BIOCHEMICAL AND MOLECULAR CHANGES IN AGED PEANUT (ARACHIS HYPOGAEA L.) SEEDS

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KEYWORDS

Seed ageing
Enzyme activity
Protein profiles
DNA integrity
Peanut

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Laboratory experiment was conducted at the Department of Seed Science and Technology, University of Agricultural Sciences, Raichur during 2011-2012. The seeds were subjected to natural and accelerated ageing treatments viz., T₁ - control, T₂ - 3 months (Natural Ageing), T₃ - 6 months (Natural Ageing), T₄ - 9 months (Natural Ageing), T₅ - 3 days (Accelerated Ageing), T₆ - 6 days (Accelerated Ageing), T₇ - 9 days (Accelerated Ageing). The results revealed that, seeds aged naturally up to six months and 3 days accelerated aged seeds (3 DAA) maintained same viability and seed vigour as that of fresh seeds. However, increase in ageing duration reduced all the seed quality parameters in both naturally aged, 6 DAA and 9 DAA seeds. Further, protein profiles showed alteration in their number of bands and at molecular level there was loss in DNA integrity in both naturally and artificially aged seeds.

ABSTRACT

INTRODUCTION

Groundnut (Arachis hypogaea L.) “King of oilseed crops”, is believed to be native of Brazil (South America). It was introduced in India during first half of the sixteenth century. It belongs to the family Leguminoceae. It is a valuable crop planted in dry areas of Asia, Africa, Central and South America, Australia and Caribbean in view of its economic, food and nutritional value. It is the 13th most important food crop, 4th most important source of edible oil and 3rd most important source of vegetable protein in the world. Groundnut posses high oil content (44-56%) and protein (22-30%) (Holaday and Pearson, 1974) and is also a valuable source of vitamins E, K and B. It is a richest plant source of thiamine and niacin, which are low in cereals. Groundnut seed has short life and looses viability quickly under ambient condition. Several factors affect the self life of the seed; among them infections by seed borne fungi is one of the factors for quick loss of viability of a seed (Urosevic, 1964). Ageing in groundnut seed leads to increased lipid peroxidation, decreased activities of several free radical and peroxide scavenging enzymes (Rao et al., 2006). Groundnut seeds are more sensitive to storage conditions like high temperature; high seed moisture content, light exposure. The qualitative loss of seed can be attributed to biochemical changes in protein, carbohydrates, fatty acids and vitamins (Girish et al., 1972). In recent years a rapid ageing method where seeds are subjected continuously to high temperature and in saturating humidity has been recognized as an important technique in estimating the rate of deterioration in seed storage. The rate of ageing mainly depends on genotype, moisture and temperature. In rapid and slow ageing (natural ageing), the pattern of deterioration preceding the death is the same whether seed survives for few hours or decades. Accelerated ageing (AA) is an important procedure for understanding the events that lead to the loss of seed viability. AA damages DNA and mRNA, causes biochemical deterioration of the stored material and reduces the vigour of seedling and early plantlet development shortly after germination. The consequences of ageing on cooking properties, digestibility and formation of resistant starch in seeds have also been investigated. However, little is known about the cellular alterations, storage mobilization and vigour during early plantlet growth. At a cellular level, aged seeds show a significant increase in the number and a decrease in the size of starch grains, as well as rupture of the cell walls and membrane-bound organelles, including protein bodies. However, the information related to sub cellular changes due to ageing under natural ageing/ accelerated ageing condition is much scanty. The metabolic defects that occur due to these changes can be rectified to the extent possible by the technique of seed priming; pre-soaking seeds in osmotic solutions has been demonstrated to improve the viability and vigour of aged seeds in various crops (Bhanuprakash et al., 2010). Therefore, the present investigation was undertaken with a view to understand the biochemical and molecular changes in aged seeds of peanut.

MATERIALS AND METHODS

Laboratory experiment was conducted at the Department of Seed Science and
Technology, University of Agricultural Sciences, Raichur during 2011-2012. The seeds were subjected to natural and accelerated ageing treatments viz., T₀ - control, T₁ - 3 months (Natural Ageing), T₁ - 6 months (Natural Ageing), T₁ - 9 months (Natural Ageing), T₂ - 3 days (Accelerated Ageing), T₂ - 6 days (Accelerated Ageing), T₂ - 9 days (Accelerated Ageing). The observations on seed quality parameters such as electrical conductivity, dehydrogenase activity, membrane injury index, α-amylase activity, total soluble protein content, total soluble sugars content and electrophoretic analysis of soluble seed protein, in addition the biochemical observations such as, peroxidase activity, protein expression and isoenzyme-esterase expression were recorded. The observations like molecular changes with respect to DNA integrity was also noted. The data collected from experiment were analyzed statistically by the procedure prescribed by Sundarrajan et al. (1972).

RESULTS AND DISCUSSION

Ageing is a common phenomenon in all the living entities and as a result with increase in ageing progressive decline in all the vital events occur culminating to death at the end. Groundnut seeds are classified as very poor storer and storage of groundnut seeds under ambient hot and humid conditions is very problematic since these conditions deteriorate seed quality faster. Many biochemical and molecular changes are linked to the process of seed deterioration and the biochemical changes during accelerated ageing were the same as those in natural ageing with only difference being the rate at which they occur.

In the present investigation, significant changes in biochemical parameters were observed due to ageing. Changes in membrane permeability, enzyme levels, protein stability, nucleic acid integrity, ATP synthesis due to ageing were reported in various crops (Mc Donald, 1999). In the present findings all the ageing treatments resulted in membrane damage as evident from higher solute leakage in aged seeds (Table 1). The fresh seeds recorded significant lower (0.315) electrical conductivity when compared to all the natural aging treatments (Table 1), where in the values went on increasing and the maximum value (0.987) was recorded at 9 months NA. Similarly the EC values also increased from 3 days (0.569) to 9 days (1.905). Estimation of electrical conductivity of seed leachate was considered as a promising parameter in seed quality studies (Bewely and Black, 1994). The outer and inner membranes appeared as potential targets for desiccation and ageing injuries in seeds, seed ageing may cause activity alteration of low plasma membrane ATPase activity (Bardel et al., 2002). Significantly highest dehydrogenase enzyme activity was observed in the fresh seeds (0.756) and the values went on decreasing throughout the period of natural aging and reached the minimum (0.375) at 9 months of natural aging. Similarly the enzymatic activity was significantly highest in fresh seeds (0.756) and reached the lowest (0.0 or nil) at 9 days of accelerated aging. Many enzymes remain active after all viability lost, however the dehydrogenase are one group of enzymes that were shown to be directly related with loss of viability (Pandey, 1989). Reduction of 2,3,5-triphenyl–tetrazolium chloride or bromide to red coloured formazan by dehydrogenase enzymes is taken as an indication of living tissue, as ageing increases the activity of dehydrogenase is reduced. The membrane injury index significantly differed among the fresh, NA and AA seeds (Table 1). The lowest membrane injury index (27.83%) was recorded in fresh seed and NA- 3 months (28.43%) followed by NA-6 months (30.33%) and AA-3 days (29.00%). Whereas, highest membrane injury index (75.50%) was observed in AA-9 days treatment followed by AA-6 days (59.37%) and NA-9 months (34.00%). The membrane damage evident from solute leakage was more in accelerated ageing as compared to natural ageing. Loss of seed vigour and viability is associated with deterioration of membrane properties (Priestly, 1986).

Significantly highest α-amylase activity was recorded in fresh seeds (61.70) and the values went on decreasing over the period of natural aging and reached the lowest values (30.47) at 9 months after storage. Similar to natural ageing, accelerated ageing also showed lower enzyme activity. There was reduction in enzyme activity to the tune of 16.32, 46.24 and 100 per cent respectively over control due to 3, 6 and 9 days accelerated ageing. This might be due to changes in phospholipid and membrane damage due to peroxidative changes associated with ageing. These changes resulted into rapid loss of viability from 99% to 2%. Ultrastructure examination confirms that membranes undergo deteriorative changes with increasing seed age. These changes include abnormalities of mitochondrial and plastid inner and outer membranes, lobbing of the nuclear envelope, fragmentation or loss of endoplasmic reticulum and golgi bodies, dissolution of the bounding membrane of vacuoles and protein bodies, fusion of lipid droplets to form larger bodies or irregular pools, discontinuities in the plasmalemma and its withdrawal from the cell wall, occasional appearance of follicular material in the extra protoplasmic space. These deteriorative changes would have an impact on the DNA, RNA and protein synthetic system (Villiers and Edgecumbe, 1975). Increase in ageing duration decreased the α-amylase activity and dehydrogenase activity in onion seeds (Bhanuprakash et al, 2006). Since enzymes are also proteins, cross linking would seriously disturb their functioning. So it has direct correlation between loss of viability and decline in enzyme activity (Abdul Baki and Anderson, 1972). The highest total soluble proteins among natural ageing treatments was observed in NA-9 months (168.33) followed by NA-6 months (161.67), NA-3 months (158.67) and fresh seeds (155.53). In case of accelerated ageing condition, treatments AA-3 days (159.0) AA-6 days (170.0) and AA-9 days (200.67) were significant from the fresh seeds. Membrane degradation was shown as the earliest event of seed deterioration. Functional membranes are a prequisite for all viability and efficient metabolism. Membrane organization was slower or may be prevented as a consequence of ageing and death leading to loss of more electrolytes (Bhanuprakash et al., 2010). The total soluble sugars followed similar trend as that of proteins. Higher TSS was noticed in aged seeds. In fresh seeds, the TSS was 105.73 μg/mL, where in natural aged 3, 6 and 9 months, it was 125.33, 136.50 and 144.10 μg/mL respectively. Accelerated ageing also affected the TSS content in similar fashion. It was 131.27, 168.30 and 211.0 in 3, 6 and 9 days accelerated aged seeds respectively. Compared with AA conditions, the NA condition has given less total
alteration in protein profile in terms of number and intensity. Aged seeds exhibited lesser number of protein bands compared to the fresh seeds. Proteins in fresh seeds disappeared upon ageing. With respect to natural ageing treatments, the numbers of bands were decreased as prolongation in ageing period. Band 12 (Rm Value: 0.55) was absent in 9 months natural aged seeds. Band number 23 was absent at Rm value 0.90 in case of 6 months and 9 months natural aged treatments as compared to control. Similar to natural ageing, accelerated ageing also affected protein profile. Loss of bands 5, 6, 12 and 23 were noticed at Rm value 0.31, 0.35, 0.55 and 0.90 respectively in 6 days and 9 days accelerated aged seeds as compared to control. Since, specific bands are characteristic of particular ageing treatment having a different seed viability levels, these profiles can be used as criteria in assessing the seed quality. Electrophoretic variations in proteins can be used to assay the amount of deterioration of the seed during ageing. Machado et al. (2001) also reported the changes in banding pattern of protein profiles of naturally and artificially aged French bean seeds. Seed ageing affected esterase isozyme profile. Esterases are necessary for the breakdown of storage lipids in to fatty acid moieties, which provide the biosynthetic energy necessary for seedling growth, alteration in isoenzyme profiles noticed in aged seeds when compared to fresh indicate that the pattern of enzyme polymorphism changes during seed ageing. These amply demonstrate that there is qualitative change in enzymes of aged seeds and these changes leads to seed deterioration. Seeds aged for 3 months, 6 months and 9 months showed alteration in esterase profiles in terms of number and intensity. Absence of polypeptide at 0.54 Rm value was noticed in 6 months and 9 months natural aged treatments when compared to control. Similarly at 0.43 Rm value, 9 months old seeds lost a band as compared to control. In similar to natural ageing, accelerated ageing also affected esterase isozyme profile. Loss of one isoform of isozyme was noticed at 0.15 Rm value in 6 days and 9 days accelerated aged seeds as compared to rest of the treatments. Whereas, three bands were absent at Rm value 0.54, 0.60 and 0.75 respectively in case of 9 days AA seeds. Thus the present results revealed the loss of characteristics isozyme profile in aged seeds. This variation may be one of the reasons for loss of seed viability and vigour in aged seeds. In addition to above, heat shock proteins appeared in prolonged aged seeds. Many seeds exhibits increased heat shock proteins following brief exposures to high temperatures. This induced thermal

<table>
<thead>
<tr>
<th>Treatments</th>
<th>EC(dSm⁻¹)</th>
<th>DH(OD values)</th>
<th>MII (%)</th>
<th>Am act(μg starch hydrolysed/mL/min)</th>
<th>TSP (μg/mL)</th>
<th>TSS(μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>0.315</td>
<td>0.756</td>
<td>27.83</td>
<td>61.70</td>
<td>155.53</td>
<td>105.73</td>
</tr>
<tr>
<td>T₂</td>
<td>0.556</td>
<td>0.601</td>
<td>30.33</td>
<td>58.80</td>
<td>158.67</td>
<td>125.33</td>
</tr>
<tr>
<td>T₃</td>
<td>0.728</td>
<td>0.480</td>
<td>34.00</td>
<td>44.83</td>
<td>161.67</td>
<td>136.50</td>
</tr>
<tr>
<td>T₄</td>
<td>0.987</td>
<td>0.375</td>
<td>30.00</td>
<td>30.47</td>
<td>168.33</td>
<td>144.10</td>
</tr>
<tr>
<td>T₅</td>
<td>0.569</td>
<td>0.521</td>
<td>29.00</td>
<td>51.63</td>
<td>159.00</td>
<td>131.27</td>
</tr>
<tr>
<td>T₆</td>
<td>1.135</td>
<td>0.181</td>
<td>59.37</td>
<td>33.17</td>
<td>170.00</td>
<td>168.50</td>
</tr>
<tr>
<td>T₇</td>
<td>1.905</td>
<td>0</td>
<td>75.50</td>
<td>0.00</td>
<td>200.67</td>
<td>211.00</td>
</tr>
<tr>
<td>S.Em±</td>
<td>0.03</td>
<td>0.04</td>
<td>0.89</td>
<td>0.61</td>
<td>0.8</td>
<td>0.37</td>
</tr>
<tr>
<td>C.D. @ 5%</td>
<td>0.10</td>
<td>0.12</td>
<td>2.71</td>
<td>1.86</td>
<td>2.44</td>
<td>1.13</td>
</tr>
</tbody>
</table>

- Fresh Seeds; T₁ - Natural Ageing 3 months; T₂ - Natural Ageing 6 months; T₃ - Natural ageing 9 month T₄ - 3 Days Accelerated Ageing T₅ - 6 days Accelerated Ageing; T₆ - 9 days Accelerated Ageing; EC-Electrical Conductivity DH-Dehydrogenase activity MII-Membrane Injury Index; Am Act-Amylase activity TSP-Total soluble protein TSS-Total soluble sugars

Plate 1: Protein profiles of fresh and aged seeds in groundnut var. R.- 2001 – 2 in SDS Page

Plate 2: Peroxidase profiles of fresh and aged seeds in groundnut var. R.- 2001 – 2 in Native Page