected to occur after long storage, much beyond 15 months, groundnut seeds were adjusted to 13.5% moisture content. These were stored at 40°C temperature for about 3 weeks. From preliminary experiments it was found that under this process of artificial ageing groundnut seeds completely lost their viability by 20th day. Seeds of ICGS 76, ICG 156, ICG 2738 and ICGS 44 belonging to the groups Virginia bunch, Virginia runner, Valencia and Spanish respectively were subjected to accelerated ageing. The seeds were thereafter tested for viability and vigor as well as for physiological and biochemical alterations at an interval of 4 days.

Seed viability:

It was observed that groundnut seeds rapidly lost their viability during the process of accelerated ageing, and it was confirmed that the loss of viability was complete in all the genotypes within 20 days. After 16 days of accelerated ageing, ICG 2738 belonging to Valencia type lost about 78% of viability, while ICGS 76 and ICG 156 belonging to Virginia bunch and runner types lost about 70% seed viability (Table 41). The rate of decline in viability of the genotypes ICGS 44 and ICG 2738 belonging to Spanish and Valencia was more rapid than observable in ICGS 76 and ICG 156 belonging to Virginia groups (Fig. 20).

Table 41. Seed viability* (%) of four cultivated genotypes of groundnut subjected to accelerated ageing.

Duration of accelerated ageing (days)	Genotypes			
	ICGS 76	ICG 156	ICG 2738	ICGS 44
0**	100.0	97.3	100.0	98.7
4	86.0	84.0	80.0	78.0
8	72.0	70.0	60.0	62.0
12	58.7	62.3	46.3	42.0
16	36.0	30.0	22.0	20.0
20	00.0	00.0	00.0	00.0

S.E. (S) ±1.42; (G) ±1.27; G X S ±2.84; CV (%) 7.5

S=Storage, G=Genotype; * determined by germination test; ** initial value

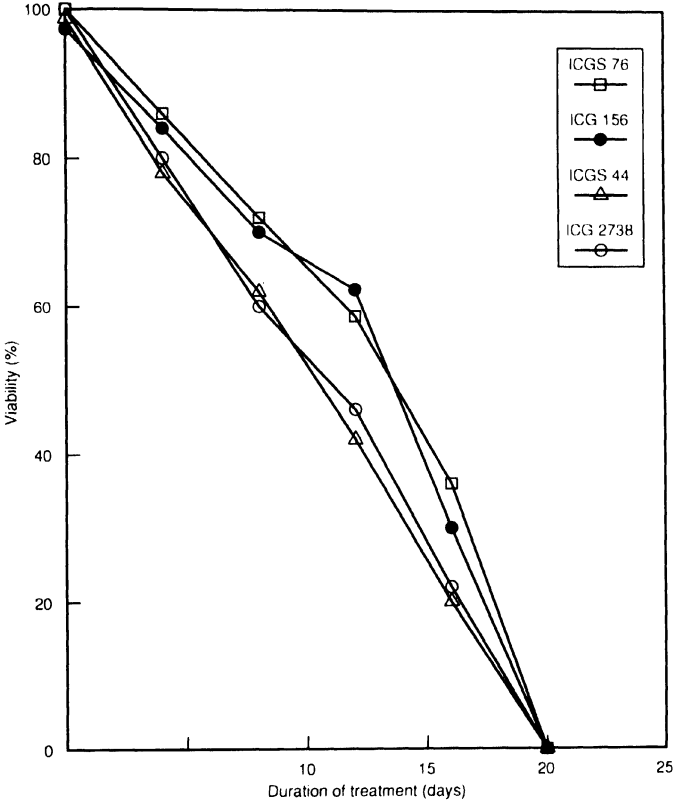

Figure 20. Decline of seed viability in relation to time due to accelerated ageing of groundnut genotypes belonging to 4 cultivar groups.

Seedling vigor:

There was considerable decline in seedling vigor as evident from reduced shoot, hypocotyl and root length of the seedlings as well as reduction in their dry weight (Table 42). As regards seedling vigor and dry weight, consistency in the genotypic differences could not be noticed. The rate of decline over the time of storage was linear as shown in Fig. 21.

Electrolyte leakage:

The seeds subjected to accelerated ageing showed a very high amount of electrolyte leakage as seen in Table 43. The rate of increase in electrolyte leakage gradually became conspicuously high during the period 8-16 days of storage (Fig. 22). Seeds of the genotypes belonging to Valencia and Spanish types showed higher amount of electrolyte leakage compared to the genotypes belonging to Virginia bunch and Virginia runner groups. The amount of electrolyte leakage was highest in the genotype ICG 156, while it was lowest in the genotype ICGS 44.

Oil content:

It was observed that the oil content of the seeds subjected to accelerated ageing rapidly declined with the period of ageing as seen in Table 44. This decline was more among the Valencia and Spanish genotypes compared to the Virginia bunch and Virginia runner genotypes. The rate of decline over the period of storage was linear as shown in Fig. 22.

Table 42. Seedling vigor (as determined from shoot length, hypocotyl length, root length and dry weight of seedlings) of different genotypes of groundnut following germination of seeds subjected to accelerated ageing.

Genotypes — DAA (days)	Shoot length (cm)				Hypocotyl length (cm)			
	ICGS 76	ICG 156	ICG 2738	ICGS 44	ICGS 76	ICG 156	ICG 2738	ICGS 44
0*	5.6	4.4	5.4	5.9	3.2	3.2	4.9	3.0
4	4.1	3.9	4.2	5.3	2.5	2.8	4.4	2.1
8	3.3	2.6	3.4	4.4	1.8	1.9	3.1	1.9
12	2.9	2.1	2.2	2.8	1.2	1.1	2.2	1.5
16	2.0	1.5	1.8	1.6	1.0	0.8	1.1	0.6

S.E. (S) ±0.15, (G) ±0.14, G X S ±0.31; (S) ±0.08, (G) ±0.07, G X S ±0.16
CV (%) 15.6 12.7

Genotypes — DAA (days)	Root length (cm)				Dry weight (g)			
	ICGS 76	ICG 156	ICG 2738	ICGS 44	ICGS 76	ICG 156	ICG 2738	ICGS 44
0*	16.4	17.6	18.2	17.4	2.52	3.44	2.25	3.03
4	12.4	13.1	16.7	15.4	2.01	3.02	1.90	2.09
8	10.8	10.2	14.2	12.6	1.87	2.56	1.65	1.95
12	6.5	7.1	8.9	8.2	1.51	1.93	1.47	1.62
16	4.4	5.0	4.2	4.0	1.01	1.57	0.87	1.07

S.E. (S) ±0.21, (G) ±0.18, G X S ±0.42; (S) ±0.09 (G) ±0.08 G X S ±0.19
CV (%) 6.6 17.2

initial value; DAA=duration of accelerated ageing

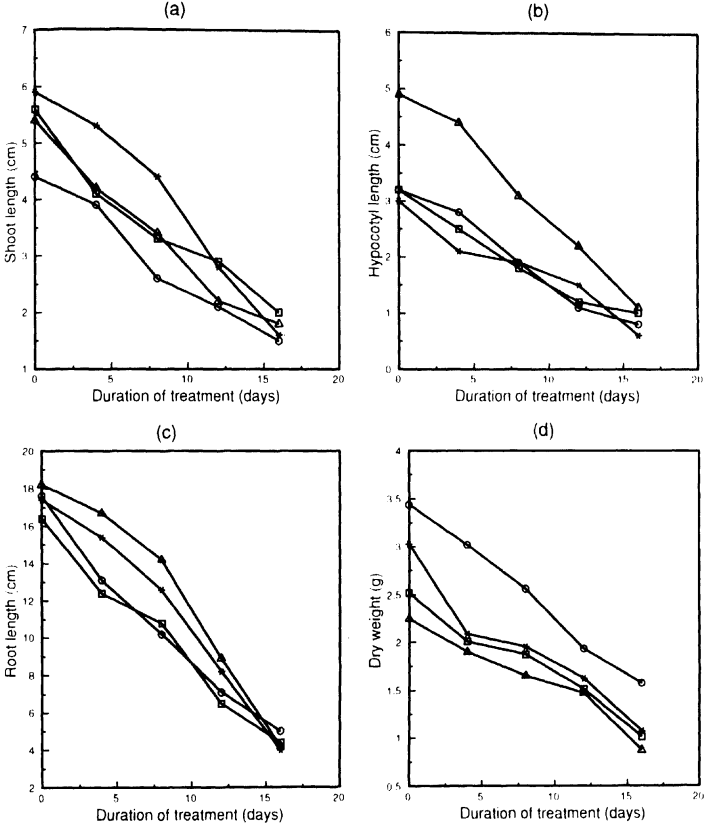

Figure 21. Seedling vigor [(a) shoot length (b) hypocotyl length (c) root length and (d) dry weight of seedlings] of groundnut genotypes subjected to accelerated ageing.

Table 43. Extent of electrolyte leakage (mmho/cm) from seeds of four cultivated genotypes of groundnut subjected to accelerated ageing.

Duration of accelerated ageing (days)	Genotypes			
	ICGS 76	ICG 156	ICG 2738	ICGS 4
0*	0.144	0.180	0.097	0.168
4	0.387	0.438	0.296	0.491
8	0.824	0.909	0.591	1.127
12	1.184	1.751	1.234	2.096
16	2.856	2.945	2.091	3.355
S.E.	(S) ±0.045	(G) ±0.040	G X S ±0.090	
CV (%)	13.1			

S= Storage, G=Genotype; * Initial value

Table 44. Changes in oil content (%) of seeds of four cultivated genotypes of groundnut following accelerated ageing.

Duration of accelerated ageing (days)	Genotypes			
	ICGS 76	ICG 156	ICG 2738	ICGS
0*	45.1	46.4	43.0	47.0
4	44.6	46.0	42.1	46.4
8	43.8	45.1	41.7	46.0
12	43.5	44.5	40.8	45.0
16	43.1	43.8	39.8	43.5
S.E.	(S) ±0.16	(G) ±0.14	G X S ±0.32	
CV (%)	1.3			

S= Storage, G=Genotype; * Initial value

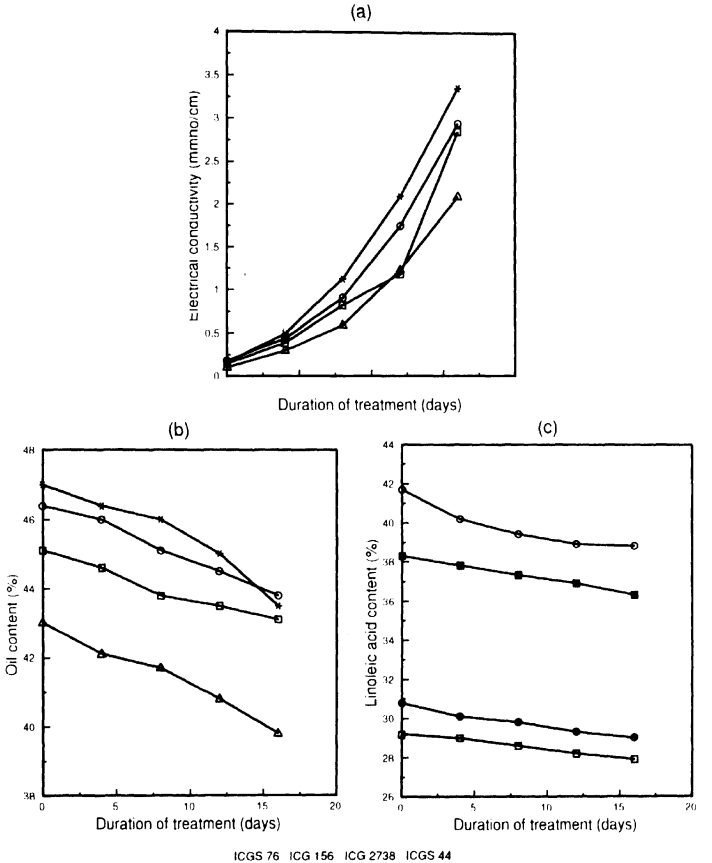

Figure 22. Extent of (a) electrolyte leakage and changes in (b) oil content and (b) linoleic acid content of the seeds of groundnut genotypes subjected to accelerated ageing.

Fatty acid composition:

Analysis of fatty acid composition showed a distinct decrease in the linoleic acid content seen in Table 45 and Fig. 22. During the entire period of accelerated ageing, the decline in the linoleic acid content was observed to be more among the genotypes belonging to Valencia and Spanish groups than the genotypes belonging to Virginia bunch and Virginia runner groups. However, the magnitude of change in linoleic acid content was much lower when compared with the changes that was observed during natural ageing of seeds under ambient condition.

Protein content:

There was a decline in the protein content of the seeds subjected to accelerated ageing as seen in Table 46. The rate of decline over the time of storage was linear observable from Fig. 23. Genotypes belonging to Valencia and Spanish types showed more reduction in the protein content compared to the genotypes belonging to Virginia bunch and Virginia runner groups.

Total soluble sugar content:

The seeds subjected to accelerated ageing showed a gradual increase in the total soluble sugar content seen in Table 47. Such increase was linear in relation to time as could be observed from Fig. 23. The increase in total soluble sugar content was more in the genotypes belonging to Valencia and Spanish types than Virginia bunch and Virginia runner genotypes.

Enzyme activities:

The activity of the enzyme lipase increased in the seeds subjected to accelerated ageing, while the activity of another enzyme peroxidase showed a decline as seen in Table 48. The rate of such changes were almost linear in relation to the time of storage. The changes in these enzyme activities were

Table 45. Changes in linoleic acid content (%) of seeds of four cultivated genotypes of groundnut following accelerated ageing.

Duration of accelerated ageing (days)	Genotypes			
	ICGS 76	ICG 156	ICG 2738	ICGS 44
0*	29.2	30.8	38.3	41.7
4	29.0	30.1	37.8	40.2
8	28.6	29.8	37.3	39.4
12	28.2	29.3	36.9	38.9
16	27.9	29.0	36.3	38.8

S.E. (S) ±0.12; (G) ±0.11; G X S ±0.25; CV (%) 1.3

S=Storage, G=Genotype; * Initial value

Table 46. Changes in protein content (%) of seeds of four cultivated genotypes of groundnut following accelerated ageing.

Duration of accelerated ageing (days)	Genotypes			
	ICGS 76	ICG 156	ICG 2738	ICGS 44
0*	23.7	20.6	28.6	24.5
4	23.3	20.1	28.2	23.9
8	23.0	19.3	27.6	23.4
12	22.7	19.1	26.1	22.8
16	21.7	18.5	25.0	21.5

S.E. (S) ±0.12 (G) ±0.11 G X S ±0.25
CV (%) 1.9

S=Storage, G=Genotype; * Initial value

Table 47. Changes in total soluble sugar content (%) of seeds of four cultivated genotypes of groundnut following accelerated ageing.

Duration of accelerated ageing (days)	Genotypes			
	ICGS 76	ICG 156	ICG 2738	ICGS 44
0*	14.4	15.6	11.8	13.3
4	14.8	16.1	12.4	14.0
8	15.5	16.5	13.6	14.4
12	16.0	16.9	14.0	15.0
16	16.4	17.5	14.2	15.7

S.E. (S) ±0.16 (G) ±0.14 G X S ±0.32
CV (%) 3.8

S=Storage, G=Genotype; *initial value

Table 48. Changes in lipase and peroxidase activities of seeds of four cultivated genotypes of groundnut following accelerated ageing.

Genotypes DAA (days)	Lipase (μ eq. of free fatty acid released/3 min assay)				Peroxidase (max. O.D.\g fresh weight)			
	ICGS 76	ICG 156	ICG 2738	ICGS 44	ICGS 76	ICG 156	ICG 2738	ICGS 44
0*	1.54	1.53	1.57	1.48	0.300	0.338	0.315	0.302
4	1.92	1.99	1.91	1.84	0.205	0.267	0.215	0.204
8	2.41	2.37	2.36	2.40	0.188	0.195	0.182	0.197
12	3.33	3.19	3.61	3.32	0.122	0.122	0.117	0.110
16	4.67	4.58	4.81	4.65	0.062	0.062	0.057	0.059

S.E. (S) ±0.12, (G) ±0.11, G X S ±0.25; (S) ±0.0062, (G) ±0.0056, G X S ±0.0125
CV (%) 16.1 5.3

S=Storage, G=Genotype; * Initial value DAA=Duration of accelerated ageing

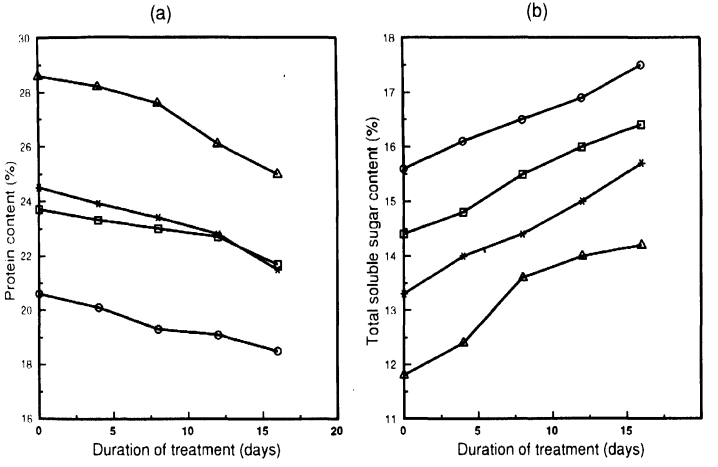

Figure 23. Changes in (a) protein content and (b) soluble sugar content of seeds of groundnut genotypes subjected to accelerated ageing.

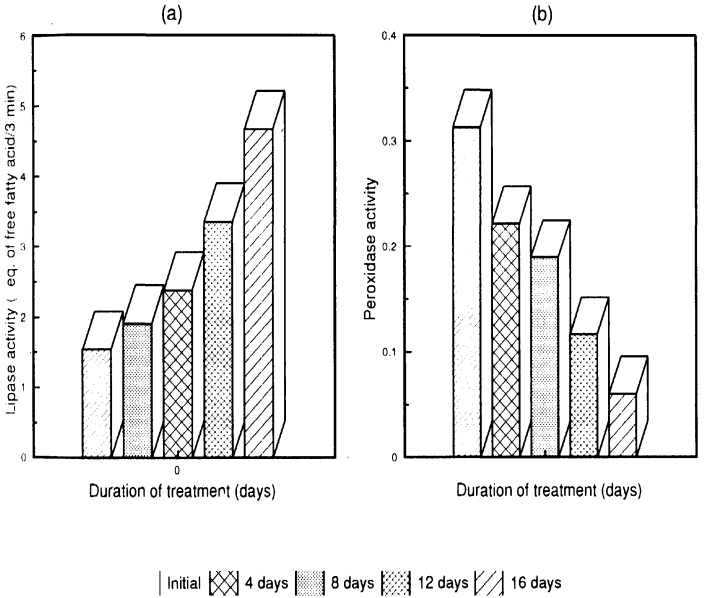

Figure 24. Changes in (a) lipase and (b) peroxidase activities in seeds of groundnut genotypes (mean) subjected to accelerated ageing.

more towards latter period of storage as could be seen in Fig. 24. No genotypic differences could be observed with regards to the changes in the enzyme activities.

Acid and Peroxide values:

It was observed that the acid value and peroxide value of the seeds increased linearly with the time of accelerated ageing seen from Table 49 and Fig. 25. Such increase was more among the genotypes belonging to Valencia and Spanish groups compared to the genotypes belonging to Virginia bunch and Virginia runner groups.

Content and Fatty acid composition of different lipid fractions:

There was considerable decline in the phospholipid and glycolipid contents of the seeds subjected to accelerated ageing as seen from Table 50 and Fig. 26. There was no differences among the genotypes in relation to changes in phospholipid and glycolipid content.

The nature of changes in the fatty acid composition were determined in the neutral lipids, phospholipids and glycolipids extracted from the seeds subjected to accelerated ageing. It was observed that in general, linoleic acid content of all these fractions decreased significantly, although such decrease was more observable in phospholipid and glycolipid fractions seen from Table 51 and Fig. 27. The decrease in the linoleic acid content was observed to be more in the genotypes belonging to Valencia and Spanish groups as compared to Virginia bunch and Virginia runner groups.

Table 49. Changes in acid and peroxide values of seeds of four cultivated genotypes of groundnut following accelerated ageing.

Genotypes DAA (days)	Peroxide value (milliequivalents of peroxide/1000 g sample)				Acid value (mg KOH per g sample)			
	ICGS 76	ICG 156	ICG 2738	ICGS 44	ICGS 76	ICG 156	ICG 2738	ICGS 44
0*	0.63	0.59	0.39	0.40	2.9	2.7	2.8	2.8
4	1.26	1.21	1.11	1.25	5.2	5.0	4.7	5.8
8	2.05	1.85	2.02	1.91	9.9	9.1	9.5	10.7
12	2.72	2.71	2.05	2.10	13.9	13.4	14.1	14.7
16	3.79	3.37	2.68	2.98	17.2	14.8	18.2	19.4
S.E.	(S) ±0.046, (G) ±0.041, G X S ±0.091;				(S) ±0.23, (G) ±0.20, G X S ±0.46			
CV (%)	7.9				8.0			

S=Storage, G=Genotype; * Initial value DAA=Duration of accelerated ageing

Table 50. Changes in phospholipid and glycolipid contents of seeds of four cultivated genotypes of groundnut following accelerated ageing.

Genotypes DAA (days)	Phospholipid (mg/g dry weight)				Glycolipid (mg/g dry weight)			
	ICGS 76	ICG 156	ICG 2738	ICGS 44	ICGS 76	ICG 156	ICG 2738	ICGS 44
0*	3.52	3.60	3.59	3.75	2.50	2.47	2.53	2.81
4	2.21	2.22	2.32	2.48	1.57	1.46	1.35	1.41
8	1.56	1.47	1.45	1.37	0.97	1.07	0.91	1.01
12	0.90	0.96	0.99	0.85	0.53	0.57	0.59	0.68
16	0.59	0.54	0.56	0.46	0.25	0.24	0.30	0.36
S.E.	(S) ±0.070, (G) ±0.063, G X S ±0.141;				(S) ±0.057, (G) ±0.051, G X S ±0.115			
CV (%)	13.8				7.4			

S=Storage, G=Genotype; * Initial value DAA=Duration of accelerated ageing

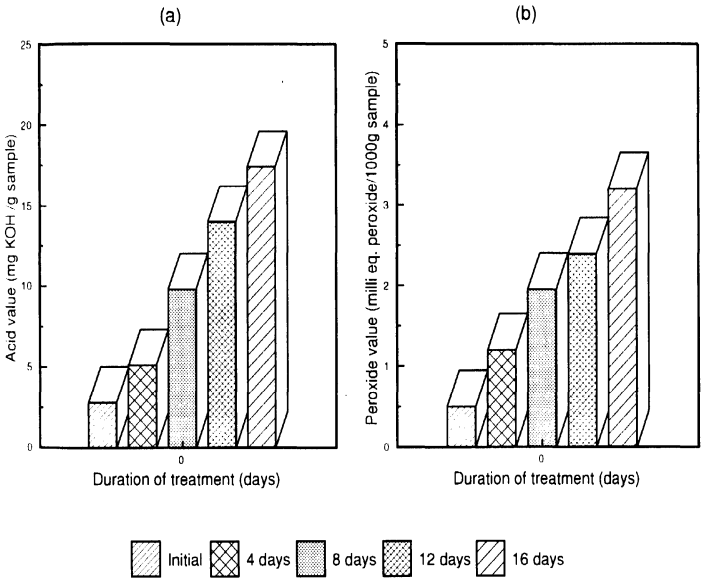

Figure 25. Changes in (a) acid and (b) peroxide values of the seeds of groundnut genotypes (mean) subjected to accelerated ageing.

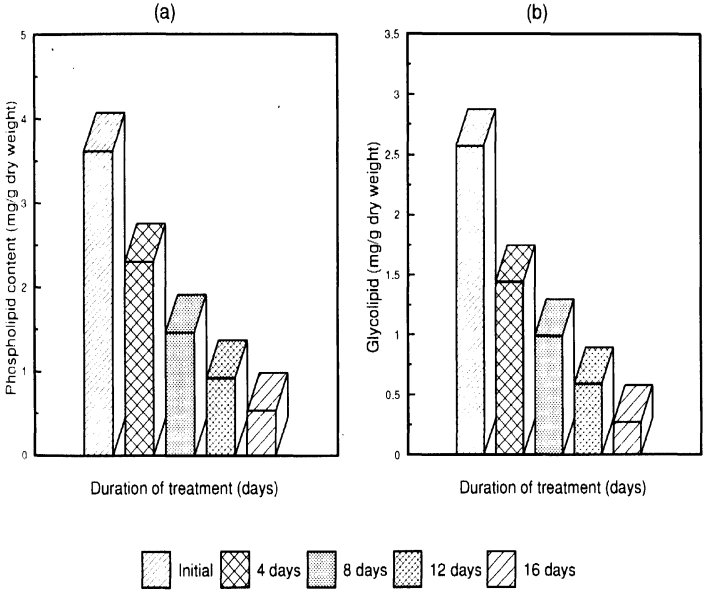

Figure 26. Changes in (a) phospholipid content and (b) glycolipid content in seeds of groundnut genotypes (mean) subjected to accelerated ageing.

Table 51. Changes in linoleic acid content of the neutral lipid, phospholipid and glycolipid of the seeds of four cultivated genotypes of groundnut following accelerated ageing.

	Neutral lipid				Phospholipid				Glycolipid			
Genotype / DAA (days)	ICGS 76	ICG 156	ICG 2738	ICGS 44	ICGS 76	ICG 156	ICG 2738	ICGS 44	ICGS 76	ICG 156	ICG 2738	ICGS 44
0*	29.2	30.8	38.3	41.7	29.9	30.7	35.9	38.1	29.3	29.5	36.6	36.7
4	29.0	30.3	37.7	40.0	29.4	29.1	33.5	37.7	27.8	28.2	32.3	34.3
8	28.8	30.0	37.5	39.8	29.0	28.0	33.1	37.1	25.9	27.6	30.1	34.0
12	28.6	29.6	37.0	39.7	28.7	27.4	33.0	34.5	25.4	25.0	30.0	33.8
16	28.5	29.3	36.2	39.0	27.3	27.0	32.6	32.7	24.4	24.7	28.1	30.0
S.E.	(S) ±0.054, (G) ±0.049; G X S ±0.109;				(S) ±0.077, (G) ±0.069; G X S ±0.155;				(S) ±0.074, (G) ±0.066; G X S ±0.149;			
CV (%)	0.6				0.8				0.9			

S=Storage, G=Genotype; * Initial value DAA=Duration of accelerated ageing

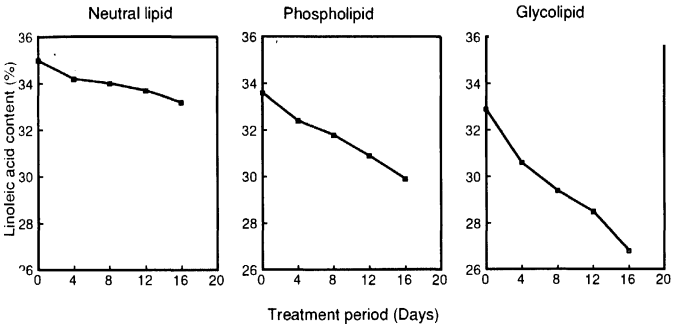

Figure 27. Changes in linoleic acid content of the neutral, phospho- and glycolipids of the seeds of groundnut genotypes (mean) subjected to accelerated ageing.

4.3 Seed deterioration consequent to ageing in wild species of groundnut

Experiments were conducted to determine the extent of seed ageing during storage of the wild species of *Arachis*. For this purpose, pods of 7 wild species viz., *A. duranensis, A. batizocoi, A. monticola, A. triseminalis, A. cardenasii, A. paraguariensis* and *A. apressipila* were stored under ambient and medium-term conditions for 15 months.

Seed viability:

It was observed that there was a rapid decline in the seed viability of all these species during storage, the extent of which is detailed in Table 52. Under ambient condition of storage there was considerable variation among the different wild species as regards their seed viability. The loss of seed viability was to the extent of 50% after 15 months of storage in case of *A. cardenasii* the best viable species, while it was as high as 90% in case of *A. paraguariensis* which was observable after 12 months of storage. Complete loss of viability was recorded in this genotype after 15 months.

When the storage was done under medium-term condition, the loss of seed viability was observed to be significantly less than under ambient condition. The loss of seed viability among the different wild species ranged from 28 to 44% under medium-term condition observed from Table 52. As regards the nature of decline in seed viability, there was no basic differences between the species during ambient storage. During medium-term storage some changes in the rate of decline was observed e.g. loss of viability in *A. apressipila, A. cardenasii* and *A. triseminalis* was relatively slow and uniform from the beginning of the storage while in other species *A. duranensis, A. batizocoi, A. monticola* and *A. paraguariensis* there was loss of viability only from 3-6 months of storage as may be observed from Fig. 28 and 29.

Table 52. Viability (%)* of the seeds of groundnut wild species following storage of pods under ambient and medium-term conditions.

Species of Arachis	Initial	Ambient storage Duration (months) 3	6	9	12	15	Medium-term storage Duration (months) 3	6	9	12	15
A. duranensis	100	82	60	52	46	32	100	90	76	60	56
A. batizocoi	100	80	66	60	52	38	100	88	80	72	60
A. monticola	90	78	60	50	40	28	90	90	70	60	50
A. apressipila	90	70	60	60	50	36	70	70	60	60	60
A. cardenasii	100	80	76	70	66	50	90	90	80	72	72
A. triseminalis	76	66	60	50	40	20	70	66	66	60	50
A. paraguariensis	70	66	50	40	10	0	70	66	50	40	40

S.E. (S) ±1.87, (G) ±2.02; (S) ±1.77, (G) ±1.91
S X G ±4.96, CV (%) 14.6; S X G ±4.69, CV (%) 11.1

S=Storage, G=Genotype; * determined by germination test

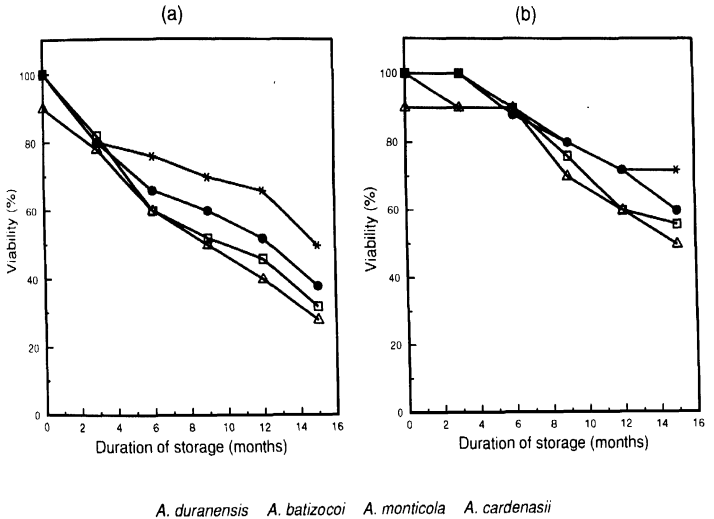

Figure 28. Decline in seed viability of the wild species of groundnut belonging to section *Arachis* following storage under (a) ambient and (b) medium-term conditions.

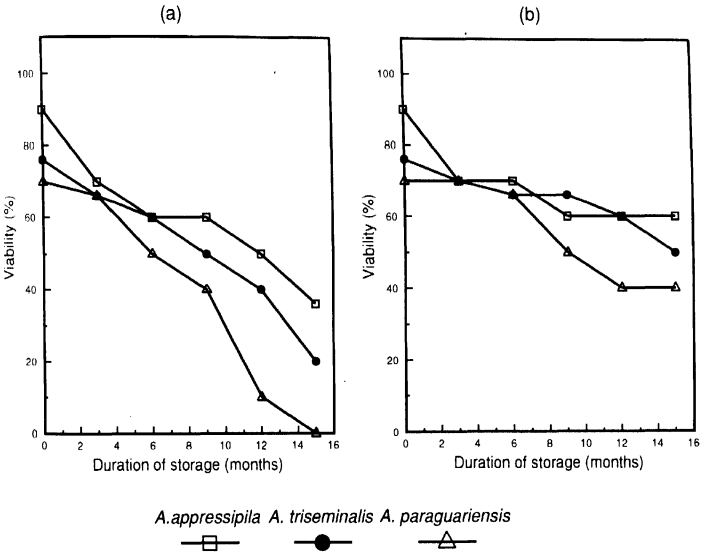

Figure 29. Decline in seed viability of the wild species of groundnut belonging to section *Erectoides* and *Triseminalis* following storage under (a) ambient and (b) medium-term conditions.

Oil content:

It was observed that during storage of the wild *Arachis* species there was a decline in the oil content of seeds. Under ambient condition, such loss of oil content extended from 1.8 to 4% in the stored seeds. The wild species *A. triseminalis* and *A. paraguariensis* showed higher amount of loss in the seed oil content compared to other wild species observed from Table 53. The loss in oil content was minimum in case of *A. apressipila* and *A. cardenasii*. Under medium-term conditions of storage the decline in the oil content of seeds was significantly less (0.6 to 2.2%) compared to the (1.8 to 4%) decrease in seeds stored under ambient conditions. The nature of decline was similar to that observed in the seeds stored under ambient condition.

Fatty acid composition:

It was observed that during storage, the linoleic acid content showed a decrease as seen in Table 55. The O/L ratio of the seeds showed an increase with the time of storage and such increase is seen from the fatty acid composition of fresh and aged seeds from Table 54. The decline in linoleic acid content was significantly more in the seeds stored under ambient conditions compared to the seeds stored under medium-term condition. Such decline was more in *A. triseminalis* and *A. paraguariensis* compared to other wild species and it was least in case of *A. cardenasii* and *A. apressipila*.

Protein content:

A decline in the protein content of the seeds of wild species of groundnut was observed with the period of ageing consequent to storage under both ambient and medium-term conditions as shown in Table 56. Such decline was slightly more in the seeds stored under ambient condition (0.6 to 1.6%) compared to the seeds stored under medium-term condition (0.4 to 0.8%). The reduction in protein content was observed to be more in the species *A.*

triseminalis and *A. paraguariensis* when compared to the other wild species.

Total soluble sugar content:

It was observed that there was an increase in the total soluble sugar content of the seeds when stored both under ambient and medium-term conditions and the changes with time of storage is shown in Table 57. However, such increase was significantly more in the seeds stored under ambient condition compared to the seeds stored under medium-term condition. Among the wild species the increase in the soluble sugar content was more in the stored seeds of *A. triseminalis* and *A. paraguariensis* compared to the other wild species.

Table 53. Oil content (%) of the seeds of groundnut wild species following storage of pods under ambient and medium-term conditions.

Species of Arachis	Initial	Ambient storage Duration (months) 3	6	9	12	15	Medium-term storage Duration (months) 3	6	9	12	15
A. duranensis	57.2	56.9	56.0	55.8	55.3	54.7	57.1	56.7	56.7	56.4	56.0
A. batizocoi	56.7	56.0	56.0	55.6	55.0	54.0	56.7	56.5	56.0	56.0	55.9
A. monticola	53.7	53.5	53.2	52.5	52.0	51.1	53.7	53.6	53.4	53.0	53.0
A. apressipila	56.6	56.0	55.5	55.2	55.0	54.8	56.4	56.4	56.1	56.0	56.0
A. cardenasii	56.8	56.6	56.4	56.1	55.7	54.8	56.7	56.3	56.3	56.0	55.8
A. triseminalis	55.8	55.6	55.2	54.2	53.5	52.2	55.7	55.5	55.0	54.5	53.8
A. paraguariensis	62.7	62.1	61.2	60.5	59.5	58.7	62.5	62.0	61.7	61.0	60.5
S.E.	(S) ±0.12, (G) ±0.13; S X G ±0.33, CV (%) 1.0;						(S) ±0.11, (G) ±0.11 S X G ±0.29, CV (%) 0.9				

S=Storage, G=Genotype

Table 54. Fatty acid composition of the seeds of groundnut wild species following storage under ambient condition.

Fatty acid/ Species		Palmitic	Stearic	Oleic	Linoleic	Arachidic	Behenic	Eicosenoic	Lignoceric	O/L ratio
A. duranensis	F	9.2	3.1	39.2	39.4	1.8	1.0	4.0	2.1	0.99
	A	9.4	3.0	40.6	37.6	1.8	1.0	4.1	2.5	1.08
A. batizocoi	F	10.2	2.8	40.2	35.3	2.8	1.6	5.0	2.0	1.14
	A	10.0	2.8	41.9	34.0	2.9	1.6	4.8	2.0	1.23
A. monticola	F	9.6	3.1	39.8	35.1	2.5	1.8	5.6	2.4	1.13
	A	9.8	3.0	41.9	34.0	2.3	1.5	5.5	2.0	1.23
A. apressipila	F	9.9	3.6	30.3	42.3	2.1	1.7	6.5	3.4	0.71
	A	10.0	3.5	32.0	41.0	2.0	1.7	6.4	3.3	0.78
A. cardenasii	F	10.6	3.2	38.9	37.2	2.5	0.7	4.7	1.8	1.04
	A	10.7	3.3	40.0	36.0	2.5	0.8	4.9	1.8	1.11
A. triseminalis	F	9.6	3.4	16.5	48.5	1.2	1.9	12.0	6.4	0.34
	A	9.9	3.5	18.2	46.4	1.2	2.0	12.6	6.2	0.39
A. paraguariensis	F	9.7	3.4	36.0	38.8	2.5	0.9	5.5	3.1	0.93
	A	10.0	3.5	39.0	35.4	2.4	1.0	5.7	3.0	1.10

F=Fresh seed; A=Aged seed; stored under ambient condition

Table 55. Linoleic acid content (%) of the seeds of groundnut wild species following storage of pods under ambient and medium-term conditions.

Species of Arachis	Initial	Ambient storage Duration (months) 3	6	9	12	15	Medium-term storage Duration (months) 3	6	9	12	15
A. duranensis	39.4	39.0	38.7	38.2	37.6	37.6	39.1	39.0	38.6	38.5	38.2
A. batizocoi	35.3	35.0	35.0	34.5	34.0	34.0	35.1	35.0	35.0	35.0	34.8
A. monticola	35.1	35.0	34.8	34.4	34.2	34.0	35.0	34.9	34.5	34.5	34.3
A. apressipila	42.3	42.0	41.5	41.0	41.0	41.0	42.1	41.9	41.7	41.7	41.6
A. cardenasii	37.2	37.0	36.6	36.6	36.3	36.0	37.0	36.9	36.8	36.8	36.8
A. triseminalis	48.5	48.1	47.4	47.0	46.4	46.4	48.3	48.3	48.1	48.0	47.0
A. paraguariensis	38.8	38.6	37.4	36.3	35.9	35.4	38.4	38.2	37.9	37.3	37.3

S.E. (S) ±0.11, (G) ±0.11; (S) ±0.08, (G) ±0.09
S X G ±0.29, CV (%) 1.3; S X G ±0.22, CV (%) 1.0

S=Storage, G=Genotype

Table 56. Protein content (%) of the seeds of groundnut wild species following storage of pods under ambient and medium-term conditions.

Species of Arachis	Ambient storage						Medium-term storage				
		Duration (months)					Duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
A. duranensis	23.9	23.7	23.4	23.4	23.1	23.0	23.7	23.7	23.5	23.5	23.4
A. batizocoi	25.9	25.7	25.5	25.4	25.0	25.0	25.9	25.9	25.4	25.3	25.3
A. monticola	21.3	21.0	20.9	20.7	20.5	20.5	21.1	21.1	21.0	21.0	20.9
A. apressipila	24.7	24.7	24.5	24.4	24.1	23.9	24.5	24.5	24.4	24.3	24.3
A. cardenasii	25.3	25.0	25.0	24.8	24.7	24.7	25.2	25.2	25.0	25.0	24.9
A. triseminalis	27.6	27.2	27.0	26.4	26.2	26.0	27.6	27.5	27.3	27.0	26.8
A. paraguariensis	19.1	19.0	18.7	18.7	18.4	18.1	18.9	18.9	18.7	18.7	18.6
S.E.	(S) ±0.08, (G) ±0.09; S X G ±0.23, CV (%) 1.8;						(S) ±0.09, (G) ±0.10 S X G ±0.25, CV (%) 1.9				

S=Storage, G=Genotype

Table 57. Total soluble sugar content (%) of the seeds of groundnut wild species following storage of pods under ambient and medium-term conditions.

Species of Arachis	Ambient storage						Medium-term storage				
		Duration (months)					Duration (months)				
	Initial	3	6	9	12	15	3	6	9	12	15
A. duranensis	7.1	7.7	8.0	8.3	8.7	9.2	7.3	7.7	8.0	8.2	8.4
A. batizocoi	6.2	6.5	6.9	7.2	7.5	7.7	6.3	6.7	7.0	7.2	7.4
A. monticola	5.9	6.2	6.4	6.9	7.0	7.3	6.1	6.4	6.7	6.8	6.9
A. apressipila	7.6	7.9	8.2	8.5	9.0	9.4	7.8	8.1	8.3	8.5	8.7
A. cardenasii	5.6	5.7	6.0	6.5	6.9	7.2	5.7	5.9	6.1	6.5	6.7
A. triseminalis	4.8	5.2	5.7	6.1	6.1	6.3	4.9	5.1	5.2	5.5	5.8
A. paraguariensis	4.6	4.8	5.3	5.7	6.0	6.3	4.8	4.9	5.3	5.5	5.5
S.E.	(S) ±0.08, (G) ±0.08; S X G ±0.21, CV (%) 5.6;						(S) ±0.07, (G) ±0.08 S X G ±0.20, CV (%) 5.4				

S=Storage, G=Genotype

Chapter 5

DISCUSSION

DISCUSSION

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseed crops of the Indian subcontinent. Often the seeds of groundnut suffer considerable damage during storage, resulting in loss of seed viability. This has not been substantiated with adequate data, or a comprehensive study on the nature and extent of deterioration. Seed deterioration is of concern to both groundnut growers who need good quality seeds for the next sowing, and to personnel involved in gene banking, whose interest lies mainly in the long-term conservation of the seeds as germplasm. The present investigation was undertaken to examine the consequences of seed ageing in both cultivated and wild genotypes of groundnut during storage and to characterize the deteriorative changes in order to find ways of arresting or slowing down the process of ageing.

The genotypes used belonged to 4 different cultivar groups viz., Virginia bunch, Virginia runner, Valencia and Spanish. Five genotypes belonging to each group were chosen randomly. Each group included genotypes with large and small seeds, with thick and thin shells, as well as a high-yielding genotype as check thus representing each cultivar group reasonably. Seeds of these 20 genotypes were stored under different conditions and seed viability was measured throughout 15 months of storage at intervals of 3 months. The term "seed viability" throughout this text, has been used with a broad meaning and refers to the ability of the seeds to germinate. It is true that seeds rendered non germinable by age may still contain viable tissues capable of metabolism with active enzymes. However, in the absence of such a test, the term "seed viability" proposed by Roberts (1972) has been used to denote seeds which can germinate under favorable conditions provided any dormancy that may be present is removed.

It was observed that under ambient storage condition (22-38°C, 44-80% RH) the loss of seed viability in groundnut can extend from 33 to 100 percent depending on the genotype, after 15 months of storage. If one accepts that the best indicator of seed deterioration during ageing is seed viability, then the observed loss in viability establishes that seed deterioration occurs in groundnut during storage and that can be regarded as a consequence of ageing. Decline in seed viability following storage has been commonly observed in many crops (Ellis and Roberts, 1981) including oilseed crops (Sardar and Islam, 1981; Minor and Paschal, 1982; Nautiyal *et al.*, 1990; Ketring, 1992). Interestingly, in groundnut, the rate of decline in viability during ambient storage was not found to be uniform, being slower in the beginning up to 9 months and becoming more rapid between 9 to 15 months. The viability curve therefore appears piece-wise linear (Fig. 1a), with a change of slope at one point, i.e., at 9 months. This is understandable if one considers that the process of ageing involves both damage and repair, which may be at different rates depending on the metabolic status and storage environment. In groundnut, both these processes might have continued at a slow speed up to 9 months (considered as the 'threshold point'), after which the deteriorative processes might have greatly accelerated, while the repairing ability rapidly diminished. The increased damage after the 'threshold point' could have been due to larger accumulation of toxic substances and/or irreparable aberrations of structural organization such as that of the membrane. This assumption receives indirect support from a sharp increase in the membrane damage evident from electrolyte leakage, and an increase in the effects of lipid peroxidation and enzyme activities observed after 9 months of storage of groundnut seeds under ambient condition (discussed at a later stage in this chapter). However, such a 'threshold point' was observed only under ambient storage condition, with high temperature and relative humidity, and was not discernible under other storage environments with a decrease in temperature

and relative humidity. Interestingly, all the 20 genotypes showed similar trends in decline of viability with the same 'threshold point' of rapid deterioration, suggesting that the event is not random. It is likely that such a point of inflection (threshold point) can also appear during other storage conditions with an environment of low temperature and low relative humidity, if the storage is allowed for a much longer period.

Of the several factors that can influence the viability loss due to seed ageing, the genetic-make up of the plant or species is certainly important because that is what governs the response of seeds to the process of ageing (Scott, 1981; Minor and Paschal, 1982). A quantitative difference in seed viability was found among the 20 genotypes of groundnut included in the experiment. The retention of viability in genotype ICG 4906 was as high as 67 percent, while the genotype ICG 10035 lost complete viability within 15 months of storage. The behavior of ICG 10035 was exceptional since no other genotype showed so much loss of viability. Genotypes which appear more vulnerable to ageing included ICG 3041, ICG 3209, the viability of which were as low as 30 and 42 percent respectively. On the other hand, the genotypes that exhibited better seed viability during ambient storage include ICG 5067, ICGS 76, ICG 4344, ICG 4236, ICG 156 and ICG 10063 in addition to ICG 4906, all of which retained 60 percent or more seed viability.

Although genotypic differences have not been very wide in response to ambient storage condition, the genetic potential to improve longevity of seeds (Ketring, 1992) during storage cannot be ignored. A comparison of the mean viability of 4 cultivar groups indicated that Valencia and Spanish groups, belonging to the subspecies *fastigiata*, are more vulnerable to seed ageing than the Virginia bunch and runner groups, belonging to the subspecies *hypogaea*. This result partly agrees with the observation of Norden (1981) and Zade *et al.* (1987). Interestingly, the differences in seed viability between the 2 groups

have been significant, suggesting that the two subspecies of groundnut are quite divergent with respect to their response to seed ageing. Two possible explanations can be offered for these differences. Firstly, the genotypes belonging to subspecies *fastigiata*, lack fresh seed dormancy unlike the genotypes of Virginia groups, belonging to *hypogaea* subspecies (Bailey and Bear, 1973), a factor that may be associated with better retention of seed viability because of delayed ageing favoured by initial dormancy. There are a few studies in rice (Chang, 1978; Siddique, 1986) which have suggested that seed dormancy contributes towards tolerance to a natural protection on storage. The evolutionary history of groundnut (Gregory *et al.*, 1973) provides the second plausible reason. Since these two subspecies are genetically isolated (Krapovickas, 1973), the *hypogaea* subspecies might have a better chance of natural selection than *fastigiata* in eliminating types that rapidly deteriorate due to ageing.

It is believed that seeds can be stored better in the form of pods (in-shell) than as shelled seeds (Navarro *et al.*, 1989). However, there have been no systematic studies to verify this general impression. Preliminary reports of Delouche *et al.* (1973) and Sankara Reddi (1988) indicated that in-shell seeds of groundnut retain viability for a longer time than the shelled seeds but no details were provided on the effects of changed storage environment. It was observed in the present investigation, that under ambient storage condition in-shell, all the 20 genotypes of groundnut stored better than the shelled seeds (kernel). The differences in seed viability were about 10 percent. This demonstrates the advantage of storing in-shell seeds (pods) of groundnut under conditions of high temperature and high humidity. The benefit is likely to have been derived from protection provided by the pods against fungal attack which is very common under high humidity. It has also been found (Woodroof, 1973; Ramamoorthy, 1977) that storage pests mostly attack kernels rather than pods in storage. However, large space required for

storing pod, can be a limitation, particularly in genebanks. The investigation of the association of pod shell thickness with capacity to retain seed viability has shown that thickness of the shell has no significant influence on the viability of in-shell seeds (pods) stored under ambient condition.

In groundnut, the seed size appears to influence the extent of storage deterioration. The viability of the small-seeded genotypes viz., ICG 4906, ICG 4344, ICG 10063 and ICG 2387 was significantly higher than the large-seeded genotypes viz., ICG 2742, ICG 4342, ICG 10035 and ICG 2959. Following storage under ambient condition, the small-seeded genotypes have showed about 11 percent more viability. In several crops such as soybean (Vyas *et al.*, 1990), chickpea (Smith *et al.*, 1987) and sorghum (Krishnasamy, 1986) there are reports that the survival of the small-seeded types during storage is higher than that of the large-seeded types. One of the probable explanations for such differences can be that large-seeded types are more prone to testa damage because of larger filling. In addition, large seeds often suffer mechanical damage during post-harvest processing. In soybean, such mechanical damage has been considered to be responsible (Dickson, 1980) for cracking and for a loosened seed coat with greater risk of microbial attack.

In addition to a loss in seed viability, a decline in seedling vigor was noticed in all the genotypes of groundnut. Loss in seedling vigor is an indicator of loss of quality and vigor of the seed (Heydecker, 1972; Roberts, 1986) and a common deteriorating effect of ageing (Abdul-Baki and Anderson, 1972; Heydecker, 1972). This is expected because of a decline in the normal physiological activities during storage along with an increase in various deteriorative biochemical changes. Loss of seedling vigor following seed storage has been reported in several crops (Priestley and Leopold, 1983; Saxena *et al.*, 1985) including groundnut (Nautiyal *et al.*, 1988; Chakraborty *et al.*, 1991). In the present investigation, seedlings of groundnut, derived from seeds stored

under ambient condition, have shown a decline in vigor noticeable through a reduction in shoot, hypocotyl and root lengths and a decrease in dry weight. The seedling vigor declined linearly with an increase in the duration of the storage period. All the 20 genotypes of groundnut suffered a considerable loss in seedling vigor even though, in some of them, the loss in seed viability was observed to be comparatively much less. For example ICGS 44 and ICG 3041 showed a loss of 10 and 18 percent seed viability after 9 months of storage under ambient condition. Correspondingly, the losses in seedling vigor of these genotypes during the same period were 24 and 33 percent, respectively. This demonstrates that seedling vigor is possibly a more sensitive measure of seed deterioration than seed viability.

Significant differences have been observed between the genotypes with respect to loss of seedling vigor. ICG 3041 and ICG 3209 showed a considerable loss in seedling vigor, while the loss was much less in genotypes ICG 4344 and ICG 156. A comparison between 4 cultivar groups showed that the genotypes belonging to the Valencia group suffered significantly more loss of vigor than the Virginia group, a trend similar to that observed in the loss of seed viability. The differences between the small-seeded and large-seeded genotypes with respect to seedling vigor was not consistent while no significant differences in vigor could be detected between seedlings derived from in-shell and shelled seeds of groundnut. This suggests that the observed differences in seed viability in this case fail to manifest themselves at the seedling stage and thereafter.

During storage, the ageing process in the seed is accelerated because of the deterioration of the cellular membrane (Delouche, 1969) which plays an important role in maintaining the integrity of cellular components. In groundnut the basic information on the influence of deterioration of the cellular membrane on loss of seed viability is lacking. The measurement of

electrolyte leakage from groundnut seeds stored under ambient condition clearly showed considerable electrolyte loss. The electrical conductivity of seed leachate was as high as 1.180 mmho/cm in genotype ICG 10035 while it was 0.249 mmho/cm in ICG 4906. The conductivity test that has been used is an accepted method to assess seed quality and to provide physiological information related to membrane integrity in seeds (Kuo, 1989). This inference of membrane damage indicated by conductivity tests has also received support from various ultrastructural studies (Fu *et al.*, 1986). The results on the leakage of solutes from groundnut seeds, evident from higher conductivity values, can represent aged, damaged or non functional cellular membranes (Simon and Raja Harun, 1972) and cellular rupture caused by imbibition damage (Powell and Mathews, 1981). The progressive loss of membrane integrity with increase of storage time that was observed in groundnut supports the findings of several other crop plants (Harman and Granett, 1972; Parrish and Leopold, 1978). It should be mentioned that even fresh undamaged seeds can show some loss of solutes as measured by the rise in conductivity of the external solution, but much of the metabolites are subsequently reabsorbed by germinating embryos of high vigor by active uptake (Pandey, 1992). But in ageing embryos with progressive impairment of the membranes, the initial loss of cytoplasmic solutes becomes much greater while the extent of active uptake becomes much less (Berjak and Villiers, 1972c). This ultimately causes loss of seed viability and/or loss of seedling vigor as occurred in the present study.

Relationship between imbibitional leakage and membrane damage has been reported by several workers (Ching and Schoolcraft, 1968; Powell and Mathews, 1977; Sreeramulu, 1983a; Siddique and Goodwin, 1985). In all the genotypes of groundnut, electrical conductivity of the seed leachate showed distinct increase with the time period of ageing. The rate of increase however was slower during the initial period of storage (0 to 6 months) and then became much more rapid (from 6 to 15 months: Fig. 5), a trend that relates to

the rate of decline in seed viability of groundnut. Membrane damage due to ageing may involve oxidative stress and free radical mediated damage, an aspect which has been discussed later in this chapter while presenting the results on lipid peroxidation. The results on conductivity testing of groundnut seeds subjected to ambient storage provide some evidence of membrane damage and deterioration in the quality of ageing seed lots. Loss in seed viability and poor seedling vigor evident among groundnut genotypes appear to be demonstrable consequence of membrane damage, and are in agreement with reports on other oil crops (Dey and Mukherjee, 1988). Leakage of electrolytes therefore is an indirect index of such a loss, a consequence of gradual weakening of cell membranes and lower retention capacity of the cell.

Groundnut is an oil rich crop. There is a widespread perception, substantiated to some extent by evidence (Spector, 1956; Gvozdeva, 1971), that lipid rich seeds tend to have a limited longevity. It is not surprising, therefore, that the hypothesis of seed ageing based on lipid degradation should be considered quite important (Priestley, 1986). A decrease in total lipids has been reported in several oil rich seeds during a prolonged storage (Sreeramulu, 1983b; Dey and Mukherjee, 1986; Subbaraman and Selvaraj, 1989; Balamurugan *et al.*, 1989; Chakraborty *et al.*, 1991). The different genotypes of groundnut showed a reduction in the oil content of the seeds following ambient storage. The reduction in total lipid ranged between 3-8 percent. The results in the present study are similar to that of Nautiyal *et al.* (1988) in groundnut. However, the rate of decline in oil content observed in the present experiment was not uniform, being slower in the beginning and becoming more rapid between 9 to 15 months of storage. The decrease in total lipid content is most likely a consequence of slow metabolism by the seeds under conditions of high temperature and humidity. The extent of this metabolic depletion, however, is unlikely to threaten viability. The differences between genotypes were mostly insignificant in respect to the loss in lipid content.

It is more likely that the loss of membrane lipid plays a more vital role than a decrease in storage reserves (Pearce and Abdel Samad, 1980). In fact, a sharp decline has been observed in the phospholipid content, an important constituent of the membrane (Fig. 16). Seed stored under ambient condition exhibited almost 50 percent reduction in the phospholipid level. This is similar to the findings of Pearce and Abdel Samad (1980) in groundnut, and of Powell and Mathews (1981) in pea. Less severe declines were noted in soybean (Priestley and Leopold, 1979), peas (Yang and Yu, 1982) and sunflower (Halder *et al.*, 1983). As phospholipids make up most of the oleosome membrane, (Singer and Nicholson, 1972) their cumulative loss means the breaking of the membrane itself (Simpson and Nakamura, 1989). Such loss of phospholipid from a cell may entail a diminution of the area of the membrane, and may affect tonoplast and plasmalemma, thereby enhancing the permeability of the cell. The cell becomes leaky which actually happened in groundnut as evident from very high solute release from aged seeds. An attempt has been made, earlier in this chapter, to correlate such leakage with the degree of seed viability.

Two suggestions have generally been offered to explain lipid degradation during seed deterioration. The lipids may have been subjected to peroxidation or else they might have been degraded by enzymes. It is known that many polyunsaturated fatty acids are susceptible to peroxidative damage and as a result not only does the lipid itself gets destroyed but a complex series of reactions generate a variety of potentially toxic products (Priestley, 1986). The results of the present investigation on groundnut show that there has been a distinct decrease in the polyunsaturated linoleic acid which may be considered as an evidence of susceptibility of the stored seeds to peroxidative degradation.

Lipid peroxidation, the oxidative destruction of polyunsaturated fatty acids, is an uncontrolled, autocatalytic process leading to the formation of fatty

acid hydroperoxides and to secondary products, including a wide range of aldehyde components. The essential mechanism of lipid peroxidation outlined by Chessman (1993) indicates that the process starts with the abstraction of H_2 atom from the target fatty acid to form a lipid (fatty acid) radical. This process is known as *initiation*. The hydroxyl ($HO^{\cdot}$) radical, most peroxyl radical ($ROO^{\cdot}$) and most alkoxy radicals ($RO^{\cdot}$) are all capable of oxidizing polyunsaturated fatty acids, while the superoxide radical ($O_2^{\cdot}$) is not. It is impossible to be certain about the relative significance of the various possible initiating agents. The product of the initiation reaction is a fatty acid radical that rapidly rearranges to form a conjugated diene structure. The extremely rapid addition of oxygen to the fatty acid radical forms a lipid (fatty acid) peroxyl radical ($LOO^{\cdot}$). This is capable of reacting with other polyunsaturated fatty acids, beginning a new chain of oxidation, thus forming a lipid hydroperoxide ($LOOH^{\cdot}$) on the original polyunsaturated fatty acid and generating a new fatty acid radical. In the propagation stage of lipid peroxidation a new chain is initiated by a lipid peroxyl radical and the breakdown of lipid hydroperoxides to more radical intermediates.

Lipid hydroperoxide breakdown is important for two reasons. It generates radicals that propagate lipid peroxidation as has been already stated, and also generates non radical fragmentation such as aldehydes, many of which are biologically active. In biological systems with mixtures of different polyunsaturated fatty acids, lipid peroxidation will generate a mixture of hydroperoxides, the breakdown of each of which can produce a variety of radical species and aldehydes. Unfortunately, it is as yet not clear which of them are actually formed in the biological system, in what quantities they are formed, and what their biological properties are.

The first indication that lipid peroxidation can be a direct cause of seed deterioration came from the report of Kaloyereas (1958) and since then there

have been several other findings that substantiate such a claim (Wilson and McDonald, 1986). The peroxidation in stored seeds has been considered to arise either through atmospheric autoxidation or through the agency of lipoxygenase, an enzyme present in many seeds (Tappel, 1962) which accelerates the rate of this reaction. Different polyunsaturated fatty acids possess different susceptibilities to peroxidation. The evidence of lipid peroxidation comes mostly from the analysis of the relative changes in the levels of unsaturated fatty acids. In most seeds the lipids that are at risk from autoxidation comprise oleate (18:1), linoleate (18:2) and linolenate (18:3) fatty acyl chains. The degree of unsaturation has considerable influence on the rate of degradation. It is stated (Schaich, 1980) that 9, 12-linoleate, with a pair of double bonds that are methylene-interrupted, is degraded about 30 to 40 times faster than 9-oleate, which has only one double bond. The present experiment demonstrated that there was a considerable decline in the linoleic acid due to ageing of groundnut seeds and an increase in the proportion of oleic acid. This was observed in all the cultivated genotypes as well as in the wild species of groundnut. The reduction in the level of linoleic acid and increase in O/L ratio were observed even after 3 months of storage which continued and became pronounced thereafter, indicating that seed tissues got increasingly peroxidized with increase in the time of storage. Priestley and Leopold (1983), employing natural ageing in soybean, also observed a gradual shift in the proportion of polyunsaturated fatty acids towards monounsaturated and saturated fatty acids that accompanied a decline in vigor and germinability. Their earlier reports on accelerated ageing of soybean (Priestley and Leopold, 1979), however, did not consider lipid peroxidation to be a major factor in seed deterioration. In this case, the reason offered was that the mechanism of accelerated ageing in soybean could be physiologically different from natural ageing. The findings of Pearce and Abdel Samad (1980) on the lipid changes during natural ageing of groundnut differ from the results of the present

experiment. They failed to observe any consistent changes in the relative fatty acid composition of the neutral, glyco- and phospho-lipid fractions and considered loss of control over subcellular compartmentation or intracellular concentration of metabolites due to breakdown of membrane lipids to be the cause of seed damage. However, much of the variability in their results might be associated with environmental effects since the seed lots were few (2 cultivars) and grown in different years. In the present experiment, all the 20 genotypes used were harvested at the same time to avoid environmental differences. Since the trend of changes in fatty acids and decline in seed viability was consistent and was observed in all the genotypes, it is difficult to exclude lipid peroxidation as an important cause of seed deterioration due to ageing.

During natural ageing of groundnut seeds, a decline in phospholipid and glycolipid contents was observed during ambient storage. The changes in the fatty acid composition of these lipid fractions, namely decrease in linoleic acid, were considerably more than those observed in the storage reserves. Although some disagreement remains on this issue of changes in fatty acid composition (Bewley, 1986; Priestley and Leopold, 1979) it is clearly seen that such changes occur in groundnut as in pea (Harman and Mattick, 1976). This has special significance when one considers that phospholipids are principal constituents of the lipid bilayer, and peroxidation of phospholipids invariably causes damage to the membrane.

Changes in enzyme activities during the ageing process in groundnut were evident from a decrease in peroxidase activity and an increase in lipase activity in seeds stored under ambient condition. The activity of peroxidase has declined steadily along with increase in the time of storage. A similar trend in the decline of peroxidase activity has been reported by other workers (Saxena *et al.*, 1985; Nkang, 1988). Peroxidase enzyme is known to catalyze the

breakdown of hydrogen peroxide to water and oxygen. By eliminating hydrogen peroxide accumulation, peroxidase prevents formation of potent free radicals. Any decrease in peroxidase activity during ageing is likely to make the seeds more sensitive to free radicals and vulnerable to lipid peroxidation. An increase in the peroxide value that has been observed in all the genotypes of groundnut indicates a certain degree of oxidative degradation. Elevation of peroxide value which correlates negatively with loss of germinability was noticed during prolonged storage of groundnut (Mathur *et al.*, 1956; Uematsu and Ishii, 1981) and other oilseeds (Sharma, 1977).

A significant increase in lipase activity was observed in the stored groundnut seeds irrespective of the genotypes. This could be another reason for the deterioration of seeds during storage since lipase is one of the two principal enzymes involved in the degradation of lipids in seeds (St. Angelo and Ory, 1983), the other being lipoxygenase. Oilseeds are rich sources of triacylglycerols and any increase in lipase activity is likely to accelerate the breakdown of triacylglycerols to glycerol and fatty acids which adversely affects the stored seeds. The observed decrease in lipid content and increase in acid value in the aged groundnut seeds could also have been due to the increased lipase activity which is known (Dey and Mukherjee, 1986) to be responsible for important changes in the lipid of deteriorating seeds of other oil crops such as mustard, corn and soybean.

In the present experiment, a decrease in the protein content of the seeds following storage under ambient condition was observed in all the genotypes of groundnut. Similar decrease has been reported earlier in groundnut (Rao *et al.*, 1970; Suneja and Nagaraj, 1988). The decrease in protein content could be due to denaturation of protein during storage undergoing the process of ageing (Roberts, 1972). Although storage causes depletion of such reserves, the loss, unless severe, is unlikely to be responsible for major damage leading to loss of

viability (Roberts, 1972).

The increase in the soluble sugar content, that has been observed in the groundnut genotypes stored under ambient condition is most likely due to impaired respiration. It is documented that respiratory changes do occur in stored seeds (Anderson and Baker, 1983) leading to various metabolic deficiencies. In soybean, a decline in respiration rate due to ageing has ben reported (Edje and Burris, 1970; Woodstock *et al.*, 1984) and similar observations have been made by Rao *et al.* (1970) in groundnut. There can be considerable changes in the respiratory characteristics of deteriorated seeds which are likely to affect the sugar level. The initial reserve of sugar, or sugars derived as a consequence of breakdown of starch, may not be effectively, metabolized.

The foregoing discussion on the results of groundnut seed deterioration due to ageing, a consequence of storage, establishes that considerable damage can occur even to freshly harvested seeds when stored under ambient condition. The deteriorative changes linked with loss of viability become apparent even after 3 months of storage and continue to progress rapidly thereafter. Any prevention of such damage therefore requires control of temperature and humidity (Ellis *et al.*, 1982). Consequences of such measures can be examined from the results of experiments in which genotypes of groundnut representing 4 cultivar groups were stored under short-term (18°C, 30% RH), medium-term (4°C, 20% RH) and long-term (-20°C) conditions and their effects compared. These are also recommended storage conditions for germplasm conservation.

It was observed that the loss of seed viability of all the 4 genotypes was considerably reduced under short-term storage condition. The loss ranged from 6 to 9 percent depending on the genotype as observed after 15 months of

storage. A better retention of seed viability with lowering of temperature and humidity is a well known phenomena and has been extensively discussed by Roberts (1986) who also provided quantitative data on longevity of seeds in storage in relation to decrease in temperature and relative humidity. The present finding in groundnut agrees with the general response, but indicates that deterioration due to ageing even under short-term storage condition has not been arrested and is likely to continue beyond 15 months of storage. This assumption was mainly derived from the nature of decline evident from the seed viability curve (Fig. 10) and also from other indices of seed deterioration e.g., loss of seedling vigor, electrolyte leakage, lipid peroxidation and enzyme activities (detailed data available in the chapter "results") all of which continued till 15 months of storage without any indication of arrest.

An important difference from the results on ambient storage was the absence of any protective effects of the pods unlike that reported by others (Hsieh, 1981; Navarro *et al.*, 1989). Under short-term storage, no differences in viability was observed between groundnut stored as pod (in-shell) or kernel (seed). This confirms our earlier opinion that pods of groundnut provide more of a physical protection from the fluctuations in external environment, and invasion of fungi or pests rather than bringing about any real difference in physiological or biochemical changes. Under conditions of low temperature and low humidity, not prevalent during ambient storage, the chances of external injury due to fungal invasion or mechanical damage diminish or no longer exist. This is the possible reason for absence of any difference in deterioration between groundnuts stored as seeds or pods under short-term or medium-term storage conditions. It also suggests the absence of any biochemical attribute specifically responsible for the differences observed under ambient storage condition. The conclusion is evident that storage of germplasm under short-, medium- and long-term makes no difference whether groundnut germplasm is stored as pod or seed.

The response of groundnut genotypes however, did change much due to differences in the storage environment. ICG 2738 and ICGS 44 belonging to Valencia and Spanish groups suffered significantly more loss of seed viability as compared to ICGS 76 and ICG 156 belonging to Virginia bunch and Virginia runner. The difference between the 4 cultivar groups continued to remain while ageing under short-term storage condition and the trend was similar to that observed after storage under ambient condition.

A considerable reduction in seed viability was observed following storage of 20 genotypes of groundnut under medium-term condition maintained at 4°C and 20% RH. The process of ageing appears to have slowed down considerably since the loss of viability ranged from only 1.3 to 4.2 percent, except in the case of the genotype ICG 10035 where the loss in viability was about 36 percent. The storage behavior of ICG 10035 indicates its vulnerability to ageing, the basis of which can only be determined through detailed genetic studies. Conspicuous differences were observed between seed viability and seedling vigor of all the genotypes. Loss in seedling vigor was considerably more than loss in seed viability suggesting that a portion of the viable seeds may not be healthy and vigorous and fail to produce good quality seedlings.

The findings of the experiments using medium-term storage condition confirm that genotypic differences exist in relation to loss of seed viability and seedling vigor due to ageing. The genotypes belonging to Valencia and Spanish groups once again prove to be more vulnerable to ageing than Virginia bunch and Virginia runner groups. The comparison between small-seeded and large-seeded genotypes stored under medium-term condition also confirms that large-seeded genotypes (ICG 5067, ICG 4344, ICG 10035 and ICG 3209) are more susceptible to ageing than the small-seeded genotypes (ICG 4906, ICG 4342, ICG 10063 and ICG 2387), a trend that has been observed during ambient storage condition. The results also indicate that even under reduced

temperature and low RH during medium-term storage condition there was considerable electrolyte leakage from the stored seeds, and there were changes in fatty acid composition, suggesting that major deteriorative processes such as membrane damage and lipid peroxidation continue even under a storage environment much more favorable than the ambient condition. However, under long-term storage condition (-20°C) none of the age-induced alterations could be detected up to 15 months.

If the deteriorative changes evidenced under three storage conditions (ambient, short-term and medium-term) are compared it would be reasonable to conclude that age-induced deteriorations can be severe under ambient storage condition with high temperature and humidity, which gets reduced under short-term condition and still less under medium-term storage condition. A quantitative evaluation is possible from the following data given in sequences of ambient, short-term and medium-term storage: seed viability - 58.0, 92.1 and 96.7 percent; electrical conductivity of seed leachates - 0.504, 0.206 and 0.176 mmho/cm; linoleic acid content of total lipid - 32.9, 34.3, and 34.6 percent; lipase activity - 2.41, 1.72 and 1.55 μ eq. of free fatty acid released/3 min assay; peroxidase activity - 0.193, 0.285 and 0.310 max. O.D./g fresh weight; phospholipid content - 1.88, 3.26 and 3.60 mg/g dry weight; and linoleic acid content of phospholipid fraction - 31.1, 33.0 and 33.3 percent.

Although a preliminary understanding of the deteriorative changes and ageing process in groundnut is possible from the present experimental data, it is suspected that with an extension of storage period beyond 15 months the deteriorative changes could have been more discernible, particularly under conditions of low temperature and humidity. The process of ageing is likely to be continuous and can possibly be arrested only by storing seeds at liquid nitrogen temperature (Benson, 1990; Jana, 1992). The present findings in groundnut and the related assumptions are likely to help in deciding the

strategy of groundnut germplasm conservation. A reduction in damage with changed environment during short-, medium- and long-term conditions could be reassuring to those involved in germplasm conservation, but it should be kept in mind that during or after collection of germplasm the seeds may be required to be kept under ambient condition for varying periods. This exposure itself can be damaging or can initiate the process of deterioration.

Groundnut seeds were also subjected to accelerated ageing to compare its effects with the consequences of natural ageing. The technique of accelerated ageing basically involves exposing the seeds to high temperature and humidity which induces rapid deterioration. It is seen that during accelerated ageing, the viability of groundnut seeds declines very rapidly and the loss becomes complete within 20 days. The question remains whether the two ageing regimes are distinctly different or they represent the same phenomena at different speeds. It seems that there is little to distinguish between the response of groundnut to the two ageing regimes (Pearce and Abdel Samad, 1980; Singh and Khatra, 1984). This was not so in case of a crop like soybean (Priestley and Leopold, 1983; Francis and Coolbear, 1988) where the response could be different depending on the ageing method used.

The relationship between the changes elicited by accelerated ageing and natural ageing in groundnut demonstrates that the deteriorative process advances in the same direction but at a much higher rate and in linear order. These changes include decline in seedling vigor, increased electrolyte leakage, increased lipid peroxidation as evident from changes in fatty acid composition, increased lipase and decreased peroxidase activities, increased acid and peroxide values, and decreased phospholipid content. The trend of changes have been similar to that observed due to natural ageing. Interestingly, the loss of seed viability due to the effects of lipid peroxidation, evidenced during accelerated ageing in groundnut, differs from the findings of accelerated ageing

in soybean (Priestley and Leopold, 1979). However, the same researchers did not notice effects of lipid peroxidation during accelerated ageing although in their subsequent experiments on natural ageing (Priestley and Leopold, 1983) they observed lipid peroxidation. They considered that in soybean, accelerated ageing might cause loss of seed viability in a manner different from natural ageing. The findings in groundnut fail to support such a contention.

The results on accelerated ageing in groundnut indicate that ICGS 44 belonging to Spanish group suffered maximum damage followed by ICG 2738 belonging to Valencia group. The deteriorative changes were much less in ICG 156 (Virginia runner) and least in ICGS 76 (Virginia bunch). These results once again demonstrate that the genotypes belonging to subspecies *fastigiata* are more vulnerable to seed ageing and lose seed viability faster than the genotypes belonging to subspecies *hypogaea*. Although it is clear from experimental findings that the rate of deterioration is much faster during accelerated ageing as compared to the natural ageing, it is not easy to quantify the damage because of the differences in the time scale in these two processes. However, comparisons of the various deteriorative changes at 50 percent survival level show that due to accelerated ageing the increase in electrolyte leakage was 700 percent more, increase in lipase activity was 70 percent more, decrease in peroxidase activity was 20 percent more, decrease in phospholipid content was 20 percent more, while the decrease in linoleic acid content was 7 percent more. The comparison, though not precise, clearly indicates that all of the deteriorative changes occur at a much faster rate during accelerated ageing, except the change in linoleic acid. The slow rate of lipid peroxidation indicates that the time required for peroxidation, and the availability of oxygen may be limiting factors during a very fast ageing process. There are reports (Ohlrooge and Kernan, 1982) that suggest that seeds tend to lose oxygen dependence during the process of accelerated ageing.

Studies on the storage behavior of the wild species of groundnut were undertaken mainly because the process of seed ageing in wild groundnut is so far unknown and, secondly, because of the expectation that wild species of groundnut may have superior resistance to seed deterioration. Such an expectation arises from the fact that wild species of cultivated crops have often provided resistance genes for the existing cultivated varieties (Stalker, 1980). In groundnut, the search seems to be of interest because groundnut has many wild relatives; a number of which are cross-compatible and have contributed to its allotetraploid origin (Singh *et al.*, 1991). More importantly, a large number of the accessions of the wild species are getting lost because of poor viability during storage (Stalker, 1992). Because of considerable sterility and constraints of low fruit and seed production, the available seeds of the wild species itself become a very important genetic resource and these seeds require the best method of conservation.

Although a limited number of wild species was randomly chosen for the present experiment, they represent different sections of genus *Arachis*, different ploidy levels, and different genomic constitution. The choice of the species could have been more systematic, but non availability of adequate seeds was a major restriction in the choice of the wild species. However, the 7 species that have been chosen provide a reasonable spectrum of the wild species of groundnut. The results of storage of wild groundnut species under both ambient and medium-term conditions showed a rapid decline in seed viability. Under ambient storage condition, even the best stored species e.g., *A. cardenasii* has shown a loss of 50 percent viability after 15 months of storage. *A. paraguariensis* proved to be much more susceptible and showed 85 percent loss in seed viability after 12 months of storage, while complete viability loss was observed within 15 months of storage. It is generally observed that seeds with low initial viability e.g., *A. paraguariensis* and *A. triseminalis* suffer greater loss during storage. Incidentally, both of these

species belong to section *Erectoides* (Gregory, *et al.*, 1973) and are diploid, whereas the more resistant species *A. cardenasii*, a diploid, belongs to section *Arachis* (Gregory, *et al.*, 1973). it appears that seed deterioration has no relation with the polyploidy level of the wild species. Considerable loss in seed viability observable among the wild species under ambient storage condition was somewhat unexpected and indicated that none of the species is likely to confer resistance towards seed deterioration or ageing in groundnut.

The loss of seed viability under medium-term storage was less than that observed under ambient condition, evidently due to lowering of temperature and humidity. A comparison of the viability of the wild species with that of cultivated groundnut provides certain interesting information. For example *A. cardenasii* (most tolerant to ageing) showed 22 percent more seed viability when stored under medium-term condition as compared to storage under ambient condition. In contrast, ICGS 76 under similar conditions showed 38.7 percent more seed viability. This indicates that the wild species of groundnut can undergo considerable deterioration due to ageing, even more than the cultivated genotypes, under medium-term storage, a condition generally maintained in genebank for germplasm conservation. In medium- term storage, the rate of decline in seed viability was uniform except in *A. batizocoi*, *A. duranensis*, *A. monticola* and *A. paraguariensis*. The results indicate that even medium-term storage is not adequate for conservation of wild germplasm of groundnut, and which requires an alternative strategy of conservation.

In the wild species, a decline in oil content was observed in the seeds stored under ambient condition, and the loss extended from 4 to 7 percent, which is not very different from the loss of oil content observed among the cultivated genotypes (3-8 percent). The loss of oil content was minimum in case of *A. cardenasii* and *A. apressipila*. Incidentally, both these species have also shown higher seed viability following similar storage. The loss of oil content

was significantly less in seeds stored under medium-term condition as compared to the loss observed due to storage under ambient condition. However, a comparison of loss in oil content of the cultivated genotypes with that of wild species stored under medium-term condition showed that the loss is much more in the wild species. This loss in lipid content may be the reason for greater loss of viability in the wild types which might not be the case for cultivated genotypes.

A change in fatty acid composition was observed in the stored seed of all the wild species with a decrease in linoleic acid content, and an increase in O/L ratio irrespective of the conditions of storage. The effect of lipid peroxidation, indicated by the loss of linoleic acid was minimum in *A. cardenasii* and maximum in *A. paraguariensis*. These findings closely correspond with the extent of loss in seed viability. Of these two species, it is evident that the effects of lipid peroxidation are responsible for seed deterioration during storage of the wild species of groundnut. The species more vulnerable to ageing viz., *A. triseminalis* and *A. paraguariensis* have shown a decline in protein content and an increase in the sugar content as in case of cultivated genotypes, where both of these events contributed towards age-induced deterioration.

Although the search for resistance among the wild species has not proved rewarding, it has provided some information that can be useful in the conservation of these species, such as the inadequacy of medium-term storage for safe conservation of the germplasm of the wild species. It is also imperative that the search among wild types for some kind of resistance to seed deterioration should continue because of two reasons. Firstly, a large number of wild species is available in groundnut and can be screened; and secondly there are instances where resistance to a character has been conferred by one or few wild species with many other wild species of the same crop remaining susceptible (Subba Rao *et al.*, 1991). A systematic search is not only desirable

for the wild species but also for the genotypes of cultivated groundnut.

In the light of the discussion above, it can be concluded that maintenance of good quality seed in groundnut remains a problem, particularly under conditions of high temperature and humidity in the subtropics. The process of ageing during storage becomes gradually rapid with inexorable trends to disorder. Although the magnitude of seed deterioration has been reasonably determined from loss in seed viability, poor seedling vigor, enhanced leakage of electrolytes, changes in lipid profile etc., the detection of the most important deteriorative mechanism has not been so definite. It is apparent that the loss of seed viability during storage is linked to a chain of complex events most of which are related. Of these events, in groundnut, lipid peroxidation and its ramifications appear to be most significant. Whether such effects of lipid peroxidation would be equally the most damaging event in other oil rich seeds is difficult to answer because certain other degradative process can become more important depending on the species and ageing environment (Priestley, 1986). The challenge is to understand how the integrated system inside a seed becomes subject to disarray due to ageing consequent to storage, and how it can be controlled. In the present investigation, a beginning has been made with a hope that satisfactory practical solutions will be forthcoming from future experiments.

Chapter 6

SUMMARY

SUMMARY

Experiments were conducted to investigate the loss of seed viability during storage of cultivated and wild species of groundnut and to ascertain the nature and extent of physiological and biochemical changes associated with the process of ageing. Different storage conditions recommended for germplasm conservation, namely short-term (18°C, 30% RH), medium-term (4°C, 20% RH) and long-term (-20°C), were used along with storage under ambient (22-38°C, 44-80% RH) conditions. Seed deterioration following accelerated ageing was compared with the findings on natural ageing.

When 20 cultivated genotypes of groundnut (*Arachis hypogaea* L.) were stored under ambient condition for 15 months, there was considerable loss of seed viability ranging from 33 to 100% depending on the genotype. The genotype ICG 10035 lost complete viability, while ICG 4906 showed minimum damage with a loss of 33% seed viability. The rate of loss in seed viability was slow in the beginning and up to 9 months, followed by a faster decline rate during the later period of storage i.e., between 9 to 15 months. Similar trend in loss of viability was noticed in all the genotypes. When groundnut was stored under medium-term condition, the loss in seed viability among the genotypes ranged mostly from 1.3 to 4.0%, except in ICG 10035 which showed 36% loss in seed viability. A comparison of the groundnut genotypes belonging to 4 cultivar groups viz., Virginia bunch, Virginia runner, Valencia, and Spanish showed that loss in seed viability was more in the Valencia and Spanish groups. Such differences were observed following both ambient and medium-term storage. The loss in seed viability during storage was more among the large-seeded genotypes than in small-seeded genotypes. Between pod (in-shell) and seed (kernel) storage, the loss in viability was more in case of kernels when stored under ambient condition but such difference was not observed when storage was done under other conditions. Pod thickness had no

influence on storability.

The effect of storage of seeds and consequent deterioration was also evident from a decline in seedling vigor. There was a distinct reduction in the lengths of shoot, hypocotyl and root, and a decrease in dry weight. Storage under ambient condition caused significantly greater loss in seedling vigor as compared to storage under medium-term condition. There existed differences between genotypes as regards loss in seedling vigor. Following ambient storage, the seedlings of genotypes belonging to Virginia bunch and Virginia runner groups showed more vigor than those belonging to Valencia and Spanish groups.

Deficiencies in membrane integrity of the aged seeds was visualized from conductrimetric analysis of leached electrolytes. The seeds stored under ambient condition showed considerable amount of electrolyte leakage. The rate of solute loss was slow in the beginning up to 6 months and increased sharply thereafter between 6-15 months, an observation that reasonably corresponds with the trend in loss of seed viability. Seed leachate measurement showed a conductivity of 1.180 mmho/cm in ICG 10035 while it was 0.249 mmho/cm in ICG 4906, demonstrating significant variation among the genotypes. Further, solute release was higher in the Valencia and Spanish groups as compared to Virginia bunch and runner groups. After ambient storage the amount of electrolyte leakage from shelled seed (kernel) was significantly more than from the unshelled seed (pod). Considerable loss in electrolyte leakage was also observed when the seeds were stored under medium-term condition.

A significant reduction was observed in the total lipid content of the seeds stored under ambient condition, the loss ranging from 1.4 to 3.6% depending on the genotypes. Reduction in phospholipid and glycolipid contents of the seeds was significant. There was no change in lipid content of groundnut

seeds stored under medium-term condition.

Significant changes in the fatty acid composition of the groundnut seeds were observed due to ageing consequent to storage. There was a decrease in the linoleic acid content in all the lipid fractions i.e., neutral, phospho- and glyco-lipids and an increase in O/L ratio. These changes were more pronounced following storage under ambient condition and much less when seeds were stored under medium-term condition. The results demonstrated that the effects of lipid peroxidation could be very important in seed deterioration of groundnut.

There were also changes in enzyme activities during storage evident from an increase in lipase activity and a decrease in peroxidase activity in the seeds stored under ambient condition, along with an increase in the acid and peroxide values. Such changes in enzyme activities were not detectable in seeds stored under medium-term condition. Other metabolic changes due to ageing of seeds while in storage included a decline in the protein content from 0.7 to 1.7 percent and an increase in the total sugar content from 1.3 to 4.7 percent. Such changes were not conspicuous when the seeds were stored under medium-term condition.

Comparisons between the effects of 4 different storage conditions viz., ambient (22-38°C, 44-80% RH), short-term (18°C, 30% RH), medium-term (4°C, 20% RH) and long-term (-20°C) showed that the loss of viability and seedling vigor, membrane damage, lipid peroxidation, enzyme activities and other metabolic changes, were severe when seeds were stored under ambient condition. Such damage was much lower in short-term and medium-term storage. Groundnut seeds stored under long-term condition failed to demonstrate any physiological or biochemical changes. The comparative values for the seeds stored under ambient, short-term and medium-term conditions

with respect to different parameters were as follows: seed viability - 58.0, 92.1 and 96.7 percent; electrical conductivity of seed leachates - 0.504, 0.206 and 0.176 mmho/cm; linoleic acid content of total lipid - 32.9, 34.3, and 34.6 percent; lipase activity - 2.41, 1.72 and 1.55 μ eq. of free fatty acid released/3 min assay; peroxidase activity - 0.193, 0.285 and 0.310 max. O.D./g fresh weight; phospholipid content - 1.88, 3.26 and 3.60 mg/g dry weight; and linoleic acid content of phospholipid fraction - 31.1, 33.0, and 33.3 percent.

When cultivated genotypes of groundnut were subjected to accelerated ageing at 40°C and 13.5 percent moisture content, the seeds lost complete viability by the 20th day of accelerated ageing. In general, the trend of various deteriorative processes observed in naturally aged seeds (under ambient storage conditions) was similar to that observed under accelerated ageing, except that in the latter process the rate of deterioration was very rapid.

Storage of seven wild species of *Arachis* (viz., *A. duranensis, A. batizocoi, A. monticola, A. triseminalis, A. cardenasii, A. paraguariensis* and *A. apressipila*) under ambient and medium-term conditions demonstrated that the extent of seed deterioration among the wild species was more than that observed in the cultivated types of groundnut stored under similar conditions. All the 7 wild species showed sharp decline in seed viability following storage, which ranged from 50 to 100% under ambient condition, and 28 to 44% under medium-term condition. In all these wild species, seed deterioration was also evidenced from reduction in oil content, decrease in linoleic acid content, decrease in protein content and increase in total soluble sugar content. These changes were observable under both ambient and medium-term storage conditions, being more pronounced under ambient condition.

From the various experiments it could be established that seeds of groundnut, the most important genetic resource, undergo ageing during storage

and shows various degrees of deterioration that affects the seed viability. The extent of ageing and consequent deterioration varies with the condition of storage, being acute under ambient condition and to a lesser degree when stored under short- and medium-term conditions. The loss of seed viability was more among the wild genotypes of groundnut as compared to the cultivated genotypes when subjected to identical storage condition. Seed deterioration as a result of ageing appears to be mainly due to membrane damage and lipid peroxidation. Any method of germplasm conservation, therefore, must aim to minimize, if not arrest these processes.

Chapter 7

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APPENDIX

Standard errors for the analysis of variance for results 4.1.1. of ambient storage

Physiological parameters

Source	Df	S.E.				
		Germination %	Shoot length (cm)	Hypocotyl length (cm)	Root length (cm)	Dry weight (g)
Storage (S)	4	0.441	0.043	0.035	0.081	0.027
Material (M)	1	0.279	0.027	0.022	0.051	0.017
Between group (Grp)	3	0.394	0.038	0.032	0.072	0.024
S X M	4	0.623	0.061	0.050	0.115	0.038
S X Grp	12	0.881	0.086	0.071	0.162	0.054
M X Grp	3	0.557	0.054	0.045	0.102	0.034
Bunch (B)	4	0.881	0.086	0.071	0.162	0.054
Runner (R)	4	0.881	0.086	0.071	0.162	0.054
Valencia (V)	4	0.881	0.086	0.071	0.162	0.054
Spanish (Sp)	4	0.881	0.086	0.071	0.162	0.054
S X Sp	16	1.971	0.194	0.160	0.363	0.122
M X Sp	4	1.247	0.122	0.101	0.230	0.077
S X V	16	1.971	0.194	0.160	0.363	0.122
M X V	4	1.247	0.122	0.101	0.230	0.077
S X B	16	1.971	0.194	0.160	0.363	0.122
M X B	4	1.247	0.122	0.101	0.230	0.077
S X R	16	1.971	0.194	0.160	0.363	0.122
M X R	4	1.247	0.122	0.101	0.230	0.077
S X M X Sp	16	2.787	0.274	0.227	0.514	0.172
S X M X V	16	2.787	0.274	0.227	0.514	0.172
S X M X B	16	2.787	0.274	0.227	0.514	0.172
S X M X R	16	2.787	0.274	0.227	0.514	0.172

Biochemical parameters

Source	Df	S.E.				
		Oil %	Protein(%)	Soluble sugars %	EC mmho/cm	Linoleic acid %
Storage (S)	4	0.047	0.064	0.048	0.0043	0.048
Material (M)	1	0.030	0.040	0.030	0.0027	0.030
Between group (Grp)	3	0.042	0.057	0.043	0.0039	0.043
S X M	4	0.067	0.080	0.068	0.0061	0.068
S X Grp	12	0.095	0.128	0.097	0.0087	0.096
M X Grp	3	0.060	0.081	0.081	0.0055	0.061
Bunch (B)	4	0.095	0.128	0.097	0.0087	0.096
Runner (R)	4	0.095	0.128	0.097	0.0087	0.096
Valencia (V)	4	0.095	0.128	0.097	0.0087	0.096
Spanish (Sp)	4	0.095	0.128	0.097	0.0087	0.096
S X Sp	16	0.212	0.287	0.217	0.0195	0.216
M X Sp	4	0.134	0.181	0.137	0.0123	0.136
S X V	16	0.212	0.287	0.217	0.0195	0.216
M X V	4	0.134	0.181	0.137	0.0123	0.136
S X B	16	0.212	0.287	0.217	0.0195	0.216
M X B	4	0.134	0.181	0.137	0.0123	0.136
S X R	16	0.212	0.287	0.217	0.0195	0.216
M X R	4	0.134	0.181	0.137	0.0123	0.136
S X M X Sp	16	0.300	0.406	0.307	0.0276	0.305
S X M X V	16	0.300	0.406	0.307	0.0276	0.305
S X M X B	16	0.300	0.406	0.307	0.0276	0.305
S X M X R	16	0.300	0.406	0.307	0.0276	0.305

Standard errors for the analysis of variance for results 4.1.1. of medium-term storage

Physiological parameters

Source	Df	S.E.				
		Germination %	Shoot length (cm)	Hypocotyl length (cm)	Root length (cm)	Dry weight (g)
Storage (S)	4	0.213	0.061	0.025	0.098	0.037
Material (M)	1	0.135	0.038	0.016	0.062	0.023
Between group (Grp)	3	0.191	0.054	0.022	0.087	0.033
S X M	4	0.302	0.086	0.035	0.139	0.052
S X Grp	12	0.427	0.122	0.050	0.196	0.074
M X Grp	3	0.270	0.077	0.032	0.124	0.046
Bunch (B)	4	0.427	0.122	0.050	0.196	0.074
Runner (R)	4	0.427	0.122	0.050	0.196	0.074
Valencia (V)	4	0.427	0.122	0.050	0.196	0.074
Spanish (Sp)	4	0.427	0.122	0.050	0.196	0.074
S X Sp	16	0.955	0.272	0.113	0.439	0.166
M X Sp	4	0.604	0.172	0.071	0.277	0.105
S X V	16	0.955	0.272	0.113	0.439	0.166
M X V	4	0.604	0.172	0.071	0.277	0.105
S X B	16	0.955	0.272	0.133	0.439	0.166
M X B	4	0.604	0.172	0.071	0.277	0.105
S X R	16	0.955	0.272	0.133	0.439	0.166
M X R	4	0.604	0.172	0.071	0.277	0.105
S X M X Sp	16	1.351	0.385	0.160	0.621	0.234
S X M X V	16	1.351	0.385	0.160	0.621	0.234
S X M X B	16	1.351	0.385	0.160	0.621	0.234
S X M X R	16	1.351	0.385	0.160	0.621	0.234

Biochemical parameters

Source	Df	S.E.				
		Oil %	Protein(%)	Soluble sugars (%)	EC mmho/cm	Linoleic acid (%)
Storage (S)	4	0.053	0.042	0.049	0.0032	0.053
Material (M)	1	0.033	0.026	0.031	0.0020	0.033
Between group (Grp)	3	0.047	0.037	0.043	0.0029	0.047
S X M	4	0.075	0.059	0.069	0.0046	0.075
S X Grp	12	0.107	0.084	0.098	0.0065	0.106
M X Grp	3	0.067	0.053	0.062	0.0041	0.067
Bunch (B)	4	0.107	0.084	0.098	0.0065	0.106
Runner (R)	4	0.107	0.084	0.098	0.0065	0.106
Valencia (V)	4	0.107	0.084	0.098	0.0065	0.106
Spanish (Sp)	4	0.107	0.084	0.098	0.0065	0.106
S X Sp	16	0.239	0.189	0.219	0.0145	0.237
M X Sp	4	0.151	0.119	0.138	0.0092	0.149
S X V	16	0.239	0.189	0.219	0.0145	0.237
M X V	4	0.151	0.119	0.138	0.0092	0.149
S X B	16	0.239	0.189	0.219	0.0145	0.237
M X B	4	0.151	0.119	0.138	0.0092	0.149
S X R	16	0.239	0.189	0.219	0.0145	0.237
M X R	4	0.151	0.119	0.138	0.0092	0.149
S X M X Sp	16	0.339	0.267	0.310	0.0206	0.335
S X M X V	16	0.339	0.267	0.310	0.0206	0.335
S X M X B	16	0.339	0.267	0.310	0.0206	0.335
S X M X R	16	0.339	0.267	0.310	0.0206	0.335

Standard errors for the analysis of variance for results 4.1.2. of four storages

Physiological parameters

		Germination %	Shoot length (cm)	Hypocotyl length (cm)	Root length (cm)	Dry weight (g)
Storage (S)	4	0.284	0.066	0.032	0.063	0.036
Temp. (T)	3	0.254	0.059	0.028	0.056	0.032
Material (M)	1	0.179	0.042	0.020	0.040	0.022
Genotype (G)	3	0.254	0.059	0.028	0.056	0.032
S X T	12	0.567	0.133	0.064	0.126	0.072
S X M	4	0.401	0.094	0.045	0.089	0.051
T X M	3	0.359	0.084	0.040	0.080	0.045
S X G	12	0.567	0.133	0.064	0.126	0.072
T X G	9	0.507	0.119	0.057	0.113	0.064
M X G	3	0.359	0.084	0.040	0.080	0.045
S X T X M	12	0.802	0.188	0.090	0.179	0.102
S X T X G	36	1.134	0.266	0.128	0.253	0.144
S X M X G	12	0.802	0.188	0.090	0.179	0.102
T X M X G	9	0.802	0.188	0.090	0.179	0.102

Biochemical parameters

Source	Df	S.E.				
		Oil %	Protein(%)	Soluble sugars %	EC mmho/cm	Linoleic acid %
Storage (S)	4	0.043	0.047	0.057	0.0026	0.046
Temp. (T)	3	0.038	0.042	0.051	0.0024	0.041
Material (M)	1	0.027	0.030	0.036	0.0017	0.029
Genotype (G)	3	0.038	0.042	0.051	0.0024	0.041
S X T	12	0.086	0.095	0.114	0.0053	0.091
S X M	4	0.061	0.067	0.081	0.0037	0.065
T X M	3	0.054	0.060	0.072	0.0033	0.058
S X G	12	0.086	0.095	0.114	0.0053	0.091
T X G	9	0.077	0.085	0.102	0.0048	0.082
M X G	3	0.054	0.060	0.072	0.0033	0.058
S X T X M	12	0.122	0.134	0.162	0.0075	0.130
S X T X G	36	0.173	0.190	0.229	0.0107	0.183
S X M X G	12	0.122	0.134	0.162	0.0075	0.130
T X M X G	9	0.122	0.134	0.162	0.0075	0.130